The epidemiology of Johne's disease in New Zealand farmed deer, including validation of abattoir-based surveillance

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Abstract

Johne's disease (JD), a fatal granulomatous enteritis predominately affecting ruminants, is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and has become a potentially serious economic risk to New Zealand deer farmers. This research investigates the incidence of clinical JD on affected deer farms, along with potential risk factors that may be adopted by farmers for its control. Additionally, research was undertaken to establish a national abattoir surveillance mechanism for MAP, and to validate criteria used for that purpose.

Personal interview of 174 farmers in a 2005/2006 national case-control study, followed by a 2007 longitudinal postal questionnaire, allowed characterization of clinical JD at the herd- and age-class levels and identification of risk factors significantly associated with herd-level MAP infection and farmer-diagnosed clinical JD in young deer. The mean within-herd clinical JD annual incidence rate was 1%, with age-class incidence rates highest (2.0%) in yearling hinds and stags. The proportion of herds affected with clinical JD increased from 2005 to 2007, showed a seasonal trend, and was higher in the South versus North Island. Species other than deer, specifically beef cattle and sheep, were variably associated with herd-level MAP infection and clinical JD in young deer. As the first investigation of herd-level MAP and clinical JD in farmed deer, this research considerably increased our knowledge specific to this species. This information may inform control measures and direct further research aimed at reducing the prevalence and transmission of MAP and development of clinical JD in farmed deer.

Another research aim was to validate abattoir MAP surveillance in deer through meat inspector identification of 'abnormal' lymph nodes (LN). A pilot cross-sectional study found 94.6% of 'abnormal' LN were MAP-positive through culture or histopathological examination. A 55 mm circumference cut-point defining an 'abnormal' LN was then established and the sensitivity, specificity and level of agreement of inspector detection were estimated at 13.3%, 99.9% and 'fair' ($\kappa = 0.32$), respectively. As an adjunct to this validation, the prevalence of and risk factors associated with seven histopathological features of grossly 'normal' LN from herds classified at 'low' and 'high' MAP risk were described. This research allows confident and informed use of

conclusions drawn from the national abattoir-based surveillance scheme. However, animal-level prevalence is currently underestimated with use of 'abnormal' LN as the sole criterion and further inspector training is required.

The research presented in this thesis provides the foundation for future examination of MAP and clinical JD in farmed deer, its control and surveillance.

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Seven years ago, when it all began, I could have never imagined the challenging journey that I was about to undertake. One advantage of spending such a (statistically?) significant period of time completing a PhD is that I can say, with complete certainty, that it is a life-changing experience. Please forgive me if I claim the right to thank the following with special enthusiasm as they have largely stuck with me for seven, long years. My achievement is not held in these pages, but in the friendships I have made.

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As lastly, to my family. Louisa Mary Alcott said 'We all have our own life to pursue, our own kind of dream to be weaving. And we all have some power to make wishes come true, as long as we keep believing.' You believed in me – thank you.

Declaration

Each chapter in this thesis was formatted in the style required of the journal to which it was submitted at the date of thesis submission. As a result, there is some repetition, particularly in the methods, and there are some small inconsistencies with style and format between chapters.

Chapter 7 has been published as a Short Communication:

Hunnam JC, Wilson PR, Heuer C, Mackintosh CG, West DM, Clark RG. Histopathology of Grossly Normal Mesenteric Lymph Nodes of New Zealand Farmed Red Deer (*Cervus elaphus*) Including Identification of Lipopigment. *Veterinary Pathology*. 48(2): 525-529, 2011.

The full, published article is reproduced in Appendix 1.

All work contained herein is original and has not been used for the award of any other degree. All co-authors to all papers have been acknowledged.

Nomenclature

AHB	Animal Health Board
bTb	Bovine tuberculosis
CCT	Comparative cervical test
CFU	Colony Forming Units
CI	Confidence interval
DFA	Deer fenced area
DINZ	Deer Industry New Zealand
DSP	Deer slaughter premise
ELISA	Enzyme-linked immunosorbent assay
H & E	Haematoxylin & Eosin
HSe	Herd-level sensitivity
HSp	Herd-level specificity
IFC	Individual faecal culture
JD	Johne's disease
JML	Johne's Management Limited
JRG	Johne's Research Group
LN	Lymph node
LRP	Lateral retropharyngeal
MAP	*Mycobacterium avium* subsp. *paratuberculosis*
MCF	Malignant Catarrhal Fever
MCT	Mid-cervical test
MLN	Mesenteric lymph node
MRP	Medial retropharyngeal
OR	Odds Ratio
PAS	Periodic Acid-Shift
PCR	Polymerase chain reaction
PFC	Pooled faecal culture
RR	Risk ratio
Se	Sensitivity
SOTD	Species Other Than Deer
Sp	Specificity
SU	Stock Units
TC	Tissue culture
ZN	Ziehl-Neelsen

List of Publications

2005:

Glossop, JC. The epidemiological investigation of Johne's disease in deer. In: *Proceedings of a Deer Course for Veterinarians, Deer Branch of the New Zealand Veterinary Association*. Pp 70-72, 2005.

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Preface

“If” – Rudyard Kipling

If you can keep your head when all about you
Are losing theirs and blaming it on you;
If you can trust yourself when all men doubt you,
But make allowance for their doubting too;
If you can wait and not be tired by waiting,
Or, being lied about, don't deal in lies,
Or, being hated, don't give way to hating,
And yet don't look too good, nor talk too wise;

If you can dream - and not make dreams your master;
If you can think - and not make thoughts your aim;
If you can meet with triumph and disaster
And treat those two imposters just the same;
If you can bear to hear the truth you've spoken
Twisted by knaves to make a trap for fools,
Or watch the things you gave your life to broken,
And stoop and build 'em up with wornout tools;

If you can make one heap of all your winnings
And risk it on one turn of pitch-and-toss,
And lose, and start again at your beginnings
And never breathe a word about your loss;
If you can force your heart and nerve and sinew
To serve your turn long after they are gone,
And so hold on when there is nothing in you
Except the Will which says to them: "Hold on";

If you can talk with crowds and keep your virtue,
Or walk with kings - nor lose the common touch;
If neither foes nor loving friends can hurt you;
If all men count with you, but none too much;
If you can fill the unforgiving minute
With sixty seconds' worth of distance run -
Yours is the Earth and everything that's in it,
And - which is more - you'll be a Man my son!

CHAPTER 1: Review of Johne's disease in deer: Occurrence, epidemiology, clinical disease, pathology, economic cost, surveillance and potential risk factors

1.1 Introduction

Johne's disease (JD, paratuberculosis) is a fatal granulomatous enteritis and lymphadenitis affecting domestic and wild ruminants, caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). The clinical stage of disease ('clinical JD') has become a potentially serious economic burden to a number of New Zealand deer farmers, due to an apparent increasing prevalence since the 1980s, particularly in young deer. Moreover, as lymphadenitis typical of MAP infection in slaughtered deer is difficult to distinguish from pathology due to infection with *Mycobacterium bovis* (*M. bovis)*, the causative agent of bovine tuberculosis (bTb), costly diagnostic sampling and testing has been required to differentiate the two bacteria (Anonymous 2005b; Griffin et al 2006b). Infection with MAP also reduces the specificity of tests for *M. bovis* in deer (Mackintosh and Wilson 2003; Mackintosh et al 2004a; Bell 2006). Despite this, the epidemiology of MAP infection and clinical JD has not been examined at the herd-level in any farmed deer industry, including New Zealand and this, therefore, represents a primary thesis aim. The other is to validate abattoir-based surveillance for MAP in commercially slaughtered deer in New Zealand through the identification of 'abnormal' lymph nodes (LN).

In cattle, the stages of JD from initial infection to advanced clinical disease have been outlined by Whitlock and Buergelt (1996) and described in detail in section 1.3.2 of this review. In short, 'MAP' or 'MAP infection' refer to infection of the host ruminant organism with the bacteria, regardless of the disease state, whilst animals with 'subclinical JD' and 'clinical JD' are those MAP-infected animals that are free or affected by clinical signs of disease, respectively.

Although the epidemiology of MAP infection and clinical JD in sheep and dairy cattle, at the herd-level, has been comprehensively reviewed (Harris and Barletta 2001; Collins 2003a; Nielsen and Toft 2009), comparatively little has been published regarding farmed deer. This chapter reviews available literature on the occurrence, clinical and subclinical signs, typical gross and histopathological changes, economic cost and risk factors associated with the presence of MAP infection and/or clinical JD, with particular reference to farmed deer. The use of abattoir-based surveillance for MAP in a number of domestic species internationally is reviewed and the potential for a similar scheme in commercially slaughtered New Zealand farmed deer is outlined. Relevant literature from sheep and cattle is included briefly for comparison where appropriate. While this paper reviews literature from international sources, its primary focus is that of relevance to JD in farmed deer in New Zealand.

1.2 History of deer farming

1.2.1 Worldwide

The first evidence of deer farming was found within a tomb of the Han Dynasty in China (202 B.C. to A.D. 200) where three deer carcasses were buried with other domestic animals (Kao 1973). A scroll found within the tomb contained the first known records of the prescription of deer products for treatment of medical conditions (*pênts'ao*), ranging from boils and carbuncles to epilepsy (Kong and But 1985). In the 9th century, the Sami people of northern Scandinavia herded reindeer for needs ranging from meat to tallow for light (Putman 1988). In Europe, deer farming can be traced back to the Ancient Greek and Roman civilisations (Fennessy and Taylor 1989) and deer parks became established throughout mediaeval Europe for hunting for sport and to provide a consistent supply of venison to the owners, with elements resembling modern farming (Putman 1988).

Deer farms are now located throughout Europe, Asia and Australasia, including Denmark, Germany, China, the United Kingdom, Australia and New Zealand. Based on the latest published estimates (Table 1.1), there are more deer farmed or kept within fences in New Zealand (>1,100,000) than in any other country in the world.

Table 1.1 Current published estimates of number of deer farms and farmed deer, by country.

Country	Year estimated	No. Deer farms	No. Deer	Average herd size	Reference
New Zealand	2010	3,000	1,123,600	374	(Anonymous 2010b)
China	1998	Unknown	500,000	Unknown	(Anonymous 2007b)
Russia	1998	Unknown	400,000	Unknown	(Anonymous 2007b)
USA	1998	Unknown	250,000	Unknown	(Anonymous 2007b)
Australia	1998	1,200	200,000*	167	(Anonymous 2007b)
Korea	1993	8,300	143,000	17	(Kwon 1993)
Canada	2003	1,931	118,941	62	(Anonymous 2003)
Germany	1998	4,500	104,000	23	(Anonymous 2007b)
Mauritius	2005	60	75,000	**	(Ramchurn 2005)
France	1998	907	58,000	64	(Anonymous 2007b)
Austria	1999	1,950	39,371	20	(Vogelmayer 1999)
United Kingdom	2003	4,500	36,000	8	(Anonymous 2003)
Taiwan	1998	Unknown	36,000	Unknown	(Anonymous 2007b)
Denmark	1998	650	31,000	48	(Anonymous 2007b)
Sweden	2005	370	20,000	54	(Thoen et al 2006)
Ireland	2008	230	Unknown	Unknown	(Anonymous 2008a)
Poland	2008	50	2,750	55	(Janiszewski et al 2008)
Argentina	2008	44	17,000	386	(Mereb 2008b)

* Severe droughts since 1998 have markedly reduced the Australian farmed deer population.

** 15,000 deer are intensively farmed on 60 farms. The remainder are farmed extensively on ranches.

1.2.2 New Zealand deer farming

Of the ten deer species introduced into New Zealand by the late 19th century, seven, namely red deer (*Cervus elaphus*), elk (*Cervus elaphus canadensis* ssp. group), rusa (*Cervus timorensis*), sika (*Cervus nippon*), fallow (*Dama dama*), sambar (*Cervus unicolor*), and white-tailed deer (*Odocoileus virginianus*), established and currently maintain one or more wild populations (Challies 1985; Yerex 2001). The first deer to set foot on New Zealand soil was believed to be a red stag introduced into the Nelson Province in April 1851 (Caughley 1983) and, from the mid 1920s, over 850 red deer were transported from Britain to New Zealand (Chapman 1991). From 1981, this rate increased markedly with 883 deer imported over a five year period (Yerex and Spiers 1987). Red deer are highly adaptable and, as the mountain forests and lowland pastures of New Zealand provided an optimum habitat, wild populations in both the North and South Islands increased rapidly (Challies 1985). This population growth was aided by a ban on deer hunting until 1870 and a subsequent restriction of hunting to a short season of March to July with a limited number of hunting licenses. As a result, by the mid 1920s, the national wild deer population numbered in the hundreds of thousands.

In 1927, the Forest Service, in an attempt to turn 'the deer liability into an asset', encouraged a private firm to develop an overseas market for the export of red deer carcasses (Caughley 1983). However, this early experiment failed as most killing was done in localities far removed for easy transport to the freezing-works. In 1959, Henry Buchanan and Malcolm Forsyth developed a market for venison in the US and, by 1963, several companies had built game packing houses to supply the wild venison export market (Caughley 1983). A helicopter was first used to recover a deer shot in the hills around Wanaka in April 1963 and hunting on the ground quickly gave way to 'gunshipping' where helicopters were used as shooting platforms. Although the use of helicopters became highly profitable, the practice was dangerous, with all helicopters undertaking venison recovery in 1975 involved in accidents, a number of them fatal (Caughley 1983).

However, the harvesting of venison by helicopter proved too efficient to result in long term sustainability of the wild deer population (Yerex 2001). Even cessation of helicopter use for short periods was insufficient to guarantee consistent supply to the burgeoning venison market and an alternative supply of venison was urgently required. In 1963, a Parliamentary Select Committee had received the first submissions to permit the farming of deer (Yerex 2001). By 1968, despite strong opposition from deerstalkers, the Forest Service and catchment authorities, the committee had approved deer farming in principle. 'Rahana Station', north of Taupo, was the first deer farm to be licensed under the Noxious Animals in Captivity Regulations and Deer Farming Regulations (Moore et al 1985). The first official slaughter of farmed red deer was completed on that property in March 1970. Although the 'red tape' surrounding deer farm licensing was described as 'magnificent in concept', the number of farmed deer herds increased exponentially (Caughley 1983). By 1980, 1591 deer farms were operating in New Zealand, containing approximately 120,000 deer of which 85% were red, 14% fallow and the remaining sika, wapiti, wapiti-red hybrids, sambar, rusa or white-tail deer.

In the early years of deer farming, stock were typically sourced from the wild, with deer trapped in fenced enclosures and then shifted to the existing herd. However, it became apparent that the required numbers of breeding stock could not be met using this method. Consequently, helicopters were modified to allow the live capture of wild deer (Yerex 2001).

From the outset, deer farmers faced numerous challenges, including matching the nutritional needs of a natural browser with the intensive, pastoral systems typical of New Zealand farming. Management techniques traditionally used in cattle and sheep farming were found to be not always applicable to deer. Fences surrounding pastures previously grazed by sheep and cattle were increased in height to 1.9 metres and holding facilities and yards required innovative modifications to allow efficient deer handling. Despite an early assertion that red deer required little on-going maintenance and suffered from fewer health conditions relative to other livestock (Yerex and Spiers 1987), Audigé *et al.* (2001) provided evidence for a age-class mortality rate of up to 5.9%, due predominately, at that time, to misadventure, yersiniosis (weaner deer) and malignant catarrhal fever (MCF).

A red deer hind worth NZ$250 in 1976, rapidly increased in value to NZ$3,000 by 1979. By August 1980, New Zealand had 259 registered civilian helicopters, many of which were involved in live deer capture. By 2004, the number of deer farms had reached an all-time high of approximately 4,500 (Anonymous 2007a). However, by 2007, deer numbers had decreased to less than 1.4 million and this trend has continued with an estimated 3,000 farmers grazing deer in 2010, commonly as part of a diversified livestock portfolio with other species, such as sheep and cattle. Despite this, the deer farming industry continues to make a significant contribution to the New Zealand economy with over NZ$276 million of product exported, including venison, velvet and hides, in the 12 months ending June 2010 (Anonymous 2010a).

Table 1.2 illustrates the number of deer farmed, by region of New Zealand, in 1993, 1999 and 2007. Although North Island herds contained the highest percentage of national farmed deer until 1993, particularly in the Waikato, Hawkes Bay and Manawatu-Wanganui regions, by 2007, 69% of deer were farmed in the South Island (Anonymous 1999, 2007a).

Table 1.2 Number of deer farmed in New Zealand, by region, in 1993, 1999 and 2007, and the percentage difference (diff.) from 1993 to 2007.

Region	No. (% total) Deer (1993)[A]		No. (% total) Deer (1999)[A]		No. (% total) Deer (2007)[B]		diff. (1993-2007)
Northland	15,517	(1.4%)	27,577	(1.6%)	7,566	(0.5%)	-0.9%
Auckland	38,002	(3.4%)	29.346	(1.8%)	12,304	(0.9%)	-2.5%
Waikato	159,461	(14.9%)	153,651	(9.2%)	116,554	(8.3%)	-6.6%
Bay of Plenty	77,935	(7.2%)	71,618	(4.3%)	54,296	(3.9%)	-3.3%
Gisborne	18,976	(1.8%)	25,113	(1.5%)	26,694	(1.9%)	+0.1%
Hawke's Bay	96,037	(8.9%)	109,489	(6.5%)	88,408	(6.3%)	-2.6%
Taranaki	20,363	(1.9%)	14,427	(0.9%)	4,456	(0.3%)	-1.6%
Manawatu-Wanganui	104,163	(9.7%)	149,948	(8.9%)	103,908	(7.4%)	-2.3%
Wellington	25,800	(2.4%)	26,441	(1.6%)	15,985	(1.1%)	-1.3%
TOTAL North Island	**556,274**	**(51.6%)**	**607,589**	**(36.3%)**	**430,171**	**(30.8%)**	**-20.8%**
Tasman	29,668	(2.8%)	35,227	(2.1%)	20,632	(1.5%)	-1.3%
Nelson	694	(0.0%)	C		C		
Marlborough	17,603	(1.6%)	21,110	(1.3%)	C		
West Coast	28,168	(2.6%)	34,244	(2.0%)	41,755	(3.0%)	+0.4%
Canterbury	213,305	(19.8%)	432,737	(25.8%)	394,833	(28.3%)	+8.5%
Otago	70,951	(6.6%)	151,052	(9.0%)	188,103	(13.5%)	+6.9%
Southland	161,816	(15.0%)	393,371	(23.5%)	307,524	(22.0%)	+7.0%
TOTAL South Island	**522,205**	**(48.4%)**	**1,069,199**	**(63.6%)**	**965,852**	**(69.2%)**	**+20.8%**
TOTAL New Zealand	**1,078,479**		**1,676,788**		**1,396,023**		

[A] (Anonymous 1999)
[B] (Anonymous 2007a)
C = unavailable

Other deer species, in addition to red deer, have been imported into New Zealand, with varying levels of success as farmed animals (Yerex 2001). Since 1981, wapiti bulls have been imported for use as terminal sires with a substantial proportion of red deer herds now containing wapiti genetics. In 1980, over 14,000 European fallow deer (*Dama dama dama*) were farmed in New Zealand and, in 1982, fallow bucks were first slaughtered in an export licensed abattoir. Despite this, fallow deer have been relatively unsuccessful as a farmed breed (O'Neill 1981; Challies 1985). Although over 20,000 fallow deer were slaughtered in the mid 1990s, less than half that number were processed in 2000 (Asher 1985; Yerex 2001). The relatively low value of fallow as a farmed species has been compounded by the virtually negligible value of fallow velvet. A small number of Mesopotamian fallow deer (*Dama dama mesopotamica*) were also introduced in the 1990s, with stags used to hybridise with the European fallow in an attempt to increase body size. A small herd of pure Mesopotamian fallow deer remain at AgResearch Invermay in Mosgiel.

Sika deer were successfully introduced into New Zealand in 1905 from Woburn Abbey Park in England and, although a small number were slaughtered in the 1970s, this species was found to be difficult to handle and relatively costly to farm (Yerex 2001). Therefore, although sika are now the second most common wild deer species in New Zealand, with populations in the Kaimanawa, Ahimanawa and Kaweka ranges, they are farmed infrequently.

Wild populations of sambar deer are maintained in forested areas in the Horowhenua, Manawatu, Rangitikei and Wanganui districts and a number of companies advertise hunting safaris for sambar stags. The first rusa deer were introduced into the North Island in 1907 from New Caledonia (Long 2003) and a small wild population still exists in a narrow belt of country in the Urewera ranges. Père-David's deer (*Elaphurus davidianus*), which have been described as having the antlers of a deer, the neck of a camel, the tail of a horse and hooves like cattle, are called 'milu' or 'the four unlikes' by the Chinese. In the 19th century, the only Père-David's deer in existence were held by the Chinese emperor. Prior to slaughter of the emperor's herd during the Boxer Rebellion (1898-1901), Père David, a Jesuit priest, sent some live animals to Europe for exhibition. These animals were then collected into a single herd for breeding purposes at Woburn Park in the United Kingdom and deer derived from this herd are now located in zoos

throughout the world. In 1984/85, approximately 70 Père-David's deer were imported onto five New Zealand properties. However, within 6 months, half of the imported animals had died from MCF (Orr and Mackintosh 1988). The remaining Père-David's deer population of less than 40 animals are now quarantined on Mount Hutt station in the Canterbury region of the South Island (Pearse, T; *pers. comm.*).

1.3 Johne's disease

Although reports of livestock with clinical signs typical of JD have been published since the 1820s, the condition was not recognized until 1894 when Dr Heinrich Albert Johne described a 'peculiar case of tuberculosis' in a cow with chronic enteritis (Johne and Frothingham 1895). Diffuse thickening and corrugation of the intestinal wall was observed on post-mortem examination and found to contain acid-fast bacilli. Recognizing the disease was likely not tuberculosis, Bang (1906) later termed the condition 'Johne's disease'. In 1910, Twort provided evidence of a causal relationship between MAP and JD by applying Koch's postulates and reproducing the disease in experimentally infected cattle (Harris and Barletta 2001).

1.3.1 Biological characteristics of *Mycobacterium avium* subspecies *paratuberculosis*

Mycobacteria are aerobic, non-motile bacteria of the genus *Mycobacterium*. The high mycolic acid content of their cell walls is responsible for a characteristic staining pattern with Ziehl-Neelsen or Kinyoun's stains, of poor absorption followed by high retention. While some mycobacteria can cause disease in mammals, such as bovine tuberculosis (bTb; *Mycobacterium bovis*) and leprosy (*Mycobacterium leprae*), others colonize their hosts without adverse consequences (Ryan and Ray 2004). MAP, the etiological agent of JD, is a subspecies of *Mycobacterium avium* (*M. avium*) (Thorel et al 1990). MAP is a small (0.5 x 1.5 micron), weakly gram-positive, slow-growing, rod-shaped bacterium that is facultatively intracellular, believed to be able to multiply only within the macrophages of a susceptible host (Gay and Sherman 1992).

Although MAP DNA is 98% identical with that of *M. avium*, the subspecies can be differentiated through identification of multiple copies of a 1.4 Kb insertion sequence (*IS900*) using

polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) or pulsed-field gel electrophoresis (PFGE) (Thorel et al 1990; Stevenson et al 2002; Collins 2003a). Although MAP can also be differentiated through the use of a mycobactin dependency test which requires comparative culture in media with and without mycobactin (Clarke 1997), one study has reported MAP-positive cultures from clinically affected sheep without the aid of mycobactin (Aduriz et al 1995).

Over 20 genotypes of MAP have been isolated internationally, with grouping into three strains based on genotypic and phenotypic characteristics: Type I (S or ovine/sheep) strain; Type II (C or bovine/cattle) strain; and an intermediate Type III strain (Whittington et al 2011). Dohmann *et al.* (2003), using representational difference analysis (RDA-PCR), found the Type II strain has undergone more deletions and rearrangements than the Type I strain, indicating the latter may be an evolutionary intermediate between the former and *M. avium*. The Type III strain, identified from a single Canadian sheep, has a restriction pattern most similar to the Type I strain and is referred to as the 'sheep variant' strain (Collins et al 1990).

Traditionally, at the phenotypic level, MAP strains have been differentiated using several criteria, namely the degree of colony pigmentation (Stevenson et al 2002), ease of culture and degree of cell clumping in saline suspensions (Reddacliff et al 2003). The Type I strain has been found to be slow-growing on solid media, forming pigmented, smooth and uniform colonies (Stevenson et al 2002). In contrast, the Type II strain has been faster growing and forms non-pigmented, rough, non-uniform colonies. However, Whittington *et al.* (2011) recently indicated that previous difficulties observed in culture of Type I MAP may have been strongly influenced by the type of culture medium used, with an optimal medium of Modified Middlebrook medium 7H10 with mycobactin J (7H10 + MJ) now recommended.

Although exhibiting a strong host preference, MAP strains are not exclusively host-specific. For example, in Australasia, sheep are predominantly infected with the Type I strain, whereas in Europe, the Type II strain is more commonly isolated from sheep as well as from cattle and non-ruminant hosts (Whittington et al 2000c; Stevenson et al 2002; Whittington et al 2011). Sequencing of the entire Type II MAP genome (Li et al 2005) has allowed recent detailed

examination of the genetic diversity of MAP subtypes through novel molecular typing methods, in particular multilocus short sequence repeats (MLSSR), mycobacterial interspersed repetitive units (MIRU) and variable number tandom repeats (VNTR) (Amonsin et al 2004; Thibault et al 2007; Douarre et al 2011). Genotyping data is now permitting tracking of epidemiological parameters specific to a particular MAP genotype, including transmission and persistence (Douarre et al 2011). For example, in a recent study, van Hulzen *et al*. (2011) demonstrated that multiple MAP subtypes can co-exist within Dutch dairy herds.

Deer are susceptible to both the Type I and Type II strains of MAP. Seventeen of 20 MAP isolates identified in farmed deer from 1985 to 1991 and, more recently, 91 of 95 isolates identified from MAP-infected deer herds were of the Type II strain (de Lisle et al 1993; de Lisle et al 2006). However, these results may not be representative of the entire farmed deer population as they were based on potentially biased subpopulations of pathological LN sampled on suspicion for *M. bovis*. A structured, national, randomised typing study is required to determine the relative prevalence of each strain type. Preliminary results of a 2010/2011 national survey using pooled faecal culture has found 95% of MAP isolates from 63 deer herds located throughout New Zealand were of the Type II strain (Wilson, PR; *pers. comm.*).

Although both strains of MAP can infect deer, Type II appears to cause a higher early incidence and severity of grossly visible pathology at slaughter (Mackintosh et al 2007a) and higher immunological reactivity (O'Brien et al 2006) relative to Type I. However, as both Mackintosh *et al.* (2007) and O'Brien *et al.* (2006) utilised Type I and Type II inoculums sourced from a single, clinically affected Merino sheep and red deer, respectively, results may not be fully representative of the effects of each MAP substrain in deer. Therefore, research examining the relationship between a wider range of molecular subtypes of MAP and pathogenicity and immunological responses in deer is needed. Moreover, longitudinal studies concurrently evaluating multiple MAP subtypes relative to disease occurrence over a number of years are necessary to assess comparative virulence between strains in the long-term. Such knowledge may inform control strategies for MAP, allowing the development of more cost-effective management techniques specific to deer. This could include identification of subtypes as possible diagnostic markers for the likelihood of clinical disease development. Such information

could then be used to prevent the entry of a virulent subtype of MAP into a deer herd through purchased animals.

1.3.2 Infection with MAP and clinical Johne's disease

'Infection' has been defined as 'invasion by and multiplication of pathogenic microorganisms in a bodily part or tissue, which may produce subsequent tissue injury and progress to overt disease through a variety of cellular or toxic mechanisms' (Anonymous 2000). After ingestion, MAP enters the ileal and jejunal epithelium through organised mucosa-associated lymphoid tissue, known as Peyer's patches (PPs). The bacterium is initially endocytosed by microfold (M) cells prior to being phagocytosed by sub- and intraepithelial macrophages that migrate into local lymphatics spreading infection to regional LN. Mycobacteria are known for their ability to circumvent the bactericidal mechanisms of macrophages and, as MAP targets the intracellular compartments of macrophages, it is also shielded somewhat from the host's immune system and can remain dormant for weeks to years (Momotani et al 1988; Lugton 1999).

In cattle, MAP infection and it's progression to clinical disease has been categorized into four stages based on the presence and severity of typical clinical signs, the rate of environmental shedding and likelihood of MAP detection with current diagnostic tests (Whitlock and Buergelt 1996). Animals infected with MAP that do not demonstrate overt evidence of disease and are undetectable with currently available diagnostic tests are in Stage I ('silent infection'). Stage II ('subclinical') animals show an immune response to MAP infection and shed low but infectious levels of MAP into the environment. These animals can exist within this Stage for their lifetime or remain free of clinical signs of disease for months to years until periods of stress or other factors cause the bacteria to emerge and replicate. Stages III and IV refer to the proportion of animals that develop 'clinical JD' where signs progress from diarrhoea and weight loss to eventual death due to dehydration and cachexia. Only a minority of MAP-infected sheep and cattle will progress to clinical disease, with the majority remaining subclinical with no apparent pathology for their lifetime (Koets et al 2002; Smeed et al 2007; de Silva et al 2010). It is estimated in cattle that for every clinical case of JD, there are up to 25 herdmates that are MAP-infected, creating an 'iceberg' effect within an individual herd. Whether a similar ratio of infected to clinical animals exists within MAP-infected deer herds is yet to be established.

Whitlock and Buergelt (1996) estimated that up to 50% of a cohort may be MAP-infected if one or more cattle are faecal-culture positive.

In a population of MAP-infected animals, a spectrum of immunological responses will be seen (Caldow and Gunn 2003). Stage I cattle mount a pro-inflammatory, cell-mediated (Type I) immune response which is largely unable to eliminate MAP due to a number of effective evasive mechanisms employed by the bacteria (Sohal et al 2008). Therefore, a MAP-infected cattle beast can remain subclinical for up to 10 years, as the cell-mediated response is eventually replaced by an antibody-mediated (Type II) immune response which is ineffective for controlling intracellular pathogens such as MAP (Kurade et al 2004; Tiwari et al 2009). In contrast, both Type I and Type II immune responses in sheep will give rise to disease states with progression from paucibacillary (Type I) to multibacillary (Type II) disease recorded (Dennis et al 2011).

1.3.2.1 Clinical Johne's disease in deer

Anecdotally, two presentations of clinical JD have been described in deer; 'sporadic' cases in adult deer (greater than 24 months old) and 'outbreaks' in weaner (3 to 12 month old) and/or yearling (12 to 24 month old) deer with up to 15 to 20% of a mob affected (Mackintosh 2000; Bell 2005). The clinical expression of JD in deer manifests as weight loss over weeks to months, with occasional non-haemorrhagic, non-mucoid diarrhoea of varying intensity pasting the hocks and perineum, as illustrated in Figure 1.1 (Stehman 1996). Deterioration of coat quality due to winter coat retention and submandibular oedema ('bottle-jaw') caused by a protein-losing enteropathy also occur. The differential diagnoses list for one or more of these clinical signs in New Zealand deer include yersiniosis (infection with *Yersinia pseudotuberculosis*) (weaner deer only), abomasal parasitism, fading elk/Wapiti syndrome, bTb, *M. avium*, copper deficiency and chronic Malignant Catarrhal Fever (MCF) (Mackintosh and de Lisle 1998).

An artificial challenge model for MAP infection in red deer was successfully developed by Mackintosh *et al.* (2003) to mimic, within 20 weeks after bacterial inoculation, the pathology and clinical signs observed in natural outbreaks of clinical JD in weaner deer (Mackintosh et al 2003). The model has subsequently been utilised to evaluate a number of epidemiological parameters, including the comparative virulence and immune response elicited by the MAP types

(O'Brien et al 2006; Mackintosh et al 2007a; Robinson et al 2008) and the efficacy of vaccines for MAP (Mackintosh et al 2003; Mackintosh et al 2005; Mackintosh et al 2008). Mackintosh *et al*. (2010), using this model, reported a time-dependant relationship between the age of a naïve deer and early clinical JD progression, faecal shedding of MAP and development of gross pathology, with weaner deer apparently more susceptible relative to yearling and adult deer. However, the authors did not incorporate a control group into the study design, leading to a potential confounding effect of age by herd of origin. In addition, the possible influence, if any, of natural infection with MAP, in addition to artificial inoculation, on the study outcomes could not be evaluated. Moreover, as the study was limited to 50 weeks, no conclusions can be drawn regarding subsequent clinical expression of disease related to age at challenge. Longitudinal studies of greater than 50 weeks are required to establish the likelihood that a naïve deer infected as a yearling or adult will develop clinical JD within its lifetime.

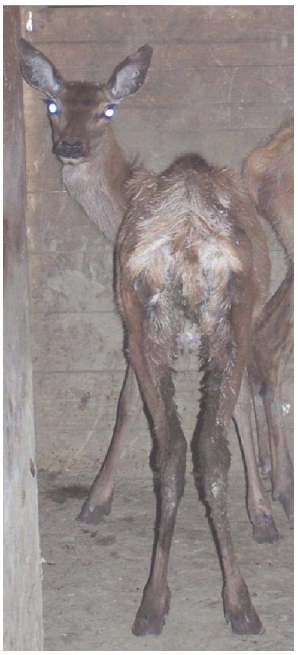


Figure 1.1 Yearling (12 to 24 month old) red deer (Cervus elaphus) with clinical Johne's disease.

However, in cattle, susceptibility to MAP infection and development of clinical JD also appears to be age-dependant (Windsor and Whittington 2010), with the likelihood of infection highest in calves less than 30 days of age and signs of clinical disease not typically observed until the animal is at least 18 to 24 months of age (Rankin 1962; Larsen et al 1975; Williams et al 1983). Similar research in sheep indicates this species may be susceptible to infection with MAP

throughout life, although lambs and hoggets may be relatively more susceptible (Begg et al 2005).

1.3.2.2 Subclinical Johne's disease in deer

Since January 2007, Johne's Management Limited (JML) has managed a national surveillance system for MAP through identification of 'abnormal' visceral LN (enlarged and/or with macroscopically visible pathology) in commercially slaughtered New Zealand farmed deer. Antemortem inspection ensures any deer demonstrating overt signs of disease, including comparatively low body condition score and/or diarrhoea, are identified and removed. Therefore, deer subsequently identified at slaughter with macroscopically visible carcass pathology have been classified as suffering from subclinical JD (Hunnam et al 2009; Wilson et al 2009). To date, approximately 40% of deer herds have had one or more slaughtered deer identified with an 'abnormal' LN (Norton, S; *pers. comm.*). As illustrated in Figure 1.2, carcass weights of deer identified with 'abnormal' LN between January 2007 and December 2008 were 2.4 to 3.5 kg lower in young (generally <2 years old) and 7.3 to 26.8 kg lower in adult (>2 years old) deer, compared with carcasses without 'abnormal' LN ($p<0.01$) (Hunnam et al 2009). This carcass weight effect is the first published evidence of production loss due to subclinical MAP infection in deer.

The effect of subclinical JD on other production traits in deer remains unclear. Stringer *et al.* (2010b) found there was no significant difference in weight between carcasses with macroscopically 'normal', MAP-positive versus MAP-negative LN ($p = 0.5$). Vaccination of weaner deer for MAP after natural and artificial bacterial exposure did not result in a significant difference in the average daily liveweight gain between cohorts (Mackintosh et al 2008; Stringer et al 2009a). Although Thompson *et al.* (2007), in a study of intrauterine transmission of MAP in red deer, incidentally found that apparently healthy, ELISA- positive hinds had a lower pregnancy rate (69%) relative to their ELISA-negative herdmates (85 to 90%)(Thompson et al 2007), the statistical significance of this result was not assessed. Additionally, there was a potential confounding environmental effect since those animals were separated from their cohorts for management after diagnosis of infection status. In contrast, a recent nationwide study found no significant difference in pregnancy rates between MAP-

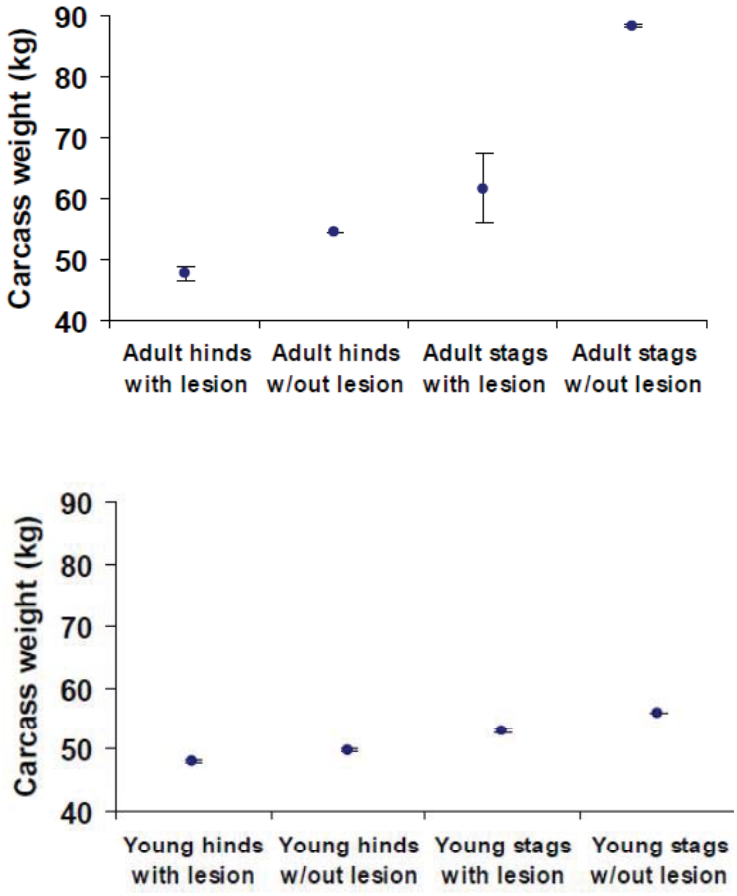


Figure 1.2 Carcass weight of commercially slaughtered adult hinds (n = 195,335) and adult stags (n = 67,731) (above) and young hinds (n = 354,989) and young stags (n = 399,421) (below) identified with ('with lesion') and without ('w/out lesion') 'abnormal' visceral lymph node(s) from January 2007 to December 2008. Vertical bars represent 95% confidence intervals around the mean. All differences with age groups were highly significant ($p < 0.01$) (published with permission from Johne's Management Limited).

positive deer herds or those with farmer-diagnosed clinical JD and a negative, control population (Verdugo, C; *unpublished data*). However, that study did demonstrate a small but significantly lower weaning rate in deer herds with clinical JD (86.1%; 95% CI: 85.4 to 86.9%) relative to herds without observable disease (87.4%; 95% CI: 86.6 to 88.2%), indicating further research into the possible subclinical effects of MAP infection to deer is warranted to determine more robust data on potential production effects.

1.3.3 Worldwide occurrence in deer

Table 1.3 outlines published investigations for MAP and clinical JD in farmed, wild, zoo and captive deer species, by country. The first putative diagnosis of clinical JD in deer was made in the UK by McFadyean in 1907 (Twort and Ingram 1912). The first published observation of clinical JD in red deer was in Scotland and Denmark, although it is unknown whether diagnostic testing for MAP was undertaken (Krogh and Jensen 1988; Fawcett et al 1995). MAP has subsequently been diagnosed in wild, zoo or farmed red deer populations in Austria, the Czech Republic, Germany, Ireland, Italy, New Zealand, USA, Norway and Spain using a variety of diagnostic techniques including tissue/faecal culture, PCR, RFLP and histopathology. Published studies of three populations of Tule Elk in the United States have identified varying proportions of MAP infection, ranging from 0% (0/289) to 100% (8/8) (Jessup et al 1981; Rhyan et al 1997; Manning et al 2003b; Crawford et al 2006). The only published study of MAP in wild Pampas deer was undertaken from 1995 to 1998 in Argentina with no positive serological reactions diagnosed (Uhart et al 2003). Similarly, of 17 wild Bolivian Grey Brocket deer tested, none were AGID-positive for MAP in the only published study of this pathogen in this deer species. Although annual surveillance in all nondomestic ruminant species over 10 months of age at the San Diego Zoo via individual faecal culture between 1991 and 2007 diagnosed MAP in a range of deer, including Indian hog deer (*Axis porcinus*), Western Tufted deer (*Elaphodus cephalophus*) and Père-David's deer (*Elaphorus davidianus*), the following species were MAP-negative; Calamian deer (*Axis calamianensis*), Kuhl's deer (*Axis kuhlii*), Pampas deer (*Ozotoceros bezoarticus*), White-lipped deer (*Pnzewalskium albirostris*) and Reindeer (*Rangifer tarandus*) (Witte et al 2009).

1.3.3.1 Occurrence of Johne's disease in New Zealand deer

The first reported diagnosis of MAP in a New Zealand deer was in 1979 (Gumbrell 1986). Diagnosis was based on histopathological features in the mesenteric LN and ileum of an adult Rusa deer. It was not reported whether this deer was feral or farmed.

Table 1.3 Published reports of *Mycobacterium avium* subspecies *paratuberculosis* and JD diagnosis in farmed, captive, zoo and/or wild deer species globally, including Fallow deer (*Dama dama*), Sika deer (*Cervus nippon*), Pampas deer (*Ozotoceros bezoarticus celer*), White-tailed deer (*Odocoileus virginianus*), Axis deer (*Axis axis*), Rusa deer (*Cervus timorensis*), Roe deer (*Capreolus capreolus*), Wapiti (*Cervus canadensis)* and Red deer (*Cervus elaphus*); and Tule elk (*Cervus elaphus nannodes*), Moose (*Alces alces*) and Reindeer (*Rangifer tarandus tarandus*) from 1955 to present, by country.

Country	Year	Deer species	Farmed/ captive/wild	Diagnostic test(s)	No. positive/ No. study animals	Clinical signs of JD	Pathology	Reference
Argentina	1992	?	Farmed	?	?	Yes	?	(Mereb 2008a)
	1995-1998	Pampas	Wild	Serology	0/ 14	?	N/A	(Uhart et al 2003)
Austria	2001-2004	Red	Wild	Tissue culture/PCR	3/ 14	Yes	Yes	(Glawischnig et al 2006)
	2002-2004	Red, Roe, Fallow	Wild	PCR/Culture	129/ 483	Yes	Yes	(Deutz et al 2005)
Belgium	1976-2002	Elk	Zoo	ELISA/PCR/culture	2/24	No	No	(Vansnick et al 2005)
		Red deer	Zoo	ELISA	0/1	No	No	(Vansnick et al 2005)
		Fallow deer	Zoo	ELISA/PCR/culture	1/2	No	No	(Vansnick et al 2005)
		Père-David's	Zoo	ELISA	1/29	No	No	(Vansnick et al 2005)
		Reindeer	Zoo	ELISA/PCR/culture	2/23	No	No	(Vansnick et al 2005)
		Dybowski's Sika deer	Zoo	ELISA	0/4	No	No	(Vansnick et al 2005)
		Sika Deer	Zoo	PCR/ELISA	1/1	No	No	(Vansnick et al 2005)
Bolivia	2004	Grey Brocket	Wild	AGID	0/ 17	No	?	(Deem et al 2004)
Czech Republic	1995-1998	Red; Roe	Wild	RFLP	12/ 175; 9/ 401	?	?	(Pavlik et al 2000)
	1997-1998	Fallow	Wild	RFLP	7/ 178	?	?	(Pavlik et al 2000)
Denmark	1985-1987	Red, Fallow, Sika, Roe	Farmed	?	2/ 105	Yes	Yes	(Krogh and Jensen 1988)
Germany	2000	Red	Wild	Histopathology /Faecal culture	1/ 1	Yes	?	(Glawischnig and Khaschabi 2001)
Ireland	1993	Red	Farmed	?	3/ 3	?	Yes	(Power et al 1993)
Italy	1995-1998	Red	Wild	PCR	17/ 19	?	Yes	(Nebbia et al 2000)
	1998-2002	Red	Wild	Tissue culture	201/ 399	No	?	(Fraquelli et al 2005)
	2006	Red	Wild	?	0/61	No	?	(Andreoli et al 2010)
New Zealand	1979-1987	Rusa; Wapiti; Red	?	Histopathology	1/ 1; 1/ 1; 8/ 8	Yes	?	(Gumbrell 1987)
	1986-1994	Red, Wapiti	Farmed	Tissue culture/PCR	93/ [a]	?	Yes	(de Lisle et al 2003)
	1994-2000	Red, Wapiti	Farmed	Tissue culture/PCR	526/ [a]	?	Yes	(de Lisle et al 2003)
	2001-2005	Red, Wapiti	Farmed	Tissue culture/PCR	1141/ [a]	?	Yes	(de Lisle et al 2006)
	2007	Fallow	Farmed	Farmer diagnosis	0/ 4266	No	N/A	(Hell et al 2008)

Country	Year	Deer species	Farmed/ captive/wild	Diagnostic test(s)	No. positive/ No. study animals	Clinical signs of JD	Pathology	Reference
	2007	Red, Wapiti	Farmed	Farmer diagnosis	2408/ 270936	Yes	N/A	(Hell et al 2008)
	2008/2009	Red, Wapiti	Farmed	Tissue culture	98/251	No	No	(Stringer et al 2009b)
Norway	1992-1999	Moose	Wild	ELISA	10/ 537	?	N/A	(Tryland et al 2004)
	1994	Reindeer	Captive	ELISA	11/ 325	?	N/A	(Tryland et al 2004)
	1996	Reindeer	Wild	ELISA	0/ 91	?	N/A	(Tryland et al 2004)
	1997	Roe	Wild	ELISA	6/ 38	?	N/A	(Tryland et al 2004)
	1998	Red	Wild	ELISA	14/ 371	?	N/A	(Tryland et al 2004)
Scotland	1985	Red	Farmed	?	?/ 16	Yes	?	(Fawcett et al 1995)
Spain	1997-1998	Fallow	Wild	Histopathology/PCR	7/ 8	Yes	Yes	(Marco et al 2002)
	2000-2009	Roe	Wild	ELISA	48/519	No	No	(Boadella et al 2010)
	2001-2003	Red; Fallow	Wild	Tissue culture/PCR	0/ 101; 1/ 94	No	No	(Alvarez et al 2005)
	2004-2007	Fallow	Wild	ELISA/Histopathology	28/ 95	No	Yes	(Balseiro et al 2008)
	?[b]	Red	Wild/farmed	ELISA	257/ 852	No	N/A	(Reyes-Garcia et al 2008)
Sweden	1955-1964	Roe	Wild	Histopathology/Tissue culture	5/ 1302	Yes	Yes	(Hillermark 1966)
USA	1971-1976	Fallow; Sika	Captive	Histopathology	28/ 62; 19/ 38	Yes	?	(Temple et al 1979)
	1975	White-tailed	Wild	Histopathology	1/ 1	Yes	Yes	(Libke and Walton 1975)
	1979	Fallow; Axis	Captive	Faecal culture	5/ 37; 3/ 52	?	N/A	(Riemann et al 1979)
	1980-1982	Tule elk	Captive	Tissue/Faecal culture	8/ 8	?	Yes	(Jessup et al 1981)
	1983	White-tailed	Wild	Tissue culture	10/ 10	?	?	(Chiodini and Van Kruiningen 1983)
	1983	White-tailed	Wild	ELISA	24/ 954	No	N/A	(Shulaw et al 1986)
	1991-1995	Elk	Wild	Histopathology	0/ 289	No	?	(Rhyan et al 1997)
	1991-2007	Indian axis	Zoo	Faecal culture	3/34	No	?	(Witte et al 2009)
		Indian hog deer	Zoo	Faecal culture	2/56	No	?	(Witte et al 2009)
		Roe deer	Zoo	Faecal culture	1/15	No	?	(Witte et al 2009)
		Red/wapiti	Zoo	Faecal culture	1/153	No	?	(Witte et al 2009)
		Sika subsp.	Zoo	Faecal culture	2/118	No	?	(Witte et al 2009)
		Fallow subsp.	Zoo	Faecal culture	1/26	No	?	(Witte et al 2009)
		Western tufted deer	Zoo	Faecal culture	2/29	No	?	(Witte et al 2009)
		Père-David's deer	Zoo	Faecal culture	3/33	No	?	(Witte et al 2009)
		Calamian deer	Zoo	Faecal culture	0/60	No	?	(Witte et al 2009)
		Kuhl's deer	Zoo	Faecal culture	0/1	No	?	(Witte et al 2009)
		Pampas deer	Zoo	Faecal culture	0/9	No	?	(Witte et al 2009)
		White-lipped deer	Zoo	Faecal culture	0/38	No	?	(Witte et al 2009)

Country	Year	Deer species	Farmed/ captive/wild	Diagnostic test(s)	No. positive/ No. study animals	Clinical signs of JD	Pathology	Reference
		Reindeer	Zoo	Faecal culture	0/5	No	?	(Witte et al 2009)
	1997	Tule elk	Captive	Faecal culture	4/ 100	?	N/A	(Cook et al 1997)
	1998	Elk	Farmed	?	4/ 4	?	?	(Manning et al 1998)
	1998-2002	White-tailed	Wild	ELISA/Tissue culture	1/ 313	No	?	(Davidson et al 2004)
	1998-2006	Elk	Wild	ELISA/AGID/Faecal culture/Tissue culture	1/214	No	?	(Corn et al 2010)
	2000	Tule elk	Wild	Tissue culture	1/ 37	No	Yes	(Crawford et al 2006)
	2000-2003	White-tailed	Captive	Tissue culture	2/ 160	?	?	(Hattel et al 2004)
	2000-2003	White-tailed	Wild	ELISA	2/ 114	No	N/A	(Wolf et al 2008)
	2002	White-tailed	Wild	Faecal culture	2/ 309	?	N/A	(Raizman et al 2005)
	2003	Tule elk	Captive	ELISA/Faecal culture	22/ 45	No	N/A	(Manning et al 2003b)
	2005-2006	Key	Wild	Tissue culture	2/ 90	Yes	Yes	(Pedersen et al 2008)
	2006	White-tailed	Wild	Histopathology/Tissue culture	1/83	Yes	Yes	(Sleeman et al 2009)

? = Not stated in reference
N/A = Not applicable
PCR = Polymerase Chain Reaction
AGID = Agar Gel Immunodiffusion
RFLP = Restriction fragment length polymorphism
ELISA = Enzyme-linked immunosuppression assay
[a] All slaughtered deer in that time period
[b] Article published in 2008

From the mid 1980s, identification of MAP in farmed deer was a consequence of LN culture for *M. bovis* at slaughter as part of the national eradication program for bTb (de Lisle et al 1993). The organism was confirmed by culture in a farmed deer for the first time in 1985 and, between 1992 and 2000, one or more commercially slaughtered deer from 296 herds were culture- and/or PCR-positive for MAP (de Lisle et al 2003). The herd-level incidence of MAP infection increased on an annual basis, as illustrated in Figure 1.3 (de Lisle et al 2005). Although this data indicate an increase in the herd-level prevalence of MAP infection in farmed deer, it cannot be used to determine a national herd-level prevalence figure as samples were limited to culture-positive LN detected as a by-product of the national bTb surveillance scheme, a biased subsample.

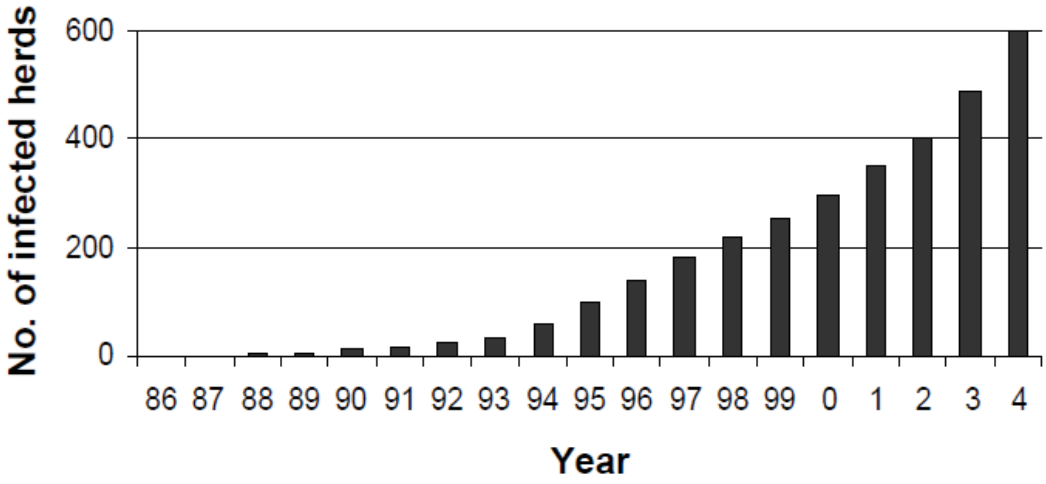


Figure 1.3 Cumulative number of MAP-infected New Zealand deer herds, identified by *IS900* PCR (first used in 1994) or by culture applied to lymph nodes with pathological lesions resembling bTb from slaughtered deer, from 1986 to 2004 (from de Lisle *et al*., 2005).

From the early 1990s, there were anecdotal reports of clinical JD 'outbreaks' in weaner (3 to 12 month old) deer, with mortality rates of up to 20% (Mackintosh and de Lisle 1998; Black and Orr 1999). Preliminary results of a 2007 postal survey found 40% of herds contained at least one deer with farmer-diagnosed clinical JD with a mean within-herd prevalence of 0.9% (Hell et al 2008). A 2008/09 nationwide study showed 56.6% of 99 deer herds sampled were MAP-positive on pooled faecal culture and/or an ELISA, with an annual mean clinical JD incidence of 0.23% (Verdugo et al 2011). Stringer *et al*. (2009b) recently found 45% (95% CI = 30 to 60%) of grossly 'normal' mesenteric LN of commercially slaughtered farmed deer

were culture-positive for MAP, with a national herd-level prevalence of 59% (95% CI = 41 to 78%). Compared to the 1970-1983 survey, these results indicate that the current apparent widespread prevalence of MAP in the New Zealand farmed deer industry is a recent phenomenon. It also appears that clinical JD exists at relatively low levels within deer herds and published case studies of clinical disease 'outbreaks', particularly in young deer, may be the exception.

1.4 Transmission

1.4.1 Faecal-oral transmission

The primary transmission pathway of MAP between susceptible individuals is believed to be via the ingestion of bacteria from a number of possible sources, including faecal-contaminated soil, pastures or water (Sweeney 1996). Ingestion of a dose of 100 MAP organisms per gram administered weekly for 10 weeks or a single innocula of between 10^8 and 10^{10} MAP per gram is sufficient to infect both neonatal lambs and calves (Kluge et al 1968; Gay and Sherman 1992; Sweeney et al 1992a; Stabel et al 2003), shown via positive faecal and/or tissue culture for MAP post-inoculation. Diarrhoeic cattle, deer and sheep affected with clinical JD can excrete at least 10^5, 10^6 and 10^8 MAP per gram of faeces, respectively, and are an important source of environmental contamination (Whittington et al 2000d; Schroen et al 2003b). Cattle, deer and sheep with subclinical JD can also shed MAP for up to 18 months before clinical signs become apparent, due to the long incubation period of the disease.

Despite the general acceptance of faecal-oral transmission as the predominant route of MAP transfer (Chiodini et al 1984; Whittington et al 2004), Corner *et al.* (2003) have questioned the validity of this assumption and hypothesized that significant transfer may also occur via the respiratory tract. In support of this was an early study by Kluge *et al.* (1968) where lambs were successfully infected with MAP via intratracheal inoculation, with eventual granuloma development in lung tissue similar to that seen in the intestines. Corner *et al.* (2004) also cited examples of successful infection via the respiratory route utilised by other mycobacterial species, such as *M. bovis* and *Mycobacterium leprae*. However, the authors accepted that further

experimental work was required to confirm both the presence and significance of respiratory transmission of MAP.

The thick capsule of MAP confers significant resistance to environmental effects, enabling survival for up to nine, 11 and 17 months in manure pats, soil and tap water, respectively, and for at least 47 months when dried (Gay and Sherman 1992). This indicates that MAP-contaminated pastures may provide an on-going source of infection to grazing ruminant livestock, complicating the successful management of Johne's disease. Whittington *et al.* (2004) found MAP could survive for up to 55 weeks in a dry, fully shaded environment but with shorter survival times in unshaded environs of 12 weeks. Recent studies have confirmed that the level of soil and plant contamination with MAP is influenced by prevailing environmental conditions, including moisture/rainfall, soil clay content and the depth at which samples were collected (Khol et al 2010; Pribylova et al 2011; Salgado et al 2011).

1.4.1.1 Transmission to deer from other livestock

The natural transmission of MAP to deer from sheep, cattle and/or goats through co- or alternate grazing warrants further investigation. While cross-species grazing spreads financial risk, allows optimal pasture use and has a positive influence on vegetation management, particularly control of the weed *Senecio jacobaea* ('ragwort') (Griffiths et al 2006), its role in transmission of disease-causing organisms, such as MAP, is unknown. Approximately 85% of New Zealand deer herds were established on farms that previously grazed cattle and/or sheep and 75% currently graze sheep, cattle and/or goats on the deer fenced area (DFA) (Griffiths et al 2006; Hell et al 2008). Figure 1.4 illustrates the number of deer farmers that grazed each age-class of species other than deer (SOTD), including sheep, dairy and beef cattle, on their DFA in 2007, with sheep and beef cattle predominating (Hell et al 2008). Figure 1.5 shows, more specifically, the age-classes of SOTD co- and alternately grazed with red/wapiti/elk weaner deer and breeding hinds. An exposure ratio of greater than 1, such as hoggets co-grazed with breeding hinds (Figure 1.5A), indicates that deer pastures were exposed more (in terms of number of animals and/or time grazed) to the former species relative to the latter species. Breeding hinds were predominately exposed to hoggets and lambs through co-grazing, while weaners were largely exposed to ewes, hoggets, lambs and goats by both co- and/or alternate grazing.

Verdugo *et al.* (2008) found farmers grazing sheep and deer were significantly less likely to have deer identified with JD-suspect LN at slaughter relative to farmers grazing deer alone or deer and beef cattle.

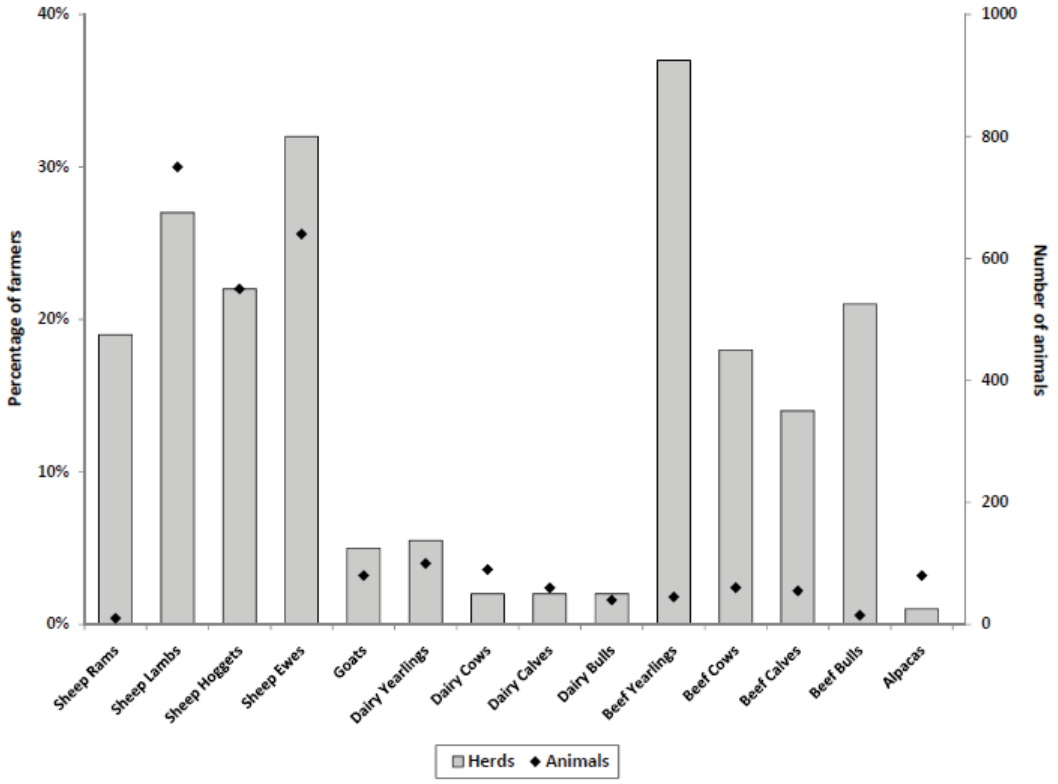


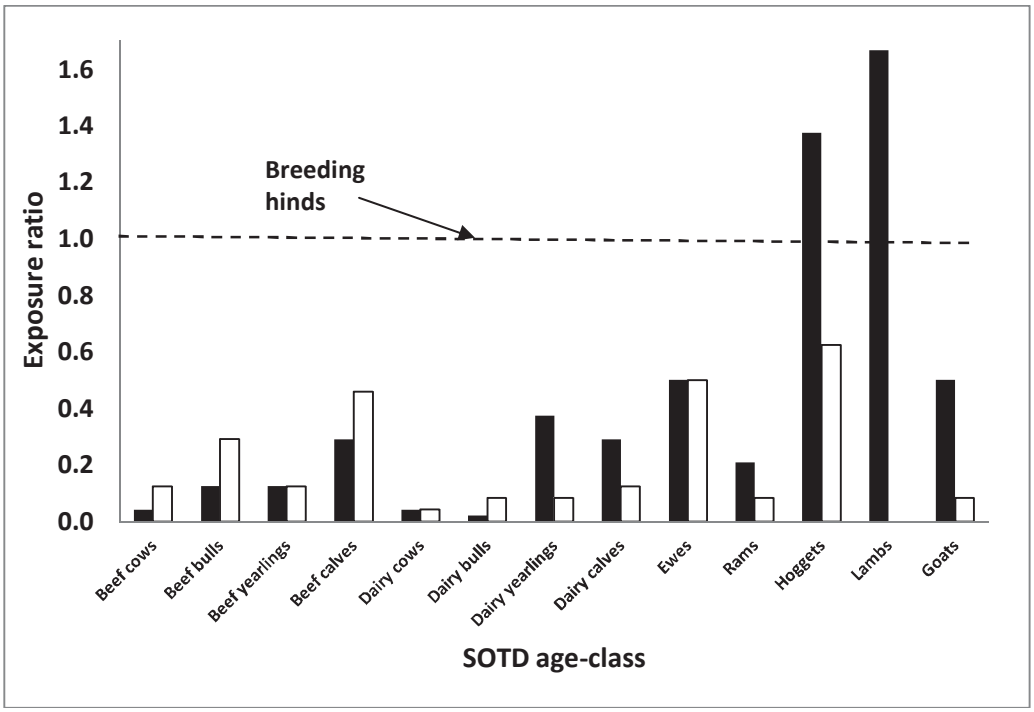
Figure 1.4 Percentage of New Zealand red deer/elk/wapiti farmers that grazed species other than deer on their deer fenced area and the mean number of animals per class of species other than deer (modified from Hell *et al.* 2008).

1.4.1.2 Transmission from wildlife

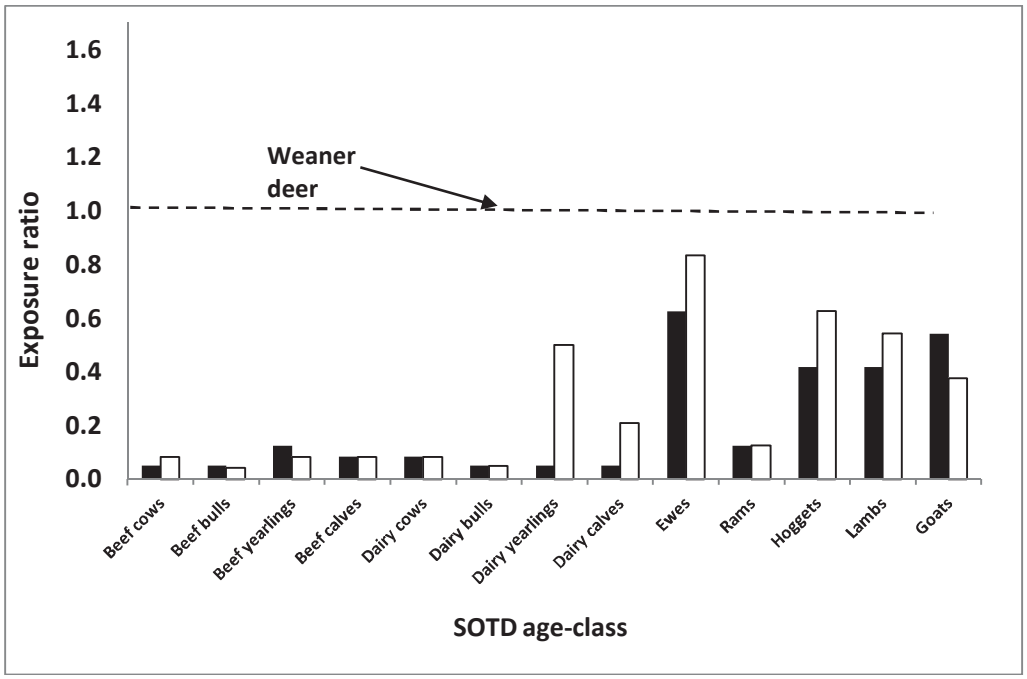
Although MAP can infect both ruminant and non-ruminant wildlife species, including monkeys, stoats, rabbits, foxes, cats, big horn sheep and bison, it has not been proven whether wildlife act as dead-end hosts of MAP, are part of a separate cycle of MAP transfer unconnected with JD in livestock and/or are directly linked with interspecies MAP transmission to livestock (Williams et al 1979; McClure et al 1987; Beard et al 1999; Buergelt et al 2000; Zwick et al 2002; Daniels et al 2003; Palmer et al 2005; Anderson et al 2007; Judge et al 2007). In 2004/2005, a range of wildlife species were surveyed for MAP on three New Zealand properties grazing deer herds with a chronic high prevalence of clinical JD (Nugent et al 2011). Analyses found 12% of birds (n = 32) and 19% of sampled mammals (380), including 36% of hedgehogs (42); 26% of rabbits (113); 25% of brushtail possums (73); and 17% of feral cats, had

culture-positive gut or gut-associated LN. However, only three MAP-infected animals had visible carcass pathology and only eight of 64 (13%) MAP-infected animals (five hedgehogs and three rabbits) from which faeces were collected were found to be also shedding MAP in their faeces.

Rabbits, in particular, have become a focus for research of the possible transmission of MAP from wildlife to livestock, particularly as this species can contaminate pastures with thousands of faecal pellets per rabbit per day. Mokresh *et al.* (1989) successfully infected 9 of 21 neonate rabbits with MAP, resulting in an estimated faecal shedding rate of 10^2 to 10^6 MAP/gram of faeces. More recently, on Scottish farms, up to 67% of rabbits have been found to be MAP-infected, with a significant positive association between clinical JD in cattle and presence of rabbits on the same property (Greig et al 1997; Greig et al 1999; Judge et al 2005; Judge et al 2006). Moreover, MAP-infected cattle and rabbits sourced from the same farm have been identified with the same strain of MAP and calves artificially inoculated with MAP isolated from a free-living rabbit developed histopathological and/or microbiological evidence of infection within 6 months post-inoculation (Greig et al 1999; Beard et al 2001). However, prior to recommending the control of rabbits or any wildlife species as part of a management plan for JD on deer farms, investigation of epidemiology of the infection in wildlife and its transfer to domestic animals, the relative bacterial challenge provided by the faeces of each species and the likelihood of avoidance behaviour of wildlife faeces by grazing deer is merited (Daniels et al 2001).



A



B

Figure 1.5 The proportion of time (in days) red deer/elk/wapiti breeding hinds (A) and weaner deer (B) were co- (■) or alternately (□) grazed with each age-class of species other than deer (SOTD) relative to each day grazed by the breeding hinds/weaners ('Exposure ratio') (from Hell *et al.* 2008).

1.4.2 Intrauterine transmission

Intrauterine transmission of MAP in deer was first described in wild red hinds in Austria (Deutz et al 2003). van Kooten *et al.* (2006) found the lung and/or intestinal tissue from nine foeti from 10 late-stage pregnant New Zealand farmed hinds with clinical JD were culture-positive for MAP. Thompson *et al.* (2007) confirmed pregnant hinds subclinically affected with JD can also infect their foetus with an estimated transmission rate of 78% (95% CI = 58 to 98%). However, the importance of intrauterine transmission of MAP in deer is difficult to establish as, to date, there has been no published investigation of whether deer foeti infected *in utero* remain subclinical carriers throughout life or progress to clinical JD. Dunkin (1935) and Manning *et al.* (2003a) both described cases of subclinical disease development in individual dairy calves that were born to a dam with clinical JD. These reports indicate that intrauterine MAP transmission may result in the development of subclinical JD in the offspring, with possible progression to clinical JD. However, in contrast, Bielanski *et al.* (2006) observed a Friesian calf for five years, born from an embryo exposed to MAP *in vitro* prior to transfer into a MAP-negative recipient dam, that did not develop clinical signs of JD. Further understanding of the long-term effects of intrauterine transmission is required, particularly if a proportion of offspring infected *in utero* become immunotolerant after birth. Although it may be less likely for these animals to develop clinical JD within their lifetime, the diagnosis of MAP infection in this subpopulation would be problematic.

MAP has been detected in the cotyledons, foetal membranes and uteri of pregnant cattle and the liver, kidneys, spleen and/or mesenteric LN of up to 40% of late-gestation foeti of cows with advanced clinical JD (Lawrence 1956; Doyle 1958; Seitz et al 1989; Sweeney et al 1992c). Lambeth *et al.* (2004) found all foeti of five pregnant ewes with clinical JD were MAP-infected. A recent meta-analysis by Whittington and Windsor (2009) found the prevalence of MAP-infected foeti within cows with subclinical JD and clinical JD were 9% (95% CI: 6 to 14%) and 39% (95% CI: 20 to 60%), respectively. The exact mechanism for MAP transfer *in utero* has not been determined, but may be through macrophages containing the bacterium passing through placentomes into foetal blood circulation (van Kooten et al 2006).

1.4.3 Transmammary transmission

Although MAP has been cultured from the milk, mammary gland and associated LN of hinds with clinical and subclinical JD (van Kooten et al 2006; Kopecna et al 2008), there has been no research on the presence or significance of transmammary transmission of MAP in deer. MAP has been detected in mammary tissue, supramammary LN and milk of sheep, goats and cattle (McDonald et al 2005; Nebbia et al 2006; Salgado et al 2007). Up to 35% of cows with clinical JD, 12% of cows with subclinical JD, and approximately half of subclinically affected sheep and goats may shed detectable levels of MAP in their milk (Taylor et al 1981; Sweeney et al 1992b; Nebbia et al 2006). Feeding of raw colostrum has been hypothesized as one of the earliest transmission routes of MAP to calves, with colostrum sourced from low-risk cattle a commonly proposed management technique for MAP in dairy cattle internationally (Dieguez et al 2008; Nielsen et al 2008; Pithua et al 2009; Pillars et al 2011).

1.4.4 Venereal transmission

The potential risk of venereal transmission of MAP from an infected stag to a naïve hind remains unknown. However, MAP detection in seminal fluid, sperm and testes from infected rams and bulls indicate that venereal transmission may be possible (Larsen and Kopecky 1970; Meat & Livestock Australia 2000; Ayele et al 2004; Herthnek et al 2006). Notwithstanding this, bovine uterine tissue was culture-negative four weeks post-insemination with MAP-infected semen, suggesting that infected bull semen may represent a low risk for transmission to recipient cows (Merkal et al 1982). Although this conclusion was supported after a formal analysis of the risk of MAP transfer via semen by the European Food Safety Authority (2004), that report also highlighted the need for further investigation of the effect of semen processing on MAP viability and transmission once infected semen is inseminated in order to develop a more robust assessment of the risk.

1.5 Pathology

1.5.1 Macroscopic Pathology

The predominant pathological change due to MAP infection described in a range of deer species is enlarged mesenteric LN with or without necrosis and/or mineralization

(Figure 1.6) (Libke and Walton 1975; Williams et al 1983; Manning et al 2003b; Crawford et al 2006). Granulomatous lesions in the retropharyngeal and mediastinal LN are also not uncommon (de Lisle et al 1993; de Lisle et al 2003). Small intestinal wall thickening does not appear with the same frequency in deer as typically observed in cattle and sheep (de Lisle et al 1993). Typical gross pathology due to MAP infection in cattle and sheep is a chronic, granulomatous enterocolitis, with a thickened, corrugated intestinal wall, and regional lymphangitis and lymphadenitis, due to infiltration by lymphocytes and macrophages (Carrigan and Seaman 1990; Clarke 1997; Amemori et al 2004). Calcification and focal necrosis of the cardiac endocardium and aorta have also been described in cattle and goats (Majeed and Goudswaard 1971; Buergelt et al 1978).

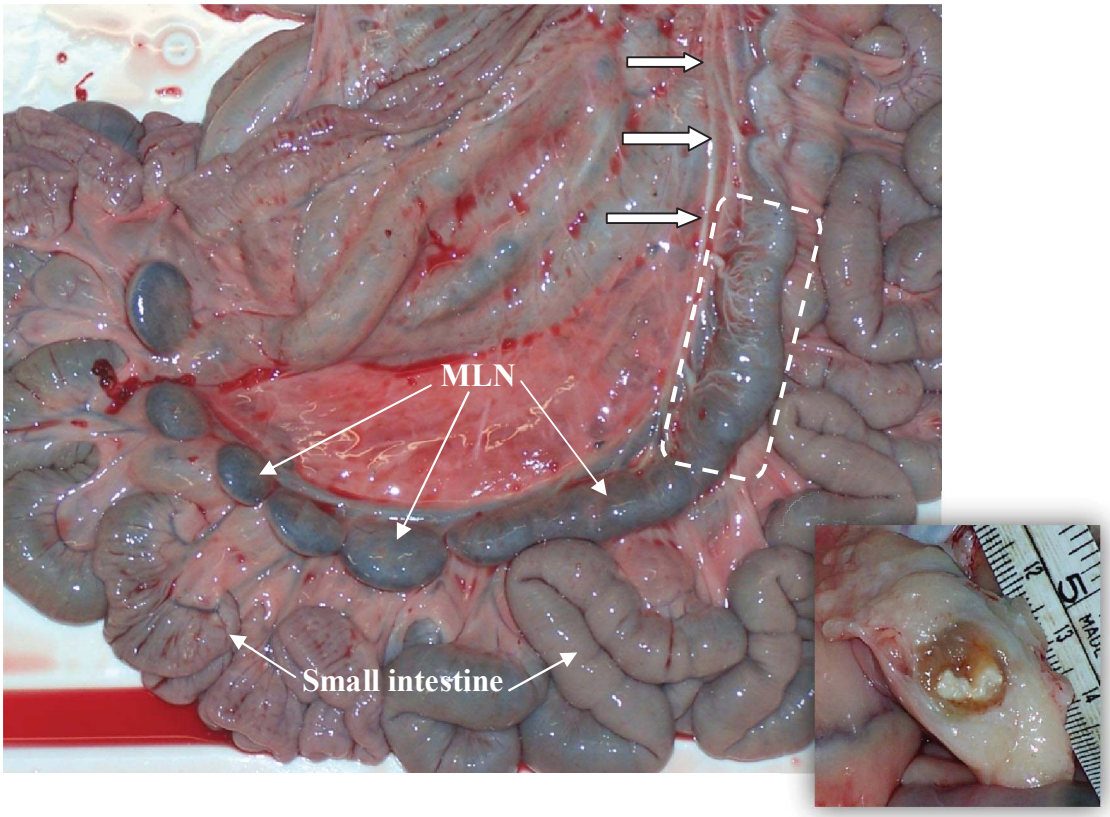


Figure 1.6 Lymphadenitis of the mesenteric lymph node (MLN) of a red deer (*Cervus elaphus*) (dashed rectangle) and lymphangitis of the distal lymphatics (open arrows) due to infection with *Mycobacterium avium* subspecies *paratuberculosis* (Inset = cut surface of enlarged MLN section showing core of caseous material).

1.5.2 Histopathology

An animal's immune response plays an important role in determining the appearance of histopathological changes as a result of MAP infection. 'Paucibacillary' changes, described in sheep (Pérez et al 1996) and goats (Corpa et al 2000), are associated with

a strong peripheral cellular immune response with lymphocytes as the predominant inflammatory cell and few to no discernable acid-fast bacilli present. In contrast, 'multibacillary' types are associated with a marked humoral immune response with infiltration of large numbers of swollen, foamy macrophages and abundant, intracellular, acid-fast bacilli.

MAP-associated histopathological changes in deer are non-homogeneously distributed between and within tissues, with the mesenteric LN, upper ileum and jejunum most commonly affected (Manning et al 1998). However, large numbers of MAP in the retropharyngeal LN have also been detected in deer, a observation which has not been described in other species (de Lisle et al 2003). The histopathological appearance of lesions, that are visible on gross examination, includes extensive caseous or pyocaseous necrosis, with or without calcification (de Lisle et al 1993; Quigley et al 1997; de Lisle et al 2003). Histopathological changes in grossly 'normal', MAP-infected LN have also been described, categorised and scored, based on the location, frequency, and type of inflammatory cell present and the number of acid-fast organisms observed (Mackintosh et al 2007a; Balseiro et al 2008; Mackintosh et al 2008; Clark et al 2010). Clark *et al.* (2010), in particular, developed a disease severity score to increase the diagnostic sensitivity of histopathological examination of deer LN for MAP. However, histological features typically observed in grossly normal LN at 'low' risk of MAP infection have not been described. The latter is required to act as a baseline against which changes in grossly 'normal', MAP-infected LN can be compared.

1.6 Risk factors for MAP and clinical Johne's disease

Although a number of risk factors associated with either the herd-level presence of MAP and/or clinical JD have been identified in cattle (Collins et al 1994; Johnson-Ifearulundu and Kaneene 1999; Wells and Wagner 2000; Hirst et al 2004; Roussel et al 2005) and sheep (Lugton 2004a; Dhand et al 2007), there are no published risk factor studies associated with these two outcomes in deer. Similarly, although discussion and evaluation of management practices for the control of, ostensibly, MAP or clinical JD, has been comprehensively reviewed in cattle (Burton and Voges

2002; Benedictus and Kalis 2003; Collins 2003b; Dorshorst et al 2006), sheep (Stehman 1996; Kennedy and Allworth 2000; Prowse 2000) and goats (Gezon et al 1988), there are few published reviews specific to farmed deer (Mackintosh and de Lisle 1998; Mackintosh 2002; Wilson and Castillo-Alcala 2004). These studies highlight the need to formulate a clear definition of the desired outcome of control (i.e. a reduction in MAP of clinical JD presence or prevalence) as a number failed to do so.

A long incubation period, the long environmental survival of MAP, uncertainty regarding the importance of various transmission routes, bacterial shedding by subclinically affected animals and the low sensitivity of current diagnostic tests in the detection of subclinical disease combine to make JD control challenging (Armstrong 1956). To date, attempts to control clinical JD on deer farms have principally relied on methods developed to control the disease in sheep and cattle, but robust, peer-reviewed reports of their effectiveness are limited (Mackintosh 2002; West 2002; Wilson and Castillo-Alcala 2004; Bell 2005, 2006). Moreover, deer are challenged by unique stressors as farming of this species has required the development of numerous novel management techniques, such as velvet removal, as well as modification of facilities used traditionally in sheep and cattle farming. Therefore, the development of effective control methods for JD in farmed deer requires research of risk factors specific to this species. The following addresses some potentially important risk factors.

1.6.1 Calf-cow contact

The frequency of contact, whether direct or indirect, between adult dairy cattle and calves has been found to have a significant association with the herd-level presence of MAP and/or clinical JD (Collins et al 1994; Obasanjo et al 1997; Wells and Wagner 2000; Berghaus et al 2005; Nielsen and Toft 2007). Therefore, JD management in dairy cattle focuses on the prevention of contact between adult cattle and calves from birth until 6 months of age (Collins and Morgan 1992; Dorshorst et al 2006). Other elements of calving and calf management, such as use and type of group housing pre- and post-weaning; poor hygiene levels in calf feeding areas; and a low amount of straw in calf bedding, also have a variably significant association with within-herd MAP transmission (Johnson-Ifearulundu and Kaneene 1998). However, given the

nature of deer farming and the behaviour of deer, it is not feasible to adopt control measures related to dam-offspring contact. Specifically, as New Zealand deer are predominately farmed extensively, this precludes the removal of fawns soon after birth as an economically or logistically viable management practice.

1.6.2 Herd geographic location

A significant positive association has been identified between spatial herd distribution and MAP infection in a number of species, including dairy cattle in the United States and Norway (Wells and Wagner 2000; Fredriksen et al 2004), wild rabbits in Scotland (Judge et al 2005) and black goats in North Korea (Lee et al 2006). Reasons for this relationship remain contentious and an association between iron availability, soil pH and MAP survival has been widely discussed. To scavenge iron in limiting environments, most mycobacterial species produce the lipid-soluble siderophore mycobactin and the water-soluble siderophore exochelin (Harris and Barletta 2001). However, MAP is a mycobactin auxotroph, synthesizing exochelin only, and so appears to be susceptible to a lack of iron. It has been assumed that, as the solubility of iron increases as pH decreases, iron is more readily available to all microorganisms, including MAP, in an acidic environment (Johnson-Ifearulundu and Kaneene 1997). Soils are ingested with pasture and it was hypothesized that the dietary intake of iron could promote the survival of MAP in the environment and so improves the likelihood of bacterial transmission.

In the first report of an association between geographic region and the occurrence of JD, Smythe (1935) claimed the disease did not occur in cattle along the southeast coast of England due to the predominant fine Aeolian sand soil type (pH = 7.0). Evidence of a positive relationship between acidic soils and the herd-level presence of MAP has subsequently been published by researchers investigating JD in cattle, sheep and goats in the United States, Australia and Spain (Johnson-Ifearulundu and Kaneene 1999; Reviriego et al 2000; Whittington et al 2004). Moreover, Johnson-Ifearulundu and Kaneene (1998) reported the application of lime to pastures resulted in a ten-fold decrease in the odds of a US dairy herd being positive for MAP. However, this relationship could not be replicated in a later Australian study in the context of survival of MAP in the environment rather than the occurrence of clinical JD (Whittington et al 2004). Lugton *et al.* (2004b), in a comprehensive review of the

effect of micro- and macronutrients on clinical JD, concluded that 'the fact that an excess of iron [was] insufficient ... to exacerbate clinical expression [of JD] in cattle, suggests that other unidentified nutritional factors may be involved'.

Anecdotal reports indicate there is a higher prevalence of clinical JD in farmed deer in the South Island of New Zealand relative to the North Island, but this has not been formally evaluated. Glossop *et al.* (2005) found, in 2003 and 2004, LN sourced from farmed deer commercially slaughtered in the South Island were more likely to be culture-positive for MAP than those slaughtered in the North Island. However, in that study, a maximum of three suspicious (pathological or enlarged) LN per line could be submitted for initial histopathological examination and only a small proportion of these were cultured. Secondary ELISA testing of blood samples collected from deer positive to a primary intra-dermal test for bTb detected antibodies to MAP in 63.2% (n = 525) and 62.3% (n = 122) of North and South Island herds, respectively (Griffin et al 2006a). However, the interpretation of these figures is difficult to assess as the reactor rate for MAP in herds negative to intra-dermal tests for bTb in the same time period was not available.

1.6.3 Purchased animals

MAP can be introduced into a naïve herd through the purchase of apparently healthy, but MAP-infected animals (Sweeney 1996). Hirst *et al.* (2004) found importation of >8% of dairy herd size annually for five years resulted in a 3.3 times higher likelihood of MAP infection in that herd, relative to herds with an importation rate of <8%. Similarly, the source of dairy herd replacement stock, including bulls, was significantly associated with the likelihood of development of clinical JD in herds in the United Kingdom (Cetinkaya et al 1997b) and New Zealand (Norton et al 2009). Therefore, a recommended management practice for New Zealand deer herds, whether MAP-infected or considered at 'low risk' of MAP infection, has been to bar the entry of purchased deer through maintenance of a 'closed' herd or by careful screening of purchased animals (Wilson and Castillo-Alcala 2004; Mackintosh 2005). However, the on-going relevance of this recommendation will depend on future research to establish whether the comparative virulence of MAP substrains is an important risk factor. Market assurance programs for JD, such as the United States Voluntary JD Control Program, the Australian JD Market Assurance Program for

Cattle ('CattleMAP') and the Alberta (Canada) Voluntary JD Cattle Herd Status Program, include screening of purchased animals. Within 'CattleMAP', participating farmers are required to undertake a management plan which reduces the likelihood of MAP entry via purchased stock. A similar national market assurance program involving a formal herd classification scheme within the New Zealand deer industry may give farmers confidence in the likelihood of purchasing MAP-infected deer. In addition, a 'low risk' or test-negative classification may enhance the value of the herd and enable deer to be sold at a premium (Mackintosh 2002).

1.6.4 Water

MAP can remain viable in water for extended periods of time. Lovell *et al.* (1944) and Larsen *et al.* (1956) recovered MAP for nine and 17 months, respectively, from spiked water samples, while Whittington *et al.* (2005) found water spiked with MAP and stored in shaded and unshaded water troughs was culture-positive for up to 48 and 36 weeks, respectively. Roussel *et al.* (2005) found the presence of running streams as a source of drinking water was associated with increased odds of a seropositive beef cattle herd for MAP, likely due to runoff from an infected herd transmitting MAP to a neighbouring naïve property. Similarly, catchment areas for water running from hillsides in South Wales have been found to be more likely to be culture-positive for MAP than water samples derived from higher altitudes (Pickup et al 2005).

1.6.5 Herd type and size

Cross-sectional surveys of US dairy herds have found that larger herd size has a significant positive association with the herd-level presence of MAP (Collins et al 1994; Wells and Wagner 2000; Hirst et al 2004), while commercial herds were 38.4 times more likely to be MAP-infected relative to registered (breeding) herds (Obasanjo et al 1997). It was hypothesized that farming for commercial milk production was more likely to result in the retention of infected animals within the herd as long as their production was acceptable. New Zealand has the largest farmed deer population in the world, with an average herd size of 374 animals (Table 1.1) (Anonymous 2010b). This is second only to Argentina with an average deer herd size of 386 animals in 2008 (Mereb 2008b). However, deer were farmed in only 44 Argentinian herds at that time, relative to approximately 3,000 deer herds in New Zealand.

1.6.6 Stress

Stress, induced by management, sub-optimal nutrition, adverse climatic conditions or other factors, has been found to exacerbate infectious disease, such as yersiniosis and tuberculosis, in deer (Mackintosh and Henderson 1984; Griffin and Buchan 1994). Similarly, Dhand *et al.* (2007) found a higher likelihood of MAP infection in sheep whose dams had been in relatively poor condition and kept at high stocking rate during lambing. It was hypothesized that a greater number of infected animals per unit area led to nutritional stress which increased dam faecal shedding of MAP and pasture contamination. Additionally, poor dam body condition due to MAP infection may have resulted in an increased likelihood of bacterial transmission to their progeny *in utero*. Although Cetinkaya *et al.* (1997a) found supplementary feeding of dairy calves with hay and coarse mix decreased the risk of JD at the herd-level, these associations could not be reproduced in the next year.

1.6.7 Presence and prevalence of wildlife

In New Zealand, an impediment to control of bTb is re-infection of livestock with *M. bovis* by infected wildlife, particularly brush-tail possums, a reservoir host for the disease (Lugton et al 1997). Two studies have found wildlife access, specifically to food stores of Norwegian dairy herds by wild birds and Scottish cattle herds by rabbits, had a significant positive association with the herd-level presence of MAP (Daniels et al 2002; Fredriksen et al 2004). However, case farms were either defined based on veterinary records only (Daniels et al 2002) or based on a high level of antibodies to MAP which were not supported by positive culture (Fredriksen et al 2004).

1.6.8 Breed

Whether an increased susceptibility to MAP infection and development of clinical JD exists in certain breeds is still unclear. Published references and anecdotal reports indicate Channel Island breeds, such as Jersey (Norton et al 2009) and Guernsey (Withers 1959; Cetinkaya et al 1997a), Brahman (Olcott et al 1991; Roussel et al 2005) and Shorthorn cattle and Scottish Blackface, Shetland, Colbred (Cranwell 1993) and fine wool breeds of sheep (Mainar-Jaime and Vazquez-Boland 1998; Lugton 2004a) have an increased susceptibility to the development of clinical JD relative to other breeds (Clarke 1997). Conversely, a higher prevalence of MAP

infection *per se* detected within a particular breed in the absence of higher rates of clinical JD may indicate an increased resistance to infection, particularly if lower levels of clinical disease are detected.

1.7 Economic cost of Johne's disease

Brett (1998) published the first economic evaluation of clinical JD in New Zealand farmed deer, based on a model developed for the Victorian (Australia) dairy industry. The estimated annual economic impact of clinical JD on whole-herd profitability ranged from NZ$3,149 (annual clinical JD prevalence of 0.5% of breeding hinds) to NZ$33,465 (7% of breeding hinds clinically affected annually), with no culling program utilized. A national herd-level clinical JD prevalence of 7% represented an annual cost to the industry of NZ$341,222. However, if clinical JD spread to every deer herd in New Zealand, the maximum productivity cost was estimated at NZ$4.9 million. These are now considered underestimates as the report did not include potential losses due to subclinical JD, such as increased reactivity to intradermal tests for bTb. A formal evaluation of the economic cost of MAP infection, including subclinical and clinical JD, to the New Zealand farmed deer industry, allowing for any uncertainty associated with parameter estimation, is urgently required to allow prioritisation of the importance of clinical JD relative to other diseases and to provide a benchmark against which the success, or otherwise, of national control programs can be monitored.

Published estimates of the cost of clinical JD in dairy cattle, focused particularly on the US dairy industry, have varied widely, ranging from US$145 to US$1,094 per infected cow (Buergelt and Duncan 1978; Abbas et al 1983; Chiodini and Van Kruiningen 1986; Benedictus et al 1987; Ott et al 1999). In 2003, the discounted per herd total loss due to JD over a 20 year period in a midsize US dairy herd without a disease control program was estimated at US$49,112, with suboptimal culling and reduced milk production the leading contributors (Ott et al 1999; Groenendaal and Galligan 2003). In 1998, Brett estimated the cost of clinical JD to the New Zealand dairy industry at NZ$18.9 million annually, with NZ$532 (one clinical case every three years) to NZ$18,550 (nine clinical cases annually) loss per herd. In 2001, the total annual cost of clinical JD to the Australian sheep industry was estimated at

AU$60 million, predominately due to on-farm sheep mortalities (Topp and Bailey 2001). Annual per farm production loss estimates ranged from AU$400 to AU$16,750 depending on mortality rate, geographic location and flock production type. In 1998, the total cost of clinical JD to the New Zealand sheep industry was estimated at NZ$9.9 million, with per flock annual loss estimates ranging from NZ$653 (1 clinical case every two years) to NZ$13,039 (five clinical cases annually), due predominately to disruptions in wool and lamb production, culls to sell and rearing and retention of replacement animals (Brett 1998).

1.8 Abattoir surveillance for *Mycobacterium avium* subspecies *paratuberculosis* in commercially slaughtered New Zealand farmed deer

One aim of this thesis was to validate the establishment of a national, abattoir-based surveillance scheme for MAP in deer. The process for development of the scheme, including potential limitations, is outlined in detail in Chapter 8, the general discussion to this thesis.

Thurmond (2003) defined surveillance for infectious diseases as 'an active, ongoing, formal, and systematic process aimed at early detection of a specific disease or agent in a[n animal] population'. 'Passive' surveillance is the examination of clinically affected cases only, while 'active' surveillance also involves examination of clinically normal animals in the population of interest (Thrusfield 2005a). Although both involve gathering, recording and analysis of data, 'passive' surveillance is not suitable for documentation of low prevalence, subclinical or non-notifiable/endemic diseases, such as JD (Stark 1996).

In 1964, the Danish pork industry began the first national comprehensive data collection scheme of animal disease based on routine slaughter inspection (Willeberg et al 1984). Subsequent studies of abattoir-based surveillance, including for salmonella in Danish pigs (Goldbach and Alban 2006; Baptista et al 2010), contagious bovine pleuropneumonia in Swiss cattle (Stark 1996) and bTb in various cattle

populations worldwide (Kaneene et al 2006; Frankena et al 2007; Muller et al 2008; Biffa et al 2010), have found this a socio-economically profitable method for disease identification relative to interventions at the herd-level. Since the commencement of abattoir-based surveillance, the proportion of human cases of Salmonella attributable to Danish pork has decreased significantly (Baptista et al 2010).

Australia initiated a surveillance scheme for ovine JD (OJD) in 25 abattoirs nationwide in 1999 (Sergeant and Baldock 2002; Bradley and Cannon 2005). Although this program has now been limited to a small number of abattoirs in New South Wales and Victoria (Citer, L; *pers. comm.*), recently Western Australian farmers acknowledged the worth of slaughter surveillance for OJD and have called for re-expansion of the scheme (Huxley 2011). In 2003, the JRG and Massey University identified the need for a national surveillance scheme for MAP infection and/or clinical JD in the New Zealand farmed deer industry and the following describes the development of a database recording 'abnormal' LN suspicious for MAP infection in commercially slaughtered deer.

To ensure 'fitness for intended purpose' (Anonymous 2005b), every deer carcass commercially slaughtered in New Zealand is assessed by an accredited meat inspector as required by Regulation 123 of the Meat Regulations 1969 and Regulation 112 of the Game Regulations. A prominent function of carcass inspection in both deer and cattle is surveillance for macroscopically visible carcass abnormalities due to *M. bovis* infection. Under the national bovine tuberculosis pest management strategy, as administered by the Animal Health Board (AHB), a 'bTb-suspect' lesion is defined as a LN containing a core of caseous, necrotic or mineralised material. Up to three carcasses per line with 'bTb-suspect' lesions (where a 'line' is a group of animals sourced from the same vendor and slaughtered on the same day in the same abattoir) were traditionally submitted for diagnostic testing, primarily via histopathological examination and secondarily by BACTEC culture, if deemed necessary. These 'bTb-suspect' lesions are now tested primarily using polymerase chain reaction (PCR).

Infection with MAP in deer can cause a lymphadenopathy with or without caseation, necrosis and/or mineralization, that is macro- and microscopically indistinguishable from pathology due to *M. bovis* infection (Mackintosh et al 1999; de Lisle et al 2003;

Crawford et al 2006). The inability of most diagnostic tests, including intradermal tests, carcass inspection and histopathological examination, to differentiate between *M. bovis* and MAP infection in deer confounds the AHB's mission of eradicating bTb from New Zealand and has resulted, in the past, in detainment of suspect deer carcasses until confirmation of the causative agent, with delayed schedule payment to the farmer (Campbell 1995). Consequently, since 1990, all 'bTb-suspect' mesenteric LN have also been cultured for MAP, initially by inclusion of an additional culture slope and then a BACTEC vial containing egg yolk and mycobactin (de Lisle et al 2005). Therefore, an indirect benefit of the reduced specificity of *M. bovis* diagnosis within the national surveillance scheme has been the diagnosis of MAP as a 'by-product' via culture.

In 1994, only 5.5% of 505 'bTb-suspect' LN were culture-positive for MAP, whilst 33.1% and 7.7% were culture-positive for *M. bovis* and *M. avium* subspecies *avium* (*M. avium*), respectively (Mackintosh and Carter 1999). In contrast, in the 2003/2004 season, 35.9% of 'bTb-suspect' LN from four deer abattoirs were culture-positive for MAP, relative to 23.9% and 9.0% culture-positive for *M. bovis* and *M. avium*, respectively (Glossop et al 2005). Therefore, the existing abattoir-based surveillance system for *M. bovis* in deer may be utilized to identify MAP at the animal- and herd-level, particularly as the latter MAP prevalence was likely underestimated with LN tested only after evaluation of previous evidence for JD within the herd of origin.

1.9 Thesis Aims and Objectives

The primary aims of this thesis were to substantially further our knowledge of MAP and clinical JD at the herd-level in the New Zealand farmed deer population, and to validate a national abattoir-based surveillance scheme for MAP in farmed deer. These aims were achieved through completion of two main sub-objectives, namely:

1) MAP/clinical JD at the herd-level:

   i) Characterisation of clinical JD in deer using a cross-sectional study approach.

   ii) Risk factors associated with the herd-level presence of MAP, using a case-control study approach.

   iii) Environmental and management risk factors associated with clinical JD incidence in young farmed deer, using a case-control study approach.

2) Validation of a national abattoir surveillance scheme for MAP in farmed deer:

   i) Prevalence of MAP in 'abnormal' deer mesenteric lymph nodes and association between MAP and deer lymph node size.

   ii) Sensitivity, specificity and level of agreement of meat inspector detection of 'abnormal' deer lymph nodes.

   iii) Histopathology of grossly 'normal' mesenteric lymph nodes of New Zealand farmed red deer (*Cervus elaphus*) including identification of lipopigment.

The scientific work is described in chapters 2 to 7, which also includes discussion of the individual studies. A general discussion of the overall work and outcomes, along with establishment of the national database for surveillance as a significant practical outcome of this research, are discussed in chapter 8.

The p-value is the probability that the test statistic would be as large or larger than the computed test statistic, if the null hypothesis was true (Dohoo et al 2003d). Although an arbitrary p-value threshold of <0.05 has been traditionally used to reject the null hypothesis, a p-value of <0.10 was used in all thesis chapters as <0.05 was found to be too narrow to identify numerous risk factors associated with each sudy

outcome.Chapter 2 quantifies Johne's disease incidence, including long-term trends, in known *Mycobacterium avium* subspecies *paratuberculosis*-infected farmed deer herds and has been submitted to the New Zealand Veterinary Journal.

CHAPTER 2: Characterisation of clinical Johne's disease in farmed deer (*Cervus elaphus*) herds in New Zealand

JC Hunnam, PR Wilson, C Heuer, F Castillo-Alcala, DM West, CG Mackintosh

2.1 Abstract

AIMS: To describe and quantify clinical Johne's disease incidence, including long-term trends, in known *Mycobacterium avium* subspecies *paratuberculosis* (MAP)-infected farmed deer herds and evaluate associations with age, sex, geographic location and season.

METHODS: Retrospective data, including the number of deer with farmer-diagnosed clinical Johne's disease, from 1 January to 31 December 2005, were obtained by personal interview of 174 farmers. These herds contained 290,818 deer, 17.1% of the published national figure, and were located in all regions of New Zealand, except Taranaki. The herd-level MAP status was determined through tissue or pooled faecal culture. Subsequently, a postal questionnaire was received from 81 survey herds, assessing the change in herd disease status and the incidence of clinical cases from 2005 to 2007. Attributable risk and attributable fraction were used to assess the misclassification rate of farmer-diagnosed clinical JD at the herd-level, based on observation of typical clinical signs. A logistic regression model was used to assess the significance of associations between the annual incidence of clinical Johne's disease and island, age-class and season.

RESULTS: In MAP-positive herds in 2005, the median incidence of Johne's disease was 1.2%, 2.0%, 2.0%, 0.9% and 1.3% in weaners, yearling hinds, yearling stags, adult hinds and adult stags, respectively. Overall, Johne's disease incidence was associated with age-class and geographic location, being higher in South Island versus North Island herds containing weaner deer, yearling hinds or adult hinds ($p<0.05$). Johne's disease incidence was greater in winter (yearling hinds, adult hinds, adult stags) or winter and spring (weaner deer, yearling stags) than in other seasons. Sixty-one percent of farmers with herds without Johne's disease in 2005 reported one or

more clinical case(s) in either 2006 or 2007, implying that the rate of herds reporting Johne's disease was almost seven times that of herds no longer reporting Johne's disease. The misclassification rate of Johne's disease at the herd level by farmers was estimated at 60%.

CONCLUSIONS: The majority of study herds had a Johne's disease annual incidence rate of less than 1%. The proportion of herds with Johne's disease appeared to increase over time, showed a seasonal trend, and was significantly higher in the South than in the North Island. Yearling hinds and stags had the highest incidence of Johne's disease. The rate of misclassification of Johne's disease diagnosis at the herd-level by farmer observation was moderately high.

2.2 Introduction

Johne's disease is a chronic, granulomatous enteritis of domestic and wild ruminants, caused by infection with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) (Collins 2003a). The clinical expression of Johne's disease in deer, the focus of this paper, manifests as individual cases or outbreaks of progressive weight loss, with or without diarrhoea, from 8 to 15 months of age and is invariably fatal (Mackintosh et al 2007a). The first putative diagnosis of MAP in a farmed deer in New Zealand was in 1979, based on histopathology considered typical of Johne's disease in the ileum of an adult Rusa deer (*Cervus timorensis*) (Gumbrell 1987). Since that time, MAP infection has developed into a significant economic burden for the farmed deer industry in New Zealand, due largely to clinical disease and mortalities in young deer (Mackintosh and Wilson 2003; Mackintosh et al 2004a). However, there has not been a comprehensive investigation of the incidence of Johne's disease in the national farmed deer population or in confirmed MAP-infected herds. Although some research has incorporated monitoring of Johne's disease in deer, the focus of those studies was exploration of a specific research question such as vaccine efficacy and diagnostic test accuracy, rather than estimation of national incidence rates *per se* (Griffin et al 2005; Mackintosh et al 2007a).

Development of a national control programme for MAP in farmed deer, if deemed appropriate in the future, will require knowledge of the baseline incidence of Johne's disease at the levels of herd and age-class (i.e. weaners, yearling hinds, yearling stags,

adult hinds, adult stags). Although MAP has been confirmed in deer herds throughout New Zealand (Glossop et al 2006), anecdotally there appeared to be a higher incidence rate of Johne's disease in South Island herds, suggesting that incidence estimates should be stratified by Island. There was also some anecdotal evidence that the occurrence of Johne's disease in deer is seasonal, most commonly observed in weaners in winter and spring of their first year (Mackintosh et al 2004a).

This paper describes the occurrence of Johne's disease by herd, age-class, season and geographic location, including longitudinal changes from 2005 to 2007. It also compares the frequency of farmer diagnosed Johne's disease in infected and non-infected herds to evaluate potential misclassification.

2.3 Materials and methods

The population of interest comprised New Zealand farmed deer herds with ≥ 20 red deer (*Cervus elaphus*), North American elk (*Cervus elaphus canadensis* subspecies group) and/or red deer/elk crossbreds ('wapiti'). A 2005 Deer Industry New Zealand postal survey, with a 60% response rate, estimated 3,762 herds contained deer (Anonymous 2006a). An initial retrospective study of 174 New Zealand deer herds in 2005 (6.1%) ('survey') was followed by a longitudinal study of a subset of 81 herds from 2005 to 2007 (2.2%). A property was defined as a single geographic location on which deer were grazed. The 'deer fenced area' (DFA) was an area within fences as defined by the Wild Animal Control Act 1977 (Anonymous 1977). A herd was defined as all deer grazed on the same DFA. Deer were classified according to age as 'weaner' (>3 months and ≤12 months), 'yearling' (>12 and ≤24 months) and 'adult' (>24 months). Female deer were referred to as hinds and male deer as stags. Deer were classified into age-classes based on age and sex as weaner deer (both sexes combined), yearling hinds, yearling stags, adult hinds or adult stags.

2.3.1 2005 survey

2.3.1.1 Selection of deer herds

Selection and enrolment of deer herds began with a call for participants by promotion at two national deer farming conferences and notification in two issues of a New

Zealand Deer Industry magazine ('Deer Industry News') (July 2004; December 2004) circulated to an estimated 4,000 deer farmers. On 15 March 2005, a short postal questionnaire was sent to 311 respondents requesting confirmation of participation in the study, their intended deer herd size and composition at 31 July 2005 and whether one or more deer within their herd had a positive tissue or faecal culture for MAP if sampled between 15 March 2004 and 15 March 2005. From 193 (62.1%) farmers initially confirming their participation, 10 farmers were excluded due to small herd size (≤ 20 weaner deer). Nine other farmers withdrew during the period of data collection. The remaining 174 (55.9%) farmers were visited by the first author for interview and sample collection between 23 July 2005 and 16 March 2006.

2.3.1.2 Determination of the herd infection status

Fifty-nine of the 174 participating herds were categorised as culture-positive for MAP based on one or more positive radiometric faecal and/or tissue cultures (usually lymph nodes for investigation of bovine tuberculosis (*M. bovis;* bTb) at slaughter), sampled between 15 March 2004 and 15 March 2005 as determined by AgResearch Wallaceville Animal Health Laboratory (Upper Hutt, Wellington).

Sixty adult breeding hinds, or otherwise an available age-class, from each of the remaining 115 herds were sampled by the first author or the herd's veterinarian, either while standing in pens or using a restraining device. A minimum of 5 faecal pellets, or the equivalent of two grams of faeces, was collected through careful insertion of a methylcellulose lubricated, gloved finger into the deer's rectum. A new glove was used for each sample. Samples were stored in a separate, sterile 70 ml medical specimen container. The age was determined from the ear tag if the year of birth was listed, or was estimated by teeth wear. Signs of diarrhoea and/or weight loss were noted. The bTb reactor status (positive or negative) to the last intra-dermal test was retrieved from farmer records. Samples were immediately refrigerated (4^{o}C) and couriered within 24 hours to Massey University (Palmerston North, New Zealand) where they were selectively pooled as below. Faeces from deer with diarrhoea, weight loss and/or bTb-reactivity were combined into the same pool(s). The remaining samples were pooled based on age. Samples were then either chilled at 4^{o}C for a maximum of 48 hours or immediately couriered to AgResearch Wallaceville (Upper Hutt, New Zealand) for culture.

Culture of 6 pools of 10 faecal samples each per herd was estimated to be the most cost-effective method for the herd-level diagnosis of MAP. Animals with the highest probability of infection (i.e. with diarrhoea and/or weight loss and non-specific reactivity to a previous bTb intra-dermal test) were targeted for sampling to optimise herd-level sensitivity (Smith and Slenning 2000). Few or no false positive herd diagnoses were expected since the specificity of faecal culture was assumed to be close to 100% (Merkal 1984). Herds with negative cultures for MAP were termed 'culture-negative', whilst herds with one or more pools culture-positive for MAP were termed 'culture-postive'. Farm owners were informed of culture results for their herd within three (3) months of sampling.

2.3.1.3 Questionnaire

Between 23 July 2005 and 16 March 2006, data were collected using a comprehensive questionnaire (see Appendix 2), including information at the herd and individual animal levels, for the period 1 January 2005 to 31 December 2005 by personal interview of each study farmer by the first author. Information provided to researchers was based on farmer recollection and/or written farm records. Properties visited prior to 31 December 2005 were contacted by phone in early 2006 to complete records and information to the end of the 2005 calendar year and to be informed of any test results.

Johne's disease data were based largely on farmer diagnosis as veterinary confirmation was infrequent. The number of each age-class of deer observed with diarrhoea and/or weight loss and/or the number diagnosed with Johne's disease, yersiniosis (due to infection with *Yersinia pseudotuberculosis*) and/or a chronic copper deficiency based on the observation of these clinical signs, including those that had died, was provided. The number of deer with diarrhoea and/or weight loss that were administered an anthelmintic for gastrointestinal parasitism and the number which responded without recurrence of clinical signs was also recorded. In addition, the year that Johne's disease was first observed in their deer herd was recorded, based predominately on farmer recall.

2.3.1.4 Determination of animal-level Johne's disease status

An individual deer was diagnosed as 'Johne's disease-positive' if the farmer had diagnosed it as such and/or a deer was observed with diarrhoea and/or weight loss that the farmer or veterinarian had not diagnosed as yersiniosis, chronic copper deficiency or other disease, and there was incomplete resolution of clinical signs if an anthelmintic drench, trace mineral and/or antibiotic had been administered. An individual deer was defined as 'Johne's disease-negative' if it had not been observed with diarrhoea and/or weight loss or had been diagnosed with yersiniosis or chronic copper deficiency and/or had responded to an anthelmintic drench, trace mineral and/or antibiotic, with persistent resolution of clinical signs. Each farmer indicated which season(s) Johne's disease or diarrhoea/weight loss was observed in deer of each age-class.

2.3.1.5 Determination of herd-level Johne's disease status

Figure 2.1 illustrates the decision pathway used to classify the herd MAP status as 'positive' with various risk categories ('high', 'low' and 'suspect'), and the Johne's disease status of each herd. Herds were classified as 'Johne's disease-positive' if they were culture-positive for MAP and contained one or more 'Johne's disease-positive' deer. All other herds were classified as 'Johne's disease-negative', including herds that contained deer with diarrhoea and/or weight loss that had been classified as 'Johne's disease-negative'.

2.3.2 Longitudinal study

2.3.2.1 Follow-up questionnaire

On 22 November 2007, 170 of the 174 farmers in the above survey were mailed a follow-up questionnaire (see Appendix 3) to gather data on the longitudinal pattern of Johne's disease. Seven questionnaires were returned as address unknown. Of the 84 farmers (51.5%) who responded, three no longer farmed deer. Thus, 81 farmers provided information on the size and composition of their deer herd grazed at 30 June 2007, the number of new cases of farmer-diagnosed Johne's disease, including those that had died, and the method of diagnosis, such as clinical signs (as above), abnormal lymph nodes sampled as suspicious for bTb at slaughter (information provided to the

farmer by the Animal Health Board) and/or a blood test (by routine screening or ancillary testing for bTb) or faecal culture. Observations recorded from 1 January 2006 to 15 November 2007 were considered for analysis. If Johne's disease was not diagnosed, the number and age-class of deer with diarrhoea and/or weight loss was recorded. Date of treatment for the prevention and remedy of gastrointestinal parasitism, yersiniosis and/or copper deficiency, such as an anthelmintic, antibiotic and/or copper supplement, to animals with diarrhoea and/or weight loss, and response to treatment were also recorded.

2.3.2.2 Determination of animal-level Johne's disease status

An individual deer was classified as 'Johne's disease-negative' or 'Johne's disease-positive' using the criteria as above. None were confirmed by blood tests and/or faecal culture as data provided was retrospective.

2.3.2.3 Determination of herd-level Johne's disease status

The Johne's disease status of longitudinal study herds was determined as shown in Figure 2.1. Herds were classified as at 'high risk' for MAP if 'culture-positive' in 2005. Longitudinal herds 'culture-negative' in 2005 were also termed at 'high risk' of MAP if one or more deer were positive to a MAP-specific blood test, faecal culture for MAP and/or abnormal lymph node(s) at slaughter in 2006 ± 2007 or one or more deer with clinical signs did not respond when treated with an anthelmintic drench, trace mineral and/or antibiotic or clinical signs recurred post-treatment. Herds were classified as at 'low risk' for MAP if 'culture-negative' in 2005 and no deer were observed with clinical signs in 2006 and/or 2007 or deer with clinical signs had responded completely to treatment. Longitudinal herds 'culture-negative' in 2005 and had one or more deer with clinical signs that were not treated were classified as 'suspect'.

2.3.3 Statistical analysis

Herds were aggregated into regions defined in 1989 according to the Local Government Act 1974 (Harris 2004). Auckland and Northland were combined and termed 'Northland'. Similarly, Tasman, Nelson and Marlborough were combined and

termed 'Nelson-Marlborough'. Herds were then aggregated into either North or South Island populations.

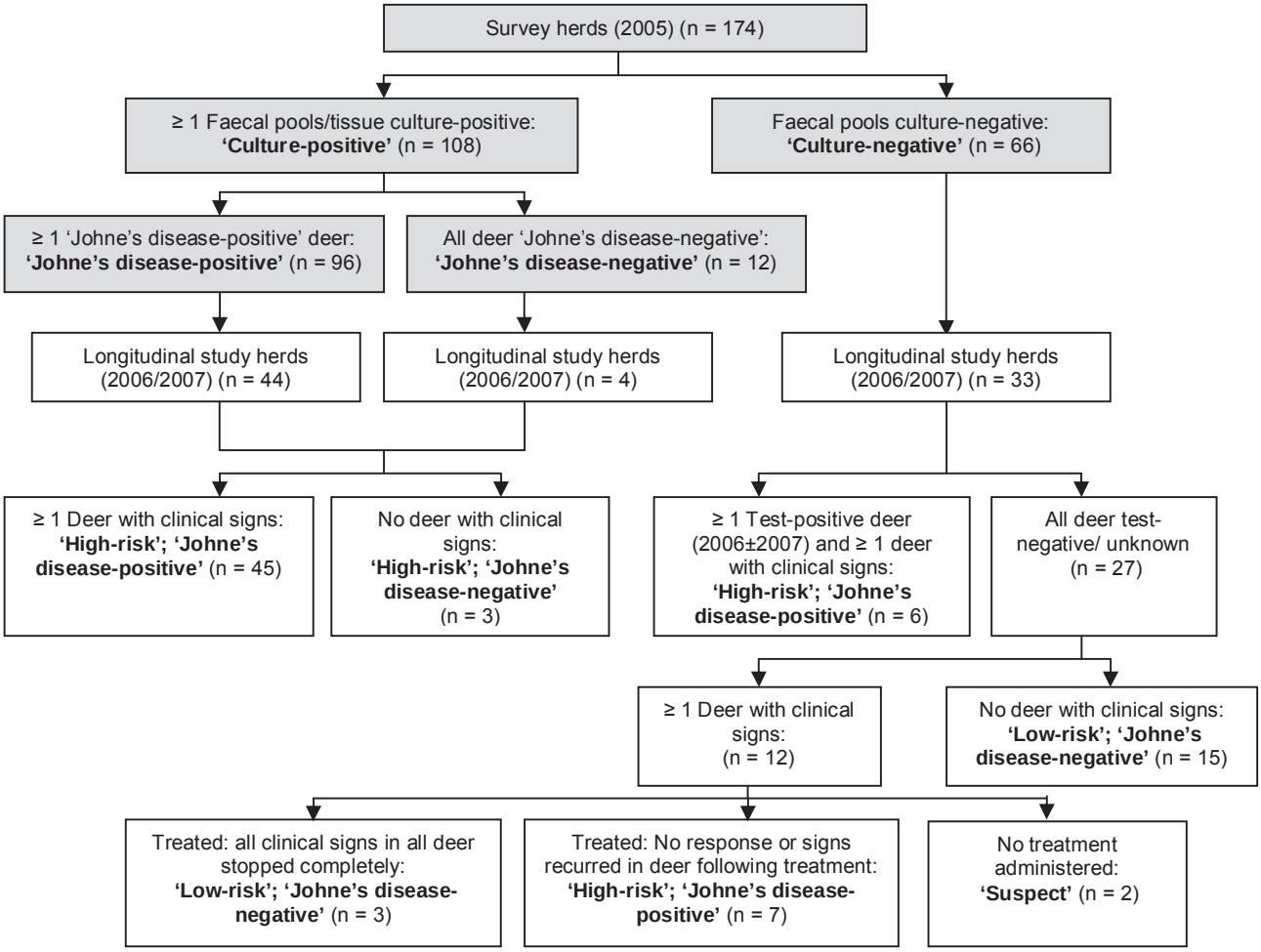


Figure 2.1 Decision tree classifying farmed deer herds in New Zealand for a 2005 survey (n = 174) (shaded) and a subsequent longitudinal study from 1 January 2006 to 15 November 2007 (n = 81) to characterise Johne's disease. (Note: herd-level classifications as referred to in the text are in bold font) MAP = *Mycobacterium avium* subspecies *paratuberculosis*

The regional distribution of the 174 survey herds was compared with 2005 and 2007 national deer herd statistics to assess to what extent the study herds represented the national population. A similar analysis was undertaken for the longitudinal study herds using 2007 figures.

Associations among the annual incidence of Johne's disease and age-class, Island and season were evaluated at animal and herd levels. Logistic regression was used with Johne's disease cases per age-class as the outcome divided by the size of the age-class. The size of each age-class was based on the number wintered as at the 30 June

2005. Introductions of deer prior to and after this date were not accounted for. Associations with season were calculated by age-class and region was included as a herd-level fixed effect. The seasons were categorised as follows: 'summer' (01 December to 28 February), 'autumn' (01 March to 31 May), 'winter' (01 June to 31 August) and 'spring' (01 September to 30 November). Correlations of animals within age-class, and age-classes within farm were adjusted using a scale parameter calculated as residual deviance divided by the degrees of freedom for error (McDermott et al 1994). Risk ratios (RR) were calculated from the logistic model and used to compare age, season and Island strata.

The known MAP infection status (i.e. 'culture-positive' or 'culture-negative') of 'Johne's disease-positive' herds was compared with reported annual incidence of Johne's disease to evaluate the misclassification rate of Johne's disease diagnoses by farmers. It was assumed that diarrhoea and/or weight loss in herds with a 'culture-negative' MAP status was unlikely to be due to Johne's disease, whilst those clinical signs in a 'culture-positive' herd were likely due to Johne's disease. The probability of farmer-diagnosed clinical signs in 'culture-positive' herds above the baseline risk (i.e. the probability of farmer-diagnosed clinical signs in 'culture-negative' herds) was calculated using attributable risk, overall and stratified by Island. An attributable fraction was then calculated to determine the proportion of clinical signs in 'culture-positive' herds likely due to MAP infection.

A customized database (Microsoft Access for Windows; Microsoft Corporation, Washington, USA) and spreadsheet software (Microsoft Excel; Microsoft Corporation) were used for data management. Statistical hypothesis tests were performed in R (version 2.4.1; The R Foundation for Statistical Computing), using a significance level of $P < 0.05$.

2.4 Results

2.4.1 Study population

The number and regional distribution of deer in the 2005 survey and 2007 longitudinal study herds are presented in Table 2.1, with national figures for

comparison. The 290,818 deer in the 2005 survey herds represented 17.1% of farmed deer (Anonymous 2005a) and were from 6.1% of New Zealand deer herds as of June 2005 (Anonymous 2006a). Fifty-five (32%) and 119 (68%) of 174 survey herds were located in the North and South Islands, respectively, and 25 (31%) and 56 (69%) of 81 longitudinal study herds were in the North and South Islands, respectively. The 2005 survey included 108 'culture-positive' and 66 'culture-negative' herds, while the 2007 longitudinal study included 58 'high-risk, Johne's disease-positive' herds, three 'high-risk, Johne's disease-negative' herds, two 'suspect, Johne's disease-positive' herds and 18 'low-risk, Johne's disease-negative' herds.

Table 2.1 Percentage of deer in a 2005 survey (n = 174 herds) and a 2005 to 2007 longitudinal study (n = 81 herds) characterising Johne's disease by region of New Zealand compared with national statistics (Statistics New Zealand Agricultural Production Survey (APS)) and the difference (diff.), for both

Region	APS 2005 (n = 1,705,000)	Survey 2005 (n = 290,818)	diff.	APS 2007 (n = 1,396,000)	Longitudinal 2007 (n = 89,617)	diff.
	% total	% total		% total	% total	
Northland	U	2.4		1.4	2.0	+0.6
Waikato	8.3	14.7	+6.4	8.4	9.4	+1.0
Bay of Plenty	3.5	1.0	-2.5	3.9	0.9	-3.0
Gisborne	1.8	0.5	-1.3	2.0	2.2	+0.2
Hawkes Bay	7.0	6.4	-0.6	6.3	5.6	-0.7
Taranaki	0.3	0.0	-0.3	0.3	0.0	-0.3
Manawatu-Wanganui	8.7	2.6	-6.1	7.4	3.7	-3.7
Wellington	U	0.3		1.1	0.8	-0.3
Total North Island	29.6	27.9	-1.7	30.8	24.6	-6.2
Nelson-Marlborough	1.9	0.7	-1.2	2.3	1.0	-1.3
West Coast	U	8.6		3.0	4.9	+1.9
Canterbury	28.3	28.3	0.0	28.3	27.0	-1.3
Otago	11.7	15.1	+3.4	13.5	20.8	+7.3
Southland	21.8	19.4	-2.4	22.1	21.7	-0.4
Total South Island	63.7	72.1	+8.4	69.2	75.4	+6.2
Total New Zealand	93.3[A]	100.0		100.0	100.0	

U = Unavailable

[A] % total did not sum to 100% as statistics for Northland, Wellington and the West Coast were unavailable

Forty-eight (44%) of the 108 'culture-positive' 2005 survey herds responded to the longitudinal study questionnaire, with 45 (94%) farmers observing clinical signs in their deer in 2006 and/or 2007. Thirty-three (50%) of the 66 'culture-negative' 2005 survey herds responded, with six (18%) herds reporting a positive test for MAP in 2006 and/or 2007. Of the remaining 33 herds, 18 (55%) herds continued to be at 'low-risk' of MAP infection. Seven herds (21%) were at 'high-risk' of MAP

infection in 2006 and/or 2007 with 'Johne's disease-positive' deer observed, while the remaining two (6%) herds were classified as 'suspect' in 2006 and/or 2007.

2.4.2 2005 survey

2.4.2.1 Year of onset of Johne's disease

The number of farmers with 'Johne's disease-positive' herds who first observed Johne's disease in their deer herd in each year from 1988 to 2005, inclusive, was presented in Figure 2.2. Sixty percent of farmers first observed Johne's disease prior to 2005. The earliest year of observation was 1988, reported by one farmer.

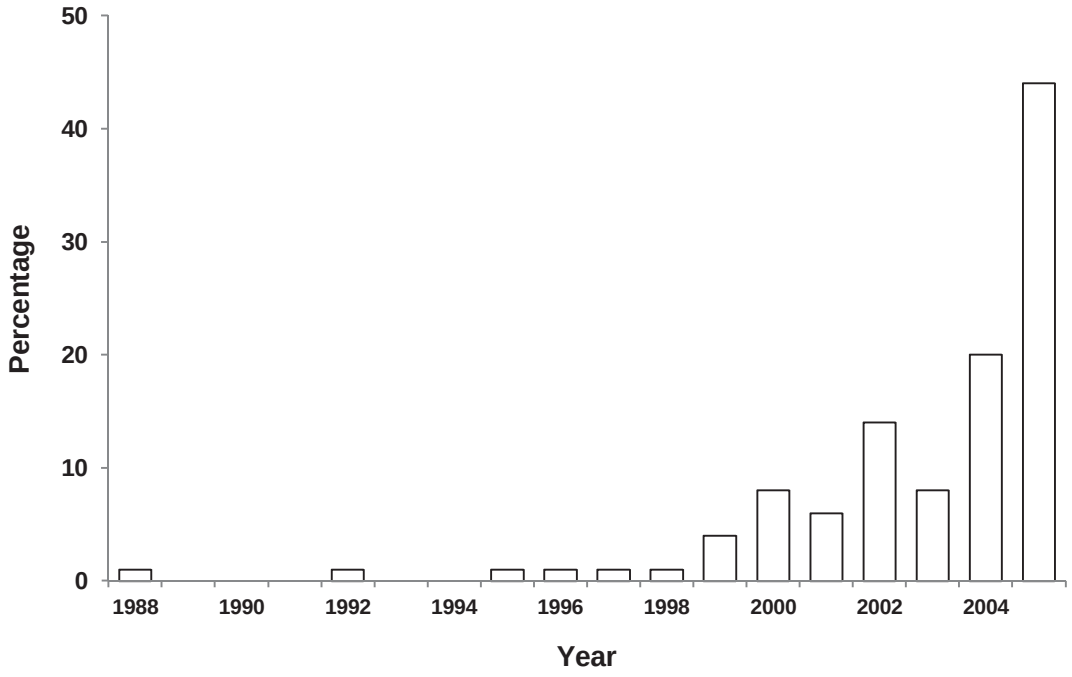


Figure 2.2 Percentage of New Zealand deer farmers who first observed Johne's disease in their deer in each year prior to and including 2005 (96 'culture-positive' herds).

2.4.2.2 Age-class incidence of Johne's disease in 'Johne's disease-positive' herds

Summary statistics for the incidence of Johne's disease by age-class (≥ 30 animals per mob) in the 96 'culture-positive', 'Johne's disease-positive' herds are presented in Table 2.2. The incidence was higher in both yearling hinds and yearling stags than in weaners, adult hinds or adult stags ($p<0.05$). The minimum incidence ranged from 0.1 to 0.2%, while the maximum incidence varied from 8.9% (adult stags) to 21.5% (weaners).

Table 2.2 The minimum, maximum and quartiles for the incidence (%) of Johne's disease in each age-class in 96 'culture-positive, 'Johne's disease-positive' survey deer herds (age-class mobs of ≤ 30 deer excluded)

	Herds	Johne's disease incidence (%)				
Age-class	**n**	**Minimum**	**25**	**50**	**75**	**Maximum (Age-class/Herd size)**
Weaner deer	71	0.1	0.4	1.2	2.7	21.5 (372)
Yearling hinds	45	0.2	1.3	2.0	4.6	20.0 (50)
Yearling stags	19	0.2	1.1	2.0	3.7	13.2 (136)
Adult hinds	64	0.1	0.5	0.9	1.7	20.8 (260)
Adult stags	10	0.2	0.8	1.3	2.4	8.9 (56)
Whole herd	**96**	**0.04**	**0.4**	**1.0**	**1.8**	**11.9 (506)**

2.4.2.3 North vs South Island age-class incidence of Johne's disease in all herds

The number of deer of each age-class (and percent 'Johne's disease-positive') and the number of herds containing each age-class (and percent with one or more 'Johne's disease-positive' deer in that age-class) in the North and South Island in the 2005 survey herds were presented in Table 2.3. The risk of Johne's disease at the animal-level was 2.8 times higher in South Island adult stags relative to North Island adult stags ($p<0.05$). There was no significant difference in the animal-level annual incidence of Johne's disease between the North and South Island populations of the other age-classes ($p>0.05$).

South Island herds containing weaner deer, yearling hinds and adult hinds were 2.8, 2.7 and 2.0, respectively, times more likely to have animals with Johne's disease, relative to North Island herds ($p<0.05$). There was no between-Island difference in clinical Johne's disease incidence in herds containing yearling stags or adult stags ($p>0.05$).

2.4.2.4 Association between season and observation of Johne's disease

Data on Johne's disease by season and age-class were available for 83 of the 96 'culture-positive' 'Johne's disease-positive' herds in the 2005 survey. Figure 2.3 shows the herd-level incidence of Johne's disease in each age-class, by season. The herd-level incidence of Johne's disease in all age-classes was lowest in summer and autumn and highest in spring and/or winter.

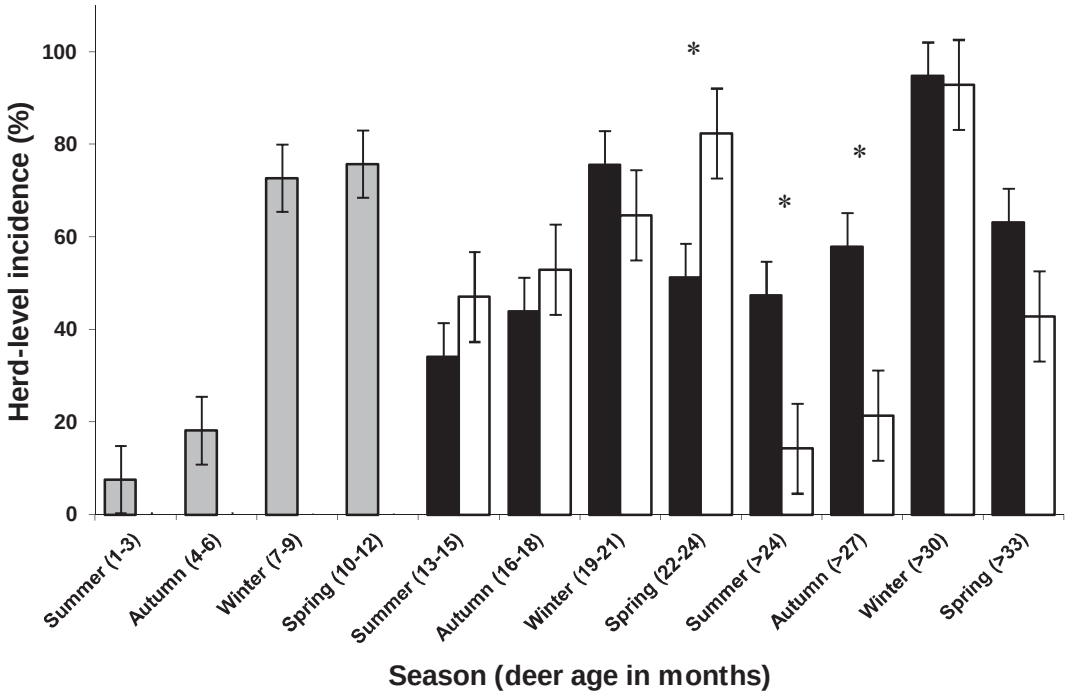


Figure 2.3 Herd-level incidence (n = 83 confirmed infected with *Mycobacterium avium* subspecies *paratuberculosis*) of clinical Johne's disease in weaner hinds and stags combined (▨), hinds (■) and stags (□) of each age-class, by season (months of age in brackets).
(*Statistically significant difference ($p<0.05$) between hinds and stags)

The age-class analysis showed there was a 9.6 (95% CI = 8.6 to 10.6) and 4.0 (95% CI = 3.3 to 4.7) times higher risk of Johne's disease in weaners in the winter of their first year, relative to summer and autumn, respectively. In the second year of life, there was a significantly higher risk of Johne's disease being observed in yearling hinds in winter, relative to summer (RR = 2.2; 95% CI = 1.4 to 3.0) and autumn (RR = 1.8; 95% CI = 1.0 to 2.4). However, there was no significant difference between the seasons in yearling stags. In both adult hinds and adult stags, the risk of Johne's disease was higher in winter than summer (RR = 2.0; 95% CI = 1.5 to 2.9 and 6.5; 95% CI = 5.4 to 7.0, respectively) and autumn (RR = 1.6; 95% CI = 1.1 to 2.0 and 4.3; 95% CI = 3.9 to 4.8, respectively).

2.4.2.5 Attributable risk of farmer-diagnosed Johne's disease in 'culture-positive' herds:

Owners/managers of 94 of 108 'culture-positive' herds (87%) reported Johne's disease, whereas the owners/managers of 31 of 66 'culture-negative' herds (47%) reported clinical signs consistent with Johne's disease in their deer. Assuming that all of the latter farmers had misdiagnosed clinical signs as being due to Johne's disease,

Table 2.3 The number (n) of deer and deer herds and incidence (%) (95% CI) of Johne's disease, adjusted for herd, for each age-class in 2005, by Island in New Zealand.

	Deer				Herds			
	North Island		South Island		North Island		South Island	
Age-class	n	Incidence	n	Incidence	n	Incidence	n	Incidence
Weaners	31,054	0.69 (0.30-1.55)	94,254	0.96 (0.65-1.40)	53	15.1 (5.1-25.1)	119	42.6 (33.9-51.8)
Yearling hinds	9,180	0.70 (0.22-2.14)	20,535	1.42 (1.02-1.98)	48	16.7 (6.0-27.3)	107	37.7 (28.4-47.1)
Yearling stags	4,569	0.62 (0.17-2.18)	8,361	0.97 (0.58-1.59)	41	14.6 (3.7-25.6)	91	20.9 (12.5-29.3)
Adult hinds	40,125	0.41 (0.19-0.89)	67,577	0.69 (0.51-0.94)	50	29.2 (16.2-42.2)	107	59.3 (49.9-68.6)
Adult stags	5,870	0.22 (0.10-0.49)	9,293	0.61 (0.34-1.09)	49	10.2 (1.6-18.8)	108	17.6 (10.4-24.8)

overall 40% of observed clinical signs, at the herd-level, were attributable to infection with MAP and thus were likely to be correct. Clinical signs in 46% of 'culture-positive' 'Johne's disease-positive' herds were attributable to MAP infection.

2.4.3 Longitudinal study

2.4.3.1 Herd-level Johne's disease status

The percentage of longitudinal study herds classified as 'Johne's disease-negative' or 'Johne's disease-positive' in 2005, in which the clinical status persisted or changed in 2006 and/or 2007, was shown in Table 2.4. Thirty-six longitudinal study herds were classified as 'Johne's disease-negative' in 2005 and 45 as 'Johne's disease-positive'. Sixty one percent of 'Johne's disease-negative' herds in 2005 contained one or more positive or suspect deer in either or both of 2006 and 2007. Ninety one percent of 'Johne's disease-positive' herds in 2005 had deer with signs of disease in 2006 and/or 2007.

Table 2.4 The percentage of survey deer herds in New Zealand with no Johne's disease and herds with one or more deer with Johne's disease in 2005 that retained or changed disease status in 2006 and/or 2007.

Johne's disease-negative in 2005 (n = 36)		**%**
2006	**2007**	
Johne's disease-negative	Johne's disease-negative	39
Johne's disease-negative	Johne's disease-positive	22
Johne's disease-negative	Suspect	11
Johne's disease-positive	Johne's disease-positive	28
Johne's disease-positive in 2005 (n = 45)		
2006	**2007**	
Johne's disease-negative	Johne's disease-negative	9
Johne's disease-negative	Johne's disease-positive	20
Johne's disease-negative	Suspect	9
Johne's disease-positive	Johne's disease-negative	4
Johne's disease-positive	Johne's disease-positive	58

The risk of a 'Johne's disease-negative' herd in 2005 having one or more 'Johne's disease-positive' deer in either 2006 and/or 2007 was not significantly different from a 'Johne's disease-positive' herd in 2005 being classified as 'Johne's disease-positive' in either 2006 and/or 2007 (RR = 1.5; 95% CI = 0.8 to 2.2). However, the risk of a 'Johne's disease-negative' herd in 2005 being classified as 'Johne's disease-positive' in both 2006 and 2007 was 3.9 (95% CI = 1.2 to 15.0) times higher than a 'Johne's disease-positive' herd in 2005 being classified as 'Johne's

disease-negative' in both 2006 and 2007. This means farmers of infected herds not observing Johne's disease in 2005 were about four times as likely to report Johne's disease in the two subsequent years as were farmers of infected herds becoming clear of Johne's disease. This suggests that the net rate at which farmers of infected herds reported Johne's disease was increasing from 2005 to 2007.

2.4.3.2 Age-class annual incidence of Johne's disease

Four herds which had fewer than 30 deer in each age-class were removed from the age-class specific analysis. The mean percentage of 'Johne's disease-positive' deer in each age-class in 2005 to 2007, in 77 herds and percentage difference between the 2005 and 2007 estimates are presented in Table 2.5. The mean incidence of Johne's disease by age-class decreased by 20% in weaners and by 25% in adult stags, and increased by 25% in adult hinds and 318% in yearling stags over that period.

Table 2.5 The mean percentage (and 95% CI) of deer with clinical Johne's disease in a longitudinal study of New Zealand deer herds from 2005 to 2007, by age-class, and the difference (Diff.) from 2005 to 2007.

Age-class	Number of herds	Percentage (%) of deer with clinical Johne's disease			Diff. (%) 2005 to 2007
		2005	2006	2007	
Weaner	67	1.2 (0.5 - 1.9)	1.1 (0.5 - 1.6)	1.0 (0.3 - 1.6)	-20
Yearling hinds	51	0.9 (0.4 - 1.5)	1.0 (0.3 - 1.8)	1.6 (0.3 - 2.8)	+71
Yearling stags	20	1.4 (0.0 - 2.8)	2.1 (0.2 - 4.1)	5.7 (0.0 - 13.1)	+318
Adult hinds	67	0.9 (0.3 - 1.6)	0.4 (0.2 - 0.7)	1.2 (0.3 - 2.1)	+25
Adult stags	21	0.2 (0.0 - 0.3)	0.2 (0.0 - 0.6)	0.1 (0.1 - 0.3)	-25
All deer	77	0.31 (0.1 - 0.5)	0.4 (0.2 - 0.7)	0.5 (0.2 - 0.9)	+74

2.5 Discussion

There has been anecdotal evidence that Johne's disease had become a serious disease on a number of New Zealand deer herds in the decade preceding this study (de Lisle et al 2006). However, this was the first characterisation of Johne's disease in known MAP-infected farmed deer herds in New Zealand or internationally, at either age-class or herd level. It was also the

first evaluation of a possible association between season or Island and the presence of Johne's disease in deer. The median annual incidence of disease in known 'culture-positive', 'Johne's disease-positive' herds ranged from 0.9 to 2.0% in most herds, but reached levels up to 8.9 to 21.5% in various age-classes in individual herds. Across all herds, the animal-level annual incidence of Johne's disease was higher in South than North Island adult stags, whilst the herd-level annual incidence was significantly higher in South Island herds containing weaner deer, yearling hinds and adult hinds. The annual incidence of Johne's disease showed a strong upward trend in the longitudinal study as 61% of the herds that were 'Johne's disease-negative' in 2005 had one or more cases reported by 2007, while 91% of 'Johne's disease-positive' herds in 2005 had further cases in the following two years. This study also demonstrated that the disease incidence dropped during that period in weaners and adult stags but increased in other age-classes. The reasons for this pattern are unknown, but could be associated with climate and/or management affecting exposure to MAP. Overall, 40% of farmer-observed clinical signs, at the herd-level, were attributable to infection with MAP. Clinical signs in 46% of 'culture-positive' 'Johne's disease-positive' herds were attributable to MAP infection. This suggests that diagnosis based on farmer-diagnosed clinical signs and lack of response to anthelmintic, trace mineral and/or antibiotic treatment overestimates the annual incidence of Johne's disease.

'External validity' relates to the appropriateness of extrapolation of study results to a reference population and was particularly important in descriptive, population-based observational studies such as this (Dohoo et al 2003e). The distribution of study herds generally matched the published national figures for the regional distribution of deer in 2005 (Anonymous 2005a) and 2007 (Anonymous 2007a), with study herds recruited from all regions except Taranaki. Thus, it was proposed that data presented here were likely to reasonably represent the geographical distribution of disease occurrence.

The Johne's disease annual incidence figures presented in this paper described the occurrence of disease as diagnosed by the farmer and will likely act as a 'benchmark' against which the effect of future national and herd-level control programmes can be assessed. However, since the primary purpose of data collection was for a case-control study designed to determine risk factors for the disease to be published elsewhere, herds were not selected at random because

enrolment depended on participant consent. As farmers generally have different motivations for participation in studies like this, it could not be accurately determined whether the study population was truly representative of the deer industry, in terms of Johne's disease occurrence. Therefore, while we believed the data presented in this paper could be used with confidence as a description of the expression of Johne's disease, it was acknowledged that further studies with randomised sampling strategies would be required to definitively measure the herd-level incidence of Johne's disease in New Zealand deer.

Pooled faecal culture has been applied to detect MAP at the flock/herd level in sheep (Whittington et al 2000a; Sergeant et al 2002; Dhand et al 2007), cattle (Tavornpanich et al 2004) and goats (Eamens et al 2007). However, to the authors' knowledge, this paper describes the first use of pooled faecal culture in the field as a herd-level diagnostic test for MAP in farmed deer. Schroen *et al.* (2003) mixed 1 gram of faeces from known MAP-infected deer with 4, 9 and 19 grams of faeces from culture-negative deer. Using this methodology, the sensitivity of detection of a single infected deer through the use of pooled faecal culture was estimated by those authors at 27 to 32% when tissue culture was used as the gold standard. At the animal-level, the sensitivity of pooled faecal culture was affected by the Johne's disease status of the animals contributing to the pool and the level of faecal bacterial shedding (Sykes et al 2000). At the herd-level, the sensitivity was also reliant on the number of samples per pool, number of pools tested, the true incidence of MAP infection in the herd and the number of organisms being shed by individual animals (Christensen and Gardner 2000). In this study, the herd-level sensitivity of pooled faecal culture was likely increased by the targeted sampling of deer with the highest probability of MAP infection, such as animals with diarrhoea, weight loss and/or a false positive diagnosis to a bTb intra-dermal test. Moreover, faeces from those deer were selectively pooled to increase the probability of culturing MAP from at least one pool since establishment of herd Johne's disease status required only one pool to be culture positive. The outcome of pooled faecal culture with targeted sampling and pooling in this study supports its proposed use as a diagnostic tool in a future market assurance programme for Johne's disease in farmed deer (Mackintosh 2005), since it was a low-cost test at the herd-level.

The first published diagnosis of Johne's disease in a New Zealand farmed deer was in 1983, after observation of emaciation, diarrhoea and sudden death in a red deer/elk hybrid yearling stag (Gumbrell 1987). Since that time, there has been no published estimate of the national herd-level incidence of Johne's disease. From 1986 to 2004, over 600 deer herds were identified as infected with MAP (de Lisle et al 2003; de Lisle et al 2006), with over half diagnosed between 2000 and 2004. However, those figures are considered an underestimate of the true herd-level incidence of MAP as they were derived from culture and polymerase chain reaction (PCR) of tissues with gross pathology, tested on suspicion for *M. bovis,* largely from material collected in Deer Slaughter Premises.

Of the 108 'culture-positive' survey herds, 96 (89%) had one or more deer with signs of Johne's disease in 2005. Of the 58 (60%) farmers who owned/managed a 'Johne's disease-positive' herd and observed the signs of Johne's disease in their herd prior to 2005, 49 (85%) reported the first case occurred between 2000 and 2004. This data, although derived from a possibly biased sample of herds, indicate the herd-level incidence of Johne's disease had increased consistent with the herd-level incidence of MAP described by de Lisle *et al.* (2006). An apparent increasing trend of 'Johne's disease-positive' deer herds, as well as the low proportion of 'culture-positive', 'Johne's disease-negative' survey herds, indicated that development of management practices to aid in the control of Johne's disease in New Zealand deer herds was desirable.

An epidemiologically and commercially important finding of this study, in contrast to anecdotal belief, was that in 'Johne's disease-positive' herds, yearling hinds and stags had a significantly higher incidence of disease than weaners (median 2.0 vs 1.2%, respectively; $p<0.05$). Although outbreaks of Johne's disease in weaner deer have been described (Mackintosh et al 2004a), there have been few reports of significant levels of disease in yearling deer, hence creating the popular belief, presumably based on observation of the number of animals *per se* rather than incidence, that the disease was manifest principally in weaners. Research focusing particularly on yearling deer is required since Johne's disease in this age-class has a greater financial impact than in weaners because of the greater investment involved in growing a deer to its second year of life.

Within a population, diseases are commonly clustered both spatially and in age strata of the host species. The present study found the animal-level incidence of Johne's disease was significantly more prevalent in South Island adult stags, relative to the North Island. It was also more likely in the South Island to have a herd with one or more weaner deer, yearling hind(s) or adult hind(s) observed with Johne's disease than in the North Island. Further research is required to ascertain whether a true association exists between Island and MAP infection and/or Johne's disease.

This study was based largely on farmer-diagnosis of Johne's disease since veterinary necropsy or culture confirmation of individual cases was rarely undertaken in participating herds. Furthermore, to perform a study involving diagnostic confirmation of all clinical disease resembling Johne's disease was not financially feasible. Thus, evaluation of the misclassification rate of farmer-diagnosed Johne's disease was undertaken to assist interpretation of the validity of findings in this study. The correct diagnosis of Johne's disease by the farmer based on diarrhoea and/or weight loss and lack of response to anthelmintic, trace mineral and/or antibiotic treatment was reliant on their skill in detecting these clinical signs, their ability to recall cases which occurred up to 12 months prior to the interview, their experience of observing Johne's disease in their deer and the occurrence of other diseases with similar signs. Diarrhoea and/or weight loss as a result of chronic abomasal parasitism, copper deficiency, yersiniosis in weaner deer and malignant catarrhal fever in adult deer are difficult to distinguish from Johne's disease on visual examination alone and may result in false positive farmer diagnoses (Mackintosh et al 2004b). As a result, reliance on farmers to diagnose Johne's disease may have introduced bias, including reporter bias, recall and misclassification bias (Dohoo et al 2003e). However, personal interview of each farmer by a single interviewer reduced the possibility of interviewer bias and was efficient in preventing missing values in the data. Furthermore, the interviewer repeatedly asked for the number of deer exhibiting diarrhoea and/or weight loss through questions which covered a variety of topics, such as treatment and clinical signs, and this data were consistently checked against the original number of deer recalled by the farmer.

Overall, 60% of observed cases, at the herd-level, were found to be non-attributable to infection with MAP. Moreover, clinical signs in 54% of 'culture-positive' 'Johne's disease-positive' herds were found to be non-attributable to MAP infection. The prior confirmed or suspected

knowledge of many survey farmers regarding the MAP status of their herd may have led to an increased intensity of observation of their deer, resulting in misclassification of Johne's disease and overestimation of the association between Johne's disease observation and MAP herd-level infection. Moreover, a herd was classified as 'Johne's disease-positive' even if only a single deer had been observed with clinical signs typical of Johne's disease within a 12 month period. Increasing the minimum cut off to two, three or more affected deer for classification of a 'Johne's disease-positive' herd may have reduced the possible level of misclassification observed. The rate of misclassification determined in this study must be taken into account when the Johne's disease incidence estimates derived from the survey are considered for comparative purposes. Further research utilising methods that are not reliant on the use of a gold standard are required to more accurately evaluate the accuracy of farmer-diagnosed Johne's disease.

Although the incidence of the clinical stage of many infectious diseases has been associated with season (Grassly and Fraser 2006), there has been no published investigation into the possible seasonality of Johne's disease in deer. Audigé *et al* (2001) reported a seasonal pattern in the mortality rates of weaner (<15 months) and adult deer (>15 months) on 15 North Island deer herds, with higher mortality rates in weaners in the autumn and winter of their first year, and adult deer deaths more commonly observed in winter and spring. Our data were partially consistent with that pattern in that Johne's disease in all age classes was more frequent in winter and spring. However, Mackintosh *et al.* (2010) found three-month-old weaner deer artificially inoculated with MAP did not show clinical signs of Johne's disease until 20 to 28 weeks post-inoculation. This indicates that the farmer-observed clinical signs reported in this study in the summer and autumn of a weaner's first year may be due to causes other than Johne's disease. A 3-year study of adult sheep in 12 Australian flocks found a higher number died due to ovine Johne's disease in winter and spring than in summer and autumn ($p<0.001$) (Bush et al 2006). A seasonal pattern has also been observed in the diagnosis of MAP in dairy cattle in the United States of America and Canada, with a decrease in the proportion of test positive animals during the summer months (McKenna et al 2004; Strickland et al 2005).

The progression of Johne's disease from infection to the manifestation of clinical signs is dependent on many factors such as host susceptibility, age and dose at infection, and possibly,

bacterial strain and stress (Mackintosh 2005). Similarly, the seasonal occurrence of Johne's disease may also depend on a number of factors, such as time of calving, contact between young and adult deer, and MAP survival on pasture. Stags have a voluntary reduction in feed intake over winter and stags entering the winter post-rut may have a low body condition score. This, in association with the typical low fat reserves and relatively sparse coat available to deer over winter, may contribute to making deer susceptible to the seasonal development of Johne's disease. Whether the association between the incidence of Johne's disease in New Zealand farmed deer and season is due predominately to stress, feed or other factors related to disease epidemiology and pathogenesis is unknown and warrants further investigation.

Longitudinal monitoring of the herd-level Johne's disease status of 81 deer herds found that the number of farmers reporting Johne's disease increased over the three year period of 2005 to 2007. The increase was observed in yearling hinds, yearling stags and adult hinds. Although this data were from a small number of herds, it may be interpreted as indicative of a continuation of the increasing incidence of Johne's disease in farmed deer, both as herd and animal incidence, as earlier hypothesised by de Lisle *et al.* (2006), and supported by anecdotal observations. However, the apparently increasing incidence could be viewed as a consequence of higher interest and awareness of Johne's disease by the deer farming community in the previous five years, fostered by the publication of a Johne's disease manual for farmers (Bell et al 2006). In addition, these analyses have not taken into account seasonal and unforeseen weather changes and adjustments in management patterns and/or herd composition and size in response to fluctuations in the venison schedule. However, the apparent trend identified in this paper calls for more efforts to determine risk factors for MAP infection and Johne's disease as well as to develop effective management practices. Moreover, monitoring individual animals over a longer time period may be required to fully understand the dynamics of Johne's disease, patterns of infection and options for control in deer herds.

2.6 Conclusion

The data presented in this paper provide descriptive information on Johne's disease on New Zealand deer herds, focusing on herd- and age-class level incidence and associations between the

presence and incidence of Johne's disease and age-class, season and Island. Overall, 62% herds were confirmed by culture to be infected with MAP and 72% of herds reported signs of Johne's disease, groups of yearling hinds and stags being most frequently affected. At the animal-level, adult stags in the South Island were more likely to suffer from Johne's disease than in the North Island. The frequency of both animals and herds in which farmers had observed Johne's disease increased over a three-year period. Johne's disease was significantly more likely to be observed in winter and spring. Data were consistent with other published findings of an increasing trend of Johne's disease, raising industry concerns about animal welfare and economic loss. Continued research about its dynamics and control is therefore warranted in New Zealand.

2.7 Acknowledgements

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Chapter 3 describes risk factors significantly associated with *Mycobacterium avium* subspecies *paratuberculosis* presence in New Zealand farmed deer herds and has been prepared for submission to the Journal of the American Veterinary Medical Association (JAVMA).

CHAPTER 3: Risk factors associated with herd-level presence of *Mycobacterium avium* subspecies *paratuberculosis* in New Zealand farmed deer

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3.1 Abstract

Objective To determine risk factors significantly associated with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) presence in New Zealand farmed deer herds.

Study Design Case-control study.

Sample population 108 MAP-positive case herds and 66 control herds.

Procedures Herd-level MAP status was determined either by tissue culture or culture of 5 to 6 pools of 8 to 10 faecal samples. Herd and animal-level data from 1 January 2005 to 31 December 2005 were collected by personal interview. Multivariable models identified risk factors significantly associated with herd-level presence of MAP.

Results A pooled faecal sample had a 9.3 times higher likelihood of being culture positive if faeces from one or more young (3 to 24 months old) deer were included. Sampling of deer with signs typical of Johne's disease (JD) did not significantly increase the likelihood of positive pooled faecal culture (PFC). Risk factors positively associated with a culture-positive herd were herd regional location; deer fenced area (DFA) consisting of hill/high country; diarrhoea/wasting in cattle grazed within previous two years on DFA; and increasing relative proportion of grazing days by young deer. Risk factors positively associated with a culture-negative herd were ≥ one dam(s) as a deer water source; >5% deer herd consisting of adult stags; and no clinical JD in deer in previous year.

Conclusions and Clinical Relevance Results suggest there are a number of uncorrelated risk factors variably associated with MAP presence in deer herds. These risk factors may aid

determination of control measures and inform further research aiming to reduce the prevalence and transmission of MAP in farmed deer.

3.2 Introduction

New Zealand has become the largest producer of venison worldwide with over 1.15 million deer, predominately red deer (*Cervus elaphus*), elk (*Cervus canadensis*) and/or red deer/elk crossbreds (i.e. 'wapiti'), farmed as of 30 June 2009 (Anonymous 2009). The first reported diagnosis of JD, a chronic granulomatous enteritis of ruminant livestock caused by infection with MAP, in a New Zealand farmed deer was in 1979 (Gumbrell 1987; Collins 2003a). Between January 2001 and October 2005, 299 infected deer herds, approximately 6% of the national total, were identified as infected with MAP (de Lisle et al 2003; de Lisle et al 2006). However, this is likely an underestimate of the true national prevalence of MAP since samples were derived largely from routine slaughterhouse surveillance for *Mycobacterium bovis* as part of a national control program for bovine tuberculosis (bTb) (de Lisle et al 2006).

Deer with MAP infection can remain free of clinical signs of JD (i.e. have subclinical JD) for weeks or years following infection, similar to sheep and cattle (Whitlock and Buergelt 1996; Mackintosh et al 2007a). A proportion of infected deer will develop clinical JD, which typically begins with intermittent diarrhoea and/or weight loss and progresses to lethargy, emaciation and profuse diarrhoea, prior to death (Mackintosh et al 2004a). Clinical JD has emerged as a significant economic burden to some deer farmers and the New Zealand deer industry with published cost industry-wide estimates ranging from $NZ340,000 to over $500,000 per annum (Brett 1998; Mackintosh and Wilson 2003). However, the true cost of MAP infection, incorporating the probable effects of subclinical JD, is likely to be considerably higher. Although a number of risk factors have been found to be significantly associated with either the herd-level presence of MAP or clinical JD in cattle and sheep, there is a dearth of epidemiological studies investigating risk factors associated with these two outcomes in deer (Wells and Wagner 2000; Dhand et al 2007).

The faecal-oral route may be an important method of MAP transmission in deer as indicated by the successful inoculation of young deer with bacteria collected from cervine lymph nodes (Mackintosh et al 2007a). However, definitive proof through inoculation of deer with MAP collected from faeces is yet to be determined. MAP can also be transmitted between a hind and her offspring *in utero* (van Kooten et al 2006) and transmammary transmission (Thompson et al 2007) may be significant based on research undertaken in sheep, goats and cattle (Grant et al 2001; Grant et al 2002; Singh and Vihan 2004). While the thick capsule of MAP confers significant resistance to environmental effects, enabling the organism to survive for up to 9 months in manure and slurry (Jorgensen 1977; Olsen et al 1985) and 11 months in soil (Whittington et al 2004), exposure to direct sunlight and alkaline soils has been shown to decrease the longevity of MAP under field conditions (Johnson-Ifearulundu and Kaneene 1997; Whittington et al 2005). Management practices and environmental conditions specific to deer farms which may permit or control the transmission of MAP have not been investigated. Of particular interest are those practices which have a high likelihood of allowing entry of MAP onto deer pastures, and practices or conditions which may contribute to the maintenance of MAP in a deer herd.

The objective of this study was to identify farm management practices and environmental factors positively or negatively associated with the presence of MAP in deer on New Zealand farms.

3.3 Materials and Methods

3.3.1 Study design

A retrospective case-control study was conducted in the period 1 January to 31 December 2005 to identify management practices and environmental factors associated with the presence of MAP in New Zealand farmed deer herds. The population of interest was New Zealand farms grazing 20 or more red deer (*Cervus elaphus*), fallow deer (*Dama dama*), elk (*Cervus canadensis*) and/or red deer/elk crossbred (commonly termed wapiti-cross). A property was defined as a single geographic location on which deer were grazed with a fenced area complying with the Wild Animal Control Act 1977 termed the 'deer fenced area' (DFA) (Anonymous 1977). A herd was defined as all deer grazed on the same DFA. Deer were classified according

to age: >3 and ≤12 months ('weaner'), >12 and ≤24 months ('yearling') and >24 months ('adult'). Female deer are referred to as hinds and male deer as stags.

3.3.2 Selection of case and control herds

Case and control herds were recruited from throughout New Zealand by personal call for expressions of interest at two national deer farming conferences and in two issues of the New Zealand Deer Industry magazine ('Deer Industry News') (Anonymous 2004a, 2004b) targeting an estimated 4,000 deer farmers. On 15 March 2005, a short postal questionnaire was sent to 311 respondents requesting confirmation of interest, their intended deer herd size and composition at 31 July 2005 and whether any deer in their herd had a positive tissue or faecal culture for MAP from 15 March 2004 to 15 March 2005. Although 193 (62.1%) farmers confirmed their initial interest, 10 farmers intended to graze <20 weaner deer in 2005 and were therefore excluded. Nine farmers withdrew during the period of data collection, resulting in 174 study herds.

3.3.3 Determination of herd-level MAP status

Fifty-nine herds of the 174 herds were classified as MAP positive based on one or more positive BACTEC faecal and/or tissue cultures, usually lymph nodes, for investigation of bTb at slaughter, between 15 March 2004 and 15 March 2005, recorded by the AgResearch Wallaceville Animal Health Laboratory. Culture of 5 to 6 pools of 8 to 10 faecal samples preferentially from adult breeding hinds, or an available age-class, was undertaken from the remaining 115 properties. Animals with the highest probability of infection such as those with diarrhoea and/or wasting and/or non-specifically positive to the previous bTb intra-dermal test were targeted for sampling to optimise herd-level sensitivity (Smith and Slenning 2000).

Deer were sampled by the first author or the farmer's veterinarian while standing in pens or a restraining device. Animal sex and age, determined either from the ear tag which listed the year of birth or estimated based on teeth wear, the presence of diarrhoea and/or wasting, and the outcome of the previous intra-dermal bTb test were noted at the time of sampling. A minimum of 5 faecal pellets (2 g) was collected per rectum using a new glove for each animal, and stored in a sterile 70 ml sample container. Samples were immediately chilled and within 24 hours were couriered to Massey University (Palmerston North, New Zealand) where they were pooled.

Where available, samples from deer with diarrhoea, weight loss and/or bTb test positive were pooled together. The remaining samples were pooled based on age. Samples were either chilled at 4^{o}C for a maximum of 48 hours or immediately couriered to AgResearch Wallaceville (Upper Hutt, New Zealand) for culture. A liquid culture system using BACTEC 12B vials (Becton Dickinson, Sparks, Maryland, USA) supplemented with 0.8 ml of egg yolk, mycobactin (Allied Laboratories, Fayette, Missouri, USA) and antibiotics (PANTA, Becton Dickinson, Sparks, Maryland, USA) was used, as described by Whittington et al (1999)

3.3.4 Case definition

Case herds were those culture-positive for MAP in one or more pooled faecal and/or tissue samples between 15 March 2004 and 31 December 2005 (n = 108), while control herds (n = 66) were those PFC negative.

3.3.5 Data collection and management

A detailed questionnaire (Appendix 2) was pre-tested in a pilot study of 22 deer farmers in November 2004 who were not included in the final study population. Between 23 July 2005 and 16 March 2006, the questionnaire was conducted by personal interview of each study farmer by the first author. The questionnaire included information at the property, herd and individual animal levels, from 1 January to 31 December 2005. Properties visited prior to 31 December 2005 were contacted by phone in early 2006 to update records and information to the end of the calendar year.

Data collected included property and DFA dimensions, herd characteristics, weaner deer and breeding/adult hind information, the feed, disease and vaccination status (for prevention of leptospirosis, clostridial diseases and yersiniosis) of each age-class and the number of each age-class purchased from 2003 to 2005, inclusive. If available, soil test results from the DFA from 2003 and onwards were also collected. The presence of high country on the DFA was indicated by the farmer where ‘high country’ is a topographical term specific to New Zealand indicating elevated pastoral land, generally > 600 m (2000 ft) above sea level.

3.3.6 Calculation of risk factors

The number of stock units (SU) including dairy, beef, sheep, goats, deer and/or alpaca present on the DFA during the study period was calculated taking into account the age and breed of animal (Woodford 2004). For species other than deer (SOTD), the approximate number of days each animal was grazed on the DFA was estimated by the farmer. For deer, the number of days was calculated for each age-class. The number of days weaner deer were present on the DFA was calculated from the date of weaning (if bred on the property) or date of purchase, until 31 December 2005. If the weaning date was after 1 May, this date was assigned as the weaning date since it was assumed weaner deer were predominately grazing pasture at this age. If weaner deer left the property prior to 31 December such as for slaughter or live sale or death, the grazing day count ended on that date. If the date was not available 31 March, 31 June and 15 October for live sales, deaths and transport to slaughter were assigned, respectively. The number of days yearling and adult deer were present on the DFA was calculated in a similar manner adjusted for animal purchases, deaths and removal for live sale or slaughter.

The number of days each farmed livestock spent on the DFA in 2005 was then multiplied by the appropriate SU. This value was totalled for all grazed livestock on the DFA to create the variable of stock unit days (SUDays), incorporating both time and SU. This variable was divided by the DFA size in hectares (ha) to create an overall stocking rate variable, namely stock unit days per hectare (SUDays/ha). This represented the mean number of days each SU grazed on each deer fenced ha. The total SUDays contributed by all livestock on the DFA was divided by the SUDays contributed by each SOTD and each age-class of deer. This determined the percentage contributed by each livestock and age-class to the overall stocking rate.

An individual deer with diarrhoea and/or weight loss was classified as suffering from clinical JD if the farmer or their veterinarian had not diagnosed the condition as abomasal parasitism, yersiniosis, chronic copper deficiency or other disease, and there was incomplete resolution of clinical signs if anthelmintic drench, copper or an antibiotic had been administered. Sheep or cattle grazed on the DFA were classified as suffering from clinical JD if diagnosed as such by the farmer.

Herd geographic location was categorised as North Island or Canterbury; Nelson-Marlborough/West Coast; Otago; and Southland in the South Island.

3.3.7 Statistical Analysis

A multivariable logistic regression random-effects model was developed with a positive or negative faecal culture at the pool-level as the outcome. Assessed binary variables were inclusion of faeces from one or more weaner and/or yearling deer in each pool ('Age') and whether each pool contained faeces from one or more deer with signs typical of clinical Johne's disease, such as diarrhoea and/or weight loss ('Clinical'). Herd was included in the model as a random effect to adjust variance estimates for correlation at this level of aggregation. The potentially confounding variable of island was forced into the model. An interaction between the fixed effects was tested for significance at $p < 0.10$ and model fit and residual variances were evaluated for distributional assumptions and influential observations.

In the second analysis, the binary outcome was a positive or negative culture for MAP at the herd-level and all variables were aggregated to the level of herd. Continuous variables that were non-normally distributed were transformed or categorized; while normally distributed variables were summarized using the mean and standard deviation. Potential risk factors were screened using univariable logistic regression with a probability of $p<0.2$ used to select factors for inclusion in the subsequent multivariable model. The Fisher's exact test was used to test for significance if $\geq$ one cell within a contingency table was ≤ 5. A backwards stepwise model-building process was then used to develop a multivariable model, and variables were retained in the model if they were associated with $p < 0.1$, derived from a likelihood-ratio test. Biologically plausible interaction terms between main-effects variables were then considered for inclusion in the multivariable model. Summary measurements of model goodness-of-fit included comparison of deviance to the degrees of freedom (df), and Pearson χ^2 statistics. Regression diagnostics included evaluation of standardized Pearson residuals.

All analyses were performed in R (version 2.4.1; The R Foundation for Statistical Computing).

3.4 Results

The study included 108 case herds and 66 control herds located throughout New Zealand (Figure 3.1). The number and regional distribution of deer in the 2005 survey are presented in Table 2.1 (Chapter 2), with national figures for comparison. Deer herd size ranged from 76 to 19,720 deer and the mean DFA (323 hectares) was 66.9% (95% CI = 61.9 to 71.9%) of the mean effective area of study properties.

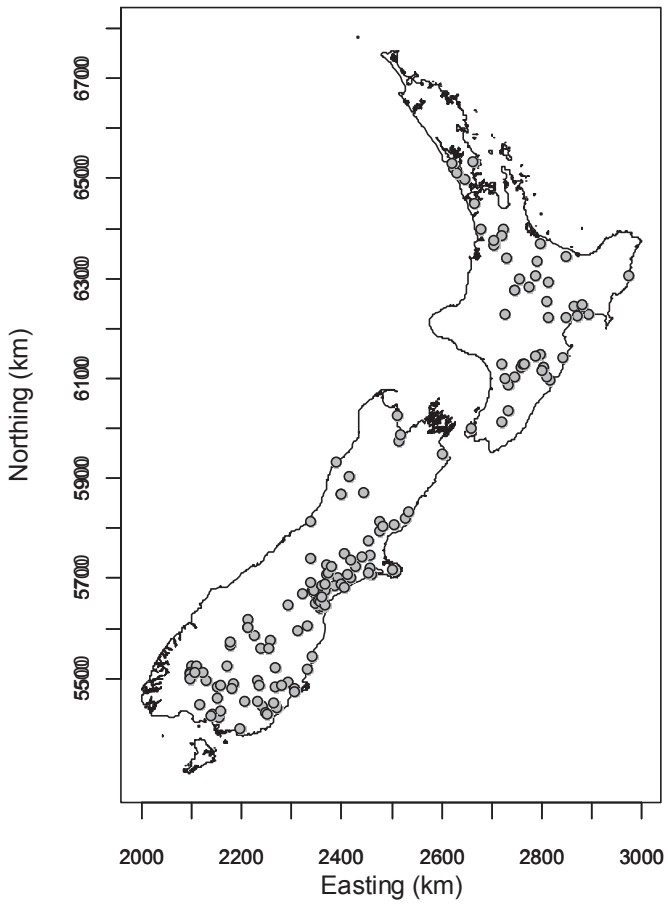

Figure 3.1 Map of the North and South Islands of New Zealand illustrating the geographic location of deer herds (n = 174) evaluated for risk factors associated with the herd-level presence of MAP (grey circles).

The MAP status of 115 herds was determined through pooling of faeces from a total of 6,138 deer into 632 pools. One hundred and thirteen pools (17.9%) contained faeces from one or more weaner and/or yearling deer, whilst 86 (13.5%) pools contained faeces from one or more deer

with signs of clinical JD (Table 3.1). Pooling of faeces from weaner and/or yearling deer was 9.3 (95% CI: 1.9 to 46.5) times more likely to result in a positive culture relative to pooling of faeces solely from adult deer ($p < 0.01$). Targeted pooling of faeces from deer with signs of clinical JD was not significantly associated with a positive culture result ($p = 0.17$).

Table 3.1 Associations between the *Mycobacterium avium* subspecies *paratuberculosis* (MAP) status of pooled faecal culture (PFC) at the pool-level and the age and clinical status of sampled deer from 115 New Zealand herds.

		MAP status of PFC		
Factor		**Negative**	**Positive**	***P* value**
Age	Weaner/Yearling	77 (68%)	36 (32%)	
	Adult	415 (80%)	104 (20%)	<0.01
Clinical	Negative	430 (79%)	116 (21%)	
	Positive	62 (72%)	24 (28%)	0.17

Twenty-five uncorrelated potential variables from 250 screened by univariable logistical regression analysis were associated with the odds of a farm being MAP positive ($p \leq 0.20$) (Table 3.2) and were included in the multivariable model. The Pearson χ^2 statistic was 30.20 ($p = 0.20$) indicating there that there is no reason to assume that the model was not correct. The proportion of SUDays contributed by weaner deer was categorized into <20%; 20 to 24%; 25 to 29%; and >30%. Variables significantly positively associated with a herd being faecal or tissue culture positive for MAP were: an increasing proportion SUDays contributed by weaner deer; the presence of hill and/or high country on the DFA; regional location of the herd and farmer-diagnosed clinical JD in cattle grazed on the DFA in 2004 and/or 2005 (Table 3.3). Variables positively associated with a herd being culture negative for MAP were: no farmer-diagnosed clinical JD in the deer herd in 2004; adult (> 24 month old) stags constituting more than 5% of the deer herd; and the presence of one or more dams as a water source to the deer herd in 2005.

3.5 Discussion

This paper presents the first investigation of associations between herd-level culture status for MAP and potential risk factors that may be applied for the control of MAP in farmed deer herds. An increasing proportion of total livestock days contributed by weaner deer; herds located in the Canterbury and Southland regions; the presence of hill and/or high country on the DFA; and farmer-diagnosed clinical JD in cattle grazed on the DFA had a positive association with a MAP-

positive herd. No clinical JD observed in deer by the farmer during the previous year; the use of one or more dams as a water source to deer; and adult stags comprising greater than 5% of the deer herd were positively associated with a MAP-negative herd.

Table 3.2 Results of univariable analysis of potential risk factors for the presence of MAP infection in 174 New Zealand deer herds ($p<0.2$). All factors refer to 2005 unless indicated.

Factor	*P* value	Odds Ratio	95% CI
Presence of lambs/hoggets on DFA	0.18	1.57	0.81-3.04
Presence of hill/high country on DFA	0.12	1.70	0.86-3.33
Purchased deer sourced from Otago and/or Southland	0.04	1.98	1.04-3.79
Presence of yearling beef cattle on DFA	0.02	2.14	1.09-4.18
Abundant observation of hares by farmer	0.01	2.20	1.17-4.10
Farmer-diagnosed clinical JD in sheep in 2004/2005	0.05	2.25	1.02-4.98
Adult stags purchased	0.03	2.34	1.07-5.10
% deer herd = hinds	0.19	3.93	0.49-31.41
% total livestock SUDays= weaner deer[a]	<0.01	4.41	1.84-10.59
Purchased yearling deer sourced from Canterbury	0.03	4.77	1.05-21.69[c]
Regional location of property:			
North Island	Ref	-	-
Nelson-Marlborough/West Coast[b]	0.38	2.70	0.68-10.71
Canterbury[b]	<0.01	8.28	3.45-19.87
Otago[b]	0.02	3.60	1.41-9.17
Southland[b]	<0.01	5.10	1.73-15.01
Farmer-diagnosed clinical JD in cattle in 2004/2005	0.04	6.63	1.21-53.06[c]
Purchased weaner deer sourced from Canterbury	<0.01	8.19	1.86-36.08[c]
No farmer-diagnosed clinical JD in deer in 2004	<0.01	0.06	0.03-0.12
% breeding hind herd ≥ 3 and ≤ 5 years of age	<0.01	0.10	0.02-0.48
Purchased yearling deer sourced from North Island	<0.01	0.17	0.06-0.48[c]
> 5% of deer herd = adult stags	<0.01	0.22	0.11-0.43
Purchased weaner deer sourced from North Island	<0.01	0.26	0.09-0.74
Farmer had >15 years experience in deer industry	<0.01	0.43	0.23-0.80
Average days (all livestock) / total livestock SU	0.17	0.46	0.15-1.39
≥ 1 deer grazed off-property	0.07	0.46	0.20-1.08
≥ 1 dam available as water source for deer	0.02	0.49	0.26-0.91
% total livestock SUDays = yearling deer	<0.01	0.54	0.34-0.86
Number years deer grazed on property	0.03	0.58	0.36-0.95
Mean SUDays grazed by each weaner deer/hectare	<0.01	0.63	0.46-0.86

[a] = Continuous variable categorized to <20%; 20 to 24%; 25 to 29% and >30%

[b] = Region in South Island

[c] = Fisher's exact test

Table 3.3 The results of a multivariable logistic regression model for the relationship between potential risk factors and presence of MAP in 174 New Zealand farmed deer herds.

Factor	*P* value	Odds Ratio	95% CI
% total livestock SUDays = weaner deer	0.08	1.5[a]	1.0 - 2.4
Regional location of herd:			
North Island	Ref	-	-
Nelson-Marlborough/West Coast	0.18	3.4	0.5 - 20.2
Canterbury	<0.01	9.3	2.3 - 38.0
Otago	0.31	2.1	0.6 - 8.4
Southland	0.05	4.4	1.0 - 20.5
Presence of hill/high country	<0.01	4.9	1.7 - 14.7
Farmer-diagnosed clinical JD in cattle in 2004/2005	0.06	13.7	1.0-212.2
No farmer-diagnosed clinical JD in deer in 2004	<0.01	0.1	0.03 - 0.2
> 5% deer herd = adult stags	<0.01	0.2	0.05 - 0.5
≥ 1 dam available as water source for deer	0.01	0.3	0.1 - 0.8

[a] = Continuous variable categorized to <20%; 20 to 24%; 25 to 29% and >30%

To date, attempts to control clinical JD on deer farms have principally relied on methods developed to control JD in sheep and cattle, such as test-and-cull, but robust, peer-reviewed reports of their effectiveness are limited (Burton and Voges 2002; Mackintosh 2002; West 2002; Wilson and Castillo-Alcala 2004). The development of effective control methods for MAP and clinical JD in farmed deer may require research specific to this species. There is some evidence to suggest that certain characteristics of MAP infection in deer, such as strain pathogenicity and virulence (Mackintosh et al 2007a), gross pathology (Clark et al 2010) and incubation period (Mackintosh et al 2007a) differs from other livestock species. Moreover, it is likely that farmed deer are challenged by unique stressors as farming of this species is recent and has required the development of numerous novel management techniques, such as velvet removal, as well as modification of facilities used traditionally in sheep and cattle farming.

Determination of herd infection status was integral to this study, which is the first to use PFC in the field as a herd-level diagnostic test for MAP in farmed deer. Pooled faecal culture has been previously applied to detect MAP at the flock/herd level in sheep, cattle and goats (Whittington et al 2000b; Tavornpanich et al 2004; Dhand et al 2007; Eamens et al 2007). Schroen *et al.* (2003) evaluated PFC in a farmed deer herd through the combination of one culture-positive gram of faeces with 4, 9 and 19 grams of faeces from culture-negative deer. The sensitivity of

PFC 5, 10 and 20 was estimated to be 32, 27 and 27%, respectively, with culture of mesenteric lymph node, ileum, ileo-caecal valve and colon applied as the gold standard.

Targeted sampling and pooling were utilised in this study to attempt to increase the predictive value of PFC through maximizing the probability of infection in sampled animals and targeted pools (Christensen and Gardner 2000; Williams et al 2009). At the animal-level, the sensitivity of PFC in the detection of MAP increased if the animal was affected with clinical JD and, in previous studies, the targeted sampling of sheep with signs of clinical JD has been utilised to determine herd/flock status as these animals are more likely to be shedding MAP relative to their flockmates (Sykes et al 2000; Sergeant et al 2002; Schroen et al 2003a). However, this study found that the targeted sampling and pooling of faeces from deer with signs typical of clinical JD was not significantly associated with a culture-positive result at the pool level ($p<0.05$). Although typical of clinical JD in deer, diarrhoea and weight loss are also associated with other conditions, such as yersiniosis (weaner deer only), abomasal parasitism and chronic copper deficiency (Mackintosh and de Lisle 1998). Therefore, evaluation of the likely effectiveness in deer of administered anthelmintics and/or trace minerals in herds of unknown MAP status may be recommended prior to targeted sampling of diarrhoeic and/or low weight deer for MAP. It has been observed that apparently healthy deer with normal faecal consistency may also shed large numbers of MAP (Griffin JFT, unpublished data).

This study also found PFC was significantly more likely to be positive for MAP if the faeces from one or more weaner and/or yearling deer was included in the pool relative to faeces solely from adult deer (OR = 9.3; $p = 0.01$). This is in contrast to studies of dairy cattle and sheep where targeted sampling of adults has been utilised to increase the probability of MAP detection at the herd/flock-level (Muskens et al 2000; Tavornpanich et al 2006). Although the likelihood of clinical JD increases with age in both cattle and sheep, outbreaks of up to 20% mortality due to MAP infection have been reported in farmed weaner and yearling deer (Mackintosh et al 1999; de Lisle et al 2003; de Lisle et al 2006). A separate analysis of data derived from this study found that, in clinically affected herds, the median incidence of yearling deer with signs of clinical JD (2.0%) was significantly higher ($p<0.05$) than that observed in adult hinds or stags (0.9 and 1.2%, respectively) (Chapter 2). Therefore, the targeted sampling of weaner and/or

yearling deer, rather than adults, may increase the sensitivity of PFC in the detection of MAP in this species.

Herd regional location

Study herds located in the Canterbury and Southland regions of the South Island were significantly more likely to be culture positive for MAP (OR = 9.3 and 4.4, respectively), relative to the North Island. Although a number of studies in farmed deer have informally assessed the effect of geographic location on the presence of MAP, this is the first comprehensive evaluation of this risk factor in deer (Glossop et al 2005; Griffin et al 2006a). Published international reports have linked geographic herd location and the presence of MAP in a number of other species, including sheep, cattle and rabbits (Collins et al 1994; Judge et al 2005; Coelho et al 2006).

There are several possible explanations for the apparent spatial clustering of MAP-infected deer herds in New Zealand, including environmental factors, such as soil iron content and pH (Kopecky 1977; Johnson-Ifearulundu and Kaneene 1997; Ward and Perez 2004), localised rather than between-island deer trading (Ward and Perez 2004), or an increased prevalence of other domestic livestock grazing with and/or in proximity to deer herds. The geographic distribution of farmed deer in New Zealand changed within the decade to 2007, with the majority (62.9%) of deer grazed in the South Island, and possible causes for the apparent clustering of MAP-infected deer herds in this geographic location merits further investigation (Anonymous 2007a).

Presence of hill and/or high country on the deer fenced area (DFA)

A herd was 4.9 (95% CI = 1.7 to 14.7) times more likely to be culture-positive for MAP if the DFA contained hill and/or high country. Approximately six million hectares, over 22% of New Zealand's total land mass, is high country, of which approximately 2.5 million hectares is farmed. Grazing of deer under hill and high country conditions has become increasingly common over the last decade, particularly as competition for the lower altitude farming country from other livestock industries has increased. An average of 54.5% (5% -- 100%) of the DFA was hill and/or high country on the 32.8% of study herds (57/174) that had deer grazing on this land topography type. There has been limited research undertaken on the unique stressors

experienced by deer farmed in this extensive environment, such as those due to the prevailing extreme conditions, including a harsh mountainous climate, steep topography and alpine flora and fauna (Peoples and Asher May 2009). The likely importance of a significant positive association between grazing deer on hill/high country and the herd-level presence of MAP requires further research in this unique environment.

Proportion of total livestock SUDays contributed by weaner deer

This study found the odds of a herd being culture-positive with MAP increased 1.5 times as the proportion of total livestock SUDays contributed by weaner deer increased in 5% intervals from <20 to >30%. The aim of many New Zealand deer farmers is the production of venison from deer less than 12 months of age to take advantage of a seasonal premium paid for carcass weights of 50 to 65 kilograms. To achieve this, some farmers 'finish' young deer by purchasing and intensively grazing 3 to 12-month-old animals on improved pastures. Therefore, the practice of buying 3 to 4 month-old weaner deer for 'finishing' may increase the risk of entry of MAP into a deer herd. However, although 38.0% of case herds purchased one or more weaner deer in 2005 compared to 28.8% of control herds, this difference was not significant ($p = 0.22$).

In addition to purchase policies, management practices involving weaner deer may also contribute to the risk of herd-level MAP infection. Management practices typically utilised to maximise the growth of weaner deer, at the same time as maximising pasture utilisation efficiency, may contribute to within-herd cycling of MAP. Older deer or other livestock are commonly grazed on pastures after young deer in a rotational grazing system with the former usually required to graze pasture lower to soil than the latter. A separate analysis of data derived from this study reported a significant positive association between the use of irrigation on deer pastures and ≥ 0.4% of weaner deer with clinical JD (Chapter 4). While the predominant diet of New Zealand farmed deer consists of perennial ryegrass/white clover pastures (Barry et al 2002), weaner deer are commonly grazed on improved, irrigated pastures or forage crops such as red clover and chicory, during the dry summer months. As MAP can remain viable in water for extended periods of time, irrigated pastures may provide optimal conditions for its survival and result in a high bacterial intake by weaner deer (Whittington et al 2005). Weaner deer are also commonly fed supplements, such as hay and grain, directly onto the ground thereby potentially

increasing the intake of MAP via contaminated pasture and soil. Combinations of these factors could contribute to higher cycling of MAP through a deer herd that contains a high proportion of weaner deer than in a herd comprising predominately of adult deer, yearling deer, cattle, sheep and/or goats.

Although, the median incidence of clinical JD has been found to be significantly higher in yearling (2.0%) versus weaner deer (1.2%) ($p<0.05$), the proportion of days contributed by the latter age-class only was significantly associated with the herd-level MAP infection status (Chapter 2). On average, there were 54.3% (95% CI = 48.1 to 60.5%) fewer yearling than weaner deer in study herds. Therefore, overall, weaner deer shed a larger volume of MAP relative to yearling deer, likely resulting in a larger contribution to pasture contamination and MAP cycling within a herd.

Farmer-diagnosed clinical JD in deer in 2004

There was a higher probability that a deer herd was culture-positive for MAP if there had been at least one deer observed with signs of clinical JD in the previous year. The accuracy of farmer observation of diarrhoea and weight loss in their deer at any one time was assumed to be high. However, diarrhoea and/or weight loss are not pathognomonic for clinical JD in deer, with differential diagnoses including abomasal parasitism and copper deficiency (Mackintosh et al 2004a). For this reason, the history and success of treatment with an anthelmintic, antibiotic and/or copper was incorporated into the study case definition of an animal suffering from clinical JD. Clinical trials in cattle with clinical JD found typical signs, such as diarrhoea and weight loss, recurred after cessation of treatment (Merkal and Larsen 1973; Belloli et al 1994). Therefore, it is unlikely that administration of an anthelmintic drench or copper would result in resolution of clinical JD in deer. As 94% of New Zealand deer farmers administer an anthelmintic drench and 88% administer copper to their deer, examination of treatment success allowed easy differentiation of signs likely due to clinical JD from those due to abomasal parasitism and copper deficiency, thereby increasing the specificity of farmer diagnosis of clinical JD (Castillo-Alcala et al 2007; Wilson et al 2008).

Animals demonstrating clinical signs of JD are considered the most important source of environmental contamination and, subsequently, animal infection with MAP. Although MAP is an obligate parasite, only replicating in an animal host, its thick capsule allows it to survive for up to 11 months in soil and 17 months in water (Larsen et al 1956; Whittington et al 2004). Deer affected with clinical JD excrete, on average, 5 x 10^6 colony forming units (CFU) of MAP per gram of faeces and 31% (5/16) of 4-month-old deer artificially inoculated daily with 10^3 CFU of MAP over a 4 day period developed clinical JD (Schroen et al 2003a; Mackintosh et al 2007a). Therefore, it is likely that the presence of deer with diarrhoea due to JD will result in considerable short and long-term contamination of pastures, soil and waterways and, consequently, increase in the likelihood of infection in herd-mates grazing the same pastures in the following year.

Adult (>24 month old) stags comprising more than 5% of deer herd

This study demonstrated a lower risk of herd-level infection with MAP if adult stags comprised >5% of the deer herd. Research in cattle has shown that MAP infection is subject to an age barrier at approximately 6 to 10 months, beyond which an animal is less susceptible to becoming infected and subsequently developing clinical disease (Clarke 1997). Similar research in sheep indicates this species may be susceptible to infection with MAP throughout life, although lambs and hoggets may be relatively more susceptible (Begg et al 2005). Mackintosh *et al*. (2010) demonstrated a time-dependant relationship between the age of a susceptible deer and development of clinical JD, through observation of 30 weaner, 20 yearling and 20 adult red deer for 50 weeks after inoculation with 10^9 CFU of MAP. The study showed that while 33% of the weaner deer succumbed to clinical JD, there were no clinical cases observed in either the yearling or adult deer within the study period. The study also found that significantly more subclinically infected weaners (13/19) and yearling deer (6/20) were faecal culture positive at some time during the study period ($p<0.05$), while 8/20 weaner and 2/20 yearling deer had visible lymph node lesions at slaughter. This compares to the adult deer of which one was faecal culture positive and none had visible lesions, suggesting that lesion development and MAP faecal shedding may also be age-dependent. As reported in Chapter 2, the national prevalence of clinical JD in adult stags (0.4%) was significantly lower than that reported for weaner deer (0.7%), yearling deer (1.4%) and adult hinds (0.5%) ($p<0.05$). Thus, adult stags may be less

susceptible to infection and faecal shedding of MAP, relative to other age-classes of deer. However, longitudinal studies are required to establish the likelihood that a naive deer infected as a yearling or an adult will, within its lifetime, shed MAP in their faeces, develop lymph node lesions and/or develop clinical JD.

In study herds where adult stags comprised >5% of herd size, on average, 56.4% (95% CI = 46.5 to 66.3%) of farming operations involving this age-class was devoted to velvet production. In contrast, herds consisting of <5% adult stags devoted, on average, only 7.4% (95% CI = 3.5 to 11.3%) to velvet production. 'Velveting' is the surgical amputation of velvet antler, which is the growing phase of bony structures that develop on the heads of yearling and adult stags (Wilson and Stafford 2002). The average age of a velvetting stag is six to seven years and they are generally grazed in separate bachelor groups with minimal contact with other deer. This may contribute to the negative association found between a high proportion of adult stags and the herd-level presence of MAP. More detailed data is required of the pathogenesis and epidemiology of infection in stags before recommendations can be made concerning the role of stag management, including velveting could have in the control of infection in deer herds.

Farmer-diagnosed clinical JD in cattle in 2004 and/or 2005

This study has shown that deer herds are more likely to be MAP culture-positive if clinical JD had been recently observed in cattle by the farmer. It was assumed that the sensitivity and specificity of farmer diagnosis of clinical JD in cattle was high. In addition to clinical JD, differential diagnoses for diarrhoea and weight loss in New Zealand dairy and/or beef cattle are malnutrition, gastrointestinal parasitism and coccidiosis (Black and Orr 1999).

MAP isolates are categorised into ovine (S or Type I) or bovine (C or Type II) types, on the basis of pulsed-field electrophoresis (PFGE) and IS900-restriction fragment length polymorphisms (RFLP) (Dohmann et al 2003). An estimated 67% of New Zealand deer farmers graze livestock species in addition to deer on their DFA and although there is some evidence to suggest the bovine strain of MAP is more prevalent and pathogenic than the ovine strain in New Zealand deer herds, dedicated research utilising strain typing is required to definitively demonstrate this (de Lisle et al 2006; Griffiths et al 2006; Mackintosh et al 2007a).

One or more dams available as a water source for deer

Dams are constructed to hold water for stock water and irrigation and this study has shown that farmers utilising dams as water sources for deer had a lower probability of having a culture-positive herd. This finding is inconsistent with the results of a previous study in South Wales where catchment areas for water running from hillsides were found to be more likely to be culture positive for MAP than water samples sourced from higher altitudes (Pickup et al 2005). However, a number of dams in New Zealand are used to collect water from springs at higher altitude rather than run-off from pasture and farmers are encouraged to fence livestock out of waterways, thereby reducing faecal contamination. MAP can remain viable in water for extended periods of time, thereby acting as a potentially important vehicle for the transmission of the bacteria. Whittington *et al.* (2005) found water spiked with MAP and stored in shaded and unshaded water troughs was culture positive for up to 48 and 36 weeks, respectively, while faecal and soil samples from the same environs were culture positive for 12 weeks. Similarly, a study of 23 Californian dairy herds found MAP could be isolated from the wastewater storage lagoon (15/23; 65%) significantly more often than from composite manure samples collected from the sick cow pen (8/22; 36%) or from the alleyway (9/23; 39%) (Berghaus et al 2006). Further research is required into the significance of water in the transmission of MAP both within and between New Zealand deer properties.

This paper involved the evaluation of a large number of predictor variables (250). There are a number of methods to reduce the number of variables that need to be considered for inclusion in a regression model, including screening variables based on descriptive statistics, correlation analysis of independent variables and creation of indices (Dohoo et al 2003a). Each of these methods were utilised in the analysed for both Chapters 3 and 4, ensuring the models did not contain multicollinearity, resulting in unstable estimates of coefficients and incorrect standard errors,

This paper presents risk factors significantly associated with the herd-level culture of MAP from New Zealand farmed deer herds. As the status of each study herd was determined during a single sampling period, the significance of risk factor associations with the herd MAP status could only be evaluated for a single point in time. Although highlighting areas for further

detailed research, including geographic herd location and deer herd age-class composition, it would be premature to recommend management practices based solely on the results of this study. Alternatively, longitudinal studies, with herd MAP status monitored over a number of years, could identify risk factors significantly associated with a naive herd becoming infected with MAP and changes in the prevalence of MAP infection within a deer herd. This study identified risk factors which may be evaluated in longitudinal studies of MAP in farmed deer.

3.6 Acknowledgements

The authors gratefully acknowledge the participation of study farmers and veterinarians who assisted.

Chapter 4 describes risk factors associated with a within-herd clinical Johne's disease annual incidence of greater than 0.4% of farmed weaner (3 to 12 month old) deer. The manuscript is prepared in the style of the American Journal of Veterinary Research and has been submitted for publication.

CHAPTER 4: Environmental and management risk factors associated with clinical Johne's disease incidence in young farmed deer

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4.1 Abstract

The objective was to identify and quantify risk factors associated with a within-herd clinical Johne's disease (JD) annual incidence of greater than 0.4% of farmed weaner (3 to 12 month old) deer. A retrospective case-control study was conducted from 1 January to 31 December 2005. In 107 deer herds positive for MAP by tissue or pooled faecal culture, a farmer-diagnosed clinical disease incidence of more than 0.4% or less than/equal to 0.4% was observed in 53 and 54 herds, respectively. Data on potential environmental and management risk factors were collected by personal interview. Multivariable logistic regression analysis found that grazing of beef yearlings on the deer fenced area (DFA) and a greater presence of sheep on the DFA, relative to other livestock, had significant positive (OR = 4.55; 95% CI: 1.70 to 12.20) and negative (OR = 0.94; 95% CI: 0.89 to 0.99) associations, respectively, with greater than 0.4% incidence of weaner deer having clinical JD. The odds of greater than 0.4% of weaner deer experiencing clinical JD increased when irrigation was used ($p = 0.01$). The odds of clinical disease incidence being greater than 0.4% decreased when a higher proportion of the herd's breeding female deer were less than five years of age ($p < 0.01$) and when yearling (12 to 24 month old) deer were purchased ($p = 0.02$). This study suggests factors that may be incorporated into herd management to reduce the incidence of clinical JD, but causation remains to be established.

4.2 Introduction

Many species of deer, including red deer (*Cervus elaphus*), elk (*Cervus elaphus canadensis*) and fallow deer (*Dama dama*), are farmed for the production of meat and antler in countries such as New Zealand, China, Russia, Korea and Germany (Reinken 1998).

Johne's disease (JD; paratuberculosis) is a chronic, granulomatous enteritis of domestic and wild ruminants caused by infection with the bacteria *Mycobacterium avium* subspecies *paratuberculosis* (MAP) (Collins 2003b). While MAP infection has been variably diagnosed in farmed, zoo and/or wild deer in the UK (Gilmour and Nyange 1989; Fawcett et al 1995), Ireland (Power et al 1993), Italy (Nebbia et al 2000), Austria (Glawischnig et al 2006), Norway (Tryland et al 2004), Spain (Marco et al 2002; Balseiro et al 2008; Reyes-Garcia et al 2008), Belgium (Godfroid et al 2000), the Czech Republic (Machackova et al 2004), Germany (Commichau 1982), Ireland (Power et al 1993), the USA (Libke and Walton 1975; Temple et al 1979; Manning et al 1998; Pedersen et al 2008), Canada (Starke 1991), Denmark (Krogh and Jensen 1988), Sweden (Hillermark 1966), and Argentina (Mereb et al 1994), published reports of clinical JD in deer outside New Zealand are less common (Libke and Walton 1975; Temple et al 1979; Fawcett et al 1995; Manning et al 1998; Marco et al 2002; Deutz et al 2005; Glawischnig et al 2006; Mereb 2008a). Since the first putative diagnosis of MAP in a New Zealand farmed deer in 1979 (Gumbrell 1987), there has been an apparent increasing prevalence of mortalities as a result of MAP infection in young deer (de Lisle et al 2003; de Lisle et al 2006).

Johne's disease in cattle has been categorized into four stages (Sweeney 1996), with Stages 1 and 2 describing subclinical disease that can last for months to years or indefinitely, and Stages 3 and 4 describing clinical disease, where diarrhoea and/or weight loss progresses to advanced disease, with signs of lethargy, emaciation and profuse diarrhoea (Whitlock and Buergelt 1996). Unlike cattle, MAP-infected deer undergo a relatively short subclinical stage of typically weeks to months, before a proportion are observed with diarrhoea and/or weight loss that is invariably fatal (Mackintosh et al 2004a).

A 1998 economic impact assessment of clinical JD in the New Zealand deer industry was estimated at NZ$0.20 to 0.34M per annum, assuming a national herd-level prevalence of 4.2 to 7.0% (Brett 1998; de Lisle et al 2003). However, this is likely to be an underestimate of the present impact of clinical JD (Mackintosh and Wilson 2003) as the incidence rate of disease appears to have increased with some outbreaks of up to 20% reported in deer 9 to 24 months of age (Mackintosh et al 1999).

There is limited information on the epidemiology of clinical JD in farmed deer, with previous studies in New Zealand focused on a specific research question, such as virulence of MAP type (Mackintosh et al 2007a) and diagnostic test validation (Griffin et al 2005). This paper investigates environmental and management risk factors potentially associated with a within-herd annual incidence rate of greater than 0.4% in weaner deer with clinical JD. This age of deer was studied because the incidence rate in this group is relatively high, disease in this age-group is of particular concern to producers and the dataset from which analyses were performed was restricted to one year production cycle only, precluding analyses for other ages.

4.3 Materials and Methods

This was a retrospective case-control study of 107 confirmed MAP-infected deer herds, with a stratified, two-stage sampling strategy. The population of interest was New Zealand red deer; fallow deer; elk and/or red deer/elk crossbred (i.e. 'wapiti cross') herds with culture evidence of infection with MAP. The eligible herds were those with MAP infection grazing 20 or more weaner deer between 1 January 2005 and 31 December 2005. A property was defined as a single geographic location on which deer were grazed. The 'deer fenced area' (DFA) was that area fenced according to standards defined by the New Zealand Wild Animal Control Act 1977 (Anonymous 1977). A herd was defined as all deer grazed on the same DFA. Deer were classified according to age as 'weaner' (>3 months and ≤12 months), 'yearling' (>12 and ≤24 months) and 'adult' (>24 months). Female deer are referred to as hinds and male deer as stags. Deer were classified into age-classes based on age and sex as weaner deer (both sexes combined), yearling hinds, yearling stags, adult hinds or adult stags.

4.3.1 Selection of deer herds

From June 2004 to February 2005, deer farmers were recruited from throughout New Zealand for inclusion in the study via promotion at two national deer farming conferences and notification in a New Zealand Deer Industry magazine, circulated to approximately 4,000 farmers. In March 2005, a questionnaire was posted to 311 farmers requesting confirmation of interest in participation in the study and their intended deer herd size and composition at 31 July 2005. Although 193 (62.1%) confirmed their interest, 10 were excluded as they intended to

graze fewer than 20 weaner deer in 2005. A further nine farmers later withdrew, resulting in final enrolment of 174 herds of which 107 confirmed MAP-infected herds were derived for the present analysis.

4.3.2 Determination of positive herd-level MAP status

Fifty-nine herds (33.9%) were determined as MAP-positive based on a positive BACTEC culture of one or more enlarged and/or pathologic lymph node lesions identified at slaughter between 15 March 2004 and 15 March 2005. For herds without this prior evidence of infection, culture of five to six pools of 8 to 10 faecal samples from adult breeding hinds was chosen as the optimum method (based on a balance between sensitivity and cost) for the herd-level diagnosis of MAP.

Deer were faecal sampled by the first author or the farmer's veterinarian while standing in pens or a restraining device. A minimum of 5 faecal pellets (2 g) was collected per rectum using a new glove for each animal, and stored in a new 70 ml sample container. Samples were immediately chilled and within 24 hours were couriered to Massey University (Palmerston North, New Zealand) where they were pooled. Where available, samples from deer with diarrhoea, weight loss and/or those previously positive to an intra-dermal test for bovine tuberculosis (bTb) were pooled together. The remaining samples were pooled based on age. Samples were then either chilled at 4^{o}C for a maximum of 48 hours or immediately couriered to AgResearch, Wallaceville (Upper Hutt, New Zealand) for culture. A liquid culture system using BACTEC 12B vials (Becton Dickinson, Sparks, Maryland, USA) supplemented with 0.8 ml of egg yolk, mycobactin (Allied Laboratories, Fayette, Missouri, USA) and antibiotics (PANTA, Becton Dickinson, Sparks, Maryland, USA) was used, as described by Whittington et al (1999)
Forty-eight sampled herds had one or more faecal pool(s) culture positive for MAP, resulting in a total study population of 107 MAP-positive herds. Animal sex and age (determined either from the ear tag which listed the year of birth or estimated based on incisor teeth number and wear), the presence of any deer on the farm with diarrhoea and/or wasting, and the outcome of the previous intra-dermal bTb test were noted at the time of sampling.

4.3.3 Data collection and management

A comprehensive questionnaire (Appendix 2), including demographic data, disease occurrence, management, environment and potential risk factors for disease, was constructed and pre-tested in a pilot study of 22 deer farmers who did not participate in the final study. Between 5 August 2005 and 16 March 2006, data were collected at the farm, herd and animal level for the time period 1 January 2005 to 31 December 2005, through personal interview by the first author. Properties visited prior to 31 December 2005 were contacted in early 2006 to update information to the end of the calendar year.

Data for this analysis were from weaner deer. The number observed with diarrhoea and/or weight loss and the number specifically diagnosed with clinical JD, yersiniosis and/or a chronic deficiency of copper based on the observation of these clinical signs was estimated by the farmer. The number of diarrhoeic and/or wasting deer treated with an anthelmintic and the number which responded completely without recurrence of clinical signs was also provided. Additional data such as property and DFA dimensions, herd characteristics, grazing of species other than deer (SOTD), such as dairy cattle, beef cattle, sheep and goats and the number of deer purchased from 1 January 2003 to 31 December 2005, inclusive, were also collected. Results of soil testing on the DFA from 2003 onwards were also provided. The data were entered into a hierarchical database using Microsoft Access Version 3 (Microsoft Corporation, Washington, USA).

4.3.3.1 Definition of the outcome variable

Within each herd, an individual weaner deer with diarrhoea and/or weight loss which had not responded to an anthelmintic drench and/or the farmer had not diagnosed as due to yersiniosis or chronic copper deficiency was classified as affected with clinical JD (Mackintosh et al 2004a; Handeland et al 2008). The incidence of weaner deer with clinical JD was calculated by dividing the number of weaner deer with clinical JD by the total number of weaner deer grazed on the DFA in 2005. Case (n = 53) and control (n = 54) herds were defined as those above or below/equal to, respectively, the median incidence rate of 0.4% clinical disease for the year of study. A cut-point of 0.4% was selected to differentiate clinical incidence between case and

control herds as there was insufficient zero incidence herds to allow an evaluation of risk factors significantly associated with clinical JD.

4.3.3.2 Calculation of potential explanatory variables

The number of stock unit equivalents (against the standard sheep stock unit used in New Zealand) (SU) represented by each farmed ruminant species grazed on the DFA was calculated according to Woodford (2004), accounting for the species, age and breed. For SOTD, the number of days each animal was grazed on the DFA was approximated by the farmer. For deer, the approximate number of days was calculated by the first author, by age-class.

The number of days that each deer was grazed on the DFA was calculated by subtracting the date of weaning for those weaned prior to May 1 (progeny still with their dams after this date were assumed to be predominately grazing pasture) or date of purchase, from the 31 December. If a weaner deer left the property prior to 31 December, the grazing day count was to that date, but with a default to 31 March, 31 June and 15 October for live sales, deaths and transport to slaughter, respectively, if the actual date was not available. The number of days yearling and adult deer were grazed was calculated in a similar manner. A similar calculation was undertaken for other livestock species grazed at any time during the year of study on the DFA. The number of days each livestock class and species spent on the DFA in 2005 was then multiplied by the appropriate SU. This value was totalled for grazed livestock, incorporating all species and classes, on the DFA to create the variable of 'stock unit days' (SUDays), incorporating both time and SU. This variable was divided by the DFA size in hectares (ha) to create an overall stocking rate variable, namely stock unit days per hectare (SUDays/ha).

Study herds were aggregated into regions as defined in 1989 according to the provisions of the Local Government Act 1974 (Harris 2004). Auckland and Northland were combined into one region termed 'Northland' and Tasman, Nelson and Marlborough were combined into 'Nelson-Marlborough'. Herds were aggregated into North and South Island populations, where appropriate.

4.3.4. Statistical Analysis

Potential risk factors (n = 186), aggregated to herd-level, were screened for association with a age-class-level incidence rate of greater than 0.4% clinical JD using either a t-test, Chi-squared test or Fisher's exact test, as appropriate. Risk factors associated with this outcome with a p-value of <0.2 were included in a multivariable regression analysis. Meaningful interactions between main effects were tested for significance and model fit and residuals were evaluated for distributional assumptions and influential observations. All analyses were performed in R (version 2.4.1, The R Foundation for Statistical Computing), with significance declared at p <0.05, and results presented as OR and 95% CI.

4.4 Results

4.4.1. Descriptive analysis

The study included 211,022 deer from 107 herds, representing 12.4% of farmed deer and 3.8% of New Zealand deer herds as at June 2005. Eighteen (16.8%) and 89 (83.2%) herds were located in the North and South Island, respectively, with herds located in all regions except Northland, and Taranaki (Figure 4.1). Of the eighteen North Island study herds, six (33.3%) were case herds and 12 (66.7%) control herds. Of the 89 South Island study herds, 47 (52.8%) were case herds and 42 (47.2%) were control herds. The incidence of weaner deer with farmer-observed clinical JD across all study herds is illustrated in Figure 4.2, with an overall median annual incidence of 0.4%. Median incidence in control and case herds was 0.0% (0.0 -- 0.4%) and 1.8% (0.5 -- 21.5%), respectively.

4.4.2 Univariable analysis

Of the 186 potential risk factors screened for inclusion in the multivariable model, descriptive statistics for the seven uncorrelated continuous and 10 uncorrelated categorical risk factors which had a p-value of <0.20 in the univariable analysis, are presented in Tables 4.1 and 4.2, respectively.

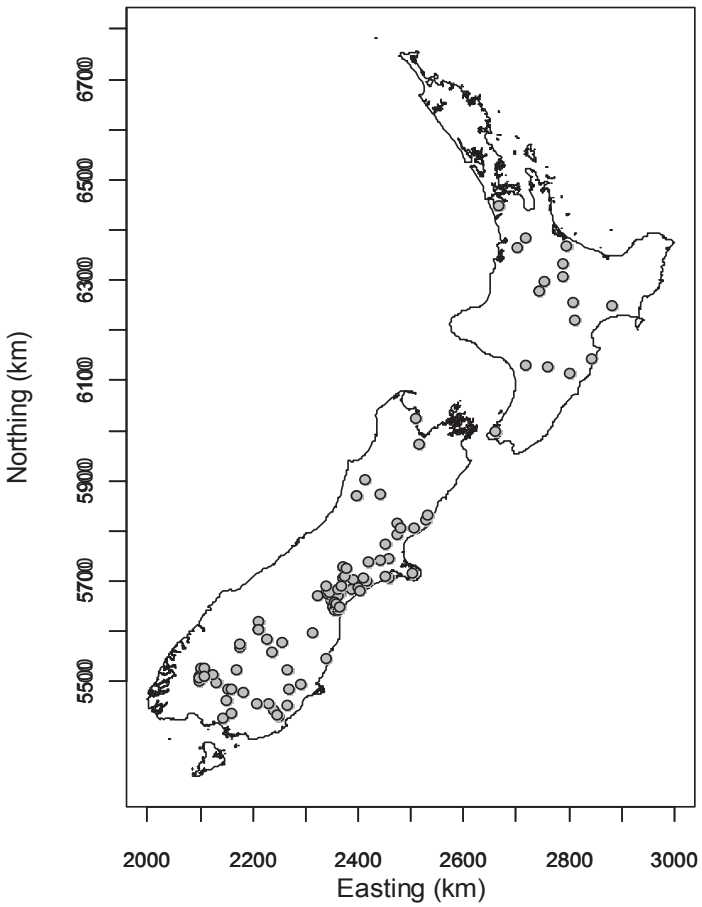


Figure 4.1 Distribution of study deer herds (n = 107) evaluated for risk factors associated with greater than 0.4% incidence of farmer-observed clinical Johne's disease in 3 to 12 month old deer.

4.4.3 Multivariable analysis

The Pearson χ^2 statistic was 45.65 (p = 0.31) indicating there that there is no reason to assume that the multivariable model was not correct. Variables significantly associated with a clinical JD incidence in weaner deer of greater than 0.4% were: the presence of yearling beef cattle on the DFA (OR = 4.55, p<0.01); and the use of irrigation on the DFA (OR = 5.18, p = 0.01) (Table 4.3). Variables significantly associated with a clinical JD incidence of equal to/less than 0.4% were: an increasing presence (i.e. proportion of total SUDays) of sheep on the DFA relative to other livestock (OR = 0.94, p = 0.03); an increasing percentage of breeding hinds less than five

years of age (OR = 0.06; p <0.01); and the purchase of yearling deer in 2005 (OR = 0.29, p = 0.02).

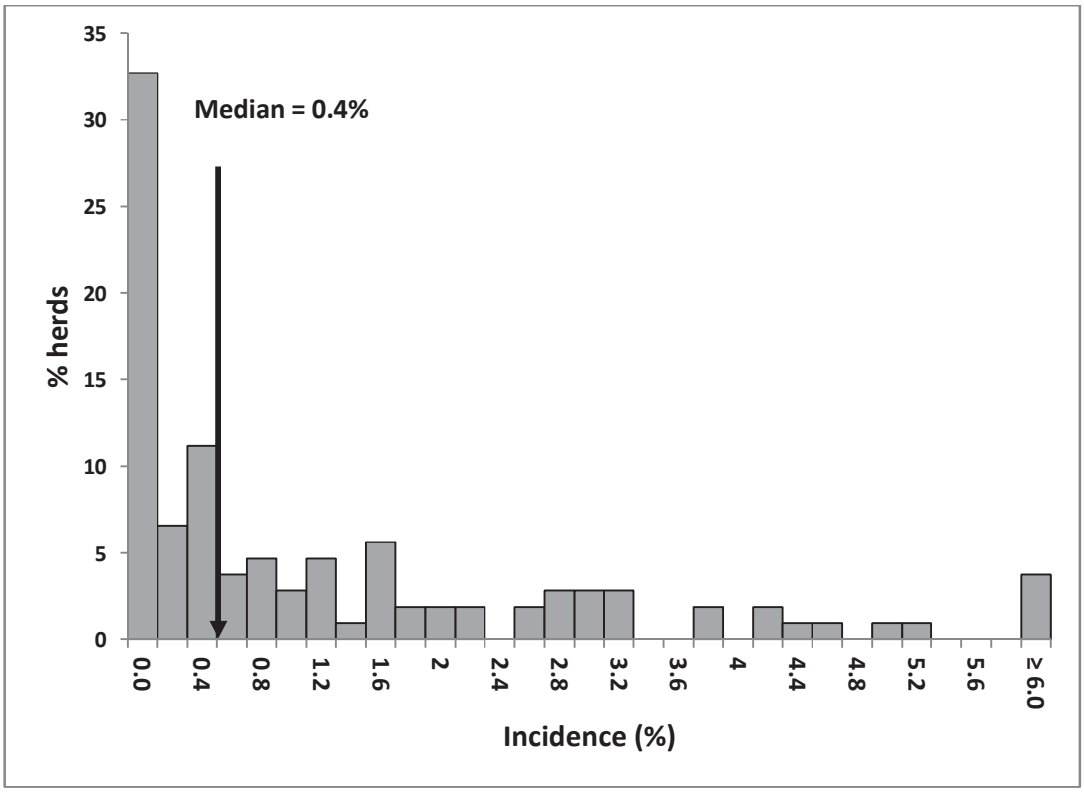


Figure 4.2 Frequency histogram of the incidence of farmer-observed clinical Johne's disease in 3 to 12 month old deer in 2005 on New Zealand deer farms culture-positive for *Mycobacterium avium* subspecies *paratuberculosis* (MAP) (n = 107).

Table 4.1. Description, mean and standard deviation (SD) of continuous variables significant (p<0.20) after univariable analyses, in case (n = 53) and control (n = 54) herds.

	Case			Control			
Continuous predictor variables	n	Mean	SD	n	Mean	SD	**p**
Total Deer SU/ha contributed by yearling deer (%)	48	12.95	11.26	50	16.60	13.68	0.13
Total Deer SUDays contributed by weaner deer (%)	53	40.77	25.10	54	32.76	15.37	0.05
Total SUDays contributed by sheep (%)	33	4.62	7.80	35	7.80	10.86	0.09
Total SOTD SUDays contributed by SOTD <2 years old (%)	41	44.53	38.49	33	27.57	33.52	0.02
Soil pH	53	6.20	0.32	54	6.04	0.31	0.03
Number of breeding female deer <5 years old (%)	41	27.70	23.32	46	40.81	26.02	0.01
Hectares with lime application on DFA (%)	25	16.64	29.55	25	9.13	18.46	0.12

SOTD = ruminant species other than deer
SU = stock units
ha = hectare

Table 4.2. Description of categorical variables significant ($p<0.20$) after initial univariable analyses, in case (n = 53) and control (n = 54) deer herds.

Categorical predictor variables	Yes/No	Case herds n	Control herds n	OR (95% CI)	p
Yearling deer purchased	Y	10	20		
	N	43	34	0.40 (0.16, 0.96)	0.04
Irrigated pasture on DFA	Y	12	6		
	N	41	48	2.34 (0.81, 6.79)	0.11
Beef yearlings grazed on DFA	Y	28	18		
	N	25	36	2.24 (1.03, 4.89)	0.04
>20% DFA flat (including irrigated land)	Y	31	24		
	N	22	30	1.76 (0.82, 3.79)	0.15
Natural springs on DFA	Y	21	32		
	N	32	22	0.45 (0.21, 0.98)	0.04
Stream(s)/ river(s) on DFA	Y	12	14		
	N	32	40	1.07 (0.44, 2.64)	0.13
Hill/high country used for fawning	Y	14	23		
	N	32	26	0.49 (0.21, 1.15)	0.09
Weaner deer fed hay	Y	9	3		
	N	44	51	3.48 (0.89, 13.65)	0.07[A]
Breeding hinds fed bailage in the previous year	Y	10	4		
	N	43	50	2.91 (0.85, 9.94)	0.09[A]

[A] Fisher's exact test
OR = Odds Ratio
DFA = Deer fenced area

4.5 Discussion

While there have been reports of clinical JD in wild and/or farmed deer in the USA (Libke and Walton 1975; Temple et al 1979), Argentina (Mereb 2008a), Austria (Deutz et al 2005; Glawischnig et al 2006), Spain (Marco et al 2002), the UK (Fawcett et al 1995) and New Zealand (de Lisle et al 2003), this is the first investigation of risk factors significantly associated with the presence of the disease in this species.

In the last decade, clinical JD has emerged as a potentially serious economic burden to a number of farmers in the New Zealand deer industry (de Lisle et al 2006), and there is concern that disease incidence is increasing. From 1986 to 2004, over 600 deer herds were identified as MAP-infected, with over half diagnosed between 2000 and 2004. However, those results may be biased as samples were derived from culture and PCR of tissues tested on suspicion for *M. bovis*, largely from material collected at commercial deer slaughter premises (de Lisle et al 2003; de Lisle et al 2006). As outlined in Chapter 1, from 1988 to 2005, there was an exponential-like

increase in the initial detection of clinical JD by New Zealand farmers in their deer, with 40% first observing disease in 2005.

Table 4.3. Results of a multivariable logistic regression analysis for associations between herd-level variables and an annual incidence of clinical JD of greater than 0.4% in farmed weaner deer.

Likelihood ratio, 59.7; df, 106; p-value of the final model, <0.0001, adj R^2 = 34.02

Predictor variables	Yes/No	Beta Coefficient	Standard error (Beta)	p	Adjusted OR	95% CI
Total SU Days contributed by sheep (%)[a]		-0.06	0.03	0.03	0.94	0.89, 0.99
Breeding hind herd <5 years old (%)[b]		-2.80	0.97	<0.01	0.06	0.01, 0.40
Yearling deer purchased	N	-	-	-	Ref	-
	Y	-1.22	0.53	0.02	0.29	0.10, 0.84
Beef yearlings grazed on DFA	N	-	-	-	Ref	-
	Y	1.51	0.50	<0.01	4.55	1.70, 12.20
Irrigated pasture on DFA	N	-	-	-	Ref	-
	Y	1.65	0.67	0.01	5.18	1.40, 19.23

[a] An increase in the total SU Days contributed by sheep from 50% to 75% decreased the log odds of clinical JD in >0.4% of weaner deer by 1.5 units.
[b] An increase in the breeding hind herd <5 years old from 25% to 50% decreased the log odds of clinical JD in >0.4% of weaner deer by 70.0 units.

Identification of risk factors may contribute to the control of the disease in farmed deer herds. It is stressed that the risk factors presented in this paper are established by statistical association only, and cannot be taken as causative unless they are shown subsequently to be so. However, where biologically plausible, these factors may provide hypothesis for research testing, or be incorporated into management practices that will possibly affect the incidence rate of clinical JD.

This study targeted young deer to 12 months of age, in contrast to previous studies of dairy cattle and sheep that focused on risk factors associated with clinical disease JD in adults (Cetinkaya et al 1997a; Lugton 2004a; Dhand et al 2007). Clinical JD in sheep and cattle is not typically observed until the animal is at least 18 to 24 months of age (Rankin 1962; Larsen et al 1975; Begg et al 2005). In contrast, deer from 6 to 8 months have been observed with clinical JD, with outbreaks of up to 20% recorded in this age-class (Mackintosh 2000). Mackintosh *et al.* (2007a) found that weaner red deer experimentally challenged with MAP had a 33% incidence of clinical disease compared with no deaths in yearlings and adults within a study period of 50 weeks.

Since a large proportion of deer are slaughtered for meat by 12 months of age, this is an important age-group to target for disease control.

To date, management practices that may influence the long-term prevalence of clinical JD in deer herds, particularly in weaners and yearlings, have been based on knowledge from the sheep and cattle and first-principles veterinary medicine (Wilson and Castillo-Alcala 2004). Given the different age distribution of clinical JD in deer compared with sheep and cattle, it is probable that factors specific to this age group may be important in determining disease expression. Hence a generic management program may be less likely to be effective than one specifically targeted at weaner deer. This is particularly applicable to 'finisher' properties which purchase young deer for slaughter at 9 to 15 months of age.

Diagnosis of clinical JD in this study, and therefore determination of incidence status, relied on farmer detection of diarrhoea and/or weight loss and recollection of the number of cases during the year of study. The accuracy of farmer observation of diarrhoea and weight loss in their deer at any one time was likely to be high since those signs are easily apparent. However, as discussed in Chapter 2, chronic abomasal parasitism, copper deficiency, yersiniosis and malignant catarrhal fever are difficult to distinguish clinically from JD, potentially reducing the specificity of farmer diagnosis (Mackintosh 2000), but to an unknown extent.

To increase the specificity of diagnosis, classification of clinical JD was restricted to deer with diarrhoea and/or weight loss that the farmer had not previously diagnosed as due to yersiniosis/copper deficiency and/or had not responded to an anthelmintic, antibiotic or copper supplement if administered. Clinical trials in cattle with clinical JD have found diarrhoea and weight loss will recur or continue after those treatments (Merkal and Larsen 1973) and clinical observations suggest the same for deer. Inclusion of this criterion of non-responsiveness to these treatments should have increased the specificity of diagnosis. Although other conditions, such as malignant catarrhal fever and infection with campylobacter, coronavirus or rotavirus, can cause diarrhoea and/or weight loss in deer, these are rare and/or do not affect deer less than 12 months of age (Mackintosh and Wilson 2005).

Association with sheep

This study found that a greater presence of sheep on the DFA lowered the risk of clinical JD in weaners above the median incidence. MAP is classified as either Type I (S or ovine/sheep) or Type II (C or bovine/cattle). Although not fully host-specific, MAP exhibits a strong host preference, with current evidence suggesting sheep, at least in Australasia, are predominantly infected with Type I and cattle with Type II (Whittington et al 2000c). Although deer can be infected by both types, 17 of 20 MAP isolates identified in farmed deer from 1985 to 1991 and, more recently, 91 of 95 isolates identified from MAP-infected deer herds were Type II (de Lisle et al 1993; de Lisle et al 2006). However, these results were based on biased subpopulations of lymph nodes sampled on suspicion for *M. bovis*. A 2007 study found artificial inoculation of deer with Type I resulted in a lower incidence and severity of clinical disease and grossly visible pathology at slaughter compared with those challenged with Type II (de Lisle et al 2006; Mackintosh et al 2007a). Thus, the evidence available suggests that the sheep type may be less pathogenic in deer than the cattle type. Evaluation of the relative infection rate and pathogenicity of each type in the New Zealand farmed deer population requires further research.

There is some evidence to suggest that grazing with sheep could reduce the survival of MAP on pasture, thereby providing a possible explanation for the association described here between sheep and clinical JD in weaner deer. Whittington *et al.* (2004) reported that the longevity of MAP was greatest in a fully shaded environment and least where soil and faecal material were fully exposed to environmental effects, such as direct sunlight. As sheep are managed in a way that tends to graze pasture close to the soil, the likelihood of MAP being exposed to a high intensity of direct sunlight is increased, thereby potentially decreasing the contamination levels of MAP on pasture over time. However, recent research has found that shared grazing of pastures by sheep with clinical JD, compared with subclinical infection, and weaner deer resulted in an increased risk of signs of clinical JD in the latter (Verdugo C, *pers. comm.*), indicating this risk factor warrants further research in relation to strain, grazing associations and links between clinical disease and subclinical infection.

Association with grazing younger hinds

The risk of clinical JD was lower when there was a high proportion of the breeding herd less than five years of age. Vertical transmission has been shown to occur in sheep, cattle and deer (van der Giessen et al 1994; Lambeth et al 2004; van Kooten et al 2006). van Kooten *et al.* (2006) demonstrated that 89% of hinds with clinical JD had MAP-positive foetuses, while Thompson *et al*. (2007) found 78% of foetuses of subclinically infected dams were MAP-positive. However, there is no data on whether younger hinds are less likely to pass MAP onto their offspring. It is possible this risk factor represents management factors correlated with a younger breeding herd, such as improved nutrition, which may also influence the level of clinical JD seen in their offspring, rather than a direct effect of dam age on disease incidence.

Association with purchase of yearling deer

In this study, there was a lower incidence of clinical JD in herds with a higher rate of purchase of yearling deer. In contrast, Mainar-Jaime and Vazquez-Boland (1998) found a high purchase rate of replacement animals was significantly associated with a high herd-level seroprevalence for MAP in Spanish sheep flocks. As it could be hypothesized that in order to maintain herd size, yearling deer would be purchased more commonly in herds with high mortality rates as a result of clinical JD in weaner deer, it is possible that this risk factor is either spurious or representative of another unknown variable.

Association with yearling beef cattle

This study showed a greater incidence of clinical JD on farms with a higher number of yearling beef cattle. While the prevalence of MAP has been studied in dairy and beef cattle in numerous countries worldwide (Lombard et al 2006; Caldow et al 2007; Kobayashi et al 2007; Norby et al 2007), estimates of the prevalence of MAP in New Zealand dairy cattle herds to-date have been based solely on extrapolation from overseas estimates and computer simulations (Burton and Voges 2002; Soons et al 2002). Animals with clinical JD shed significantly higher levels of MAP relative to subclinically infected animals (Whittington et al 2000d). However, since clinical JD in cattle is uncommonly observed prior to 18 to 24 months of age, it would be expected that older cattle would present a higher risk of MAP infection. Therefore, a

biologically plausible link with this risk factor is difficult to establish and further study is recommended.

Association with Irrigation

This study found that irrigation of pastures on the DFA with water was positively associated with greater than 0.4% of weaner deer demonstrating clinical JD. Although MAP is an obligate pathogenic parasite of animals, only multiplying within the macrophage of a susceptible host, its thick, complex cell wall confers significant resistance to environmental effects (Whittington et al 2004). There is substantial evidence that MAP can persist for considerable periods of time in a moist environment (Lovell et al 1944; Larsen et al 1956; Pickup et al 2005; Roussel et al 2005) and irrigation is commonly applied in mid-summer and autumn in New Zealand to promote the production of high quality pasture for growth of young deer either shortly prior to or after weaning. Therefore, deer up to six months of age grazed on irrigated pasture contaminated with MAP may be exposed to this bacteria for a longer period of time, relative to weaner deer grazed on MAP-infected, un-irrigated pasture. Irrigated pastures can also generally maintain a higher stocking rate relative to un-irrigated pastures,a well-recognised driver for an increased incidence of clinical disease.

This paper reports risk factors significantly associated with an incidence rate of clinical JD of greater than 0.4% in weaner deer, identifying potential environmental and management factors that farmers may consider for reducing the risk of clinical JD in young deer. It would appear that higher grazing rates with sheep on deer pastures may reduce the risk of clinical JD in young deer, although this may apply only to sheep flocks without clinical JD as suggested by other research. Grazing yearling beef cattle on deer pasture *per se,* and grazing on irrigated pastures appear to increase the risk of a high incidence of clinical JD in young deer. While the purchase of yearling deer and an increasing percentage of the breeding hind herd being less than five years of age were both associated with a reduced risk of clinical JD, these factors may not be enduring due to possible confounding. While the risk factors identified in this paper may provide guidance for management of the risk of clinical JD, they require validation for biological causation before they can be adopted with confidence.

4.6 Acknowledgements

We acknowledge the generosity of the study herd owner/managers and the assistance of veterinary clinics in sample collection. This study was funded by the New Zealand Deer Industry Johne's Research Group1 with assistance of DEEResearch, and FRST via a subcontract with AgResearch, and Massey University.

Chapter 5 outlines development and validation of the use of 'abnormal' lymph nodes in commercially slaughtered New Zealand farmed deer for national surveillance for *Mycobacterium avium* subspecies *paratuberculosis*. The manuscript is prepared in the style of Epidemiology and Infection and, at the time of writing, permission from funders to submit the manuscript was awaited. It is intended for publication alongside a study entitled 'Prevalence of *Mycobacterium avium* subsp. *paratuberculosis* in grossly normal mesenteric lymph nodes of New Zealand farmed deer (*Cervus elaphus*)' by LA Stringer, PR Wilson, C Heuer, JC Hunnam, C Verdugo and CG Mackintosh.

CHAPTER 5: Association between *Mycobacterium avium* subspecies *paratuberculosis* and lymph node size in New Zealand farmed deer (*Cervus elaphus*)

JC Hunnam, PR Wilson, C Heuer, L Stringer, RG Clark, CG Mackintosh

5.1 Abstract

Criteria for 'abnormal' lymph nodes (LN) at slaughter were developed and validated for national surveillance for *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in New Zealand farmed deer. A cross-sectional pilot study found 94.6% of 129 'abnormal' LN, those enlarged and/or containing grossly visible pathology, from 76 deer herds were positive for MAP through culture or histopathology. Subsequently, a circumference cut point for an 'abnormal' mesenteric LN (MLN) (n = 412) was determined at 55 mm, based on >95% specificity of MAP detection. Increasing MLN circumference was positively associated with moderate follicular hyperplasia ($p<0.01$), focal granulomas ($p<0.01$) and a synergistic interaction between focal granulomas and MAP status ($p = 0.03$). Although using 'abnormal' LN as a sole criterion underestimates animal-level prevalence of MAP, this data gives sufficient confidence for its use as a marker for the purpose of national herd-level surveillance for MAP in farmed deer.

5.2 Introduction

Johne's disease (JD; paratuberculosis) is a chronic, granulomatous enteritis of domestic and wild ruminants caused by infection with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) (Collins 2003a). Johne's disease presents a potentially serious economic burden to the New Zealand farmed deer industry (de Lisle et al 2003). Concurrent with an increase in the prevalence of clinical JD on deer farms (Wilson and Mackintosh 2002), there has been an apparent increasing prevalence of grossly visible abnormalities of visceral lymph nodes (LN) of

slaughtered deer consistent with pathology typical of MAP infection in this species (de Lisle et al 2003).

As part of a national programme to eradicate bovine tuberculosis (*Mycobacterium bovis*) from domestic livestock in New Zealand, meat inspectors are trained to visually inspect, palpate and/or incise a number of deer LN, including the iliac, atlantal, mesenteric and retropharyngeal LN. A national database has been established to identify MAP-infected deer herds based on recording of 'abnormal' LN characteristics in commercially slaughtered deer by these inspectors (Lynch 2007). The primary aim of the database is to provide a practical method for monitoring the national and regional prevalence of MAP at the herd-level within the New Zealand farmed deer industry. However, use of the database for this purpose requires validation that infection with MAP is the predominant cause of 'abnormal' LN in deer since other pathogens such as *M. bovis, Mycobacterium avium* subspecies *avium*, *Actinomyces pyogenes and Rhodococcus sp* can cause a lymphadenitis that is indistinguishable grossly from that due to MAP infection (Carman and Hodges 1987; Griffin et al 2006b; Chiu et al 2009).

Assessment of the accuracy of inspector detection of grossly visible abnormalities through abattoir surveillance requires a quantitative measure that can be applied to diagnose the presence or absence of that abnormality (Hathaway and Richards 1994; Enoe et al 2003). For the national database, a useful measure of an 'abnormal' LN could be at or above a cut-point in LN circumference at which there is a high specificity of MAP detection, as an increase in LN size due to MAP infection has been noted in other ruminant species (Buergelt et al 2000; Catton 2002). Identification of animal-level risk factors, including MAP status, that are significantly associated with increasing LN circumference may also provide insights into the pathogenesis of LN enlargement in deer.

This paper is one of a series describing development and validation of parameters for the surveillance of MAP in slaughtered deer through the national New Zealand farmed deer industry database. The objectives described in this paper were to determine animal and histopathological risk factors associated with increasing LN circumference, to establish the circumference cut point for defining an 'abnormal' LN and to estimate the prevalence of MAP infection in

carcasses with 'abnormal' LN. Associated papers describe the prevalence of MAP in 'normal' lymph nodes (Stringer et al 2009b) and associated risk factors, and the specificity and sensitivity of meat inspector detection of 'abnormal' LN (Chapter 6).

5.3 Materials and Methods

Study deer were sampled at commercial deer slaughter premises (DSP). Some phenotypically red deer (*Cervus elaphus*) may have contained some wapiti (*Cervus elaphus canadensis* subspecies group) genes, as cross-breeding is common. A herd was defined as deer farmed at a single location. A line was defined as a shipment of deer sent for slaughter from the same herd to the same DSP on the same day. Female deer were referred to as hinds and male deer as stags.

5.3.1 Study I: Determination of MAP prevalence in 'abnormal' LN

The objective of this pilot cross-sectional study was to estimate the prevalence of MAP infection in 'abnormal' LN. Lymph nodes were classified subjectively as 'abnormal' by an accredited meat inspector based on grossly visible pathology, such as discolouration, focal or diffuse enlargement and/or containing a core of mineralized, caseous and/or necrotic material.

5.3.1.1 LN selection and testing

One hundred and twenty-nine 'abnormal' LN, sourced from 76 herds, were sampled from deer slaughtered in four South Island and three North Island DSP between 15 May 2007 and 23 November 2007. One-hundred and fourteen LN were sourced from 63 South Island herds in the Canterbury (28), Southland (18) and Otago (17) regions, whilst 15 LN were sourced from 13 North Island herds in the Waikato (5), Hawkes Bay (5), Bay of Plenty (2) and Manawatu (1) regions. A maximum of one LN per animal and three animals per line were sampled by the inspector. Two 15 mm MLN sections were stored in separate 70 ml sterile containers, one with 10% formalin. All tissues were collected by sterile technique using new equipment for each sample.

Fresh samples were kept chilled in an insulated container prior to courier to AgResearch Wallaceville (Upper Hutt, New Zealand) for culture. A liquid culture system, as described by

Whittington *et al*. (1999), using Bactec 12B vials (Becton Dickinson, Sparks, Maryland, USA) supplemented with 0.8 ml of egg yolk, mycobactin (Allied Laboratories, Fayette, Missouri, USA) and antibiotics (PANTA, Becton Dickinson, Sparks, Maryland, USA) was used (Whittington et al 1999). Subsequent to a positive culture result, a conclusive diagnosis of MAP was made based on the presence of a slow-growing acid-fast organism and mycobactin dependence of the isolate (de Lisle et al 2003).

Fixed tissue samples were dehydrated, cleared and embedded in paraffin. Sections were stained with haematoxylin and eosin (H & E) and Ziehl-Neelsen (ZN). All sections were examined by one histopathologist (RGC) without knowledge of the age, sex or herd of origin of the deer being examined.

5.3.1.2 Data collection and management

Herd, DSP, age, sex, and LN location and colour (normal, red, brown, green, yellow, white, other) were recorded at the time of sampling. Because of between-DSP variation in the precision of recording, age was aggregated into young and adult and combined with sex into categories of young hinds, young stags, adult hinds and adult stags. With the exception of herd location and DSP, data were recorded at the carcass-level.

5.3.1.3 Statistical analysis

The prevalence of MAP within 'abnormal' LN, nationally and by Island (North and South), was calculated by dividing the number of culture-positive LN by the total number of LN sampled, with exact confidence intervals derived from the binomial distribution (Dohoo et al 2003b). A Fisher's exact test was used to assess the significance of herd location (North or South Island). This test ignored multiple sampling per herd as few LN were sampled per herd and, thus, only a low effect was expected on the estimated variance.

5.3.2 Study II: Determination of an 'abnormal' MLN circumference cut point and factors associated with increased MLN circumference

The objective of this study was to define the MLN circumference cut point at which the specificity of MAP detection was >95%. Associations between MLN circumference and animal-level factors and histopathological features were also evaluated.

5.3.2.1 Selection of herds and MLN

Mesenteric LN samples were collected from 412 deer slaughtered from 79 herds in three South Island (n = 49 herds) and two North Island DSP (n = 30 herds) between October 2007 and January 2009. Any herd from which five or more deer were slaughtered on any sampling day was included. Four to seven MLN per line were randomly selected, with the maximum circumference of each measured using a flexible measuring tape by the primary author or a technician, and entire length incised. Two 15 mm cross-sections were taken from each LN and stored in two individual sterile containers, one with 10% buffered formalin. Samples were collected and processed for culture and histopathology as above.

5.3.2.2 Data collection and management

Carcass weight, age, sex and herd location were recorded at the time of sampling. Age was categorised as young or adult as above. The histopathological features of follicular hyperplasia, capsular infiltration by eosinophils, foci of macrophages containing lipopigments, parasitic granulomas, calcified foci, focal granulomas and/or reaction to a ZN stain were categorised, graded and coded for later analysis as outlined in Chapter 7. Mesenteric LN circumference, all histopathological features, age, sex, carcass weight and herd were recorded at the carcass level.

5.3.2.3 Statistical analysis

Mesenteric LN circumference was categorised into 5 mm increments, starting with those 35 mm and above, and used for determination of a cut point. The specificity (Sp) and sensitivity (Se) of MAP diagnosis were plotted as cumulative percentages at each MLN circumference increment. The Se was defined as the correct diagnosis of MAP infection within a MLN truly culture-positive for the bacteria, whereas the Sp was defined as the correct diagnosis of no MAP infection within a MLN truly culture-negative for the bactiera. The circumference cut point of

an ‘abnormal’ MLN was defined as the lowest MLN circumference with a Sp of MAP diagnosis >95%.

Potential factors associated with a MAP culture-positive MLN, such as carcass weight, age, sex and histopathological features, were screened for association with MLN circumference. Factors associated with MLN circumference with a p-value of <0.2 were included in a multivariable, linear mixed-effects model. Three MLN were removed from the analysis as animal age was unavailable. In order to correct for a skewed distribution of residuals, the outcome variable was log transformed. Herd of origin was included in the model as a random effect to adjust variance estimates for correlation at this level of aggregation. Age was forced into the final model to control for confounding. Biologically plausible interactions between fixed effects were tested for significance and model fit and residuals were evaluated for distributional assumptions and influential observations. A boxplot was used to show the mean and scatter of circumference in MLN positive and negative for MAP, stratified by the presence of focal granulomas. Four outlying MLN circumference measurements (culture negative MLN >90 mm circumference containing focal granulomas) were removed and the model re-run to assess their impact on the complete model. All analyses were performed in R (version 2.4.1; The R Foundation for Statistical Computing) and significance was declared at $p < 0.1$.

5.4 RESULTS

5.4.1 Study I

Table 5.1 shows the number (and percentage) of culture-positive and negative LN and their distribution by region, age-class, DSP, LN location and colour. The overall prevalence of MAP in ‘abnormal’ LN was 92.2% (95% CI: 86.2 to 96.2%). The prevalence in the North Island samples was 80.0% (95% CI: 51.9 to 95.7%) and the prevalence in the South Island samples was 93.9% (95% CI: 87.8 to 97.5%) ($p = 0.09$).

The 10 culture-negative ‘abnormal’ LN were from eight herds located in the Southland (n = 3), Canterbury (1), Otago (1), Waikato (2) and Hawkes Bay (1) regions. On histopathological examination, three of the culture-negative LN showed calcified, caseated, granulomatous

Table 5.1 Descriptive statistics for 129 'abnormal' lymph nodes (LN) positive and negative for *Mycobacterium avium* subspecies *paratuberculosis* (MAP) (Study I).

			LN MAP status	
			Positive	Negative
Variable	**Category**	**Sub-category**	**n (%)**	**n (%)**
Herd regional location	North Island	Waikato	6 (75)	2 (25)
		Hawkes Bay	4 (80)	1 (20)
		Bay of Plenty	1 (100)	0 (0)
		Manawatu	1 (100)	0 (0)
	South Island	Canterbury	42 (93)	3 (7)
		Southland	35 (92)	3 (8)
		Otago	30 (97)	1 (3)
Animal age-class	Young	Stag	66 (94)	4 (6)
		Hind	44 (92)	4 (8)
		Unknown	1 (50)	1 (50)
	Adult	Hind	6 (100)	0 (0)
	Unknown	Stag	1 (50)	1 (50)
		Hind	1 (100)	0 (0)
Deer Slaughter Plant	North Island	1	7 (100)	0 (0)
		2	3 (50)	3 (50)
		3	2 (100)	0 (0)
	South Island	1	40 (93)	3 (7)
		2	37 (93)	3 (7)
		3	28 (97)	1 (3)
		4	2 (100)	0 (0)
LN location	Mesenteric		88 (96)	4 (4)
	Hepatic		10 (83)	2 (17)
	MRP		9 (82)	2 (18)
	Mediastinal		3 (75)	1 (25)
	Prescapular		4 (100)	0 (0)
	LRP		1 (50)	1 (50)
	Ileo-caecal		2 (100)	0 (0)
	Bronchial		1 (100)	0 (0)
	Apical		1 (100)	0 (0)
LN colour	Normal		44 (94)	3 (6)
	Yellow		26 (96)	1 (4)
	Brown		17 (100)	0 (0)
	Green		8 (57)	6 (43)
	Red		11 (100)	0 (0)
	Unknown		8 (100)	0 (0)
	White		5 (100)	0 (0)

MRP = medial retropharyngeal lymph node
LRP = lateral retropharyngeal node

lymphadenitis, consistent with mycobacterial infection (Clark et al 2010). One of these lymph nodes was positive to a ZN stain. Inclusion of these LN as MAP-infected increased the overall

prevalence of the bacteria in 'abnormal' LN to 94.6% (95% CI: 89.2 to 97.3%). Additional noteworthy histopathological changes observed in the remaining culture-negative LN were follicular hyperplasia (n = 3), oedema (1) and changes consistent with immune-mediated disease (1).

5.4.2 Study II

Mesenteric LN circumference ranged from 19 mm to 124 mm with a mean circumference of 42.5 mm (± 13.0 mm) and a median circumference of 42.0 mm (Figure 5.1). The mean circumference of culture-negative and positive MLN was 40.7 mm (± 12.9 mm) and 44.1 mm (± 13.0 mm), respectively.

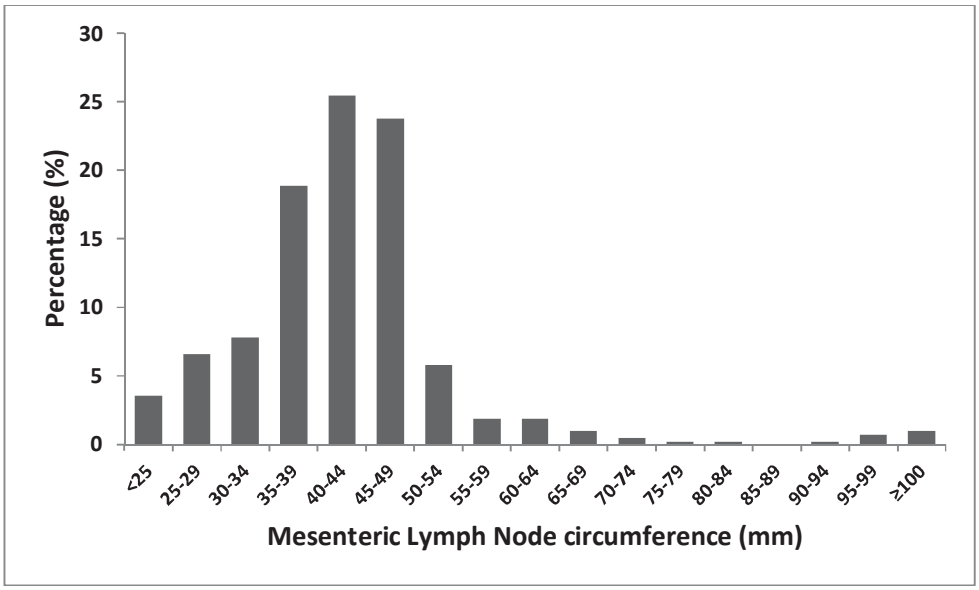


Figure 5.1 Frequency histogram of the circumference of deer mesenteric lymph nodes (MLN) (n = 412) at 5mm increments (Study II).

Figure 5.2 illustrates the cumulative sensitivity and specificity of the measurement of MLN circumference at cut-points from 35 mm to 70 mm in 5 mm increments to diagnose MAP infection. The circumference cut point for an 'abnormal' MLN was estimated at 55 mm, based on a specificity of MAP detection of >95%. The sensitivity of MAP detection at this cut point was approximately 12%.

Results of the crude analysis of association between log MLN circumference and age-class, MAP status and histopathological features with p-values <0.20 are presented in Table 5.2.

Scattered foci of macrophages containing lipopigments, parasitic granulomas, focal granulomas, calcified foci and MAP status were all significantly associated with MLN circumference. Multivariable, mixed linear regression analysis found moderate follicular hyperplasia, focal granulomas and a synergistic interaction between focal granulomas and MAP status were positively associated with log MLN circumference (Table 5.3).

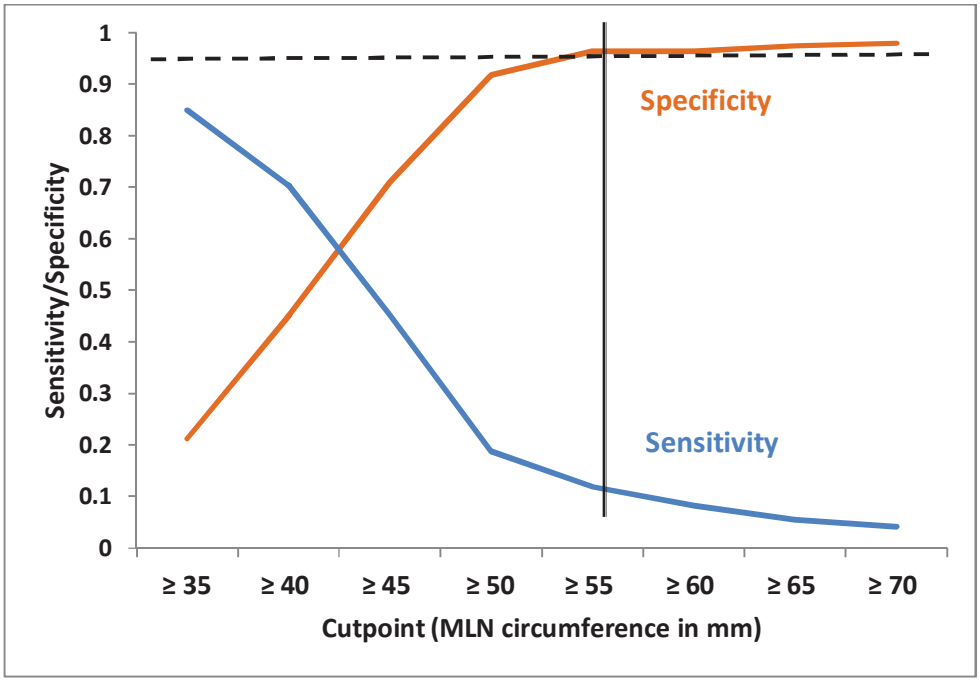


Figure 5.2 Cumulative sensitivity and specificity (%) of potential cut points based on MLN circumference for MAP determination. Dashed line = 95% Specificity. Vertical line = MLN circumference from which specificity was > 95% (Study II).

A boxplot of the circumference of MLN culture-positive and -negative for MAP, stratified by the presence of focal granulomas, is presented in Figure 5.3. The association between MLN circumference and focal granulomas became non-significant after removal of the four outlying culture-negative MLN with focal granulomas and a circumference >90 mm.

5.4.3 Potential causation of LN enlargement

The associations described above, when combined with histopathological observations from associated (Chapter 7) and other studies (deLisle et al 1993; Quigley et al 1997; de Lisle et al 2003), allowed the development of a potential qualitative causal pathway for LN enlargement as illustrated in Figure 5.4. As outlined in Chapter 7, both moderate follicular hyperplasia and focal

granulomas are significantly more common in young deer, while there are numerous reports of focal granulomas within MLN of deer caused by pathogens other than MAP, such as *M. bovis* and *Mycobacterium avium* subspecies *avium* (*M. avium*).

Table 5.2 Results of univariable analysis showing number (n), mean and standard deviation of variables significantly associated with deer MLN circumference (p<0.20) (0 = Absent, 1 = Present) (Study II).

		MLN circumference (mm)			
Variable	**Category**	**n**	**Mean**	**SD**	**p-value**
Age	Young	325	42.8	12.3	Ref
	Adult	83	41.3	15.7	0.12
MAP culture status	Negative	193	40.7	12.9	Ref
	Positive	215	44.1	13.0	<0.01
Mild follicular hyperplasia	0	250	42.9	12.7	Ref
	1	158	41.8	13.6	0.19
Moderate follicular hyperplasia	0	281	42.1	13.6	Ref
	1	127	43.3	11.7	0.16
Mild capsular infiltration by eosinophils	0	266	43.7	14.1	Ref
	1	142	40.2	10.5	0.17
Moderate capsular infiltration by eosinophils	0	284	42.2	14.1	Ref
	1	124	43.2	10.2	0.14
Scattered foci of macrophages containing lipopigments	0	229	44.2	15.6	Ref
	1	179	40.2	8.2	<0.01
Parasitic granulomas	0	369	42.1	13.0	Ref
	1	39	46.3	13.2	0.02
Focal granulomas	0	303	40.3	9.5	Ref
	1	105	48.8	18.6	<0.01
Calcified foci	0	385	42.1	12.6	Ref
	1	23	48.7	17.8	0.02

Ref = Reference category
SD = Standard Deviation

Table 5.3 Results of a mixed linear model for the relationships between animal-level predictor variables and histopathological features and MLN circumference (log [mm]) (0 = Absent, 1 = Present) (Study II).

Predictor variables	Category	β	SE(β)	p-value
Constant		3.69	0.06	-
MAP Status	Negative	Ref	-	-
	Positive	<0.01	0.03	0.89
Moderate follicular hyperplasia	0	Ref	-	-
	1	0.07	0.03	<0.01
Focal granulomas[a]	0	Ref	-	-
	1	0.22	0.06	<0.01
Focal granuloma * MAP Status		0.15	0.07	0.03

ß = Beta-coefficient
Ref = Reference category
SE = Standard Error
[a] = this predictor variable became non-significant (p = 0.21) after removal of four outlying MLN circumference measurements

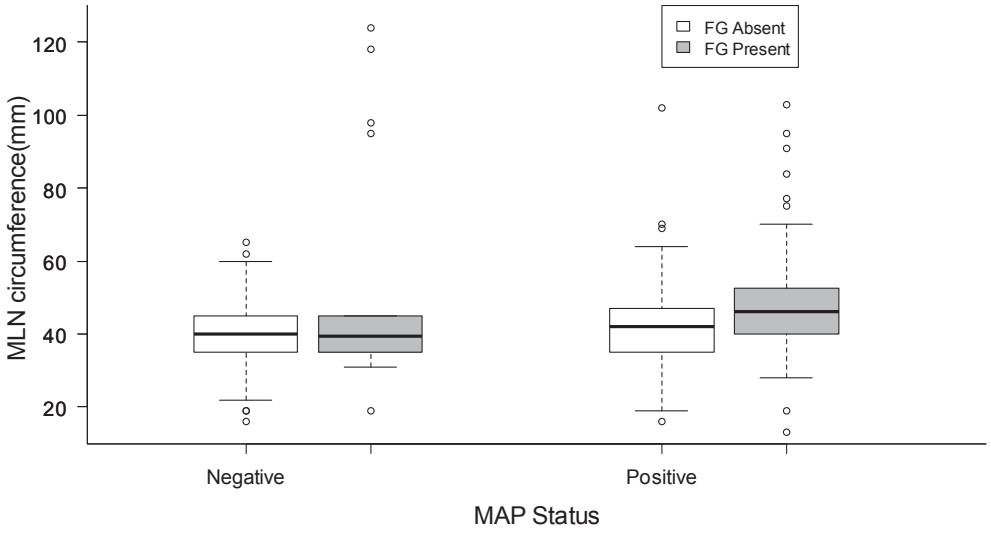


Figure 5.3 Boxplot of the circumference of mesenteric lymph node (MLN) culture negative (n = 193) or positive (n = 215) for MAP, stratified by the presence or absence of focal granulomas (FG) (Study II).

5.5 Discussion

This paper describes the first evaluation of lymph node (LN) characteristics as an indicator of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection in farmed deer and is the

first to quantitatively measure an association between increasing LN size and MAP infection in any species. Over 94% of 'abnormal' LN were categorised as infected with MAP. The circumference cut point for an 'abnormal' LN was determined at 55 mm, based on a specificity for MAP detection of >95% and a corresponding sensitivity of 12%. The presence of moderate follicular hyperplasia, focal granulomas and an interaction between MAP status and focal granulomas were positively associated with increasing MLN circumference.

MAP infection in 'abnormal' LN

A number of pathogens, including *Mycobacterium bovis* (*M. bovis;* bovine tuberculosis), *M. avium* subspecies *avium* (*M. avium*) (avian Tb), *Actinomyces* spp. (lumpy jaw), *Corynebacterium pseudotuberculosis* (caseous lymphadenitis) and chronic gastrointestinal parasitism, can cause lymphadenitis or lymphadenopathy in deer that is difficult to distinguish grossly from that due to infection with MAP (Stauber et al 1973; Mackintosh et al 2004a; Griffin et al 2006b; Chiu et al 2009). A 2003/2004 retrospective DSP study found 35.2% of 236 'abnormal' LN, cultured on suspicion for bTb, were MAP-infected, whilst 22.9 and 9.3% were infected with *M. bovis* and *M. avium*, respectively (Glossop et al 2005). In contrast, this study found the prevalence of MAP infection in 'abnormal' deer LN, based exclusively on culture, was 92.2%. Inclusion of histopathological changes typical of mycobacterial infection in culture-negative MLN increased the prevalence estimate to 94.6%.

The higher prevalence of MAP recorded in the present study may be due to meat inspectors utilizing characteristics in addition to visual inspection, such as previous knowledge of herd history, to increase the sensitivity of MAP detection. Alternatively, the prevalence of MAP infection in slaughtered deer may have increased since 2003/2004, whilst the prevalence of other pathogens, such as *M. bovis* and *M. avium*, decreased. There is some evidence of rapid MAP spread within the New Zealand farmed deer population within the last 10 to 15 years (de Lisle et al 2003; de Lisle et al 2006) while the number of *M. bovis*-infected deer herds decreased from 117 in 2003 to 18 in 2009 (source: Animal Health Board).

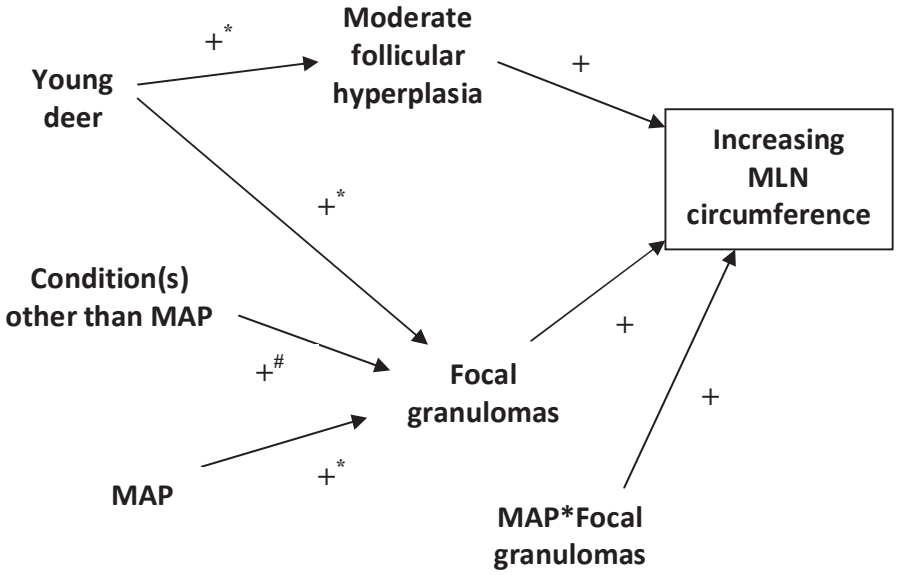


Figure 5.4 Proposed causal pathway of associations between potentially contributory variables and increasing deer mesenteric lymph node (MLN) circumference.
* data from Chapter 7
# data from de Lisle *et al.* (1993); Quigley *et al*. (1997); de Lisle *et al.* (2003)

The prevalence of infection can be influenced by covariates that affect disease risk, such as animal age and geographic location. Statistical evaluation of the effect of covariates on the prevalence of MAP in 'abnormal' LN was prevented by the low number of culture-negative LN detected. Nevertheless, culture-negative LN were 'normal', yellow or green in colour and were sourced from multiple DSP and carcass locations. As outlined in Chapter 2, the mean prevalence of clinical JD in weaner and yearling deer is higher than that observed in adult deer and further sampling of 'abnormal' LN from adult deer is warranted to ascertain whether the prevalence of MAP in those is also associated with age.

This study found there was no significant difference in the prevalence of MAP infection in 'abnormal' LN sourced from the North or South Island. However, the number of North Island LN sampled was low and further examination of a possible association between MAP prevalence and Island is warranted, particularly as a 2005 on-farm survey using pooled faecal culture found Canterbury and Southland herds were significantly more likely to be faecal or tissue culture positive for MAP, relative to North Island herds (Chapter 3). Other reports have also linked

geographic location and MAP in a number of species, including sheep, cattle and rabbits (Collins et al 1994; Judge et al 2005; Coelho et al 2006).

This study has demonstrated that inspection for 'abnormal' LN may be an appropriate marker for MAP surveillance in this species. However, this is likely to be more sensitive at the herd rather than individual animal level since herd-level sensitivity increases with the number of samples taken and this system captures information on all commercially slaughtered deer in New Zealand. The high prevalence of MAP infection in 'abnormal' LN also indicates that costly secondary diagnostic testing of individual LN post-inspection, prior to farmer notification of their presence, may be unnecessary. However, farmers notified of the presence of 'abnormal' LN through the national database are encouraged to undertake herd-level testing on-farm to confirm the presence and prevalence of MAP. In the future, if the prevalence of MAP infection in 'abnormal' LN is shown to have decreased, secondary test(s) may be required to minimize false positive diagnoses.

Cut point for determination of an 'abnormal' MLN

Gold standard tests previously used to investigate the accuracy of meat inspection of a variety of abnormalities have included pathogen culture (Elbers et al 2003), polymerase chain reaction (PCR) (Geysen et al 2007) and detailed post mortem assessment by an experienced individual (Ogunrinade and Oyekole 1990; Wanzala et al 2003; Goodwin-Ray et al 2007). Development of a benchmark measure for an 'abnormal' deer MLN was undertaken because an inspector's diagnosis of enlargement (or otherwise) is likely to be biased by a number of factors, including his/her experience of inspecting deer carcasses. The 55 mm circumference cut-point has been used to define MLN as 'normal' or 'abnormal' in companion papers assessing histopathological features typically observed in 'normal' MLN (Chapter 7), the prevalence of MAP infection in 'normal' MLN (Stringer et al 2009b), and the sensitivity and specificity of inspector detection of 'abnormal' MLN within the national surveillance database (Chapter 6).

Whilst the animal-level specificity of MAP detection through inspection of 'abnormal' LN was high at >95%, the sensitivity was low (approximately 12%), indicating that using this as the sole criterion for surveillance for MAP within the national database will underestimate animal-level

prevalence. Estimates of true prevalence of MAP in deer carcasses are around 45% based on the culture of 'normal' LN (Stringer et al 2009b). Statistical techniques, such as stochastic scenario-tree analysis and latent class analysis, can be used to adjust MAP prevalence estimates based on the detection of 'abnormal' LN, an imperfect test (Sergeant and Baldock 2002; Branscum et al 2004; Pence et al 2009). Such analyses can be applied to the data currently being collected in the national database to obtain estimates of national, regional and herd-level true MAP prevalence (Hunnam et al 2009). Estimation of the herd-level sensitivity and specificity of MAP diagnosis through detection of 'abnormal' LN is also warranted, and is being undertaken now that sufficient quantity and robustness of surveillance data is available. However, the true herd status of MAP infection *per se* may not be an appropriate endpoint for monitoring paratuberculosis as long as it has not been firmly established that MAP infection is a strong indicator of production loss and/or clinical disease in deer. Therefore, studies evaluating associations between the presence, frequency and persistence of 'abnomal' MLN, MAP infection and economically relevant outcomes are also required.

Risk factors associated with MLN circumference

To the authors' knowledge, the present study is the first to investigate possible factors associated with LN circumference in any species. The presence of focal granulomas, in the absence of MAP infection, was positively associated with increasing MLN circumference, supporting reports describing focal granulomas in deer LN resulting from infection with pathogens other than MAP, such as *M. bovis* or *Actinomyces* species (de Lisle et al 1995; Quigley et al 1997; Palmer et al 2002). This indicates 'abnormal' LN should be described as 'suspicious only' for MAP infection, particularly as Study I identified that MAP is present in less than 100% of 'abnormal' LN. However, it is possible that 'abnormal' MLN culture-negative for MAP may have been truly infected, but were erroneously found to be test-negative. Conversely, the presence of focal granulomas may have been spurious, with the development of an 'abnormal' MLN the result of an unknown factor unrelated to this histopathological feature. Further research into the cause(s) of 'abnormal' MAP-negative MLN in deer is warranted.

As outlined in Chapter 7, the presence of focal granulomas in grossly 'normal' MLN was 85.4 times more likely to be observed in herds at 'high' risk of MAP, indicating this feature may be

useful as a predictor of sub-clinical infection. However, in this study, 17.1% of focal granulomas were observed in MAP-negative MLN, suggesting that this histopathological feature should not be used in isolation as a criterion for the diagnosis of MAP infection.

A positive MAP status, in the absence of focal granulomas, was not significantly associated with an increasing MLN circumference (Figure 5.3) suggesting that MAP can exist within a MLN without causing an increase in circumference. This is supported by a recent study which detected MAP in 45% of 'normal' deer MLN (Stringer et al 2009b). Mycobacteria are known for their ability to circumvent the bactericidal mechanisms of macrophages and can remain dormant for weeks to years (Momotani et al 1988; Lugton 1999). MAP, in particular, targets the intracellular compartments of macrophages to shield itself from the immune system of its host. A synergistic interaction between a positive MAP status and the presence of focal granulomas indicates that development of the latter as a result of infection with the former causes a significant increase in MLN circumference.

Follicular hyperplasia is an increased proliferation of the 'normal' cell population within the germinal centres of lymphoid follicles located in the nodal cortex (Aughey and Frye 2001). Follicular hyperplasia within MLN occurs due to antigenic stimulation from the intestinal mucosa as a result of constant challenge by dietary antigens and commensal and pathogenic microbes and their products, including migrating parasitic larvae (Aughey and Frye 2001). As outlined in Chapter 7, moderate follicular hyperplasia was found in 51.3% of grossly 'normal' deer MLN and was significantly more common in young deer ($p<0.01$) and the anterior MLN ($p<0.01$), but was not significantly associated with a positive MAP status at the herd-level. Therefore, the positive association between increasing MLN circumference and moderate follicular hyperplasia identified in this study may have resulted from the high proportion of young deer sampled (79.7%).

A causal pathway for associations between the main effects variables, interaction term and increasing MLN circumference is proposed based on the results of this and previous studies. This pathway highlights that moderate follicular hyperplasia and focal granulomas were the only histopathological features examined in this study that were associated with increasing MLN

circumference and additional features, including capsular infiltration by eosinophils, parasitic granulomas and calcified foci, were not significantly associated with this outcome. Therefore, conditions unrelated to development of moderate follicular hyperplasia and/or focal granulomas, such as coccidial infections and nematode larval migration, do not appear to be common causes of 'abnormal' deer LN. Hence, validation of the use of the national database for MAP surveillance in deer should focus on differentiating LN enlargement due to MAP infection from that due to moderate follicular hyperplasia, particularly in young deer, and focal granulomas caused by pathogens other than MAP.

In conclusion, the prevalence of MAP infection in 'abnormal' deer LN was high. Mesenteric LN with a circumference of 55 mm or more have a 95% likelihood of being MAP-positive. Although increasing MLN circumference was positively associated with moderate follicular hyperplasia, focal granulomas and an interaction between focal granulomas and MAP status, this outcome was not associated with MAP status in the absence of histopathological features. While using 'abnormal' LN as a sole criterion will underestimate the animal-level prevalence of MAP, this data does give sufficient confidence for its use as a marker of MAP at the herd-level for the purpose of developing a national database for MAP surveillance (Hunnam et al 2009).

5.6 Acknowledgements

The authors would like to sincerely thank the management, staff and AsureQuality meat inspectors at all DSP throughout New Zealand.

Chapter 6 describes the sensitivity and specificity and level of agreement in the detection of 'abnormal' deer mesenteric lymph nodes by meat inspectors for the purpose of slaughter plant surveillance for *Mycobacterium avium* subspecies *paratuberculosis* in New Zealand farmed deer. The manuscript is prepared in the style of the New Zealand Veterinary Journal and, at the time of writing, permission from funders to submit the manuscript was awaited.

CHAPTER 6: Sensitivity, specificity and level of agreement of meat inspector detection of 'abnormal' lymph nodes of farmed deer (*Cervus elaphus*) in New Zealand

JC Hunnam, PR Wilson, C Heuer, LA Stringer, C Mackintosh

6.1 Abstract

AIMS: To assess the sensitivity (Se), specificity (Sp) and level of agreement in the detection of 'abnormal' deer mesenteric LN (MLN) by meat inspectors for the purpose of slaughter plant surveillance for Johne's disease in New Zealand farmed deer.

METHODS: To determine Se and Sp, four deer-accredited meat inspectors in two commercial deer slaughter premises (DSP) each examined between 153 and 925 MLN under normal inspection procedures (visual examination, palpation and/or incision). The inspector's diagnosis of 'normal' or 'abnormal' was sourced from the national DSP-based surveillance database for Johne's disease in deer. Truly 'abnormal' MLN were defined as those with a circumference measurement of ≥ 55 mm and/or grossly visible pathological changes, such as caseation, necrosis and/or mineralisation, while those with a circumference of <55mm and without gross lesions were defined as 'normal'.

To determine between-inspector level of agreement, 54 deer-accredited meat inspectors visually examined two photographic images of each of 19 'normal' and 10 'abnormal' MLN, and recorded their diagnosis Between-inspector agreement was calculated based on a modification of Cohen's kappa for multiple raters. Covariates, such as inspector age and experience, on the between-inspector level of agreement were assessed using the Breslow-Day statistic and test for equal Kappa coefficients.

RESULTS: The weighted average Se and Sp of inspector detection of 'abnormal' MLN was 13.3% (4.8 -- 41.2%) and 99.9% (99.5 -- 100.0%), respectively. The level of between-inspector agreement in the diagnosis of 'abnormal' and 'normal' MLN was fair ($\kappa = 0.32$). Employment

location, inspector age, experience inspecting deer or other species, and the number of shifts inspecting deer within the previous four weeks had no significant influence on between-inspector agreement ($p>0.05$).

CONCLUSIONS: Inspectors diagnosed 'abnormal' deer MLN with a high specificity, but low sensitivity. These data supported the current use of MLN characteristics for national surveillance for paratuberculosis, while highlighting the need for further training and consistent evaluation of inspectors.

6.2 Introduction

Infection with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) (Collins 2003a) can result in the development of Johne's disease, also known as paratuberculosis (Ptb), a chronic, granulomatous enteritis of domestic and wild ruminants and presents a potentially serious economic burden to the New Zealand farmed deer industry (de Lisle et al 2003). A national surveillance database has been established to assist the control of MAP in deer through detection of herds with one or more 'abnormal' lymph nodes identified in commercially slaughtered deer by accredited meat inspectors (Lynch 2007). These herds are given management advice on the control of JD to apply on a voluntary basis. An additional objective for the database is to provide a practical method for monitoring the national, regional and herd-level prevalence of MAP indicators within the New Zealand farmed deer industry and as a tool for epidemiological studies.

An accurate assessment of the prevalence of any grossly visible abnormality diagnosed through slaughter plant surveillance requires consideration of the sensitivity (Se), specificity (Sp) and level of agreement in the detection of that abnormality by meat inspectors (Enoe et al 2003). Gold standard tests previously used to investigate the accuracy of meat inspection of a variety of abnormalities have included pathogen culture (Elbers et al 2003), PCR (Geysen et al 2007) and detailed post mortem assessment by an experienced individual acting as a 'benchmark' inspector (Ogunrinade and Oyekole 1990; Wanzala et al 2003; Goodwin-Ray et al 2007).

Enlarged deer lymph nodes, and/or those with pathology, including a central core of caseous, necrotic and/or mineralised material, are commonly associated with MAP (de Lisle et al 1991). These abnormalities appear with the highest frequency in the mesenteric lymph nodes (MLN). A previous study found 94.6% of 'abnormal' LN showed evidence of infection with MAP, and defined a cut point circumference of 55 mm for an enlarged MLN with those below this circumference classified as 'normal' (Chapter 5). In this study Se is defined as the proportion of truly abnormal lymph nodes diagnosed as 'abnormal' and Sp is defined as the proportion of truly normal LN diagnosed as 'normal'.

As part of a national programme to eradicate bovine tuberculosis from domestic livestock in New Zealand, meat inspectors are trained to assess deer LN for 'granulomas [which] generally contain thick, yellow, cheese-like pus or caseous material; sometimes with a gritty texture (calcification)' (Anonymous 2008b). Thus, the sensitivity of meat inspector diagnosis of MLN with such lesions would be expected to be high. However, the accuracy of inspector determination of enlargement of MLN and the accuracy of classification of 'normal' MLN is unknown. Furthermore, the level of agreement between meat inspectors remains unknown.

This paper outlines the sensitivity, specificity and between-inspector level of agreement in meat inspector diagnosis of 'abnormal' deer MLN. It also describes the significance of association between inspector-related factors and the level of agreement in meat inspector classification of MLN.

6.3 Materials and Methods

The population of interest were meat inspectors accredited to assess deer slaughtered in Deer Slaughter Premises (DSP) between 1 January and 31 December 2007. All DSP had been visited between August and December 2006, prior to the start of data collection for the national surveillance database, to instruct meat inspectors on the correct classification of 'abnormal' and 'normal' MLN, with educational material left for replacement inspectors (Appendix 4). Although study deer had a phenotype typical of red deer, the breeding of red hinds to an elk bull (*Cervus elaphus* subsp. *canadensis*) or a red deer/elk crossbred stag is common on New Zealand

deer farms, therefore animals may have contained some elk genes. A herd was defined as a collection of deer farmed at a single location. A line was defined as a shipment of deer sent for slaughter from the same herd to the same DSP on the same day.

6.3.1 Study I: Inspector Se and Sp

The objective of this cross-sectional study was to estimate the sensitivity and specificity of meat inspector detection of 'abnormal' deer MLN. 'Abnormal' MLN were defined as those with a maximum circumference of ≥55 mm and/or the presence of grossly visible lesions, while 'normal' MLN had a maximum circumference of <55 mm and contained no grossly visible lesions (Chapter 5). Inspectors assessed MLN under normal working conditions, allowing visual examination, palpation and/or incision with visualisation of any cut surfaces. 'Palpation' was defined as the tactile manipulation of the MLN to allow detection of gross changes, such as an abrupt difference in size from one portion of the MLN to another, typical of an abscess.

6.3.1.1 Selection of inspectors and MLN. Four deer-accredited meat inspectors employed in two South Island DSP were visited between 27 and 30 November 2007. Between 153 and 925 MLN were examined per inspector, totalling 2,313 MLN from 51 lines sourced from 51 herds. The first author or an assistant measured the maximum circumference of 1,290 MLN sourced from 41 lines after inspection, using a flexible measuring tape. Ninety-five percent or more of the MLN from 27 of the lines were measured, while between 5.3 and 87.5% of MLN in the remaining lines were measured.

6.3.1.2 Data collection and management. Observations of 'abnormal' MLN by the meat inspectors were electronically recorded as positive into a national database at the point of inspection. Data were extracted for each of the four inspectors directly from this database.

6.3.1.3 Statistical analysis. Individual inspector specificity (Sp) for the diagnosis of 'abnormal' MLN was estimated by dividing the number of 'normal' MLN diagnosed by each inspector by the number of 'normal' MLN as determined by measurement. Individual inspector sensitivity (Se) for the classification of 'abnormal' MLN was the number of MLN diagnosed as 'abnormal'

by each inspector, divided by the number of 'abnormal' MLN determined by measurement. The weighted average Se and Sp, taking into account the relative proportion of MLN examined by each inspector, was calculated by multiplying the mean individual inspector Se and Sp by the number of MLN examined, with the summed total divided by the sum of the weights.

6.3.2 Study II: Between-inspector level of agreement

The primary objective of this cross-sectional study was to assess the between-inspector level of agreement in the detection of 'abnormal' and 'normal' MLN, using visual examination of photographic images. The association between inspector-factors and the level of agreement of meat inspector detection of 'abnormal' and 'normal' MLN was also assessed.

6.3.2.1 Selection of inspectors. Of the 184 meat inspectors accredited to inspect deer carcasses in April 2007, 54 (29.3%) participated. Nineteen and 35 inspectors participated in the North and South Islands, respectively, representing 26.0 and 31.5% of inspectors in those areas. Inspectors were recruited through AsureQuality New Zealand with participation being voluntary. Study inspectors were limited to those who had inspected farmed deer within the previous 12 months and were available for evaluation in April and May 2007. One inspector declined to participate despite being available at the time of testing and the remaining inspectors were ineligible or were unavailable due to holidays, sickness or other causes unrelated to the exercise. All inspectors who had completed four or more shifts inspecting deer within the month prior to evaluation (March 2007) were included in the exercise.

6.3.2.2 Evaluation. Twenty-nine MLN, based on the gross external appearance of the node, were selected from deer slaughtered at Feilding and Christchurch DSP. Each node was arranged in a white fibreglass tray with the rumen and abomasum at the top and MLN clearly visible at the bottom of the tray, arranged in a semi-circular fashion. Two images were taken using a Kodak EasyShare DX6490 zoom digital camera (Eastman Kodak Company, Rochester, NY 14650). In the first image, the entire tray was photographed to allow the viewer to assess the size of each gastrointestinal tract and MLN. The second was a close view of the MLN, excluding the rumen and large intestines. The height at which each image was photographed was similar for all

images. Before use, the colour and clarity of each image was judged to be acceptable by two experienced inspectors.

The circumference of each MLN was then measured using a flexible measuring tape by the primary author. Those with a maximum circumference ≥55 mm (n = 10), sourced from three hinds and five stags aged less than 24 months, were defined as 'abnormal' (Chapter 5). Those with a maximum circumference of <55 mm (n = 19), sourced from four hinds and four stags aged less than 24 months and seven adult (≥24 months) hinds and four adult stags, were defined as 'normal'.

All images were included in a standard sequence in a 'powerpoint' presentation (Microsoft Powerpoint®), with 'abnormal' LN unevenly spaced between 'normal' LN. Based on the first author's personal experience, 'abnormal' LN tend to be spread within a line of animals, without clustering. No alterations, such as cropping or colour enhancement, were made to any original image. The presentation was automatically timed with the first and second image of each MLN visible for three and five seconds, respectively. The timing approximated that available for inspection in DSP. Images were clearly labelled with the sequential tract number (1 to 29) and the animal age, sex and carcass weight at slaughter.

6.3.2.3 Data collection and management. Each inspector individually viewed each image and recorded the LN, as defined in Study I, as either 'normal', which would have been passed without further palpation and/or incision, or 'abnormal' which would have required further palpation and/or incision to confirm this.

6.3.2.4 Statistical Analysis. The between-inspector level of agreement for the detection of 'abnormal' and 'normal' MLN was assessed using the macro MAGREE within the SAS System®, version 8.02, for Windows (SAS Institute, Inc., Cary, NC, USA, 2001). This routine applies an expansion of Cohen's kappa statistic (κ) (Cohen 1960) and assesses the agreement by multiple raters. The interpretation of κ was based on a standard scale as described in Dohoo *et al.* (2003b).

The Breslow-Day statistic and test for equal Kappa coefficients was used to determine the influence of covariates on the between-inspector level of agreement (Breslow and Day 1980). Covariates included: Island (Upper North, Lower North, Upper South, Lower South); inspector age (<40 years, 40 to 50 years, 50 to 60 years and >60 years); number of years inspecting any ruminant stock (<10 years, 10 to 20 years, 20 to 30 years and >30 years); number of years inspecting deer (<5 years, 5 to 10 years and >10 years); and number of shifts inspecting deer in the previous 4 weeks (0 shifts, 1 to 3 shifts and >3 shifts).

Within two weeks of their assessment, Studies I and II inspectors were informed by post of their individual sensitivity, specificity and level of agreement relative to their respective study group.

6.4 Results

6.4.1 Study I

Of the 1,290 MLN assessed by the four study inspectors and subsequently measured, 84.9% were 'normal' and 15.1% 'abnormal'. Mesenteric LN circumference ranged from 20 to 118 mm, as illustrated in Figure 6.1, with a mean circumference of 46.9 mm (± 8.4 mm) and median circumference of 46.0 mm.

The total number of MLN assessed and measured, the number of 'normal' and 'abnormal' MLN measured and the Se and Sp of detection of 'abnormal' MLN, by inspector, is shown in Table 6.1. At a MLN circumference cut-point of ≥55 mm, the weighted average Sp in the detection of an 'abnormal' MLN was 99.9% (99.5 -- 100.0%), while the weighted average Se was 13.3% (4.8 -- 41.2%).

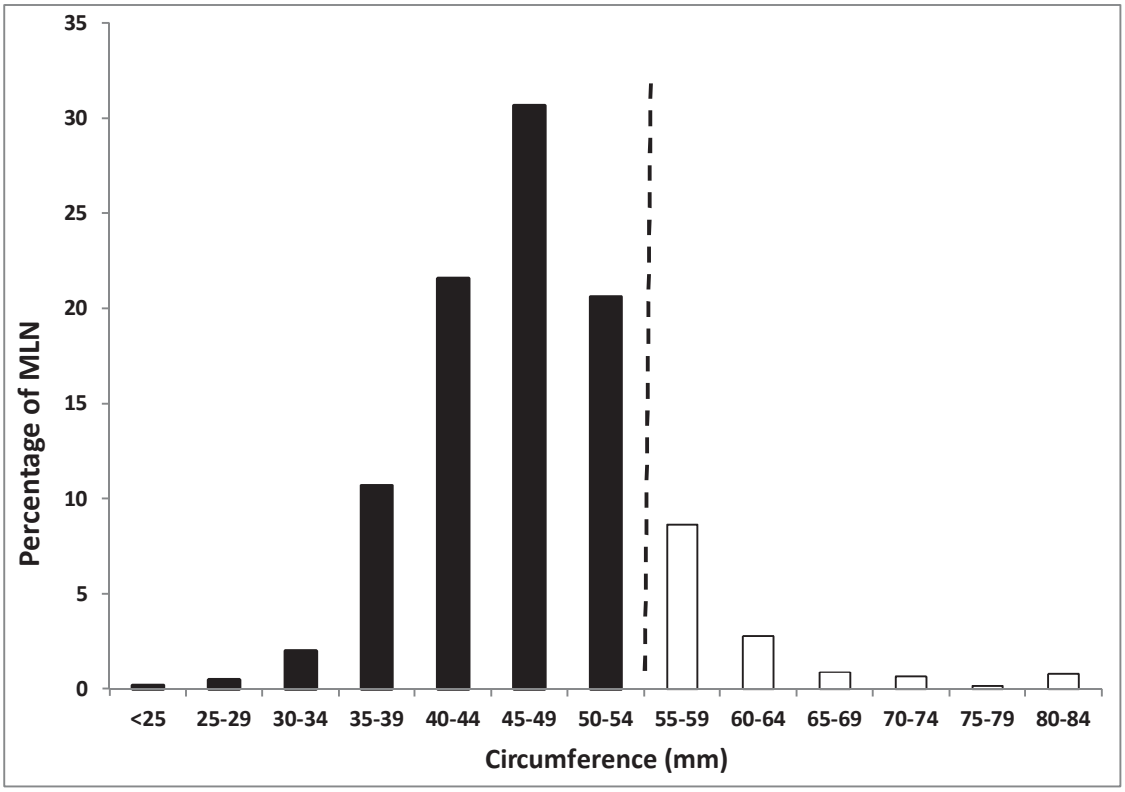


Figure 6.1 Percentage of 1,287 mesenteric lymph nodes (MLN) in each circumference category ('normal' (n = 1,095; black) and 'abnormal' (n = 195; white) based on an artificial cut-off of 55 mm (dashed line)) from 41 lines of deer slaughtered.

Table 6.1 The number of mesenteric lymph nodes (MLN) assessed, measured, and defined as 'normal' and 'abnormal', and the specificity (Sp) and sensitivity (Se) of each inspector's classification based on visual examination and/or palpation and/or incision.

	Assessed	Measured	'Normal'	'Abnormal'		
Inspector	n	n (%)	n (%)	n (%)	Sp (%)	Se (%)
1	345	247 (71.6)	216 (87.5)	31 (12.5)	215/216 (99.5)	8/31 (25.8)
2	925	688 (74.3)	605 (87.9)	83 (12.1)	605/605 (100.0)	4/83 (4.8)
3	890	236 (26.5)	172 (72.9)	64 (27.1)	172/172 (100.0)	7/64 (10.9)
4	153	119 (77.7)	102 (85.7)	17 (14.3)	102/102 (100.0)	7/17 (41.2)
Total	2313	1290 (55.8)	1095 (84.9)	195 (15.1)	1094/1095 (99.9)	26/195 (13.3)

6.4.2 Study II

The distribution of inspector age and experience is illustrated in Figure 6.2. Inspector age ranged from 24 to 66 years (mean 48 ± 11 years). On average, study inspectors had 23 years (range: 0.2 -- 46) experience inspecting any species and seven years (0.3 -- 30) experience inspecting deer.

Within the previous four weeks, inspectors had worked a mean of four day or night shifts (0 -- 20) inspecting deer.

The between-inspector level of agreement for both 'abnormal' and 'normal' MLN was fair (κ = 0.32), with the variation by individual MLN illustrated in Figure 6.3. All associations between covariates and between-inspector level of agreement were non-significant ($p>0.1$).

6.5 Discussion

This is the first evaluation of the sensitivity (13.3%), specificity (99.9%) and level of agreement (κ = 0.32) in the detection of 'abnormal' deer lymph nodes by meat inspectors under normal working conditions. These estimates underpin the interpretation of data collected into a database for surveillance for MAP in New Zealand farmed deer.

The mean circumference (46.9 mm) and circumference range (20 -- 118 mm) of the measured MLN were comparable to those reported in Chapter 5 (Study II) of this thesis for 412 deer MLN sourced from 79 herds located throughout New Zealand. Therefore, it is likely that the MLN measured were representative of those in the commercially slaughtered New Zealand deer population.

'External validity' refers to how well the sensitivity and specificity of meat inspector detection of 'abnormal' MLN, represents the performance of all inspectors. Study I inspectors were not randomly selected and although the performance parameter estimates determined are informative, they may not be representative of all inspectors. Inspector participation in Study II was also not random and involved particular emphasis on inclusion of inspectors who had completed four or more shifts inspecting deer within the month prior (i.e. March 2007). However, an effort was made to ensure reasonable geographic distribution of inspectors in both islands. Also, an approximately equal percentage of inspectors employed in the North and South

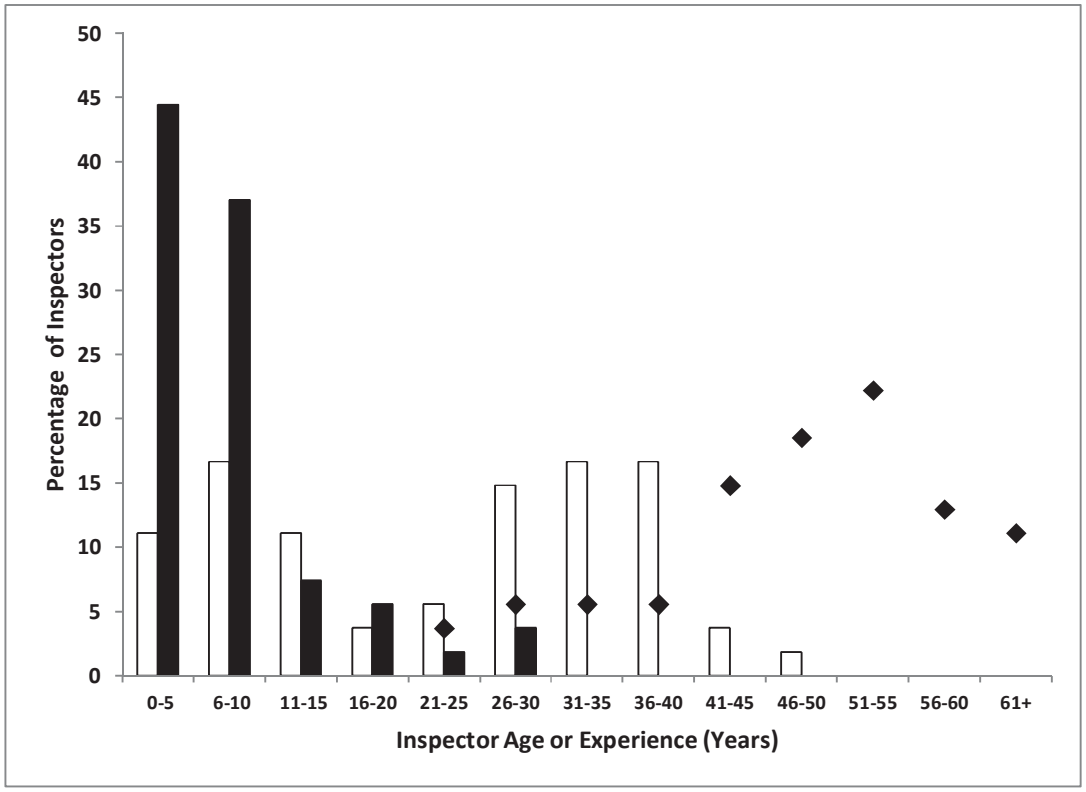
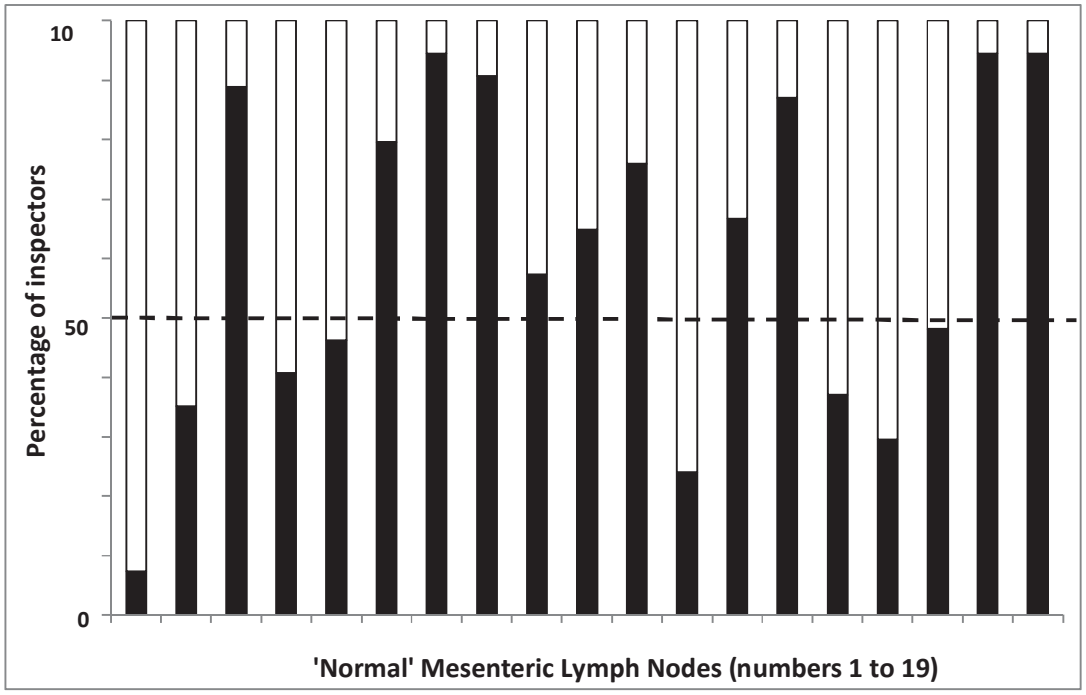


Figure 6.2 Proportion (%, n = 54) of meat inspectors with experience inspecting any species (pigs, sheep, cattle and/or deer) ( ), and deer only ( ), and inspector age in years ( ).



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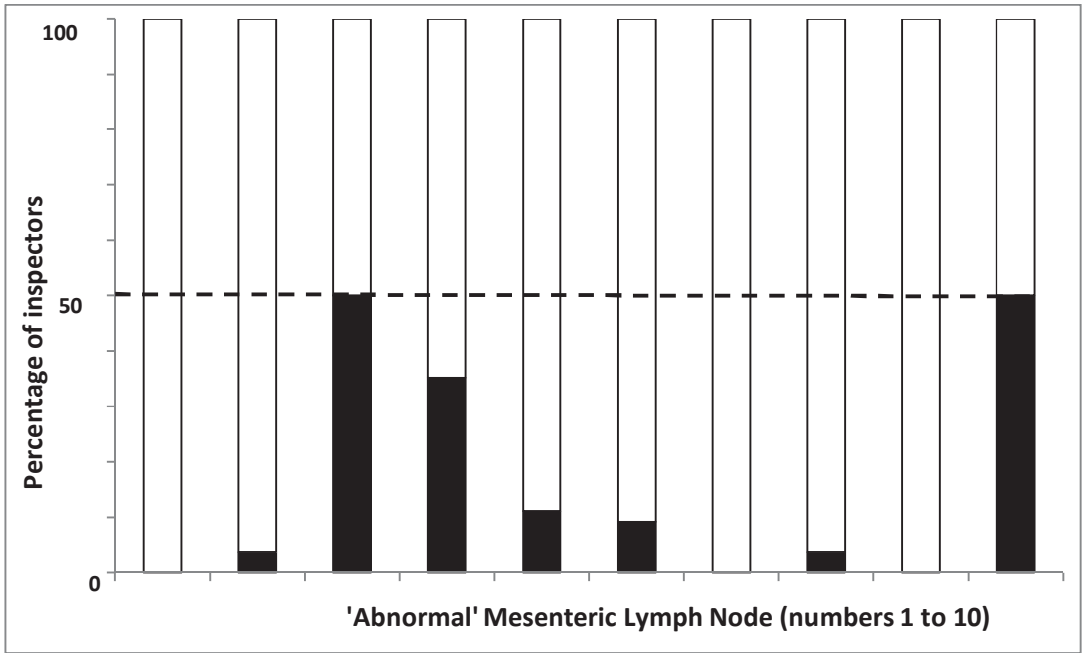

B

Figure 6.3 Percentage of meat inspectors (n = 54) who diagnosed 'normal' (n = 19) (A) and 'abnormal' (n = 10) (B) mesenteric lymph nodes as 'normal' (black bars) or 'abnormal' (white bars) based on visual examination of photographic images. Dashed line = minimum level of between-inspector agreement.

Islands (i.e. 26.0 and 31.5%, respectively) were included in the evaluation, regardless of their level of experience.

6.5.1 Sensitivity of inspection

The Se of inspector detection of 'abnormal' deer MLN using visual examination, palpation and/or incision was 13.3%. This is similar to sensitivity values reported in other studies evaluating meat inspector detection of a macroscopically visible abnormality, such as moderate-severe pneumonia in New Zealand lambs (Se = 17%) (Goodwin-Ray et al 2007) and porcine cysticercosis in Zambian village pigs (Se = 22.1%) (Dorny et al 2004). Previous evaluation of Study II data (Glossop *et al*. (2008)) found the sensitivity of meat inspector detection of 'abnormal' MLN, based on visual examination of photographic images only, was 68.0%. Assuming the two study datasets are comparable, it is likely that the lower sensitivity estimate recorded in Study I (13.3%) was because the four inspectors investigated did not record the presence of all observed 'abnormal' MLN into the database, despite possibly observing 'abnormal' MLN. The sensitivity estimate reported by Glossop *et al*. (2008) may indicate the detection rate which inspectors are capable of, whilst the sensitivity reported in this paper may indicate the actual level of recording into the national database. This conclusion has significant ramifications on the validity of conclusions drawn from analysis of the national database and requires further evaluation. In particular, inspector education is needed in the consistent entry of positive diagnoses into the national database in order to increase the level of recording and thus the sensitivity of inspector detection of 'abnormal' MLN.

The minimum circumference of an 'abnormal' MLN (55 mm) used in this study was based on a specificity of MAP detection > 95% as determined in Chapter 5 of this thesis. However, it is unlikely that inspectors under normal working conditions will be able to consistently differentiate between a 'normal' MLN circumference of 50 to 54 mm and an 'abnormal' MLN circumference of 55 to 60 mm. Over 62% of 'abnormal' MLN had a circumference between 55 and 60 mm. Therefore, while a 55 mm MLN circumference cut point is useful for assessment of inspector accuracy, achievement of a 100% sensitivity and specificity level of detection is unlikely in practice. Increasing the circumference cut point for definition of an 'abnormal' MLN

to 60 mm increased the apparent weighted average sensitivity of inspector detection to 22.6%, but decreased specificity to 98.9%.

Despite its use in studies such as this, circumference may not be a useful measurement in practice as it is difficult for inspectors to measure under normal working conditions which do not allow sufficient time to apply a flexible measuring tape. Alternatively, inspectors may use the maximum diameter of an 'abnormal' MLN with a circumference of ≥55 mm (≥17.5 mm if the MLN is assumed to be a perfect circle). A mark could be made on an inspector's knife to allow rapid measurement of the MLN and so improve the accuracy of detection of 'abnormal' MLN.

Although the effect of covariates on the sensitivity of inspector detection of 'abnormal' MLN could not be evaluated in this paper due to the small number of inspectors assessed, Glossop *et al*. (2008) found the sensitivity of meat inspection, when evaluated through visual examination of photographic images, was related to the location of meat inspector employment (i.e. upper North Island, lower North Island, upper South Island, lower South Island) and the gross appearance of 'abnormal' MLN (i.e. multiple, discrete abscesses; diffuse enlargement). Further assessment of a larger number of inspectors is required to evaluate these covariates further.

6.5.2 Inspector specificity

The specificity of inspector detection of 'abnormal' MLN on visual examination, palpation and/or incision was 99.9%. A high specificity is essential to the validity of the national database as even a low false positive rate would have a significant effect on farm-level reporting, because of the large number of farmed deer slaughtered in New Zealand.

6.5.3 Between-inspector level of agreement

The generalised Kappa statistic that is implemented in the MAGREE macro (SAS) is a generalised version of Fleiss' kappa, which is a modification of Cohen's kappa, as a measure of inter-rater reliability or level of agreement between multiple raters (Fleiss 1971). This macro can also compute kappa statistics conditionally on the response category, in this case, 'normal' and 'abnormal' MLN. The between-inspector level of agreement (κ) of both 'normal' and

'abnormal' MLN at 0.32, was fair, based on the common interpretation of kappa (Dohoo et al 2003c).

In order to have confidence in estimates of farm, regional and national MAP prevalence derived from the national DSP-based surveillance database, a high level of between-inspector agreement is required. This is particularly significant at the farm-level as a farmer will commonly slaughter deer at different DSP during a season and a single DSP may employ more than one inspector. It is possible that factors other than those evaluated were responsible for the between-inspector level of agreement found in this study. For example, MLN-associated factors, such as degree of enlargement and type of pathology.

6.6 Conclusions

New Zealand deer-accredited meat inspectors were able to diagnose 'abnormal' deer MLN with low sensitivity but high specificity. However, evaluation of inspectors employed in other South Island DSP and DSP in the North Island, utilising Study I methodology, is justified to more precisely assess the sensitivity of detection of 'abnormal' MLN and to determine the significance of the possible effect of covariates on the sensitivity estimates. Ongoing training and supervision of inspection staff will be necessary to ensure optimum performance and therefore robustness of data in the surveillance database.

6.7 Acknowledgements

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Chapter 7 describes histopathological features of grossly 'normal mesenteric lymph nodes of New Zealand farmed red deer, including identification of a unique lipopigment. Chapter 7 has been published as a Short Communication:

Hunnam JC, Wilson PR, Heuer C, Mackintosh CG, West DM, Clark RG. Histopathology of Grossly Normal Mesenteric Lymph Nodes of New Zealand Farmed Red Deer (*Cervus elaphus*), Including Identification of Lipopigment. *Veterinary Pathology*. 48(2): 525-529, 2011.

The full, published article is reproduced in Appendix 1.

CHAPTER 7: Histopathology of grossly normal mesenteric lymph nodes of New Zealand farmed red deer (*Cervus elaphus*) including identification of lipopigment

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7.1 Abstract

This paper describes histopathological features of grossly normal mesenteric lymph nodes (MLN) of New Zealand farmed red deer (*Cervus elaphus*). Eighty MLN, 40 from North and 40 from South Island herds, classified as at 'low' and 'high' risk of infection with *Mycobacterium avium* subspecies *paratuberculosis* (MAP), respectively, were randomly selected, comprising between two and 15 animals per herd of origin. Age and sex strata were yearling (12 to 24 month old) and adult (>24 month old) hinds and stags. Nodes were sectioned at 25, 50 and 75 percent of their length for histopathological examination. Fixed sections were stained with haematoxylin and eosin and Ziehl-Neelsen (ZN). Periodic-acid-shift, Perl's and Sudan black were also selectively applied to aid identification of a granular material present within macrophages.

The prevalence of seven histopathological features, namely a positive ZN stain, follicular hyperplasia, capsular infiltration by eosinophils, focal granulomas, foci of macrophages containing lipopigment, parasitic granulomas and calcified foci, were described with a subjective severity grading ascribed where appropriate. Animal age, sex, herd of origin and incision location on the MLN were variably associated with the presence of one or more features ($p < 0.05$). There was a strong association (OR = 85.4) between the presence of focal granulomas and a high risk of infection with MAP in the herd. Additional histological features of interest, such as haemosiderin-like pigment in medullary sinuses, focal granulomas containing coccidian

zoites, follicular germinal centres with perivascular fibrinoid material, trabecular fibrosis and dilated, oedema-filled sinusoids were also described

These observations allow histopathologists to differentiate between likely non-pathological histological features in deer MLN and features possibly attributable to infection with a pathogen, such as MAP.

7.2 Introduction

The New Zealand farmed deer industry is the largest producer of venison worldwide, with over 700,000 deer commercially slaughtered in 2006 (de Lisle et al 2003; Anonymous 2007a). Every deer carcass is assessed by an accredited meat inspector and lymph nodes (LN), including the mediastinal, retropharyngeal and mesenteric lymph nodes (MLN), are visually inspected, palpated and/or incised for lesions resembling tuberculosis (bTb) (*Mycobacterium bovis; M. bovis*), under a National Tuberculosis Pest Management Strategy for that disease (Anonymous 2005b). Nodes identified as suspicious for bTb, such as those with a central core of caseous, necrotic or mineralised material, are sampled and assessed, primarily by histological examination (Quigley et al 1997; O'Brien et al 2001; Palmer et al 2002).

Histopathological features of grossly abnormal deer LN infected with *M. bovis*, or the closely related bacterium *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and *Mycobacterium avium* subspecies *avium* (*M. avium*), have been well described (de Lisle and Collins 1995; Quigley et al 1997). However, to the authors' knowledge, there has not been a published description of the range and prevalence of histopathological features in grossly normal deer MLN or the typical cellular architecture of those nodes. Such knowledge may be useful to histopathologists to help differentiate between non-pathological features in grossly abnormal MLN and those likely to be related to infection with *M. bovis*, MAP or *M. avium*.

This paper describes the cellular architecture of grossly normal MLN of red deer from two populations with differing risk of infections with MAP. It determines the range and prevalence of selected histopathological features in a population of MLN, associations between each

histopathological feature and MLN, animal risk factors particularly in relation to MAP, and identifies additional histological features of interest in MLN.

7.3 Materials and Methods

7.3.1 Selection of deer herds for sampling

Herds were selected from participants in a 2005 epidemiological study of Johne's disease (JD) in New Zealand farmed deer herds (Chapters 2 to 4 of this thesis). Five North Island herds (Herds 1 to 5) were selected as 'low' risk for MAP infection on the basis of an absence of clinical disease resembling JD or lesions reported from Deer Slaughter Premises (DSP) and a negative culture of five to six pools of eight to ten faecal samples from adult hinds for MAP. It was subsequently confirmed in early 2007 that no deer on those farms had exhibited clinical signs of JD and/or grossly abnormal MLN at slaughter in the intervening two years. Five South Island herds (Herds 6 to 10) were selected as at 'high' risk for MAP infection on the basis of a positive pooled faecal culture in 2005. In addition, one or more deer had been observed with clinical JD and/or one or more deer had a false positive reaction to an intra-dermal test for *M. bovis* in the period to early 2007.

All herds had a bTb status of C7 to C10 indicating no evidence of *M. bovis* infection in the previous 7 to ≥ 10 years. Despite all sampled deer having the typical phenotype of red deer, the breeding of red hinds to an elk bull (*Cervus elaphus* subsp. *canadensis*) or a red deer/elk crossbred stag (i.e. 'wapiti') is common on New Zealand deer farms and, therefore, study animals may have contained some wapiti genes.

7.3.2 Selection of MLN

MLN were selected from deer slaughtered in DSP between 1 January 2007 and 31 December 2007. Eighty MLN, 40 from the North Island herds ('low risk' category) and 40 from the South Island herds ('high risk' category) were randomly selected, comprising between two and 15 animals per herd of origin. Age and sex strata were yearling (12 to 24 month old) and adult (>24 month old) hinds and stags. In this way, 10 MLN were obtained from 10 carcasses in each age/sex class and in each MAP risk category. Only grossly 'normal' MLN were selected,

comprising those regarded as such by meat inspectors, that is, those containing no visible pathology, such as discolouration, focal or diffuse enlargement or a core of mineralized, caseous and/or necrotic material. The entire length of each node was incised and measured at 25, 50 and 75% (anterior to posterior) of the length of each node, using a flexible measuring tape, with study lymph nodes having a maximum circumference of 55 mm.

7.3.3 Histology

A 15 mm wide cross-section was taken from each MLN at 25, 50 and 75% of MLN length and stored individually in 10% buffered formalin. Fixed tissue samples were dehydrated, cleared and embedded in paraffin. Sections were stained with haematoxylin and eosin (H & E) and Ziehl-Neelsen (ZN). Additional stains, including periodic-acid-shift (PAS), Perl's and Sudan black, were applied to sections from six animals to aid identification of a granular material present within macrophages. All sections were examined by one pathologist (RGC) without knowledge of the age, sex or farm of origin of the deer being examined. They were examined under low (2.5 x 10), 10 x 10 and high dry (10 x 40) magnification using a Leitz Wetzlar microscope. Occasionally oil emersion (10 x 100) was used if there was doubt on identification of a feature.

Additional histological features of interest were photographed using an Olympus BX51 with a mounted Olympus DP70 camera and an Olympus U-RFL-T light source. Ultra violet examination employed a mercury burner at wavelengths 365/366, 404.7, 435, 546.1 and 577/579.1 nm. For UV light an Olympus WU filter: excitation 380-385nm and emission >420nm was used.

7.3.4 Data collection and management

Carcass weight (kg) and animal age, sex and regional location of the herd of origin were obtained at the time of sampling. Histopathological features were categorised, graded and coded for later analysis (Table 7.1). All MLN circumference measurements and histopathological features were recorded at the incision level (25, 50 and 75%), while age, sex, carcass weight and herd of origin were recorded at the carcass level.

Table 7.1 Description and subjectively assessed grades of common histopathological features observed in grossly 'normal' mesenteric lymph nodes of red deer.

Histological feature Code	Grade	Description
Zeehl-Nielsen (ZN)		Positive to a Zeehl-Nielsen stain
Follicular hyperplasia (FH)	**1**	Mild follicular hyperplasia. Numerous follicles spreading deep within the cortex. Germinal centres were pale with mitotic figures, tingible macrophages and were rimmed by a mantle of small lymphocytes
	2	Moderate follicular hyperplasia. The difference between FH1 and FH2 was largely subjective based on density of follicles, depth of penetration into the medullary area and/or degree of reactivity in the follicles.
Capsular infiltration by eosinophils (CE)	**1**	Mild capsular infiltration by eosinophils
	2	Moderate-marked capsular infiltration by eosinophils
Calcified foci (CF)		Occasional-scattered calcified foci, often concentric laminated, no inflammatory changes within germinal centres
Focal granulomas (FG)	**1**	Solitary focal granuloma
	2	Occasional focal granulomas (i.e. no more than one per 10 x 10 field)
	3	Occasional-scattered focal granulomas in cortex
	5	Widespread sheets of macrophages in cortex
	7	Calcified caseated granuloma
Foci of macrophages (FM)	**1**	Occasional foci of macrophages in cortex with lipopigment
	2	Scattered foci of macrophages in cortex with lipopigment
Parasitic granuloma(s) (PG)		One or more granuloma suspected to be of parasitic origin

7.3.5 Statistical Analysis

The prevalence of each histopathological feature was summarised into a frequency table at the carcass level. Statistical analyses were performed to examine the relationships between each individual histopathological feature and risk factors (age, sex, incision site, herd-level MAP risk, remaining histopathological features) at the animal and MLN levels. For each section (n = 240), a binary outcome variable was generated for the presence or absence of each feature. Focal granulomas (scores FG1 to FG7) were in low numbers and so were combined into a single variable (FG) for analysis, whereas grades for other features described in Table 7.1 were analysed individually. Separate, multivariable logistic regression random-effects models were developed with each histopathological feature as an outcome. MLN and herd were included in each model as nested random effects to adjust variance estimates for correlation at these two levels of aggregation. Meaningful interactions between fixed effects were tested for significance

and model fit and residual variances were evaluated for distributional assumptions and influential observations. All analyses were performed in R (version 2.4.1; The R Foundation for Statistical Computing) and significance was declared at $p < 0.10$.

7.4 Results

7.4.1 Cellular architecture

The typical cellular architecture of a red deer MLN is illustrated in Figure 7.1 and was typical of that seen in other ruminants (Dellman 1971). The MLN is surrounded by an outer cortex with trabeculae extending into the parenchyma. A cross-section of an afferent lymph vessel which opens into the subcapsular sinus is visible. The outer cortex of the node contains secondary follicles separated by diffuse lymphatic tissue. Medullary cords are separated by a network of sinuses and connective tissue trabeculae. The only histopathological feature in this image which may have influenced its cellular appearance was mild follicular hyperplasia. The MLN was sourced from a herd at 'low' risk of MAP infection.

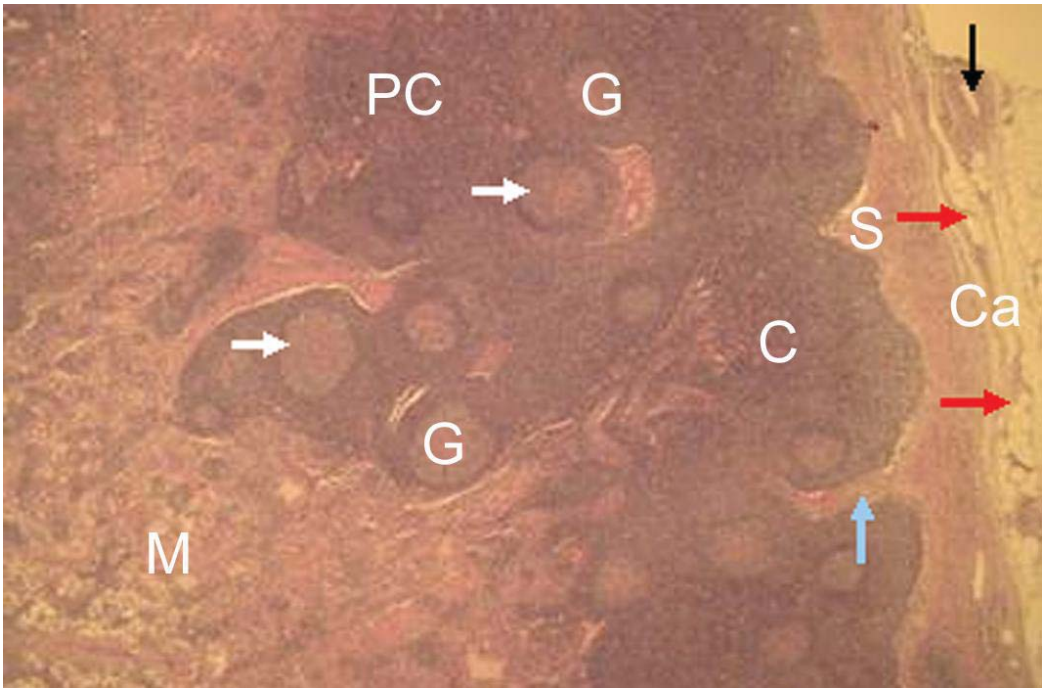


Figure 7.1 Grossly normal deer mesenteric lymph node containing mild follicular hyperplasia. Capsule (Ca) containing adipose tissue (*red arrows*); Subcapsular sinus (S); Cross-section of afferent lymphatic (*black arrow*); Connective tissue trabeculum (*blue arrow*) extending into the cortical area; Cortical sinus (C); Secondary lymphoid follicles with active germinal centres (G) and dark-staining corona/mantle (*white arrows*); Paracortical (PC) area extending between the lymphoid follicles; Medullary cords (M). HE.

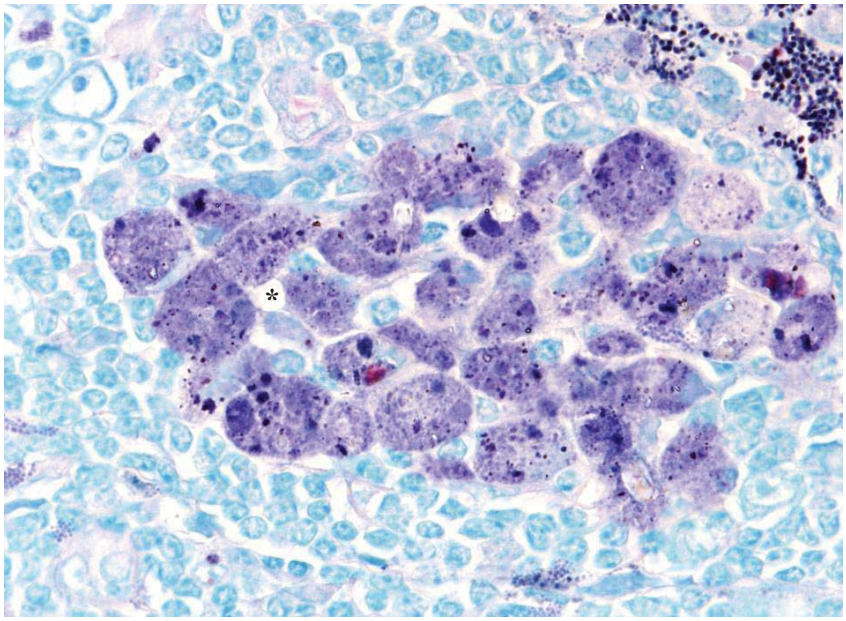
7.4.2 Additional histological features of interest

In 92% of lymph nodes, a variable amount of fine, granular, irregularly-sized material was observed within macrophages in the cortex and medullary cords. This material was often observed with a pale brown appearance on H&E, black on a ZN stain and, very rarely, contained a red granule, as illustrated in Figure 7.2A. This material was also Sudan black and PAS positive (Figure 7.2B) and was positive to a variable extent on Perl's stain, indicating the presence of iron (Figure 7.3). Some foci also contained a crystalline-like material (Figure 7.2A). On UV light examination of unstained sections, there were scattered, focal areas of a golden yellow colour, which related to focal concentrations of macrophages seen on H&E and other special stained slides (Figure 7.4). The UV light and special stain results are indicative of a lipopigment (Jolly and Dalefield 1990). The material was more commonly observed in MLN from adult rather than yearling deer.

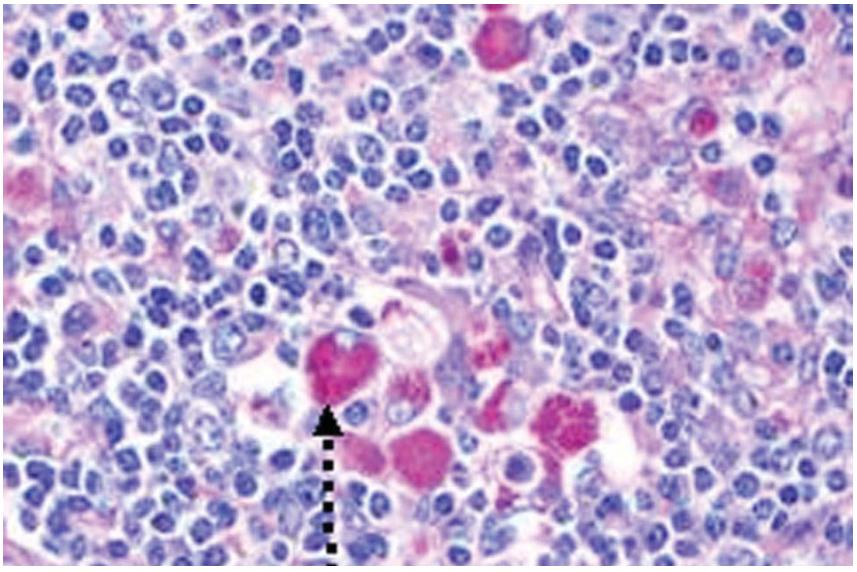
Calcified foci, often with a laminated appearance, were present within germinal centres. These generally had no inflammatory response but occasionally were associated with a multinucleated giant cell. Less common features were small, focal granulomas containing coccidian zoites and follicular germinal centres with perivascular fibrinoid material (Figure 7.5). Trabecular fibrosis was present in one or two sections in three animals, and dilated, oedema-filled sinusoids were seen in all sections from two animals. Haemosiderin-like material was present within the medullary sinusoids of a number of animals, being particularly marked in one case.

7.4.3 Prevalence of histopathological features in MLN

The animal-level prevalence of each histopathological feature is presented in Table 7.2. One or more sections in 86.3% of lymph nodes contained occasional foci of macrophages with lipopigment in the cortex, while 71.3 and 62.5% of nodes contained one or more sections with mild follicular hyperplasia or a mild capsular infiltration by eosinophils, respectively.



7.2A



7.2B

Figure 7.2 Grossly normal deer mesenteric lymph node with focal concentrations of macrophages within the cortex containing **7.2A**. fine, granular, dense, black-staining material (*asterix*) and occasional crystalline-like material. Zeehl-Neilsen. and; **7.2B.** red-staining PAS positive material (*dashed arrow*). Periodic-acid-shift.

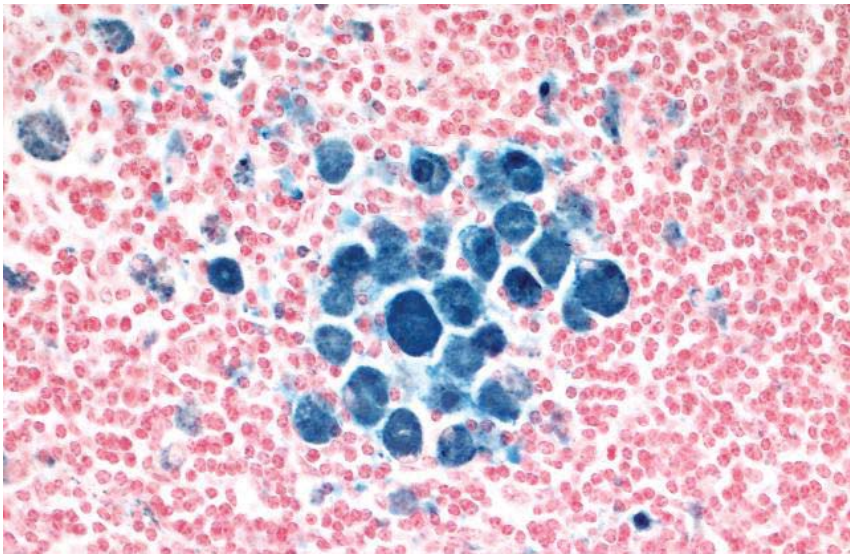


Figure 7.3 Grossly normal deer mesenteric lymph node with focal concentrations of macrophages within the cortex containing blue-staining, iron-positive material. Perl's.

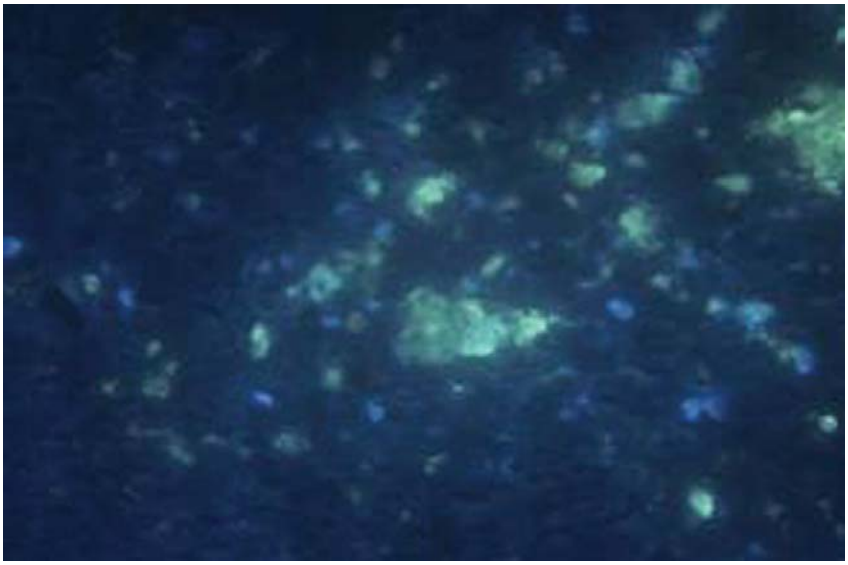
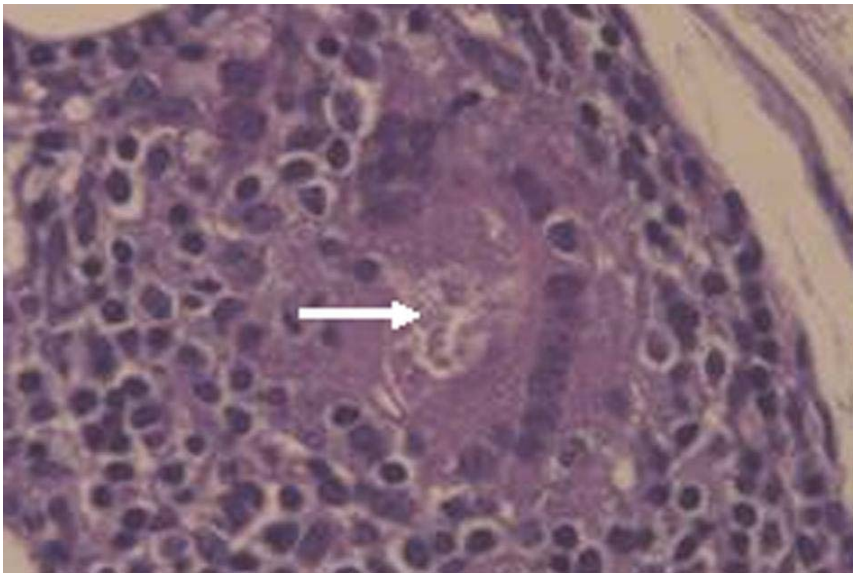
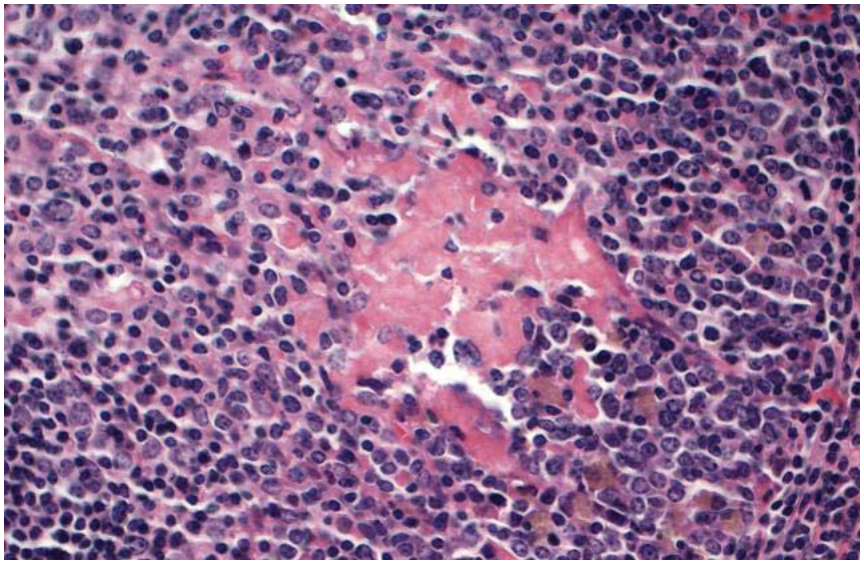


Figure 7.4 Cortex of grossly normal deer mesenteric lymph node with focal concentration of macrophages and scattered, isolated macrophages containing fluorescent material visible under UV light.



7.5A



7.5B

Figure 7.5 Grossly normal deer mesenteric lymph nodes, containing (**7.5A**) a parasitic granuloma with coccidian zoits (*white arrow*) within a giant cell (H&E stain) and (**7.5B**) perivascular fibrinoid deposits in the germinal centre of a lymphoid follicle. HE.

Table 7.2 Prevalence of each grade of histopathological feature (see Table 7.1) in grossly normal deer mesenteric lymph nodes (MLN) (n = 80). As three sections were sampled per node, >1 feature may have been observed within an individual MLN.

Histopathological feature	Grade	Prevalence (%)
Zeehl Nielsen-positive		12.5
Follicular Hyperplasia	1	71.3
	2	51.3
Capsular Infiltration by Eosinophils	1	62.5
	2	30.0
Calcified Foci		21.3
Focal Granulomas	1	13.8
	2	25.0
	3+	0.0
Foci of Macrophages	1	86.3
	2	60.0
Parasitic Granulomas		11.3

7.4.4 Association between each histopathological feature and animal risk factors

The frequency and results of multivariable logistic regression models for each histopathological feature are presented in Table 7.3. Calcified foci (OR = 0.2; $p < 0.01$) and occasional foci of macrophages in the cortex (OR 0.5; $p = 0.02$) were less common in MLN sourced from 'high' versus 'low' MAP-risk herds, while single to occasional focal granulomas within the cortex (OR = 85.4; $p < 0.01$), mild follicular hyperplasia (OR = 2.1; $p = 0.02$) and scattered foci of macrophages in the cortex (OR = 2.5; $p = 0.04$) were significantly more common in 'high' MAP-risk herds. MLN from hinds were less likely to contain scattered foci of macrophages in the cortex (OR = 0.4; $p = 0.02$) and mild follicular hyperplasia (OR = 0.4; $p = 0.01$) when compared with stags. While mild follicular hyperplasia (OR = 0.4; $p < 0.01$), moderate follicular hyperplasia (OR = 0.1; $p < 0.01$), focal granulomas (OR = 0.3; $p = 0.03$) and occasional foci of macrophages (OR = 0.3; $p < 0.01$) were less likely to be observed in MLN from adults than yearlings, scattered foci of macrophages (OR = 3.3; $p < 0.01$) were more likely in MLN from this age group.

Moderate follicular hyperplasia, moderate to marked capsular infiltration by eosinophils and occasional foci of macrophages in the cortex were more common at 25% of the length of the MLN compared with 50% and 75% ($p < 0.05$). However, occasional foci of macrophages were more significantly more common at 50% relative to 25% of the length of the MLN (OR = 3.4; p

< 0.01). Marked follicular hyperplasia had a significant positive association with a mild capsular infiltration by eosinophils (OR = 1.8; p = 0.08) and a significant negative association with the presence of calcified foci (OR = 0.4; p = 0.04). Single to occasional focal granulomas within the cortex were significantly less common if a mild capsular infiltration by eosinophils was also present (OR = 0.3; p = 0.03). A positive Ziehl-Neelsen stain had a negative association with a mild capsular infiltration by eosinophils (OR = 0.1; p = 0.08).

Table 7.3 Results of multivariable logistic regression analysis of risk factors associated with histopathological features (0 = absent; 1 = present) in grossly normal deer mesenteric lymph nodes, with odds ratio (OR), 95% confidence interval (95% CI) of OR, and p-values.

Variable	Category	0	1	OR	95% CI	p
Mild follicular hyperplasia (FH1): constant (const.) = -0.39						
Age	Yearling	57	60	Ref	-	-
	Adult	84	39	0.4	0.2, 0.7	<0.01
Sex	Stag	61	59	Ref	-	-
	Hind	80	40	0.4	0.2, 0.8	0.01
MAP risk	Low	80	40	Ref	-	-
	High	61	59	2.1	1.1, 4.0	0.02
Moderate follicular hyperplasia (FH2): const. = 0.70						
Age	Yearling	73	44	Ref	-	-
	Adult	105	18	0.1	<0.1, 0.4	<0.01
Incision site	25%	48	32	Ref	-	-
	50%	61	19	0.3	0.1, 0.7	<0.01
	75%	69	11	0.1	<0.1, 0.3	<0.01
Mild capsular infiltration by eosinophils (CE1): const. = -1.00						
FH2	0	125	53	Ref	-	-
	1	33	29	1.8	0.9, 3.5	0.08
ZN	0	147	81	Ref	-	-
	1	11	1	0.1	<0.1, 1.3	0.08
Moderate-marked capsular infiltration by eosinophils (CE2): const. = -1.77						
Incision site	25%	65	15	Ref	-	-
	50%	72	8	0.4	0.2, 1.2	0.09
	75%	72	8	0.4	0.2, 1.2	0.09
Calcified foci (CF): const. = -0.79						
MAP risk	Low	88	32	Ref	-	-
	High	109	11	0.2	0.1, 0.5	<0.01
FH2	0	141	37	Ref	-	-
	1	56	6	0.4	0.1, 0.9	0.04
Single to occasional focal granulomas within the cortex (FG): const. = -4.31						
Age	Yearling	91	26	Ref	-	-
	Adult	106	17	0.3	0.1, 0.9	0.03

Variable	Category	0	1	OR	95% CI	p
CE1	0	125	33	Ref	-	-
	1	72	10	0.3	0.1, 0.9	0.03
MAP risk	Low	119	1	85.4	8.2, 891.2	<0.01
	High	78	42			
Occasional foci of macrophages in the cortex (FM1): const. = 2.02						
Incision site	25%	24	56	Ref	-	-
	50%	41	39	0.3	0.2, 0.7	<0.01
	75%	34	46	0.5	0.3, 1.0	0.06
Age	Yearling	34	83	Ref	-	-
	Adult	65	58	0.3	0.2, 0.8	<0.01
MAP risk	Low	41	79	Ref	-	-
	High	58	62	0.5	0.2, 0.9	0.02
Scattered foci of macrophages in the cortex (FM2): const. = -2.31						
Age	Yearling	94	23	Ref	-	-
	Adult	73	50	3.3	1.5, 7.5	<0.01
Sex	Stag	75	45	Ref	-	-
	Hind	92	28	0.4	0.2, 0.8	0.02
MAP risk	Low	92	28	Ref	-	-
	High	75	45	2.5	1.1, 5.7	0.04
Incision site	25%	63	17	Ref	-	-
	50%	47	33	3.4	1.5, 7.3	<0.01
	75%	57	23	1.7	0.7, 3.6	0.21

Ref = reference level for comparison

7.5 Discussion

This paper is the first published description of the cellular architecture and prevalence of histopathological features in grossly normal mesenteric lymph nodes (MLN) of farmed red deer of either high or low risk of MAP. A variety of histopathological features were observed. The animal's age, sex and herd-level risk of MAP infection, and the anatomical site of sampling on the MLN were significantly associated (positive or negative) with the presence of one or more individual features. Lipopigment-like deposits are reported for the first time in deer lymph nodes, identified using ultraviolet light and special stains.

Histological features of grossly normal MLN

Studies illustrating histological features in grossly normal deer LN of any anatomical origin are limited. Although Barrell and Simpson-Morgan (1990) described the gross anatomy, weight and

dimensions of LN of the head of 13 fallow (*Dama dama*) stags, a detailed description of histological features was not provided (Barrell and Simpson-Morgan 1990). The histological features of deer MLN examined in this study appear to be similar to that observed in all ruminants, such as cattle and sheep (Dellman 1971).

Prevalence of histopathological features in grossly normal MLN

The prevalence of each histopathological feature in normal MLN may be used as a baseline reference by histopathologists when examining grossly abnormal MLN for possible infection with pathogens such as *M. bovis*, MAP or *M. avium*. Infiltration by eosinophils into the nodal capsule was observed in over 60% of MLN. Possible explanations include migration of nematode larva, usually liver fluke (*Fasciola hepatica*) in other species, and/or lungworm (*Dictyocaulus* spp) (Haigh et al 2002), or aberrant coccidia. However, although parasitic granulomas were observed in the capsule of over 11% of MLN, a significant relationship between this feature and a capsular infiltration by eosinophils was not found in this study.

Follicular hyperplasia was a common histopathological feature. The cortical area of a MLN contains nodular, primary and secondary lymphoid follicles separated by cortical sinuses (Ioachim 1994). In a MLN not subjected to antigenic stimulation, the cortex consists predominately of primary follicles which contain a homogeneous cell population of small lymphocytes (Dellman 1971). Follicular hyperplasia involves proliferation of the normal cell population within the lymphoid follicle's germinal centre with consequent formation of secondary lymphoid follicles with germinal centres of variable size (Henry and Farrer-Brown 1981). Follicular hyperplasia is typically seen in MLN as a result of constant antigenic stimulation from challenge of the intestinal mucosa by dietary antigens and commensal and pathogenic microbes and their products (Dellman 1971; Aughey and Frye 2001).

Calcified foci within the germinal centres of MLN are illustrated in Figure 7.6. Similar mineralised bodies of varying size and shape, including laminated corpra amalacea, are most often located in germinal centres and are occasionally observed in bovine LN. In cattle, they have been interpreted as post-reactive changes and the larger ones are considered to be an

outcome of parasitic infection (Ladds, 1986). Although this may be an explanation for this feature in this study, calcified foci have also been observed in grossly normal and abnormal MLN of known MAP-infected deer (RGC, pers. obs.).

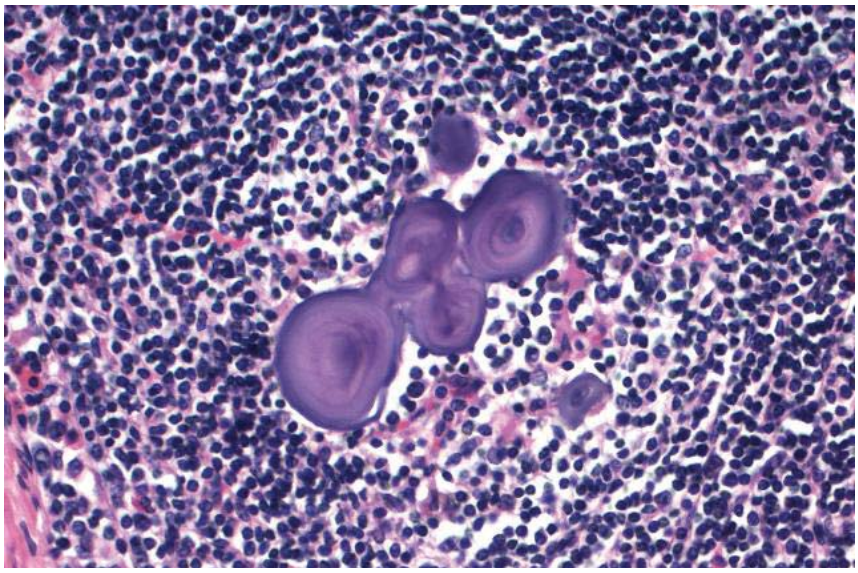


Figure 7.6 Grossly normal deer mesenteric lymph node containing calcified foci showing concentrically laminated bodies. HE.

Association between each histopathological feature and MLN incision site and animal risk factors

Only one focal granuloma (2.5%) was observed in 40 MLN sections sourced from 'low' MAP-risk herds, while 30 MLN with focal granuloma(s) (75.0%) were sourced from three (of five) 'high' MAP-risk herds. This histopathological feature has been previously described in both grossly normal and abnormal MAP-infected MLN of deer (de Lisle et al 1993; Quigley et al 1997; de Lisle et al 2003; Clark et al 2010). Granulomas have also been shown to be more evident in MLN than in the adjacent intestinal mucosa, especially in the mild stages of disease (Clark et al 2010). This study found focal granulomas were 85.4 times (95% CI: 8.2 to 891.2) as likely in grossly normal MLN from herds at 'high' risk of MAP, indicating this feature may be useful as a predictor of sub-clinical MAP infection. However, this feature can also develop in deer MLN in response to infection with other pathogens, such as *M. bovis* or *M. avium* (de Lisle et al 1995; Quigley et al 1997; O'Brien et al 2001; Palmer et al 2002). The likelihood of *M. bovis* infection in study herds was low as a selection criterion was maintenance of a bTb test-

negative herd-level status for 7 to 10 or more years. The likelihood of infection with *M. avium* in study herds was unclear as the nationwide and regional prevalence of this pathogen in New Zea land farmed deer is unknown. Assessment of the sensitivity and specificity of each histopathological feature in the detection of sub-clinical MAP infection, determined by culture or PCR, in grossly normal MLN of deer is warranted.

Both mild and moderate follicular hyperplasias were significantly less likely to be observed in MLN sourced from adult deer. This association may be explained by the 'high' antigenic stimulation of the MLN which tend to occur relatively early in life, due to constant challenge by dietary antigens, microbes and other stimuli (Henry and Farrer-Brown 1981) .

The presence of several histopathological features varied significantly depending on incision location (25, 50 or 75% of the MLN length). Moderate follicular hyperplasia, a moderate to marked capsular infiltration by eosinophils and foci of macrophages with lipopigment in the cortex were more likely to be clustered at 25% of the MLN length. However, the distribution of focal granulomas, presumably due to MAP infection, was not significantly associated with incision location on the MLN. This finding has relevance to selection of the site of the LN for investigation into MAP infection. Carrigan and Seaman (1990) found the most severe gross pathology due to MAP infection in sheep occurred in the last three to four metres of the small intestine. This lesion distribution was presumed to be associated with the varying presence of lymphoid tissue within the intestinal wall. In contrast, a recent study has found that in cases of advanced disease due to MAP infection in deer, grossly visible abnormalities are most commonly located in the first half of the MLN (Clark et al 2010).

Additional histological features of interest

Solitary to occasional focal granulomas must be differentiated from focal concentrations of macrophages with lipopigment in the nodal cortex. Although there are no published reports describing this pigment, it has been observed previously in deer MLN (RGC, pers obs). Lipopigments, also referred to as lipofuscins, include ceroid, age pigments and pigments found in the inherited storage disease ceriod-lipofuscinosis (Jolly and Dalefield 1990). As the pigment was more likely to be observed in adult deer than yearling deer, it may be an age related change

in deer, but that does not mean it is an age pigment. The iron present within the lipopigment was variable, suggesting a variable source. Possibilities included haemosiderin (e.g. from haemolysis or copper deficiency) or iron from soil ingestion. An unidentified pigment which increases with age has been reported in cattle LN, particularly in MLN (Lubis et al 1982). That pigment was positive to Schmorl's reaction and under ultraviolet light showed yellow auto-fluorescence, similar to the pigment observed in the present study. However, it was negative to PAS, ZN, Perl's Prussian blue and a fat stain Sudan IV-oil red O staining procedures. These staining reactions were different to those observed in the present study, suggesting a different pigment.

The crystalline material reported in some macrophages may be due to ingested soil, or possibly fat crystals, such as cholesterol. The dilated, oedema-filled sinusoids have been previously reported in cattle and likely causes include local obstruction or congenital defects in efferent lymphatics (Ladds 1986). A less common histopathological feature observed was follicular germinal centres with perivascular fibrinoid material. Fibrinoid foci and vascular hyalinisation within follicular germinal centres have been considered to be a post-reactive change in cattle and, in man, the latter has been shown to increase with age (Ladds 1986).

These results confirm grossly normal MLN of farmed red deer in New Zealand contain a variety of histopathological features. A number of these features, such as mild to moderate follicular hyperplasia, occur in reaction to constant antigenic stimulation, particularly early in life, and can be considered common and relatively non-pathological. However, the presence of focal granulomas, whether gross or histological, may suggest the presence of a mycobacterial infection and further diagnostic tests such as culture and PCR are warranted, regardless of the gross appearance of the node, to differentiate between MLN infected or non-infected with mycobacteria.

7.6 Acknowledgements

The authors would like to sincerely thank the management, staff and AsureQuality meat assessors at Venison Packers, Fielding and Alliance Sockburn, Christchurch. Mr Dan Lynch,

acting manager of Johne's Management Limited, is acknowledged and thanked for his tireless work to ensure the project ran smoothly.

CHAPTER 8: General Discussion

And practical application of thesis research

JC Hunnam

8.1 Introduction

The thesis has encompassed two main study areas; to further our knowledge of MAP and clinical JD at the herd-level in deer and to validate a national abattoir-based surveillance scheme for MAP in the New Zealand farmed deer population. Chapters 2 to 4 established, for the first time, baseline annual incidence rates for clinical JD at the levels of herd and age-class, as well as evaluating risk factors for the herd-level presence of MAP infection overall and clinical JD in young deer. It was confirmed, in Chapter 5, that MAP is the predominant cause of macroscopic abnormalities in visceral lymph nodes of commercially slaughtered deer in New Zealand. Subsequently, a measurable criterion for an 'abnormal' lymph node was defined and then utilised in Chapter 6 to evaluate the sensitivity, specificity and repeatability of meat inspector detection of 'abnormal' lymph nodes. The frequency of histopathological features in macroscopically 'normal' mesenteric lymph nodes (MLN) of red deer were also described (Chapter 7), including a novel lipopigment, allowing more informed assessment of histopathological changes in 'abnormal' MLN.

As each chapter has been presented as a paper for submission to a peer-reviewed journal, only restricted appraisal of the limitations, relevant literature and implications unique to each were appropriate to that format. Therefore, this final chapter expands upon the discussion within each paper providing for a broader critique, but also describes how study conclusions may contribute to the New Zealand deer industry's efforts to control MAP infection and clinical JD to date. It concludes by proposing avenues for further research.

8.2 Epidemiology of Johne's disease

Historically, identification of MAP infection or clinical JD on a property for the first time could be accompanied by a social stigma, affecting not only the producer but also his/her family and community. The Kangaroo Island (Australia) ovine Johne's Disease (OJD) committee noted 'some bitter family and neighbour feuds have resulted from differing opinions about the best way to deal with a positive diagnosis [of Johne's disease]' (Anonymous 2002). At the outset of the research described in this PhD (2004), there appeared to be a general trepidation amongst New Zealand deer farmers in their willingness to participate in paratuberculosis studies, or to discuss or even acknowledge the potential threat of MAP infection and clinical JD to their farms and industry. Forced destocking of MAP-infected beef and dairy herds and sheep flocks in Australia in an attempt to eradicate the bacteria appeared to significantly contribute to anxiety, as some New Zealand deer farmers were concerned they would be expected to follow the same path. Therefore, my discussions with each potential study farm owner/manager typically involved explaining the limited success of destocking on the elimination of MAP in Australia and the value of farmer education and on-farm management in its control (Freeman and Jordan 2005).

Prior to the research undertaken in this thesis, MAP infection and clinical JD at the herd-level had not been examined in any farmed deer industry, including New Zealand. Early anecdotal reports of 'outbreaks' or 'epidemics' of clinical JD in rising yearlings, particularly in Southland, may have inadvertently escalated farmer concerns around the potential consequences of MAP infection in their deer (Mackintosh and de Lisle 1998; Black and Orr 1999; Bell 2005). However, data presented in Chapter 2 suggests that 'outbreaks', while they did occur in New Zealand, they were the exception. Although it appears that herd-level MAP infection within farmed deer is widespread (see below), the within-herd annual incidence of clinical JD is generally $\leq$ 1% in most infected herds. Extensive engagement and education of industry stakeholders on JD, including this information, has alleviated the stigma previously associated with a herd-level clinical JD diagnosis. Thesis results have been distributed through published media, conferences, field days and the 2010 national circulation of a manual on the practical control of clinical JD in deer (Hunnam and Goodwin-Ray 2010). As a result, farmers now appear more willing to participate in surveys for MAP and clinical JD and to actively search for advice around the control of clinical JD within their herd, with the knowledge that they are not

unique and, therefore, unlikely to be unduly scrutinised or disadvantaged (Wilson, P; *pers. comm.*). There is a dearth of social science surveys examining the influence of a MAP diagnosis or clinical JD on farmers of any ruminant livestock species. An investigation evaluating the social effects, if any, of this disease on the New Zealand deer industry is warranted in its own right, but also to potentially serve as a model for the impact of other diseases.

Herd-level MAP prevalence

The 2005 estimate of herd-level MAP prevalence (62%), outlined in Chapter 3, generally matches that seen in more recent 2008/2009 surveys of MAP prevalence in deer: 59% of deer herds (n = 57) were MAP-positive through tissue culture (TC) of grossly 'normal' mesenteric lymph nodes (MLN, four per farm) (Stringer et al 2009b) and 57% of deer herds (n = 99) were MAP-positive through pooled faecal culture (PFC) and an ELISA (Paralisa™) applied in series (Verdugo et al 2011). However, the similarity in herd-level MAP prevalence estimates between these three independent studies is unexpected as, based on current information; the herd-level sensitivity of diagnostic test(s) used in each study would have differed.

Schroen *et al.* (2003), in the only published assessment of PFC for MAP diagnosis in deer, estimated a pool-level sensitivity of 32% for pools of 5 and 27% for pools of 10 and 20, in relation to TC. However, although 24 'subclinical' and eight 'clinical' deer were assessed, the authors did not evaluate whether the spectrum of disease in this study population was likely representative of that within the wider farmed deer population. Notwithstanding this, based on these estimates, the HSe of PFC used in the 2005 study (generally six pools of 10 faecal samples) was 81% while the HSe of PFC used by Verdugo *et al.* (2011) (two pools of 10 faecal samples) was 42%, based on an assumed within-herd true prevalence of 20% (Christensen and Gardner 2000). A possible reason for similar herd-level MAP prevalence estimates in the two studies, despite this apparent difference in HSe, may be that the sensitivity of PFC at a dilution rate of 10 is higher than that predicted by Schroen *et al.* (2003). This would mean that increasing the number of pools from two to six may not result in a higher sensitivity at the herd-level.

Alternatively, parallel interpretation of the PFC and Paralisa™ results by Verdugo *et al.* (2011) may have increased the HSe to a level comparable with that of the 2005 case-control study.

However, this methodology would have also decreased herd-level specificity (HSp), particularly as Stringer (2011) recently found that the specificity (Sp) of the Paralisa™ in a subclinical population of young deer is 94% (95% CI: 93 to 96%). Research investigating the sensitivity of PFC at different dilution levels in New Zealand deer herds is warranted to better evaluate its use as a diagnostic test for herd-level MAP diagnosis. This will be of particular importance if the deer industry intends to implement a national herd classification scheme for MAP.

Although HSe estimates calculated above illustrate the relative difference between the two studies, the actual figures are not directly applicable as the equation used (Christensen and Gardner, 2000) assumes a random population. Both study populations were non-random, consisting only of farmers who were willing to participate. Therefore, the true HSe of PFC in these populations were likely higher than that estimated.

Targeted faecal sampling and pooling in the 2005 case-control study

Targeted sampling and pooling were utilised in the 2005 case-control study to increase the predictive value of PFC through maximizing the probability of MAP infection in sampled animals and targeted pools (Christensen and Gardner 2000; Williams et al 2009). Sampling of adult sheep and dairy cattle has previously been used to increase the probability of MAP detection at the herd/flock-level (Muskens et al 2000; Tavornpanich et al 2006). However, as outlined in Chapter 3, targeted sampling and pooling of faeces from adult hinds (relative to weaner/yearling deer) was not significantly associated with a culture-positive result at the pool level ($p<0.05$). As a result, targeted sampling of weaner and/or yearling deer, rather than adults, has been utilised in recent studies to increase PFC sensitivity in the detection of MAP in this species (Stringer 2011; Verdugo et al 2011).

Although targeted sampling of sheep with signs of clinical JD has also previously been used to determine flock status (Sykes et al 2000; Sergeant et al 2002), inclusion of faeces from deer with signs typical of clinical JD, such as diarrhoea and weight loss, was not significantly associated with a MAP-positive PFC ($p<0.05$) in the 2005 case-control study. Diarrhoea and weight loss are not pathognomonic for clinical JD in deer, being also associated with conditions such as yersiniosis (weaner deer only), abomasal parasitism and chronic copper deficiency (Mackintosh

and de Lisle 1998). As trials in cattle with clinical JD found typical signs recurred after cessation of treatment (Merkal and Larsen 1973; Belloli et al 1994), it was considered unlikely that an anthelmintic, antibiotic or copper supplement would resolve clinical JD in deer. Over 85% of New Zealand farmers administer anthelmintics and/or copper supplements to their deer (Castillo-Alcala et al 2007; Wilson et al 2008). Therefore, subsequent development of a definition for a 'clinical' deer or herd, utilised in Chapters 2 to 4, included evaluation of the likely effectiveness of administered anthelmintics, antibiotics and/or trace minerals to increase the specificity of farmer diagnosis of clinical JD. A similar case definition was subsequently used in the 2007 longitudinal survey (Chapter 2) and has been used by Stringer *et al.* (2009a) and Verdugo *et al*. (2011) to classify clinical JD in deer based on farmer description of clinical signs and response to treatment.

8.2.1 Characterisation of clinical JD in New Zealand farmed deer herds

Chapter 2 is the first researched quantification of clinical JD annual incidence, including medium-term trends, in known MAP-infected farmed deer herds. While the estimated (farmer-diagnosed) annual herd-level clinical JD prevalence in the 2005 case-control study was 72% (125/174) (*unpublished data*), only 62% of study herds (108/174) were confirmed to be MAP-infected through TC or PFC. This disparity may indicate that MAP culture under-diagnoses herd-level infection and/or farmers over-estimate clinical JD incidence on their farms based on observation of typical clinical signs coupled with non-responsiveness to conventional treatments. The latter explanation was supported by a recent survey of New Zealand deer farmers (Verdugo, C; *pers comm*.) in which farmer-diagnosed clinical JD also occurred on more farms than were confirmed positive by culture or ELISA. This was in contrast to observation of sheep and beef cattle farms, and may have been due to heightened awareness within the deer compared with sheep and beef industries. Although used as a primary diagnostic tool in numerous studies of clinical JD in cattle (Cetinkaya et al 1997b; Naugle et al 2004; Norton et al 2009), farmer-diagnosis of disease can introduce significant bias. This has been discussed in detail in Chapter 2 and prevalence results should be interpreted with these possible limitations in mind.

8.2.1.1 Annual herd-level clinical JD incidence

The annual herd-level clinical JD incidence of 72%, estimated in the 2005 case-control study, was higher than the 40% incidence described by Hell *et al.* (2008) (n = 342 herds) in the only other national, published survey of herd-level clinical JD incidence in deer. This apparent difference may be associated with the dissimilar sample frames used, being potentially all New Zealand deer farmers (in the 2005 study) compared to only herds that had commercially slaughtered deer in the previous 12 months in the study by Hell *et al.* (2008). Moreover, although participation in both studies was voluntary, resulting in non-random sample populations, data collection in 2005 were via personal interview which may have introduced interviewer bias while data collection by Hell *et al.* (2008) were via a postal survey with possible associated selection bias. Alternatively, as the 2005 case-control study was the first national survey on JD in the New Zealand deer industry, the specificity of farmer diagnosis of clinical JD may have improved by November 2007, when data collection by Hell *et al.* (2008) were completed. In 2006/07, there was an industry-wide program of farmer education, including publication of a detailed description of the clinical signs typically observed in a deer suffering from clinical JD in a widely-distributed farmers' manual.

8.2.1.2 Annual mean within-herd clinical JD incidence

Relative to the annual herd-level incidence of clinical JD (72%), the mean within-herd incidence of disease, at 0.98% across all herds surveyed (n = 174) (*unpublished data*) in 2005, was comparatively low. As a comparison, in a 2007 survey, Hell *et al*. (2008) described a mean within-herd incidence of clinical JD of 0.90% (n = 342) and in a 2008/09 survey, Verdugo *et al.* (2011) found an annual mean within-herd (n = 99) incidence of 0.23%. The comparatively lower incidence in the latter relative to the two former surveys may indicate that either clinical JD incidence is truly decreasing or, as discussed above, that farmer diagnosis of disease is changing with time as knowledge of the disease improves, resulting in fewer false-positives. Regardless, the deer industry has devoted considerable resources to addressing its JD 'problem', including development of the abattoir surveillance system. Considering the relatively low within-herd clinical disease incidence estimates outlined above, it may now be prudent to re-evaluate the economic significance of this disease, including any subclinical manifestation, relative to other

conditions of deer, such as gastrointestinal parasitism and trace mineral deficiencies, and to consider where available resources are best devoted.

8.2.2 Risk factors for herd-level MAP infection and clinical JD in young deer

A primary aim of the 2005 case-control study, outlined in Chapters 3 and 4, was to assess whether any biologically plausible risk factors were potentially associated, whether negatively or positively, with the herd-level presence of MAP or clinical JD in young deer. A cut-point of 0.4% was selected in Chapter 4 to differentiate clinical incidence between case and control herds as there was insufficient zero incidence herds to allow an evaluation of risk factors significantly associated with clinical JD. To date, methods to control clinical JD on deer farms have been based primarily on those used for sheep and cattle, such as test-and-cull, but robust peer-reviewed reports of their effectiveness are limited. Although Chapters 3 and 4 have highlighted statistically-derived associations, each identified risk factor requires validation for plausibility of biological causation prior to its adoption as the foundation for a management practice to control MAP and/or clinical JD in deer.

As 75% of New Zealand deer farmers currently graze sheep, cattle and/or goats on their deer fenced area (DFA) (Griffiths et al 2006; Hell et al 2008), of particular interest was the identification of an association between clinical JD in young deer and grazing of species other than deer (SOTD) on the DFA. As outlined in Chapter 4, grazing of beef yearlings and a greater presence of sheep on the DFA, relative to other livestock, had significant positive (OR = 4.55; 95% CI: 1.70 to 12.20) and negative (OR = 0.94; 95% CI: 0.89 to 0.99) associations, respectively, with greater than 0.4% incidence of weaner deer having clinical JD. Verdugo *et al*. (2008) also found that farmers grazing sheep and deer were significantly less likely to have deer identified with 'abnormal' lymph nodes at slaughter relative to farmers grazing deer alone or deer and beef cattle. However, recent data suggests that the clinical status of sheep may be significant since shared grazing of pastures between weaner deer and sheep flocks with clinical JD, compared to sheep flocks with subclinical infection only, resulted in an increased risk of signs of clinical JD in the deer (Verdugo C, *pers. comm.*). Nonetheless, there has been only one published report of clinical disease in deer due to infection with Type I MAP (de Lisle et al

1993) so these associations may either be related to cross-immunity or have no biological plausibility. Further research investigating this risk factor in relation to strain, grazing associations and links between clinical disease and subclinical infection is warranted. Moreover, longitudinal studies, with herd-level MAP status and clinical JD incidence monitored over a number of years, could identify whether SOTD are significantly associated with a naive deer herd becoming infected with MAP and/or deer developing clinical JD for the first time, as well as monitoring changes in the prevalence of MAP infection/clinical JD within a deer herd. Alternatively, a more cost-effective option may be intervention studies where co- and/or alternate grazing of SOTD with deer could be manipulated and evaluated in detail.

8.3 Abattoir-based surveillance for MAP in commercially slaughtered deer

As outlined in Figure 8.1, a number of European countries have voluntary national or regional approaches to MAP and/or clinical JD control in either sheep or cattle (Benedictus et al 2000; Fridriksdottir et al 2000; Caldow and Gunn 2003; Anonymous 2006b; Nielsen 2009). Although each program involves some level of data collection, only the Netherlands has any form of continuous surveillance program (since 2006), with approximately 75% (2008 figures) of dairy herds biennially tested for MAP via bulk-milk sampling (Nielsen 2009). Currently, New Zealand, through abattoir surveillance, is the only country worldwide that has an on-going national abattoir-based surveillance scheme for MAP in any species with over 90% of deer herds consistently monitored. While Australia initiated a surveillance scheme for ovine JD (OJD) in 25 abattoirs nationwide in 1999 (Sergeant and Baldock 2002; Bradley and Cannon 2005), that program has now been limited to a small number of abattoirs in New South Wales and Victoria (Citer, L; *pers. comm.*).

Possible options for MAP surveillance in New Zealand farmed deer

Johne's disease was removed from the notifiable disease list in New Zealand in 2000. Passive surveillance for MAP and/or clinical JD through voluntary notifications made by farmers, veterinarians and laboratories, was considered unsuitable for development into a comprehensive national scheme for the deer industry. Serology has been used internationally to both confirm diagnoses of clinical JD (Weber et al 2009) and to determine the point prevalence of MAP

infection in cattle (Muskens et al 2000; Nielsen et al 2000; Wilson et al 2010), deer (Davidson et al 2004; Tryland et al 2004; Reyes-Garcia et al 2008) and zoo populations (Vansnick et al 2005).

Figure 8.1 European countries specifying to have a national or regional approach (green), or with some regional approach to MAP control (yellow-grey). Some countries did not specify any significant activities (red) whereas others did not provide information (grey) or were not included in the survey (black) (from Nielsen 2009)

However, use of the Paralisa™ (IgG1 ELISA), the most commonly used test for MAP in live deer in New Zealand, for on-going surveillance is problematical. The current population in which the Paralisa™ is being used appears biased towards two extremes (i.e. clinically diseased animals to confirm a diagnosis of clinical JD and healthy, valuable animals that are being evaluated for a test-negative status). Anecdotally, the latter population appears predominately to include rising two-year-old hinds prior to their first mating (Liggett, S; *pers. comm.*). Therefore, use of the Paralisa™ for the purpose of national surveillance for MAP would require active expansion of the sample base, with resultant increased costs, or clear acknowledgement that conclusions drawn from any data were restricted to the limited existing sample population.

Alternatively, non-specific reactivity to a primary intra-dermal test for *M. bovis*, the Mid-Cervical Test (MCT), may be used for MAP surveillance at the herd-level in deer within the national Bovine Tuberculosis Pest Management Strategy (Mackintosh et al 2007b). In 2005, 55% of 83 New Zealand deer herds enrolled in a nationwide case-control study contained one or more MCT reactors, with a maximum recorded within-herd reactor rate of 17% (Hunnam, *unpublished data*). Using the same dataset, Stringer *et al.* (2011) found MAP-infected deer herds had a 3.1 times higher odds of having one or more MCT reactor(s), relative to MAP-negative herds. Moreover, Griffin *et al*. (2006a) found 63% of deer herds contained one or more MCT reactor(s) that were also positive to the Paralisa™, with no significant between-Island difference. However, only 11% to 23% (dependant on region) of MCT-positive animals nationwide were also Paralisa™-positive, indicating MAP may not be the principal cause of MCT reactivity at the animal-level in deer.

A surveillance system was already established in New Zealand abattoirs by the Animal Health Board (AHB) for the detection of macroscopically visible abnormalities, particularly within lymph nodes, due to *M. bovis* infection in commercially slaughtered cattle and deer. This surveillance included visual inspection, palpation and, as necessary, incision of suspect lymph nodes by trained meat inspectors (Anonymous 2005b). The predominant pathological change due to MAP infection in deer is enlarged lymph nodes with or without necrosis and/or mineralization (Libke and Walton 1975; Williams et al 1983; Manning et al 2003b; Crawford et al 2006). Therefore, use of the existing abattoir surveillance system for *M. bovis* to secondarily diagnose MAP through identification of 'abnormal' lymph nodes (LN) was considered cost-effective. Although LN abnormalities due to MAP infection are indistinguishable from that caused by *M. bovis,* the effect of the latter pathogen on the specificity of abattoir surveillance for MAP was considered to be low as the national herd-level prevalence of *M. bovis* was minimal (ten deer herds in 2009, four in 2011). Small intestinal wall thickening does not appear with the same frequency in MAP-infected deer as typically observed in infected cattle and sheep and, therefore, was not considered suitable as a visual indicator for MAP surveillance (de Lisle et al 1993).

A national abattoir surveillance scheme for MAP in commercially slaughtered New Zealand farmed deer was implemented in January 2007. The primary aim of Chapter 5 of this thesis was to validate the use of 'abnormal' LN as an indicator of MAP infection within this scheme, including development of a quantitative, measurable cut-point against which a LN could be defined as 'abnormal'. Circumference measurement has proven to be a practical, rapid and cost-effective technique to assess LN size and has formed the basis of a number of recent studies, including Verdugo *et al.* (2009), Stringer *et al.* (2010), Cayol *et al.* (2011) and, more recently, Norton, S *et al.* (*unpublished data*). Although concept development and the undertaking of science to support this surveillance system were completed as part of this PhD project, it was intended that the ongoing establishment and management of the system would be handed over to an industry body once the developmental phase was complete. The database is now managed by Johne's Management Limited (JML), a company implemented and funded by the deer industry.

The primary aims of the scheme were:

- To estimate national and regional prevalence levels of 'JD-suspect' LN;
- To allow analysis of epidemiological parameters on a national and regional level;
- To monitor control programs/intervention studies through evaluation of the on-going presence or prevalence of 'JD-suspect' LN;
- To provide market assurance of a national programme to monitor and report on MAP infection in the New Zealand deer industry; and
- To provide a point of reference for detection of infected properties that could be targeted to decrease the incidence of 'JD-suspect' LN at slaughter.

Abbott and Whittington (2003), using Monte Carlo simulation, found the flock-level sensitivity of abbatoir-based surveillance for MAP in Australian sheep was dependant on within-flock prevalence, the number of animals examined and was sensitive to estimates of animal-level sensitivity and specificity. A flock-level prevalence of ≥7% was required to reach a median probability of detection of 0.95, based on the examination of one slaughter line. As illustrated in Table 2.2 (Chapter 2), the median incidence of clinical Johne's disease in MAP-infected deer herds was 1.0%. If the 'iceberg' principle of herd-level MAP infection based on the presence of

visible clinical signs exists in deer in the same way as cattle (Whitlock and Buergelt 1996) , the true- flock-level incidence of MAP within New Zealand deer herds may well be well over 7%. Moreover, as illustrated in Figure 8.2, to March 2010, JML had collated data on close to 100% of commercially slaughtered deer, including age, sex and carcass weight, providing a comprehensive dataset. Monthly proportions greater than 100% reflect the policy of some venison processors of allocating data into the previous or next month, depending on the date of final kill.

The correct diagnosis of MAP from an infected deer via abattoir surveillance requires the successful passage of that deer through a specific, sequential pathway of individual steps (Figure 8.3). There are a number of possible scenarios (A to E) when a MAP-infected deer on-farm can remain dormant or be misdiagnosed through abattoir surveillance. This thesis has contributed to validating use of 'abnormal' LN as an indicator of MAP infection and has assessed meat inspector identification of these LN. However, further research is required to evaluate the probability that MAP infection will be detected at each step and the relative importance of each to the overall ability of the pathway to act as a surveillance tool for MAP. Information pertinent to this evaluation is described below, with completed (Chapters 5, 6 and 7) and suggested future research outlined.

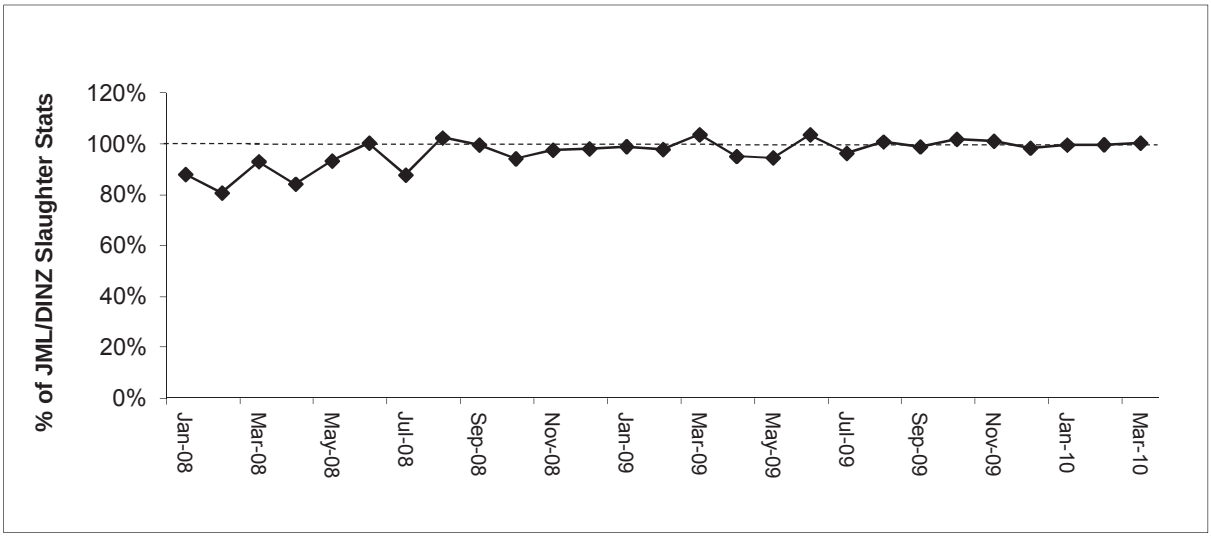


Figure 8.2 Proportion of total commercial deer kill for which data has been captured into the Johne's Management Limited (JML) database as a function of calendar time, January 2008 to March 2010 (reference population = levies collected by Deer Industry New Zealand (DINZ)) (reproduced with JML permission).

8.3.1 Scenario A: Deer are not slaughtered commercially

A MAP-infected deer will not be identified through abattoir surveillance if it is not commercially slaughtered (Scenario A; Figure 8.3). Abattoir surveillance is inherently limited to examination of a biased population, predominately being those animals that are in sufficient health to be considered for slaughter (Bradley and Cannon 2005). In New Zealand, the age distribution of the slaughter population is biased relative to the source population, as the commercial deer kill consists predominately of yearling (12 to 24 month old) hinds and stags. Moreover, data

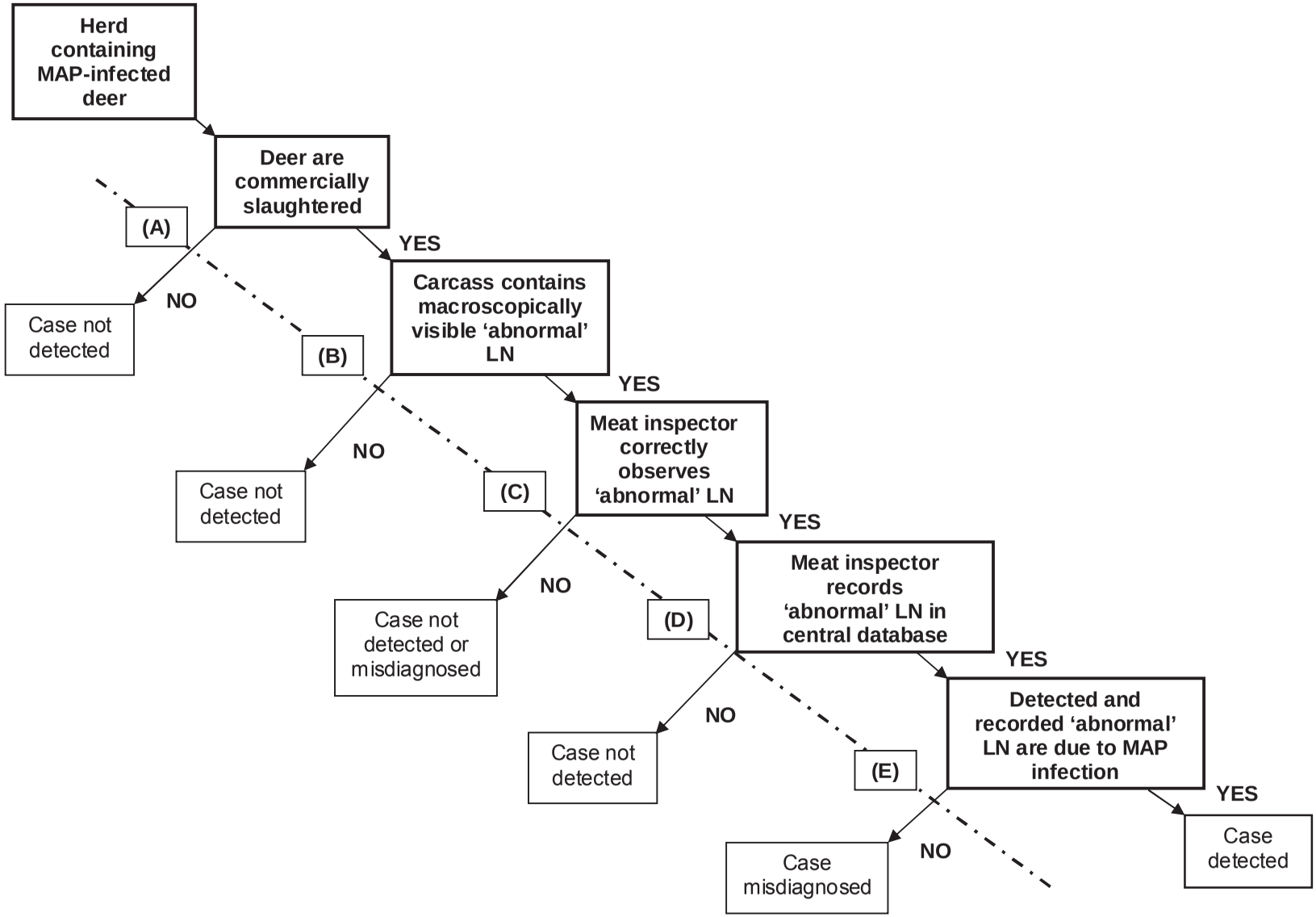


Figure 8.3 Scenario tree for herd-level active syndromic abattoir surveillance for *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection in commercially slaughtered New Zealand farmed deer through inspection for 'abnormal' lymph nodes (LN) (modified from Hadorn and Stark, 2008).

available on slaughtered adult hinds and stags are likely to be biased as a proportion of these deer are sent based on advanced age, poor body condition score, poor production and/or poor fertility relative to their herd mates. The visible spectrum of disease in animals eligible for slaughter will also vary from that in the national population, as deer affected with clinical JD are likely to die naturally or be euthanized on-farm. Moreover, wild deer are currently not included in the JML database, as the gastrointestinal organs of deer shot in the wild are left at the kill location and the LN cannot be examined for abnormalities by an accredited meat inspector. Therefore, abattoir surveillance may not provide an accurate estimate of MAP prevalence in the on-farm, live deer population in New Zealand. A study assessing the relationship between 'abnormal' LN at slaughter relative to clinical disease prevalence on-farm is now an essential step to assess the 'external validity' of data collected via abattoir surveillance (i.e. the relationship between LN status at slaughter and the clinical or subclinical disease manifestation on-farm). This research has recently been initiated by the Deer Research Group at Massey University. If this relationship is low, then an alternative method of surveillance in either live deer and/or in slaughtered deer may be required, particularly as Chapter 2 provides evidence for a clinical JD presence in adult hinds and stags.

A national, cluster-adjusted individual MAP prevalence of 45% (95% CI: 30 to 60%) was recently described by Stringer *et al.* (2009a). Although evaluation of mesenteric lymph nodes (MLN) of commercially slaughtered deer, as completed in this study, currently provides the most pragmatic method for assessing MAP prevalence, the study population differs from the live farmed deer population for the reasons outlined above. There is no published investigation of an animal-level MAP prevalence estimate in New Zealand live (i.e. non-slaughter) farmed deer, drawn from a random population. Chapter 2 of this thesis and a recent study by Verdugo *et al.* (2011) provided estimates of apparent herd-level MAP prevalence in live deer of 62% and 57%, respectively. However, herd participation in these studies was non-random, being reliant on voluntary participation, and did not allow calculation of animal-level prevalence estimates.

If MAP/clinical JD surveillance within the live deer population was deemed necessary, use of test results from existing, national active surveillance for other diseases will likely be more cost-effective than attempting to institute a surveillance system from scratch. An on-going

surveillance system would also allow more detailed monitoring of changing infection dynamics over time relative to repeated sampling surveys. There are few diagnostic tests that can be used practically and economically in the live farmed deer population for active surveillance for MAP infection. Although individual faecal culture is sensitive in the detection of MAP-infected deer, it is expensive and can take three months for results to be available. Alternatively, diagnostic tests that are currently used, or could be used, in the live farmed deer population with some consistency that diagnose MAP infection are the mid-cervical test (MCT) (intradermal test for bovine tuberculosis; bTb) and the Paralisa™ (IgG1 ELISA). Potential issues around the use of both the MCT and Paralisa™ for the purpose of national surveillance for MAP in deer have been outlined in Section 8.3.

8.3.2 Scenario B: MAP-infected deer carcasses do not contain 'abnormal' lymph nodes

A commercially slaughtered, MAP-infected deer will remain undetected through abattoir surveillance if it does not have 'abnormal' LN as a result of the infection with MAP (Scenario B; Figure 8.3). In addition to animal- and herd-level MAP diagnosis, it is intended that abattoir surveillance will allow calculation of MAP prevalence at the national-, regional- and herd-levels (Thrusfield 2005b). However, if the 'iceberg' principle of herd-level MAP infection exists in farmed deer in the same way as shown in cattle (Whitlock and Buergelt 1996), the true animal-level prevalence of MAP infection is likely to be higher than that estimated by identification of 'abnormal' LN at slaughter only. Therefore, use of 'abnormal' LN as the sole criterion for the diagnosis of MAP infection requires, as a point of reference, concurrent investigation of MAP prevalence within 'normal' LN of slaughtered deer.

An incidental finding from data collected for Chapter 6 (Study I) in two South Island abattoirs was an apparent prevalence of MAP infection in 136 'normal' MLN (circumference <55 mm) of 66.0% (*unpublished data*). This estimate was the first examination of MAP prevalence in 'normal' LN and was initially considered a likely overestimate due to possible cross-contamination at the time of sampling. However, Stringer *et al*. (2009) subsequently detected a national animal-level MAP prevalence of 45% (95% CI: 30 to 60%) in 'normal' MLN, with a significantly higher prevalence in the South versus North Island (51 vs. 29%) ($p<0.05$).

Similarly, a 2010/11 study demonstrated a 42.2% MAP prevalence in 'normal' MLN where samples were sourced from 11 abattoirs located throughout New Zealand (Norton S; *unpublished data*). The lower MAP prevalence in the latter two studies relative to that derived from Chapter 6 is likely associated with region, as samples for the latter studies were sourced from across New Zealand, whereas the former study was conducted in the lower South Island, which has a higher prevalence of MAP infection.

8.3.3 Scenarios C and D: Meat inspectors do not observe, or misdiagnose and/or do not record 'abnormal' lymph nodes

A commercially slaughtered MAP-infected deer with one or more 'abnormal' LN will remain undetected via abattoir surveillance if meat inspectors do not observe and/or record the presence of the 'abnormal' LN (Scenarios C and D; Figure 8.3). Estimates of MAP prevalence based on the JML database must take into account that the primary diagnostic tool is subject to human error. The influence and variance of this on 'abnormal' LN detection levels can be minimised through initiatives such as high quality and on-going inspector education. Towards this aim, JML has provided an operational manual to all deer abattoirs with a clear outline of the 'abnormal' LN definition (note: previously referred to as an 'enlarged visceral lymph node' or EVL) (Appendix 4). Rennie *et al*. (2007) demonstrated that the provision of clear, simple instructions can reduce the influence of human factors on the accuracy of diagnostic testing. As outlined in Chapter 6, New Zealand deer-accredited meat inspectors were able to diagnose 'abnormal' deer MLN with low sensitivity (13.3%) but high specificity (99.9%).

In order to have confidence in estimates of farm, regional and national MAP prevalence derived from abattoir surveillance, a high level of between-inspector agreement (repeatability) is required. This is particularly significant at the herd-level as farmers will commonly slaughter deer at different abattoirs during a season and a single abattoir may employ more than one inspector. Research has found that between-observer agreement of clinical signs is dependent on the cut-off applied for a positive diagnosis and is higher in the absence rather than presence of clinical signs, despite training and standardization of observers (Petersen et al 2004; Thomsen and Baadsgaard 2006).

In South Island abattoirs, 'abnormal' LN have been consistently recorded since the program's inception, indicating that this has become an accepted part of the inspection technique of most South Island meat inspectors (Hunnam et al 2009). However, there has been considerable between-abattoir variation and lower South Island inspectors appear, on average, to record a higher percentage of 'abnormal' LN relative to upper South Island inspectors. This variation may reflect differences in the prevalence of MAP infection in deer herds supplying the abattoirs and/or may be due to inspector bias (i.e. an inspector in one abattoir identifies and records 'abnormal' LN consistently more or less than an inspector in a different abattoir). Chapter 6 provided some evidence for the latter explanation as the kappa for between-inspector repeatability was only fair ($\kappa = 0.32$). As a number of abattoirs have only one meat inspector employed, this parameter can be particularly influential on the overall accuracy of abattoir surveillance for MAP.

The monthly proportion of kill recorded with 'abnormal' LN in North Island abattoirs has been inconsistent and low relative to proportions recorded in South Island abattoirs (Hunnam et al 2009). This result may reflect a true low prevalence of 'abnormal' LN in North Island deer or may indicate that North Island inspectors are not aware of the pathology typical of MAP infection in deer and/or can identify an 'abnormal' LN but are not recording the data into the database. Stringer *et al.* (2009) showed that MAP prevalence is significantly higher in 'normal' MLN sourced from the South Island (51%; 95% CI: 36 to 66%) relative to the North Island (29%; 95% CI: 16 to 45%), providing some evidence towards the first explanation. However, previous evaluation of Study II data (Glossop et al 2008) found the sensitivity of meat inspector detection of 'abnormal' MLN, based on visual examination of photographic images only, was significantly lower in upper North Island abattoirs. This indicates that the reason for the current relatively lower detection rate of 'abnormal' LN in the North Island is likely to be multifactorial.

8.3.4 Scenario E: Pathologic changes within 'abnormal' lymph nodes are not due to MAP infection

Finally, a commercially slaughtered deer with an 'abnormal' LN that has been observed and recorded by a inspector into the national database can be wrongly diagnosed as MAP-infected if

that abnormality is due to a condition other than MAP (Scenario E; Figure 8.3). A possible advantage of the potentially low specificity of the JML definition of a 'JD-suspect' LN is the likely inclusion of most 'abnormal' LN due to MAP infection. However, a possible disadvantage is a potentially high level of false-positive diagnoses being notified to farmers. Incorrect farmer notification of the presence of one or more 'JD-suspect' LN in slaughtered deer can have important repercussions if management practices are initiated or changed in response. Culture or histopathological evidence of MAP infection in over 94% of 'abnormal' MLN (Chapter 5) provides assurance that costly secondary diagnostic testing of individual LN post-inspection, prior to farmer notification of their presence, may be unnecessary. Despite this, 'abnormal' LN are still described as 'suspicious' for MAP and farmers are encouraged to undertake herd-level gold-standard (i.e. culture) testing on-farm to confirm the presence and prevalence of the bacteria.

The influence of *M. bovis* on the specificity of abattoir surveillance for MAP is likely to be low. In 2009/10, only 2% of 437 MCT reactors were found to be truly infected with *M. bovis* and, in the same time period, only 10 New Zealand deer herds were diagnosed with one or more *M. bovis*-infected animals (Anonymous 2010c). As outlined in Chapter 6, *M. bovis* was not cultured from 'abnormal' LN despite samples being sourced from throughout New Zealand. However, results outlined in Chapter 6 are limited to 2007 and repeated investigations are required to provide further confidence in the apparent low regional and national prevalence of *M. bovis* and other causative agents of LN lesions in deer, such as Rhodococcus spp., *Actinobacillus lignieresii*, Fusibacterium spp., and Corynebacterium, that may influence the specificity of MAP surveillance in deer (Campbell 1995).

Currently, diagnostic tests used for abattoir surveillance of either MAP or *M. bovis* infection in farmed deer are carcass inspection, histopathological examination and radiometric tissue culture. Although histopathological examination is relatively low-cost and has a rapid turn-around, differentiation of MAP from other mycobacteria, such as *M. bovis* and *M. avium*, is currently achievable only through culture (de Lisle et al 1993). Histopathological features of macroscopically 'abnormal' LN infected with a mycobacteria species have been described (de Lisle and Collins 1995; de Lisle et al 1995; Quigley et al 1997) and changes in macroscopically

'normal', MAP-infected LN have been categorised and scored, based on the location, severity and type of inflammatory cell present and the number of acid-fast organisms observed (Mackintosh et al 2007a; Balseiro et al 2008; Mackintosh et al 2008; Clark et al 2010). However, histopathological features typically observed in grossly 'normal' LN at 'low' risk of MAP infection had not been described. Chapter 7 may be useful to histopathologists to help differentiate between non-pathological features in macroscopically 'abnormal' LN and those likely to be related to infection with *M. bovis*, MAP or *M. avium*.

8.4 Deer-specific classification scheme for MAP infection, subclinical and clinical JD

Deer identified at slaughter with 'abnormal' LN have previously been classified as suffering from 'subclinical JD' (Hunnam et al 2009; Wilson et al 2009). However, carcass weights of those identified with 'abnormal' LN between January 2007 and December 2008 were 2.4 to 3.5 kg lower in young (generally <2 years old) and 7.3 to 26.8 kg lower in adult (>2 years old) deer, compared with carcasses without 'abnormal' LN ($p<0.01$) (Hunnam et al 2009) and lower body condition score/weight loss is traditionally associated with clinical JD (Whitlock and Buergelt 1996). Mackintosh *et al.* (2007) developed an experimental protocol whereby deer artificially inoculated with MAP were considered to be 'clinical' if they lost more than 5% of their liveweight over two successive weeks (Mackintosh et al 2007a). The following is a suggested classification scheme for MAP and clinical JD in deer, based on that outlined by Whitlock and Buergelt (1996) for cattle, focusing on the presence of clinical signs and the results of diagnostic testing. Deer can exist within Stages I or II for their lifetime or remain free of clinical signs for months to years until periods of stress or other factors cause MAP to emerge, replicate and create disease.

Stage I: 'Silent Infection'

Deer are truly infected with MAP but show no overt evidence of disease, including any deleterious production effect while live and/or carcass abnormality at slaughter. These animals remain undetectable using current diagnostic tests, including faecal/tissue culture, histopathology

or serological tests. As these animals cannot be identified, the presence and relative proportion of deer with 'silent infection' within a population remains largely hypothetical.

Stage II: 'Subclinical Disease'

Deer are truly MAP-infected and, similar to those with 'silent infection', show no overt evidence of disease, including any deleterious production effect while live and/or carcass abnormality at slaughter. However, these animals may have detectable antibodies to MAP and a proportion may be detectable on tissue/faecal culture. Deer with MAP-positive, 'normal' LN at slaughter can be classified as 'subclinical', as recent studies have found no significant difference in weight between carcasses with MAP-positive relative to MAP-negative 'normal' MLN (Stringer et al 2009b; Cayol et al 2011).

Stage III: 'Early Clinical Disease'

A truly MAP-infected animal with 'early clinical disease' has subtly lowered production parameter(s), such as reduced weight loss or velvet production, but may not appear different to its peers, based on visual examination. Deer suffering from 'early clinical disease' include those identified with 'abnormal' LN at slaughter.

Stage IV: 'Advanced Clinical Disease'

Deer are MAP-infected and demonstrate observable clinical signs such as pronounced weight loss, including loss of muscle tone and pronounced hip/pin bones, and/or diarrhoea. The definition of disease based on observation of clinical signs only, as outlined in Chapter 2, may be utilised to increase the specificity of this method of diagnosis. However, additional diagnostic tests, such as individual faecal culture, would be required to confirm a diagnosis. Deer may or may not have 'abnormal' LN at slaughter. The current published sensitivity of Paralisa™ detection of MAP infection in deer with 'advanced clinical disease' is 91% (Griffin et al 2005).

8.5 Areas for further research

This thesis presents data on the epidemiology of JD in New Zealand farmed deer and the validity of abattoir surveillance for MAP in this population. However, as is typical of foundation studies

such as this, several areas that warrant further research/evaluation have been raised, including the following:

- Evaluation of the economic cost of clinical and subclinical JD to the New Zealand deer industry, accounting for inherent parameter uncertainty. Cost-benefit analyses of the success of different potential management techniques, including test-and-cull and vaccination, on minimizing the incidence of clinical disease and/or MAP infection;
- Repeated national studies investigating between- and within-herd prevalence of MAP and clinical JD to establish the true incidence rate of clinical disease, to evaluate the on-going success of control efforts and to further evaluate risk factors for these outcomes;
- Longitudinal study to better describe the dynamics of MAP infection, including risk factors, for individual animal transfer from subclinical to clinical disease;
- Study evaluating the relationship between the presence and prevalence of 'abnormal' LN at slaughter and clinical disease on-farm to better determine the value of abattoir surveillance in predicting on-farm disease;
- Calculation of the true prevalence of MAP infection through abattoir surveillance utilising statistical techniques in the absence of a gold standard;
- Evaluation of the predictive value and cost-effectiveness of available diagnostic tests, such as IFC, Paralisa™ and PFC at different dilution levels, for the herd-level diagnosis of MAP infection under field conditions;
- MAP prevalence stud(ies) in wild deer populations; and
- A survey investigating the social impact of MAP and clinical JD, both at the instance of first diagnosis and the effects of on-going management, on New Zealand deer farmers.

8.6 Concluding statement

Over the last decade, the New Zealand deer industry has been active in developing and promoting initiatives for the control of MAP and/or clinical JD, in particular a comprehensive 2010 technical manual for veterinary advisors. Currently, there are two predominant possible costs as a result of the widespread presence of MAP in deer, namely production loss due to clinical (and possibly subclinical) JD and a potential threat to international markets from the risk of a causal association between MAP and Crohn's disease. Although the New Zealand deer

industry may aspire to eradicate clinical disease due to MAP infection, eradication of the bacteria itself is an unrealistic aim. Therefore, the question of whether national surveillance should focus on the detection of signs of clinical JD (i.e. Stages III and IV), rather than solely evidence for MAP infection (Stages I and II), arises. An economic evaluation of the true cost of clinical JD to the deer industry, with appropriate adjustment for parameter uncertainty, and investigation of the presence of subclinical effects of MAP infection in deer are urgently required to establish the true focus of surveillance.

However, potentially overriding this production loss focus is the on-going possible threat posed by Crohn's disease to New Zealand's international venison and velvet markets (Ryan and Campbell 2006). If a causal association is definitively shown between Crohn's disease and MAP, or a pervasive perception of causation develops, then the ability to prove that its' products are at lower risk of MAP contamination relative to alternatives may be advantageous for the New Zealand deer industry. However, discussion involving all stakeholders regarding the level of focus that should be devoted to this potential issue, relative to immediate production loss due to clinical JD, is required. This should include debate on the need for a national control scheme for clinical disease, an on-going surveillance system for MAP in live deer and/or evaluation of the risk of MAP transmission to humans posed by products derived from New Zealand farmed deer. To achieve cost-efficient national control of MAP and clinical JD, the deer industry must take a holistic view of past and present efforts and objectively develop a plan that places appropriate focus of time and resources into the future management of this disease.

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APPENDIX 2: Herd-level questionnaire of clinical JD in farmed New Zealand deer and common management practices - 2005

"THE EPIDEMIOLOGY OF JOHNES DISEASE IN NEW ZEALAND FARMED DEER"

PhD candidate: Dr Jaimie Glossop BSc BVSc MVSc
Supervisors: Prof Peter Wilson, Dr Cord Heuer, Dr Colin Mackintosh, Prof David West
Research Officer: Fernanda Castillo-Alcala

QUESTIONNAIRE:

Date: _____
Present: _____

Section 1: Contact details

1.1 Property name: _____

1.2 AHB (Animal Health Board) number: _____

1.3 Contact person: _____

Owner/manager (please circle)

1.4 Farm address: _____
_____

1.5 District: _____

1.6 Region: _____

1.7 North or South Island: _____

1.8 Phone (home): _____

1.9 Phone (work): _____

1.10 Fax: _____

1.11 Mobile: _____

1.12 E-mail: _____

SECTION 2: Farm information

General farm information

Please indicate years as whole numbers. If less than one year, please write <1

2.1 How many years have you spent in the **deer industry**? _____ year(s)

2.2 How many years have you spent **on this property**? ____ year(s)

Please indicate whether area values are in acres or ha

2.3 What is the **total** area of your farm (including bush)? _____ acre/ha

2.4 What is the **effective** area of your farm? _____ acre/ha

2.5 What area of your farm has been **fenced for deer**? _____ acre/ha

2.6 What **number of paddocks** are deer fenced? _____

Environment:

2.7 List the percentage (%) of each type of **land topography** found within your deer fenced area

Please ensure the percentages total 100%

Land topography type	% land topography type Deer fenced area
High Country	
Hill Country	
Downlands/Rolling	
Intensive Flat	
Intensive Irrigated	
Other	

2.8 What **environmental conditions**, including soil type(s), pH and Olsen P values are present within your deer fenced area.

Environmental conditions	Deer fenced area	Unknown
Number of snowfalls (2005)		
Soil type(s) **1**		
2		
3		
Soil pH ($CaCl_2$)		
Olsen P value		

Conversion to deer farming:

2.9 How many years has it been since your property was converted to deer farming?

_____ Years ☐ Unknown

2.10 If property conversion was **after January 2004** which species were grazed on the deer fenced area prior to conversion?

Please tick all that apply

Species	
None	
Sheep	
Beef Cattle	
Dairy Cattle	
Other	
Unknown	

Section 3: Stock numbers at 30 June 2005

3.1 DEER: How many of each deer class did you have wintered at **30 June 2005**

☐ No deer wintered on this property at June 30 2005

Deer breeds	Numbers of each deer class					
	Weaners (0-12 months)		**Yearlings (12-24 months)**		**Adult Stags (2+ years)**	**Adult Hinds (2+ years)**
	Female	Male	Female	Male		
Red						
Rakaia red						
Wapiti type						
Fallow						
Elk						
Other						

3.2 As a percentage (%), what were the <u>main</u> type(s) of deer farming operation undertaken for each deer class, in terms of **proportion of that deer class**, in **2005**

Please ensure the percentages total 100% for each deer class

☐ No deer grazing on this property in 2005

Deer farm operation type	% Deer Age Class					
	Weaners (0-12 months)		Yearlings (12-24 months)		Adult Hinds (2+ years)	Adult Stags (2+ years)
	Female	Male	Female	Male		
Breeding/stud						
Replacements						
Venison						
Live sales						
Velvet						
Game Park						
Trophy stags						
Died						
Other						

3.3 STOCK OTHER THAN DEER: What were the main breed(s) and number of livestock species (other than deer) grazed or to be grazed within your property's **deer fenced area** at any time from **January 2004 to December 2005**?

☐ No species other than deer grazed/to be grazed from Jan 2004 - Dec 2005

Species	Class	Livestock		
		Breed	Number	Time length of grazing (days)
Sheep	Ewes			
	Rams			
	Hoggets/2-tooths			
Beef Cattle	Cows (2+ years)			
	Bulls/steers (2+ years)			
	Yearlings (6 - 24 months)			
	Calves (0 – 6 months)			
Dairy Cattle	Cows (2+ years)			
	Bulls/steers (2+ years)			
	Yearlings (6 - 24 months)			
	Calves (0 - 6 months)			
Other	TOTAL			
	TOTAL			

SECTION 4: Pastures in 2004

4.1 Describe the percentage (%) of each pasture species within the deer fenced area on your property in **2005**

Please ensure the percentages total 100%

Pasture Species	% Pasture species
	Deer fenced area
Ryegrass	
White Clover	
Red Clover	
Brown Top	
Cocksfoot	
Chicory	
Plantain	
Other	

4.2 Describe the amount of each of the following on your deer fenced area in 2005:

Rushes	□ abundant	□ moderate amounts	□ rare	□ none on-farm
Thistles	□ abundant	□ moderate amounts	□ rare	□ none on-farm
Scrub	□ abundant	□ moderate amounts	□ rare	□ none on-farm
Other	□ abundant	□ moderate amounts	□ rare	□ none on-farm

4.3 Describe the quantity and area of supplementation applied to deer fenced pastures from **January to December 2005**

Please indicate whether the area is in acre or ha

□ No supplementation applied in 2005
□ unknown

Pasture supplementation type		Deer fenced pastures
Fertilizer	Type	
	Quantity (kg)	
	Area (acre/ha)	
Lime	Quantity (kg)	
	Area (acre/ha)	
Urea	Quantity (kg)	
	Area (acre/ha)	
Other	Quantity	
	Area (acre/ha)	

4.4 What percentage (%) of the deer fenced area, was managed with the following from **January to December 2005**?

□ No management applied in 2005
□ unknown

Paddock management type	% deer fenced area	Pasture/crop type:
Pasture topping		
Haymaking		
Pasture re-sow		
Cropping		
Other		

4.5 Describe any unusual environmental conditions experienced by your deer herd from **January to December 2005** (eg: drought, flood)

□ no unusual conditions observed in 2005

_____

4.6 On what dates did your mating period begin and end in **2005**?

Date stags went in: _____ □ unknown
Date stags were taken out: _____ □ unknown

□ Stags grazed with hinds year-round in 2005

4.7 In **2005**, what were the (ultrasound) pregnancy rates for:

R2YO hinds: _____ % □ unknown
MA hinds: _____ % □ unknown

Section 5: Mating, fawning and pre-weaning periods in late 2004-2005

(Note: newborn deer are described as "pre-weaning fawns" from fawning until they are physically removed from their mothers (ie: "weaned"))

5.1 Did you run breeding hinds in **2004**?

Yes
No Please go to Section 6 below

5.2 What number of breeding hinds were present within each age group at **June 30 2004:**

□ Breeding hind ages unknown

Age (years)	Number of breeding hinds
<2	
3 - 5	
5 - 10	
>10	

5.2 What was the predominant stag breed(s) used over hinds in **2004**?

_____

5.4 Describe the age, number and time length of stock grazed on any paddocks used for fawning for the **6 months prior** to the beginning of fawning in **late 2004-2005**.

Stock	Stock grazing on fawning paddocks		
	Age (0-12 months; 12-24 months; 2+ years)	Stock numbers	Time length (days)
Deer			
Sheep			
Beef cattle			
Dairy cattle			
Other			

5.5 What area was available to the fawning hind mob(s) in **late 2004-2005?**

Please indicate whether the area is in acres or ha

Area: _____ ha/acre

5.6 On what dates did fawning begin and end in **late 2004-2005**?

Fawning began: _____ □ unknown
Fawning ended: _____ □ unknown

5.7 How would you describe the general grazing strategy for your hinds (and fawns) **between fawning and weaning** in **2005**?

- □ Set-stocked for entire period
- □ Set-stocked over fawning with some rotational grazing prior to weaning
- □ Predominately rotational grazing with regular shifts
- □ Other: _____

5.8 If adult hinds and fawns were rotationally grazed between fawning and weaning in **2005**, what was the average grazing interval?

Rotation grazing length:_____ days □ Unknown

5.9 How many mob(s) were your fawning mobs grazed in **late 2004-2005**?

_____

5.10 Describe the percentage (%) of each land topography type and environmental conditions found in **fawning paddocks** in **late 2004-2005**.

Please indicate whether altitude values are in metres or feet

Land topography type	% Fawning paddocks
High country	
Hill country	
Downlands/Rolling	
Intensive Flat	
Intensive Irrigated	
Other	

Environmental characteristic	Fawning paddocks	Unknown
Predominant pasture types **1**		
2		
Average altitude (m / ft)		
kgDM/ha at fawning		
Snowfall over fawning (Y / N)		
Presence of trees/scrub (0-2)		
Presence of shade (0/1)		
Presence of shelter (0-2)		
Presence of gorse (0-3)		

Where: Presence of trees/scrub:
0 = no trees
1 = one or more isolated trees
2 = one or more groups of trees

Presence of gorse:
0 = no gorse
1 = few plants
2 = groups of plants in some areas
3 = large area of gorse

Presence of shelter:
0 = no shelter belt or less than 0.5 m high
1 = shelter belt 1-5 m high
2 – shelter belt over 5 m high

Presence of shade
0 = limited shade at most times of the day
1 = shade at all times

5.11 Describe the number of stock which grazed on pastures **prior to or with** fawns, in addition to their mothers, between fawning and weaning during **late 2004-2005** and the length of time this occurred.

Species	Age and sex	2005 grazing policy			
		PRIOR to fawns		WITH fawns	
		Number of animals	Time length of grazing (days)	Number of animals	Time length of grazing (days)
Deer	Purchased weaners (0-12 months)				
	Yearlings (12-24 months)				
	Purchased/dry adult hinds (2+ years)				
	Adult stags (ie: post-rut) (ie: 2+ years)				
Sheep	Lambing ewes				
	Dry ewes				
	Rams				
	Weaners/hoggets				
Beef cattle	Cows and calves				
	Dry cows				
	Bulls				
	Yearlings/steers				

Dairy cattle	Cows and calves				
	Dry cows				
	Bulls				
	Yearlings/steers				
Other					

□ No stock grazed with fawns (and breeding hinds) between fawning and weaning in late 2004-2005

5.12 Describe the amount, time length and season of supplementation of each supplement fed to fawns (and breeding hinds) between **fawning and weaning** in **late 2004-2005**.

Supplement type	**Fawns (and hinds) pre-weaning in 2004-2005**		
	Amount of Supplement (total)	Time length of supplementation (days)	Season of supplementation (eg: Spring)
Sward hay (bales)			
Peavine hay (bales)			
Clover hay (bales)			
Lucerne hay (bales)			
Bailage (tonne)			
Silage (tonne)			
Maize (kg)			
Barley (kg)			
Other			

□ No supplements were fed to fawns (and breeding hinds) between fawning to weaning in late 2004-2005

5.13 How are supplements fed to fawns between fawning and weaning in **late 2004-2005**

- □ On the ground
- □ Off the ground (type (eg: trough): _____)
- □ In a feed pad
- □ Other: _____

- □ No supplements fed to fawns (and breeding hinds)

Section 6: Weaning in 2005

6.1 Did you wean fawns on-farm between **January-December 2005?**
(where "weaning" = physical removal of the fawn from its mother)

- □ Yes
- □ No **Please go to section 7**

6.2 On what date(s) did you wean your fawns in **2005**?

Date(s): _____

6.3 What were your weaning rates in **2005** (ie: number of weaners divided by the number of hinds which fawned on-farm) for:

R2YO hinds: _____ □ unknown
MA hinds: _____ □ unknown

6.4 What was the kgDM/ha of pasture grazed by *adult hinds and fawns* at weaning in 2005?

kgDM/ha: _____ □ kgDM/ha unknown

6.5 On what basis did you allocate weaner mobs in **2005**?

Please leave blank or write a '0' for any characteristics which were not considered.

Weaner characteristic	Weaner mob distribution based on…
Age	
Sex	
Weight	
Condition score	
Breed	
Dam parity	
Other	

□ All weaners were grazed in one mob from weaning to December 31 2005

Weaning paddock:

6.6 Describe the age, number and time length of stock grazed on any paddocks used **at weaning** for the **3 months prior** to weaners in **2005**.

Stock	Stock grazing on weaning paddock(s)		
	Age (0-12 months; 12-24 months; 2+ years)	Stock numbers	Time length (days)
Deer			
Sheep			
Beef cattle			
Dairy cattle			
Other			

6.7 Describe the percentage (%) of land topography and environmental conditions found within the weaning paddock(s) **at weaning** in **2005.**

Land topography type	% Weaning paddocks
High country	
Hill country	
Downlands/Rolling	
Intensive Flat	
Intensive Irrigated	
Other	

Environmental characteristic	Weaning paddocks	Unknown
Predominant pasture/ crop types **1** **2** **3**		
kgDM/ha at weaning		
Soil pH		
Snowfall (Y / N)		
Average altitude (m / ft)		

Section 7: Weaners (period = weaning to December 2005)

7.1 Please describe the source of weaners run on-farm to **December 2005**

- ☐ Farm-bred weaners only **Please go to question 7.3**
- ☐ Replacement/purchased weaners only **Please go to question 7.3**
- ☐ Farm-bred **and** replacement/purchased weaners
- ☐ No weaners were run on-farm in 2005 **Please go to section 8**

7.2 For what percentage of time were farm-bred weaners grazed with purchased weaners (ie: on the same pasture) to December 2005?

Percentage: _____ %

7.3 Did you use indoor wintering for weaners in 2005?

- ☐ Yes
- ☐ No **Please go to Question 7.6**

7.4 If yes, on what date were they put indoors and then let out?

Date put indoors: _____ ☐ Unknown
Date let out: _____ ☐ Unknown

7.5 Did you use feed-lotting for weaners in **2005**?

- ☐ Yes
- ☐ No

7.6 Please list the number of stock which grazed **prior to or with** your farm-bred and/or replacement ***weaner*** **mob(s)** (ie: on the same paddock) during **2005** and the length of time this occurred.

Species	Age and sex	2005 Grazing policy			
		PRIOR TO weaner mob(s)		WITH weaner mob(s)	
		Number of animals	Day(s) grazing	Number of animals	Day(s) grazing
Deer	Yearlings (12-24 months)				
	Adult hinds (2+ years)				
	Adult stags (2+ years)				
Sheep	Lambing ewes				
	Dry ewes				
	Rams				
	Weaners/hoggets				
Beef cattle	Calving cows				
	Dry cows				
	Bulls/steers				
	Yearlings				
Dairy cattle	Calving cows				
	Dry cows				
	Bulls/steers				
	Yearlings				
Other					

☐ No other stock grazed pastures prior to or with weaner mob(s) between weaning and December 31 2005

7.7 How would you describe the grazing strategy of your weaners in **2005**?

- ☐ Set-stocked
- ☐ Predominately rotational with regular shifts
- ☐ Approximately equal mix of rotational grazing and set stocking
- ☐ Other: _____

7.8 If weaners were rotationally grazed, what was the average rotation length in **2005**?

Rotation length:_____ days ☐ Unknown

7.9 What area was available to the weaner mob(s) in **2005?**

Please indicate whether the area is in acres or ha

Area: _____ ha/acre

7.10 How many mob(s) were your weaner mobs grazed in **2005**?

_____

7.11 Describe the amount, time length and season of supplementation to **weaners** from **weaning to December 31 2005**

Supplementation type	Weaner supplementation: weaning to Dec 2005		
	Supplement amount (total)	Time length of supplementation (days)	Season of supplementation (eg: Spring)
Sward hay (bales)			
Peavine hay (bales)			
Clover hay (bales)			
Lucerne hay (bales)			
Bailage (tonne)			
Silage (tonne)			
Maize (kg)			
Barley (kg)			
Other			

☐ No supplements were fed to weaners from weaning to December 31 2005

7.12 Are supplements fed to the weaners:

- ☐ On the ground
- ☐ Off the ground (type (eg: trough): _____)
- ☐ In a feed pad
- ☐ Other: _____

☐ No supplements fed to the weaners

7.13 What was the range of hind and stag weaner weights at weaning in **2005**?

Hinds Minimum: _____ kg ☐ unknown
Maximum: _____ kg ☐ unknown

Stags Minimum: _____ kg ☐ unknown
Maximum: _____ kg ☐ unknown

7.14 How often did you **yard** the fawns/weaners between fawning in late 2004-2005 and December 2005?

- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5
- ☐ 6+

SECTION 8: Environment

8.1 Indicate the source(s) of water to your deer in 2005
Please tick all that apply

- ☐ Troughs
- ☐ Dams
- ☐ Wallows
- ☐ Natural Springs
- ☐ Stream
- ☐ River
- ☐ Irrigation ditches
- ☐ Other: _____

8.2 Indicate any livestock species upstream of each water source in **2005**
Please tick all that apply

Water source	Species upstream in 2005											
	No livestock species upstream	**Deer**		**Sheep**		**Dairy Cattle**		**Beef Cattle**		**Other**	
		own	other	own	other	own	other	own	other	own	other
Troughs											
Dams											
Natural Springs											
Stream/River											
Irrigation											
Other											

8.3 Are deer able to lie in/play in troughs?

- ☐ Yes
- ☐ No

8.4 How often are troughs cleaned?

- ☐ Once a month
- ☐ Once every 6 months
- ☐ Annually
- ☐ Once every 2 years
- ☐ Never

8.5 Indicate type(s) of irrigation used on deer paddocks

- ☐ None
- ☐ Spray
- ☐ Gravity
- ☐ Flood
- ☐ Other: _____

8.6 Do you spread manure/animal faeces on your deer paddocks?

- ☐ Yes (Source of faeces: _____)
- ☐ No

Section 9: Johne's Disease

Deer herd:

9.1 How do you rate Johne's disease as far as your deer are concerned:

- ☐ A serious concern
- ☐ A moderate concern
- ☐ A minor concern
- ☐ No concern at all

9.2 Describe details of Johne's disease suspicion or diagnosis in your deer herd **prior to January 2005**

Johne's Disease in deer prior to January 2005								
Month	Year	Sex (M/F)	Age (years)	Breed(s)	Number of deer	Diagnostic test used	Clinical signs	Purchased or farm-reared

☐ Johne's disease has **NOT** been suspected or diagnosed in deer prior to January 2005 on this property

- ☐ **Weaners** were not run on this property prior to January 2005
- ☐ **Yearlings** were not run on this property prior to January 2005
- ☐ **Adult hinds** were not run on this property prior to January 2005
- ☐ **Adult stags** were not run on this property prior to January 2005

9.3 Describe details of Johne's disease suspicion or diagnosis in your deer herd from **January 2005 to current date.**

Johne's Disease in the deer herd from January 2005 to current date							
Month	Sex (M/F)	Age (years)	Breed(s)	Number of deer	Diagnostic test used	Clinical signs	Purchased or farm reared

□ Johne's disease has **NOT** been suspected or diagnosed in deer from January 1 to December 31 2004 on this property. **See Question 9.7**

9.4 What is your level of confidence when diagnosing Johne's disease based on clinical signs alone in your deer herd?

- □ Totally confident
- □ Very confident
- □ Confident
- □ Not really confident/doubtful
- □ Not at all confident

9.5 Indicate the season(s) in **2005** when deer suspected/diagnosed with Johne's disease first showed obvious clinical signs of disease (eg: loss of condition/scouring).

Please tick all age groups that apply

Season	Deer class suspected/diagnosed with JD			
	Weaners (0-12 months)	Yearlings (12-24 months)	Adult Hinds (2+ years)	Adult Stags (2+ years)
Summer				
Autumn				
Winter				
Spring				

□ No clinical signs of Johne's disease were seen in deer herd in 2005

9.6 Indicate any possible source(s) of stress prior to onset of clinical signs of Johne's disease in your deer herd in **2005**

Please tick all that apply

- ☐ Nutritional
- ☐ Velveting
- ☐ Pregnancy
- ☐ Mating
- ☐ Weaning
- ☐ Fawning
- ☐ Other: _____

☐ No obvious source(s) of stress prior to onset of Johne's disease were seen in deer herd in 2005

9.7 Describe the management program used to keep your deer herd free of Johne's disease and/or decrease the number of clinical cases.

_____

_____

_____

_____

☐ no management

9.8 When did this management program commence? _____

☐ no management program in place in deer herd

9.9 Do you consider this management program to be:

- ☐ Highly successful
- ☐ Moderately successful
- ☐ Fairly successful
- ☐ Completely unsuccessful

☐ No management plan in place in deer herd

9.10 Outline any treatment given to deer suspected or diagnosed with Johne's disease on this property in **2005**

Treatment of deer suspected/diagnosed with JD in 2005			
Deer age (0-12 months; 12-24 months; 2+years)	Treatment type (eg: antibiotic)	Length of treatment (days)	Treatment response (0-4)

□ No treatment has been given to deer suspected or diagnosed with Johne's disease in 2005

Where: Treatment response:

0 = no response
1 = little response
2 = moderate response
3 = good response but recurrence
4 = complete recovery without recurrence of signs

9.11 What **number** of deer suspected or diagnosed with Johne's disease in **2005** experienced the following fates?

Fate	Numbers of each deer class			
	Weaners (0-12 months)	Yearlings (12-24 months)	Adult hinds (2+ years)	Adult stags (2+ years)
Shot				
Deer slaughter plant				
Found dead				
Other				

9.12. In **2005**, once a deer is suspected or diagnosed with Johne's disease how long, on average, is it until that animal is shot or sent to a deer slaughter plant?

□ Less than one week
□ Between one week and one month
□ Between one month and 6 months
□ Over 6 months

□ Animals are neither shot nor sent to the works

9.13 Indicate the movement of deer suspected or diagnosed with Johne's disease which were kept on-farm in **2005**

Please tick only one option

□ Left in/replaced into **original mob**
□ Moved into a separate, scouring or **wasting mob**
□ Placed into a **random paddock** separate from healthy animals
□ Placed into a paddock specifically isolated as a **"hospital" paddock**
□ Other: _____

9.14 Indicate, in order of prevalence (ie: 1= most prevalent; 8 = least prevalent), the clinical sign(s) which you have most commonly observed in deer suspected or diagnosed with JD.

Please leave blank or write a '0' for any clinical signs which have not been seen

Clinical signs	Prevalence of clinical signs in cases of Johne's disease		
	Weaners (0-12 months)	Yearlings (12-24 months)	Adults (2+ years)
No JD seen in age group			
Loss of condition/wasting			
Rough hair coat			
Persistent scouring			
Intermittent scouring			
No drench response			
Sudden death			
Coughing			
Discharge from eyes			
Other			

9.15 Do you actively investigate Johne's disease in your deer herd or rely on Deer Slaughter Plants to diagnose Johne's disease?

- ☐ Actively investigate
- ☐ Rely on Deer Slaughter Plants

Species other than deer:

9.16 Indicate which species other than deer which were vaccinated for Johne's disease in **2005**

Please tick all that apply

- ☐ No species other than deer were vaccinated in 2005
- ☐ Sheep
- ☐ Dairy cattle
- ☐ Beef cattle
- ☐ Other: _____

9.17 Describe Johne's disease suspicion or diagnosis in species on-farm other than deer at any time from **January 2001 to current date**

Species	Species other than deer suspected/diagnosed with JD				
	Year	Number	Age (0-12 months; 12-2 months; 2+ years)	Diagnostic test used	n/a
Sheep					
Dairy cattle					
Beef cattle					
Other					

☐ Johne's disease has **NOT** been suspected or diagnosed in species other than deer from January 2001 to current date on this property

9.18 Describe the current policy for Johne's disease management in each species other than deer on-farm

Sheep: _____ ☐ n/a
☐ no management

Beef cattle: _____ ☐ n/a
☐ no management

Dairy cattle: _____ ☐ n/a
☐ no management

Other: _____ ☐ n/a
☐ no management

Section 10: Herd health

10.1 Describe the number of deer scouring and/or wasting of an unknown cause and the management applied in each deer class from **January 2005 to current date**

Please tick all that apply

Scouring/Wasting deer management	Number of each deer class			
	Weaners (0-12 months)	Yearlings (12-24 months)	Adult Hinds (2+ years)	Adult Stags (2+ years)
Number of deer affected				
No management applied				
Drench				
Antibiotics				
Other treatment				
Shot				
DSP ASAP				
Other				

10.2 Indicate the type of vaccination administered to each deer class in **2005**.

Please tick all that apply

Vaccination regime	Deer class			
	Weaners (0-12 months)	Yearlings (12-24 months)	Adult Hinds (2+ years)	Adult Stags (2+ years)
No vaccine given				
5-in-1				
7-in-1				
Clostridia				
Yersinia				
Leptovoid				
Other				

10.3 In **2005**, what deer classes did you worm drench?

- ☐ Weaners (0-12 months)
- ☐ Yearling hinds (12-24 months)
- ☐ Yearlings stags (12-24 months)
- ☐ Adult hinds (2+ years)
- ☐ Adult stags (2+ years)

- ☐ No deer were drenched in 2005

10.4 What number of deer were diagnosed or suspected with the following conditions in **2005**?

Please note the number of animals affected

Condition	Deer class			
	Weaners (0-12 months)	Yearlings (12-24 months)	Adult Hinds (2+ years)	Adult Stags (2+ years)
No conditions diagnosed				
Yersinia				
Clostridia				
Leptospirosis				
Salmonella				
Malignant Catarrhal Fever (MCF)				
Cryptosporidia				
Pneumonia				
Facial Eczema				
Ryegrass Staggers				
Foot lameness				
Parapoxvirus				
Teeth wear				
Other				

10.5 Indicate the form(s) of trace mineral supplementation given to each deer class in 2005

Form of trace mineral supplementation	Deer class			
	Weaners (0-12 months)	Yearlings (12-24 months)	Adult Hinds (2+years)	Adult Stags (2+ years)
No trace mineral supplementation				
Copper				
Selenium				
Cobalt				
Iodine				
Other				

SECTION 11: Replacement policy

11.1 Indicate the type of replacement policy with which you managed your herd **to December 2005**

- ☐ **Closed herd** (ie: did not graze any deer other than those which were farm-bred)
- ☐ Since: _____
- ☐ **Sire stag only herd** (ie: grazed sire stags bred off-farm only with farm-bred herd)
- ☐ **Replacement herd** (ie: grazed deer bred off-farm, in addition to sire stags, with farm-bred herd)

11.2 Have you grazed your deer off this property for any length of time in the last 5 years?

- ☐ Yes Year(s): _____
- ☐ No

11.3 Indicate years when you share-grazed any stock which do not belong to you (including deer) on the deer paddocks in the last 5 years?

Leave blank if stock species not share-grazed in last 5 years

Species share-grazed	Year(s)
No species share-grazed	
Deer	
Sheep	
Dairy cattle	
Beef cattle	
Other	

11.4 Please describe the number, age class, source region and quarantine length (if applicable) of replacement deer (including sire stags) from **January 1 2001 - December 31 2005**

Year	Replacement deer						
	Month	Number of deer	Breed(s)	Sex (M/F)	Age (years)	Source region (eg: Otago)	Number of source properties
2001							
2002							
2003							
2004							

☐ No purchased deer grazed on-farm between 2001 and December 31 2005

11.5 Do you actively ask for the Johne's status/history of source properties of replacement deer?

- ☐ Yes
- ☐ Sometimes
- ☐ No

11.6 Please indicate any treatment routinely given to replacement deer on arrival to your property.

Please tick all that apply

- ☐ No treatment given to replacement deer on arrival
- ☐ Drench
- ☐ Antibiotic
- ☐ Vaccination
- ☐ Trace mineral supplementation: _____
- ☐ Other: _____

Sire stag only or replacement herds (Closed herds: go to section 12):

11.7 Do you routinely quarantine any replacement deer (including sire stags) before grazing with on-farm deer and do you have a quarantine paddock(s) set aside for this purpose?

- ☐ No **Go to section 12**
- ☐ Yes – no specific quarantine paddock(s)
- ☐ Yes – specific quarantine paddock(s) set aside

SECTION 12: Wildlife

Carcass management:

12.1 How do you dispose of your dead deer carcasses?

- ☐ Offal pit
- ☐ Burn
- ☐ Bury
- ☐ Leave in paddock
- ☐ Other: _____

12.2 Is your offal pit

- ☐ Covered
- ☐ Open
- ☐ No offal pit on-farm

12.3 Relative to your deer paddocks, is your (or your neighbour's) offal pit(s)

- ☐ Within 10 metres of your deer fences
- ☐ Within 10-100 metres of your deer fences
- ☐ Within 100-1000 metres of your deer fences
- ☐ Over 1 km from your deer fences

☐ unknown

12.4 Please indicate the abundance of wildlife species on or immediately surrounding your deer fenced area

	Abundant:	**Seen occasionally:**	**Seen rarely:**	**None:**
Ferrets	☐	☐	☐	☐
Stoats	☐	☐	☐	☐
Feral cats	☐	☐	☐	☐
Possums	☐	☐	☐	☐
Hedgehogs	☐	☐	☐	☐
Rabbits	☐	☐	☐	☐
Hares	☐	☐	☐	☐
Feral sheep/goats	☐	☐	☐	☐
Feral cattle	☐	☐	☐	☐
Wild deer	☐	☐	☐	☐
Feral dogs	☐	☐	☐	☐
Ducks	☐	☐	☐	☐
Gulls	☐	☐	☐	☐
Other:	☐	☐	☐	☐

_____

SECTION 13: Neighbours

13.1 Please indicate livestock grazed by all neighbours in direct contact (ie: across fenceline) with your deer paddocks and describe the type of boundary fencing in place

Please tick all that apply

Neighbour(s)	**Species**					**Fencing type**	
	Deer	Sheep	Dairy cattle	Beef cattle	Other	Single	Double
1							
2							
3							
4							
5							

13.2 How often does stock from neighbouring properties enter your deer paddocks:

- ☐ never
- ☐ very rarely
- ☐ occasionally
- ☐ often

13.3 Please describe your knowledge of Johne's disease in neighbouring properties:

- ☐ All neighbouring farms definitely diagnosed with Johne's disease
- ☐ Some neighbouring farms definitely diagnosed with Johne's disease
- ☐ Suspect some neighbouring farms of having Johne's disease
- ☐ Unsure
- ☐ No neighbouring farms have Johne's disease

AHB/TB herd records access: Please read and sign the following statement

I, _____, the owner/manager of _____ allow the researchers to contact the Animal Health Board in order to access and gain copies of the **TB herd records** of this property. I understand this information will remain completely confidential and will not be disclosed in any form which may identify the property owner/manager or the property involved.

Owner/Manager:

(Signature) _____

(Print name) _____

Date: _____

Property AHB number: _____

☐ I do **not** allow researchers to access or copy my AHB/TB herd records

Please describe any details which have been important in your personal experience of Johne's disease:

_____

_____

_____

_____

_____

_____

_____

Thank you. Your participation is much appreciated.

APPENDIX 3: Herd-level questionnaire of clinical JD in farmed New Zealand deer – 2006/2007

(Massey) Farm number:

Johne's Disease survey

How to fill out this questionnaire

Please provide data as requested for the years 2006 and 2007 ONLY. All you have to do is tick a box, write in the space provided or follow instructions that explain what to do. Please read the question carefully before writing your answer.

The questions examine grazing of other farmed ruminant species (e.g. sheep, beef cattle, dairy cattle) directly with and prior to weaners and breeding hinds. There are no right or wrong answers. We would just like to know your ***own personal practices***.

If you have any questions about the survey, please call Jaimie Glossop on (06) 350 5600 (Ext: 4008) or (027) 289 8508 or email on j.c.glossop@massey.ac.nz.

Returning the questionnaire

When you have completed the questionnaire, please post it back in the reply-paid envelope (no stamp is required), **as soon as you possibly can**.

Confidentiality

Questionnaires are coded and returned via Johne's Management Limited. Respondents will not be identifiable by the researchers or upon publication of results.

(Massey) Farm number:

SECTION I: Your deer herd in 2007

1. What species of deer did you graze between 1 January 2007 and 15 November 2007?

Please tick the appropriate box

Fallow deer only ☐ … *Please go to Question 18 on page 9*

Red, Wapiti/hybrid and/or elk only ☐ … *Please continue*

A mixture of the above two deer species ☐ … *Please continue*

NOTE: If you grazed **fallow deer** on your farm in 2007, **do not** include them in your answers to the remaining questions

2. How many DEER did you winter at 30 June 2007?

Please write the number of deer

	Weaners (0-12 months)		Yearlings (12-24 months)		Mature Age (2+ years)	
	Hind	Stag	Hind	Stag	Hind	Stag
Number of deer at 30 June 2007						

3. How many DEER were confirmed (proven by tests) or suspected (scouring/wasting) with Johne's Disease from 1 January 2007 to 15 November 2007 on your property and what diagnostic method(s) did you and/or your veterinarian use?

☐ No deer were diagnosed with Johne's Disease in 2007… *Please go to question 4*

Please write the number of deer affected, for weaners - include the month when the first case occurred (if applicable) and tick all diagnostic method(s) that apply

	Weaners (0-12 months)	Yearlings (12-24 months)		Mature Age (2+ years)	
		Hinds	Stags	Hinds	Stags
Number with Johne's Disease in 2007					
Month in 2007 when weaner deer first seen with Johne's Disease					
How did you diagnose the deer with Johne's Disease? (*please tick all that apply*)					
Scouring and/or wasting					
Carcass lesion(s) at slaughter					
Blood test positive					
Faecal culture positive					
Other (please specify):					

(Massey) Farm number:

4. **How many DEER have scoured and/or wasted on your property <u>for any reason (other than Johne's Disease)</u>, from 1 January 2007 to 15 November 2007 and what do you believe caused the scouring and/or wasting?**

☐ No scouring and/or wasting deer were seen in 2007 … *Please go to question 7*

Please write the number of deer that scoured and/or wasted and for weaners, the month the <u>first case occurred</u> (if applicable)

	Weaners (0-12 months): Hinds and Stags	Yearlings (12-24 months)		Mature Age (2+ years)	
		Hind	Stag	Hind	Stag
Number of deer scouring and/or wasting from 1 January 2007					
Cause(s) of scouring and/or wasting					
Month in 2007 when weaner deer <u>first seen</u> scouring and/or wasting					

5. **Did you treat any of these scouring and/or wasting animals in 2007 (e.g. with a worm drench, antibiotics or copper/selenium)?**

Yes ☐ …… *Please go to question 6*

No ☐ …… *Please go to question 7*

Unsure ☐ …… *Please go to question 7*

6. **How many deer responded to treatment and how long did that response last?**

Please write the <u>number</u> of animals against each applicable length of response

Type of response	Weaners (0-12 months)	Yearlings (12-24 months)		Mature age (2+ years)	
		Hinds	Stags	Hinds	Stags
The scouring and/or wasting stopped completely					
The scouring and/or wasting stopped at first but came back					
No response to treatment at all					
Unsure of response					

7. How many animals of other species did you graze on the <u>deer fenced area</u> at any time in 2007 and how many scoured and/or wasted for any reason?

☐ No stock other than deer grazed on the deer fenced area in 2007
…… *Please go to question 18*

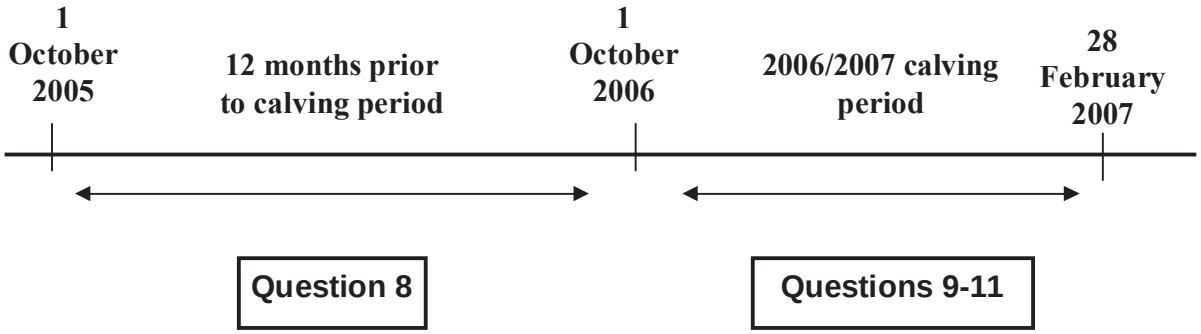
Please write the number for each age group

Species	Class	Number grazed on deer fenced area in 2007	Number scoured and/or wasted for any reason	Number confirmed with Johne's Disease by vet/slaughterhouse
Sheep	Ewes (4-tooth +)			
	Breeding rams			
	Hoggets/2-tooths			
	Lambs			
Beef Cattle	Cows (2+ years)			
	Bulls/steers(2+ years)			
	Yearlings (6-24 months)			
	Calves (0-6 months)			
Dairy cattle	Cows (2+ years)			
	Bulls/steers(2+ years)			
	Yearlings (6-24 months)			
	Calves (0-6 months)			
Goats	Total			
Alpacas/llamas	Total			

SECTION II: BREEDING HIND management: 2006/2007 calving

<u>NOTE:</u> If you did **<u>not</u>** graze breeding hinds in 2006/2007, please go to Section III on page 6

Questions 8-11 ask about the paddocks grazed by breeding hinds and calves in the 2006/2007 calving period. Please base your answers on the following time periods:



8. In the 12 months prior to the 2006/2007 calving period (i.e. from 1 October 2005 to 30 September 2006), what species were grazed on the paddock(s) that were later used for BREEDING HINDS AND CALVES over the 2006/2007 calving period?

☐ No species were grazed on breeding hind paddocks in the 12 months prior to the 2006/2007 calving period *Please go to question 9*

✔ *Please tick whether species were grazed on paddocks in the periods prior to 1 October 2006*

Species	Class	0-3 months prior	3-6 months prior	6-12 months prior
Sheep	Ewes			
	Rams			
	Hoggets/2-tooths			
	Lambs			
Beef Cattle	Cows (2+ years)			
	Bulls/steers(2+ years)			
	Yearlings (6-24 months)			
	Calves (0-6 months)			
Dairy cattle	Cows (2+ years)			
	Bulls/steers(2+ years)			
	Yearlings (6-24 months)			
	Calves (0-6 months)			
Goats				
Alpacas/llamas				

9. How many and what age class(es) of other species were <u>co-grazed</u> (i.e. *IN THE <u>SAME PADDOCK</u> at the <u>SAME TIME</u>*) with the breeding hinds and calves, within each month from 1 October 2006 to 28 February 2007?

☐ Did not co-graze breeding hinds and calves with any other species …… *Please go to question 10*

Please write the number you co-grazed with fawning hinds and fawns below

(***Example***: November 2006**:** *200 lambs and 50 dairy yearlings*)

NOTE: Please use same stock age class descriptions as listed in the table in <u>question 8, above</u>

October 2006: _____

November 2006: _____

December 2006: _____

January 2007: _____

February 2007: _____

10. How many times did you move your BREEDING HINDS WITH CALVES to a fresh paddock from 1 October 2006 to 28 February 2007?

Nil ☐ *Please go to question 12*

1-5 times ☐

5-10 times ☐

10+ times ☐

11. How many and what age class(es) of other species were <u>alternately grazed</u> (i.e. on the SAME PADDOCKS but at <u>*DIFFERENT TIMES*</u>) to the breeding hinds from 1 October 2006 to 28 February 2007?

Please write the number and age class

(***Example***: January 2007**:** *55 beef yearlings*)

NOTE: Please use same stock age class descriptions as listed in the table in the <u>table in question 8</u>

October 2006: _____

November 2006: _____

December 2006: _____

January 2007: _____

February 2007: _____

<u>Section III. WEANER (0-12 month old) deer management: 2007</u>

<u>NOTE:</u> If you did **<u>not</u>** graze weaner deer in 2007, please go to question 18 on page 9

12. If you had breeding hinds calving over 2006/2007, on what date(s) did you wean their progeny in 2007 (e.g. 15th March)?

☐ No calves were weaned on-farm in 2007 …… *Please go to question 13*

Please list the weaning date(s)

Date(s): _____

13. In what month(s) were purchased WEANERS brought onto your farm in 2007?

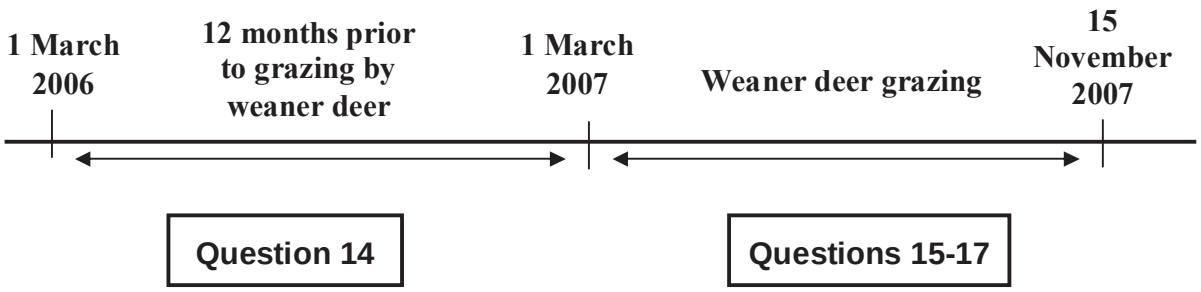
☐ No weaner deer were purchased in 2007 ……*Please go to question 14*

✔ *Please tick all that apply*

February 2007	☐	July 2007	☐
March 2007	☐	August 2007	☐
April 2007	☐	September 2007	☐
May 2007	☐	October 2007	☐
June 2007	☐	November 2007	☐

…… ..

Questions 14-17 ask about the paddocks grazed by weaner deer from 1 March 2007 to 15th November 2007. This includes paddocks grazed by weaners still with their dams after 1 March 2007. Please base your answers on the following time periods:



14. What species were grazed *up to 12 months prior* to 1 March 2007 (i.e. from 1 March 2006 to 28 February 2007) on paddock(s) which were later used for WEANER DEER in 2007?

☐ Only deer were grazed between 1 March 2006 and 28 February 2007 on paddocks later grazed by weaner deer in 2007…… *Please go to question 15*

✔ *Please tick which species were grazed on paddocks in the periods prior to 1 March 2007*

Species	Class	0-3 months prior	3-6 months prior	6-12 months prior
Sheep	Ewes			
	Rams			
	Hoggets/2-tooths			
	Lambs			
Beef Cattle	Cows (2+ years)			
	Bulls/steers(2+ years)			
	Yearlings (6-24 months)			
	Calves (0-6 months)			
Dairy cattle	Cows (2+ years)			
	Bulls/steers(2+ years)			
	Yearlings (6-24 months)			
	Calves (0-6 months)			
Goats				
Alpacas/llamas				

15. How many and what age class(es) of other species were co-grazed (i.e. *in the SAME PADDOCK at the SAME TIME*) with the weaner deer, within each month between 1 March 2007 and 15 November 2007?

Please write the number you co-grazed with weaner deer

(***Example***: August 2007**:** *20 does*)

NOTE: Please use same stock age class descriptions as listed in the table in question 8

March 2007: _____

April 2007: _____

May 2007: _____

June 2007: _____

July 2007: _____

August 2007: _____

September 2007: _____

October 2007: _____

November 2007: _____

(Massey) Farm number:

16. **How many times did you move your WEANER DEER to graze on a fresh paddock from 1 March 2007 to 15 November 2007 (even if they were still with their dams)?**

Nil ☐ *Please go to question 18*

1-5 times ☐

5-10 times ☐

10+ times ☐

17. **How many and what age class(es) of other species were <u>alternately grazed</u> (i.e. on the SAME PADDOCKS but at *DIFFERENT TIMES*) to the WEANER DEER from 1 March 2007 to 15 November 2007?**

Please write the number and age class

(***Example***: June 2007**:** *10 rams and 55 beef yearlings*)

March 2007: _____

April 2007: _____

May 2007: _____

June 2007: _____

July 2007: _____

August 2007: _____

September 2007: _____

October 2007: _____

November 2007: _____

Thankyou for completing this survey: just one more important question!

18. **<u>Future Research:</u>**

The participation of deer owners/managers is essential to the success of Deer Industry supported research at Massey University into Johne's Disease and other diseases of importance to the deer industry. This participation may include access to herds for field work or completion of questionnaires such as this one. If you would agree to be included in a list of deer herd owner/managers held by the Deer Research Group at Massey University, which may be invited to contribute to disease research in deer in the future, please tick the box below. Your inclusion will remain confidential at all times and your contact details will not be used for any other purpose or provided to a third party for any reason.

I agree to my contact details being provided ☐

NOTE: This is not binding. Any future involvement will be voluntary and entirely at your discretion

Thank you once again for your time.

APPENDIX 4: Johne's Management Limited Operations Manual

Table of Contents:

1. What is Johne's Disease and why is everyone so concerned?

Johne's disease (JD) is a chronic (long-standing) disease of the gut found in all ruminant and camelid species including cattle, sheep, deer, alpacas and llamas. It is caused by the bacteria *Mycobacterium avium* subspecies *paratuberculosis*, or MAP for short, which is closely related to *Mycobacterium bovis*, which causes TB. Some animals infected with MAP will develop visceral lymph node lesions which are indistinguishable from TB lesions on visual inspection. These animals, when alive, may or may not show typical clinical signs of Johne's Disease, such as chronic diarrhoea, emaciation and a rough coat. There is no cure for this disease and it is emerging as a serious problem on many deer farms in New Zealand.

Figure 1: **Yearling hind with clinical Johne's Disease. Note the diarrhoea/scour on the hocks and perineum and the prominent backbone**

Source: Jaimie Glossop

Johne's Disease is a serious concern to the New Zealand deer industry because:

- It causes problems in deer slaughter premises as lesions due to Johne's Disease look exactly like TB on gross inspection
- It can cause significant production losses and deaths of deer, particularly in weaners and yearlings
- There are significant welfare issues when deer become very thin
- Johne's disease interferes with current TB skin-testing protocols
- It is difficult to control on-farm
- There are negative perceptions from a few international sectors concerning a possible association between Johne's disease and human health issues. There has not been a causal link established between MAP and clinical disease in humans, however.

2. What is JML?

JML stands for Johne's Management Limited and is a company owned by New Zealand deer processors. Deer Industry New Zealand is the sole shareholder. The goal of JML is:

> ***"To provide data to industry and researchers enabling effective targeting of education and monitoring of Johne's Disease in Deer"***

JML will achieve this by monitoring all deer processed and by recording every carcass which contains lymph node lesion(s) which could be due to infection with MAP. All premises which slaughter deer in New Zealand have agreed to participate in the collection of data for the JML database.

The definition of a "suspect JD lesion" for the purposes of JML is outlined below. Properties which slaughter lines with a significant number of "lesions" will be notified and offered information and resources for the effective management of Johne's Disease on their property. Data will also be available on a confidential basis to the Epicentre at Massey University for research into Johne's Disease in deer.

2. What is do I have to do?

Meat inspectors are asked to capture key data relating to "lesions" which will allow effective monitoring.

"Suspect JD lesion" definition:
The definition of a "suspect JD lesion" which is required for the JML database:

Any lymph node which appears abnormally increased in size regardless of the believed cause of that enlargement

A JML "suspect JD lesion" will **not** include[1,2]:

a. an abscess/pyogenic lesion (ie: the lesion is accompanied by a strong putrefactive smell)
b. lumpy jaw (ie: submaxillary or parotid lymph node lesion with an actinoform lesion in the upper or lower jaw)
c. an ocular squamous cell carcinoma (and the lesion is in the parotid and/or atlantal lymph nodes)
d. a skin lesion of any kind
e. bruising
f. arthritis
g. wounds
h. injection/vaccination site lesions
i. peritonitis
j. pleurisy/broken rib
k. septicaemia
l. orchitis/ epididymitis
m. facial eczema
n. emaciation

This definition does not rule out lymph node enlargement due to conditions such as *M.bovis*, *M.avium* and Actinobacillus. The herd owner will, therefore, be informed of significant numbers of "lesions" only (ie: not a specific diagnosis) and determination of the exact cause of the lesions will be part of an on-farm management program.

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[1] As outlined in PM-13: Suspect Tuberculosis Sampling (07/08/2006)
[2] As outlined in Section 6.4 (Disease and Defect Recording Requirements), Manual 16: Post-mortem Inspection Procedures (October 2005)

Although JD lesions are most commonly located in the mesenteric lymph nodes (eg: Figure 2), they can also occur in other abdominal locations (eg: splenic and hepatic lymph nodes) and in the head and thoracic lymph nodes (eg: retropharyngeal lymph nodes). To help JML determine the most likely cause of the lesion, any "lesions" under the JML definition are to be recorded into one of three categories:

Category 1: GM (gut mesenteric) – lesions in the mesenteric and ileo-caecal lymph nodes only

Category 2: GO (gut other) – lesions in abdominal lymph nodes other than the mesenteric and ileo-caecal lymph nodes (eg: hepatic)

Category 3: HT (head/thoracic) – lesions in head and/or thoracic lymph nodes

Listed below are the tissues which apply specifically to Categories 1, 2 and 3. The inspection protocol referred to below is the same as listed in Appendix 3 of Manual 16.

Category 1: Gut mesenteric and/or ileo-caecal lymph nodes

Lymph nodes	Inspection protocol
Mesenteric	View and palpate
Ileo-caecal	Not in Appendix 3 (Manual 16)

recommend view

Category 2: Gut other (than mesenteric or ileo-caecal lymph nodes)

Lymph nodes	Inspection protocol
Hepatic	Incise
Iliac	View
Renal	View
Precrural/Subiliac	Palpate

Category 3: Head and thoracic

Lymph nodes	Inspection protocol
Left bronchial	Incise
Right bronchial	Incise
Mediastinal	Incise
Parotid	Incise

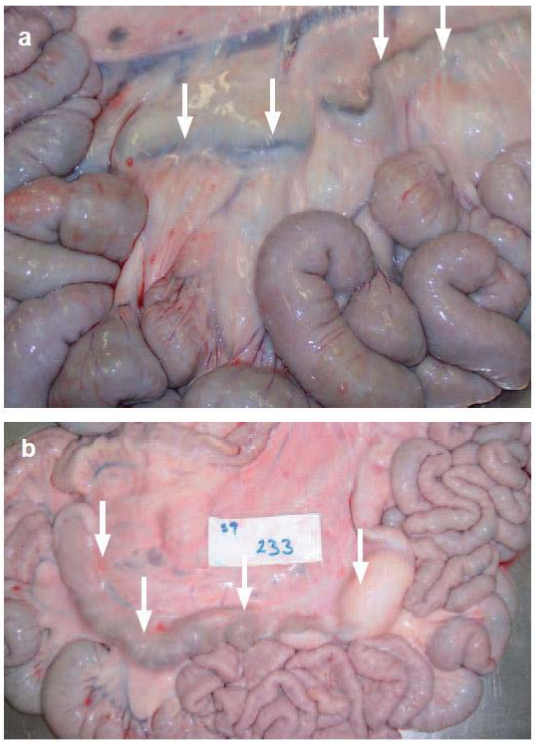


Figure 2: **An example of normal mesenteric lymph node chain (a) and swelling ("piping") of the mesenteric lymph node chain (b)**

Photos courtesy of: Colin Mackintosh

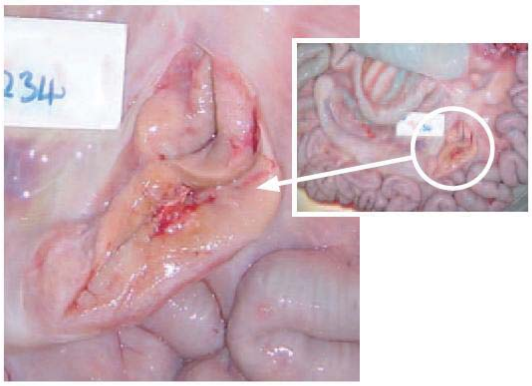


Figure 3: **A JML "suspect JD lesion" may be oedematous without visible caseation, necrosis or mineralization (insert: mesenteric lymph chain "piping" with location of incision)**

Photo courtesy of: Colin Mackintosh

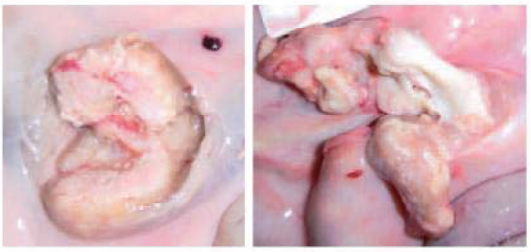


Figure 4: **JML "suspect JD lesions" include oedematous lymph nodes with central caseation and necrotic material should be recorded as JML "suspect JD lesions" regardless of believed cause**

Prescapular/ Superficial Cervical	Incise
Medial retropharyngeal	Incise
Submaxillary/ Mandibular	Incise
Apical	Incise
Atlantal/Lateral retropharyngeal	Incise

Multiple lesion locations
If there are visceral lymph node lesions in multiple location categories in one carcass record the lesions in the following priority:

1. Category 1 (GM) i.e "JD suspect" lesions in categories 1+2+3, 1+2 or 1+3
2. Category 3 (HT) i.e "JD suspect" lesions in categories 2+3

Sampling of lesions as suspicious for TB
If you have sampled an enlarged lymph node on suspicion for TB (eg: Figure 4), please also record the lesion's location under category 1, 2 or 3 as a JML "Suspect JD lesion", regardless of whether you believe the lesion is typical for TB.

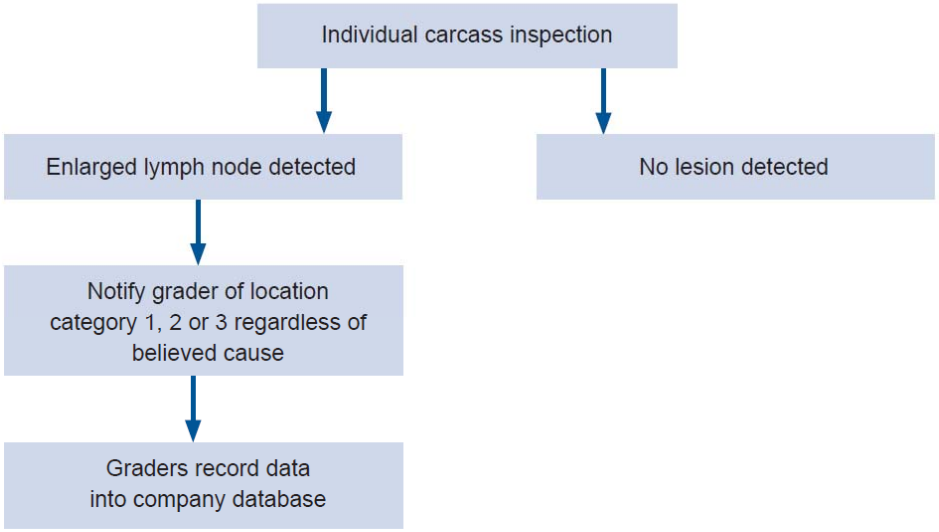
"Suspect JD lesions" can be recorded in the JML database by either:

1. **Informing the grader.** He/she can then enter the data directly into the touchpad/keyboard
2. Applying the **appropriate ticket** (if used) to the carcass which will then be read by the grader

The system of JML database recording will vary between slaughter plants depending on the distance between the inspector and grader and the presence of touchpads on the slaughterboard. Contact your supervisor to find out the system used at this plant.

Record *every* "suspect JD lesion" from *every* deer carcass inspected regardless of number affected per line

A flowchart of carcass inspection incorporating the JML database:



You are not required to sample a lesion you do not believe is suspicious for TB

4. What will happen to the data once collected?

JML aims to reduce the prevalence of carcass lesions at slaughter due to Johne's Disease by:

i) farmer feedback
ii) research

i) Farmer feedback:

The data will be collated from deer slaughter premises and from Animal Health Board's DMIS database (ie: sampled lesion data). Properties which have a significant number of "suspect JD lesions" will be informed and appropriate resources made available to them to:

i) determine the most likely cause of the lesions
ii) if appropriate, allow development of a JD management plan specific to their property.

ii) Research:

Data will also be provided to the Epicentre at Massey University. Under the privacy act, the herd owner's contact details will not be provided to the researchers and individual farm results will not be available through published results. The Massey University research team aims to:

a) monitor the regional and national prevalence levels of suspect JD lesions under the JML definition
b) determine the effect of subclinical Johne's Disease on production characteristics such as carcass weight
c) recommend and monitor the effectiveness of management programs specifically for New Zealand farmed deer

5. Who should I contact if I have questions?

If you have any questions or comments concerning the JML Database, please contact either:

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6. Privacy

JML has developed a protocol in association with participating processors to ensure the requirements of the Privacy Act 1993 are met. All data collected for the JML database is confidential to the supplier of the stock. Any information released as a consequence of this program will not identify individuals or farms.

7. JD manual

The Johne's Disease manual, which was produced by the Johne's Research Group (JRG) in 2006, is an invaluable resource for information specific to Johne's Disease in New Zealand farmed deer. If this folder does not include a copy of the manual please contact Mr Dan Lynch.

8. Acknowledgements

JML would like to acknowledge Dr Colin Mackintosh (AgResearch Invermay) for allowing use of the photographs included in this document.