

**Epidemiology of *Mycobacterium avium* subspecies
paratuberculosis infection on sheep, beef cattle and deer
farms in New Zealand**

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Summary

Paratuberculosis (Ptb) is a chronic enteric infection caused by *Mycobacterium avium* subspecies paratuberculosis (MAP), affecting wild and domestic ruminants. In domestic ruminants MAP infection is largely sub-clinical, but can result in chronic diarrhoea leading to emaciation and death. Clinical disease is commonly observed in adult cattle and sheep but in deer the disease incidence is higher in young animals (8-12 months). In the New Zealand pastoral farming system, it is common practice to co-graze Ptb susceptible livestock species (sheep, cattle, and deer) together, either concurrently or successively, on the same pasture. Thus several susceptible species have contact at farm level, being at risk of transmitting MAP between species through contaminated pasture. Johnes' Disease Research Consortium (JDRC), a partnership between livestock industries, government and research providers was created to study Ptb in an overarching approach, involving all susceptible species, aiming to generate scientific knowledge to support Ptb control policies.

The present research was implemented under the financial support of JDRC, aiming to generate epidemiological information about Ptb infection and clinical disease on mixed-species pastoral farms, grazing sheep, beef cattle, and/or deer. A total of 350 mixed-species farms (11,089 animals) were faecal and blood sampled and related epidemiological information was collected. Data was used to estimate: i) the national herd level true prevalence (HTP) of MAP infection on sheep, beef cattle and deer, ii) the risk of MAP infection and clinical disease incidence associated with species co-grazing,

iii) the association between infected and affected herds/flocks and production outputs, and iv) relationships between molecular strain types of MAP isolates and their distribution across livestock sectors and geographical areas. Finally, data and results from previous studies allowed v) the development and calibration of a two host-species (sheep & beef cattle) mathematical model, simulating MAP transmission between species and the effect of several control measures under mixed species farming.

MAP infection is widely spread in New Zealand. A Bayesian analysis to account for lack of sensitivity (Se) and specificity (Sp) of testing protocols, indicated that the highest HTP estimate for sheep flocks (75%, posterior probability interval (PPI) 68-82%), followed by deer (46%, PPI 39-54%) and beef herds (43%, PPI 35-51%). Sheep and beef cattle flocks/herds presented a higher prevalence in the North Island (NI), whereas deer infection was mainly located in the South Island (SI).

Logistic and Poisson regression models using Bayesian inference to adjust for lack of Se and Sp of diagnostic tests and of farmer's recall of clinical Ptb indicated that the shared use of pasture was associated with Ptb prevalence and incidence. When beef cattle and sheep were co-grazed, the infection risk increased 3-4 times in each species. Similarly, co-grazing of beef cattle and deer increased 3 times the risk of infection on deer. Co-grazing beef cattle with sheep, or beef cattle with deer, also was associated with increased clinical incidence in these species. Conversely, the co-grazing of sheep and deer was associated with a lower clinical disease incidence in both species.

Classical logistic and Poisson regression models indicated that MAP 'infection' status was significantly ($p = 0.03$) associated with reduced calving rates in beef cattle herds and lower culling rates in deer herds and sheep flocks. Moreover, in sheep flocks and deer herds, a significant and a marginally significant ($p = 0.05$ and 0.09 , respectively)

association were observed between ‘affected’ flocks/herds and lower tailing rates in sheep and weaning rates in deer, respectively.

Molecular analysis of MAP isolates obtained from sheep, cattle (beef and dairy) and deer, using a combination of the variable number of tandem repeats (VNTR) method and the short sequence repeat (SSR) method, rendered 17 MAP subtypes. Analysis indicated significantly higher subtype richness in dairy cattle and livestock sector as the main source of subtype variation. Moreover, similar subtypes were sourced from sheep and beef cattle, which tended to be different to the ones obtained from other livestock sectors. However, when beef cattle and deer were both present on the same farm, they harboured similar subtypes. These results provided strong evidence for transmission of MAP between species through the joint use of pasture.

Simulation results of a mathematical infectious disease model for Ptb indicated that the length of the co-grazing period was positively associated with the infection prevalence of sheep and beef cattle. Long pasture spelling periods from 9 to 15 months reduced MAP contamination up to 99%. However, the infection of naïve animals was still possible, but the prevalence remained <1% for at least 25 years. The simultaneous application of control measures on both species was the most efficient approach to reduce the prevalence and incidence. The separation of co-grazed species in tandem with an increased farmer surveillance, to reduce the time that clinical animals remained on the farm, was most effective in sheep, whereas T&C was in beef cattle.

The present research provides evidence that MAP infection is highly endemic in New Zealand farming livestock, and that the clinical disease incidence is generally low (<0.5%) in most infected farms. Moreover, inference from molecular pathogen typing of strategically collected isolates from farms across New Zealand strongly suggested that MAP is transmitted between species, mainly from sheep to beef cattle and between

beef cattle and deer, all of which are commonly grazed together in the New Zealand pastoral farming system.

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Probably this is the last part that everyone writes in their thesis. However, it is the most important part of the study because at this point you should have realized that carried out a PhD thesis; it is an impossible endeavour without the help and collaboration of other people. In my particular case, I have to say that the list of people that I have to give my most sincere thanks is quite long and without them, probably I would not be writing these words.

First, I have to acknowledge to my parents, without them I would not be here (technically) and this thesis would not have been written. I am not saying that this research would not have been done, I am pretty sure that there are several people more qualified and smarter than me, that would had been interested in take this challenge. However, it would not have been the same because this thesis reflects in certain way, the person that I am. And please do not think about the previous lines as a pointless exercise of self-adoration, rather my intention is totally the opposite. The person that I am is the outcome of all people that I have met through my life, and to all of you I am very thankful. You have made me a better person, I look back and I think that I have been lucky. During my PhD studies I have not just grown up as a professional or scientist, I have also grown up as a human being. Sorry if I am boring you but this is the only part (in more than 300 pages), where I have been allowed to divagate with my thoughts, so I am taking the opportunity.

Obviously, it is impossible to name all the people here, and I say sorry if I have omitted your name here. As I said before, my parents deserve a special thanks, despite of their humble origin they always teach me that I could be better person, that I could achieve any goal I propose and never (ever) give up. Paulina, probably the only person that really knows me, you have been my friend, my adventure partner, my wife and now the mother of my child. I am very thankful for met you, for all your support. Paulina, you are the perfect balance of my life. To my family also a very special thanks, independently that they never have understood what exactly I have been doing, they always have been there supporting and helping me.

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Probably, at this point I got you totally bored, but in my defence I have to say that I trimmed the best jokes (self-censure) because they were just too politically un-correct. After this long acknowledgement section, I am pretty sure that you have understood why this thesis has more than 300 pages.

Nomenclature

ACI	Annual clinical incidence
AFLP	Amplified fragment length polymorphism
AGID	Agar gel immunodiffusion
AMOVA	Analysis of molecular variance
AP	Apparent prevalence
AVLN	Abnormal visceral lymph nodes
BTM	Bulk tank milk
CD	Crohn's disease
CF	Complement fixation
CFU	Colony-forming unit
CG	Co-grazing
cPtb	Clinical paratuberculosis
DFA	Deer fenced area
DI	Diversity index
DIC	Deviance Information Criterion
Env	Simulation model environmental component
FC	Faecal culture
FTS	Farm type strata
GLM	Generalized Linear Models
HAP	Flock/herd level apparent prevalence

HPVj.neg	Joint herd-level predictive value negative
HPVj.pos	Joint herd-level predictive value positive
Hse	Herd-sensitivity
Hsej	Joint herd-level sensitivity
Hsp	Herd-specificity
Hspj	Joint herd-level specificity
HTP	Flock/herd-level true prevalence
IME	Individual milk ELISA
JDRC	Johne's Disease Research Consortium
LAM	Lipoarabinomannan
LIC	Livestock Improvement Corporation
MAC	Mycobacterium avium complex
MAP	Mycobacterium avium ssp. Paratuberculosis
MCMC	Markov Chain Monte Carlo
MPIL	Multiplex PCR of IS900 integration loci
NI	New Zealand North Island
OR	Odds Ratio
PCR	Polymerase chain reaction
PFC	Pooled faecal culture
PFGE	Pulsed field gel electrophoresis
POPR	Posterior probabilities
PPI	Posterior probability interval
PSI	Proportional similarity index
Ptb	Paratuberculosis
RFLP	Restriction fragment length polymorphism

RR	Relative risk
Se	Sensitivity
SI	New Zealand South Island
Sp	Specificity
SSR	Short sequence repeats
T&C	Test & cull
Th1	T-helper 1
TP	True prevalence
VNTR	Variable number of tandem repeats

List of Publications

Salgado M, Verdugo C, Castillo P, Zamorano P, Heuer C. A novel low cost method for *Mycobacterium avium* subsp. *paratuberculosis* DNA extraction from an automated broth culture system for real time PCR confirmation. *Journal of Veterinary Science*, *accepted for publication*, 2013.

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Preface

“If everyone is thinking alike, then no one is thinking.”
— Benjamin Franklin

“I can not change the world but I can change myself.”
— Alejandro Jodorowsky

“- How long will take to learn this art?
- A life, maybe a little more.”
— Choi Hon Hi

Introduction

Originally, H. A. Johne and L. Frothingham in 1895 described the case of a cow, which was not responsive to veterinarian treatment and presented tubercular bacilli at intestinal level. However, the observed bacilli differed from bacilli normally (at that time) observed in cattle, resembling to those found in chickens with tuberculosis. A few years later Bang (1906) reproduced the disease by inoculating calves with intestinal scrapings sourced from an animal with similar signs as the ones previously mentioned by Johne. Professor Bang named the disease as pseudo-tuberculosis, and McFadyean in the 1907 annual report for the Principal of the Royal Veterinary College used the name Johne's disease (Prof. S.S. Nielsen, personal communication). Since then, Johne's disease, currently denominated as Paratuberculosis (Ptb), has been the name commonly used to describe a chronic enteropathy which affect mainly domestic and wild ruminants (Harris and Barletta, 2001). The causal agent is *Mycobacterium avium* ssp. *paratuberculosis* (MAP), which is a slow-growing, gram positive, facultative intracellular, mycobactin J dependant, acid-fast bacteria (Motiwala et al., 2006b). MAP targets the terminal ileum, invading the host through the Peyer's patches, where they are phagocytosed by subepithelial and intraepithelial macrophages (Momotani et al., 1988; Fujimura and Owen, 1996; Lugton, 1999). This bacterium has the capacity to survive and reproduce inside of macrophages. The host immune response could control the infection or eventually produce an intestinal granuloma, presenting a typical corrugated intestinal epithelium at post mortem inspection. This inflammatory process leads to a protein-

losing enteropathy, which in its terminal stages is characterized by diarrhoea, ill-thrift, muscle wasting and finally death or culling (Harris and Barletta, 2001). However, several years could elapse between infection and onset of clinical signs; hence most infected animals remain subclinical for life (Nielsen and Toft, 2008). In dairy cows, paratuberculosis has been associated with production losses such as decreased milk yield, pre-mature culling, reduced carcass value, and impaired fertility and ability to rear progeny (Benedictus et al., 1987).

The epidemiology of Ptb is complex, characterized by a long incubation period, the ability to infect and survive in multiple mammalian hosts, the ability of MAP to evade host immune response, a latent period of a few months to several years, and a long survival of MAP in the environment. These features in addition to the lack of reliable test have hampered eradication attempts, and control programs have had only moderate success, despite of being implemented in developed countries that have the resources and capabilities for effective disease control (Sweeney, 2011). Paratuberculosis is present in all continents with livestock populations and in New Zealand has been diagnosed in all ruminant species (de Lisle, 2002). A particular characteristic of New Zealand farming is the presence of multiple livestock species at farm level, such as sheep, beef cattle (dairy to a lesser extent) and deer. Thus several susceptible species are in direct or indirect contact with each other through the grazing of contaminated pasture, generating opportunities for cross-species transmission of MAP. With the aim of developing options for herd control of Ptb, the New Zealand Johne's Disease Research Consortium (JDRC) was initiated. This initiative is a partnership between livestock industries, research providers and government for the research and control of Ptb (JDRC, 2013).

The research reported in this thesis has been implemented with financial support of JDRC and in collaboration with at AgResearch Ltd., Livestock Improvement Ltd., Otago University, and research collaborators of the Sydney University, University of California (Irvine), and Cornell University. The main objective was to gain epidemiological insight of MAP infection dynamics and clinical paratuberculosis (cPtB), in New Zealand commercially farmed sheep, beef cattle and deer. This thesis describes five epidemiological studies presented in the format of scientific papers for submission for publication to peer-reviewed scientific journals.

The first study, estimated the herd true prevalence (HTP) of MAP infection of three livestock species (sheep, beef cattle, deer), was based on a New Zealand wide stratified-random survey, collecting blood and faecal samples and using two diagnostics test (culture, ELISA). Results were adjusted using a novel latent class Bayesian model to account for lack of sensitivity (Se) and specificity (Sp). The second study is herd level assessment of the transmission of MAP across species on farms grazing single or mixed livestock species in isolation or jointly, and estimating its impact on prevalence of MAP infection and incidence of cPtB. In this study, farming system variables were evaluated using error adjusted logistic and Poisson regression models, based on Bayesian inference. This approach considered estimates of herd-sensitivity (Hse) and herd-specificity (Hsp) obtained in the previous chapter. The third study correlated survey questionnaire responses about farm production parameters with levels of MAP infection and clinical affection. The outcomes of interest were rates of pregnancy, culling, tailing, calving, and weaning at herd or flock level in sheep, beef cattle and deer. The fourth study is a molecular survey of MAP isolates sourced from four productive sectors

(sheep, deer, beef cattle, dairy cattle). Samples were strain typed using a combination of two molecular techniques (Collins et al., 2012), i.e. variable number of tandem repeats (VNTR) and short sequence repeats (SSR). MAP strain subtypes were analysed using a variety of molecular statistical techniques to assess their relative richness, association with host and geographical sources. Finally, in the last study, a novel two-species mathematical model is described for the study of MAP cross-species transmission simulating different grazing management options and disease control interventions. Specifically, the model simulated a farm with a sheep breeding flock and a beef breeding herd under different co-grazing regimens, where transmission occurred indirectly through contaminated pasture as the main source of infection.

The thesis begins with a review of previous paratuberculosis research. The review focused on relevant topics that will be covered on each of the scientific papers generated in the present thesis. Particular topics involve infection prevalence, production effects, genetic aspects of MAP strains, transmission, and simulation models. The thesis concludes with a general discussion of the study findings, and knowledge gaps, which preclude a fully understanding of MAP epidemiology.

Literature review

2.1 Introduction

Paratuberculosis (Ptb), also known as Johne's disease (JD), was originally reported in Germany by H. A. Johne and L. Frothingham in 1895. However, Koch's postulates were not fulfilled until 1906 by Prof. B. Bang, who replicated the disease experimentally infecting calves with intestine scraps, sourced from a disease cow (Prof. S.S. Nielsen, personal communication). However, F. W. Trowt in 1910 grew *M. paratuberculosis* (MAP) in culture and reproduced JD in experimentally infected cattle, being commonly credited as the first who replicated the disease under controlled conditions (Chiodini et al., 1984; Harris and Barletta, 2001; de Lisle, 2002), demonstrating MAP as causative agent of JD. The agent was originally named *Mycobacterium enteritidis chronicae pseudotuberculosis bovis johne*, now known as *Mycobacterium avium ssp. paratuberculosis* or simply MAP (Rowe and Grant, 2006). Paratuberculosis is an untreatable chronic inflammatory infection of the intestines, which affects domestics and wild ruminants. Additionally, the causal agent has been retrieved from a wide range of hosts including foxes, stoats, possums, crows, humans, and rabbits (Motiwala et al., 2006b).

Paratuberculosis lesions are mainly located in the terminal jejunum, ileum and their regional lymph nodes (Payne and Rankin, 1961b). Infected animals could remain

subclinical for several years before the onset of clinical signs, which mainly involve chronic diarrhoea, weight loss and death or premature culling. Sub-clinical Ptb can be associated with a variable extent of production decrease (Berghaus et al., 2005). Despite already being discovered and described over 100 years ago, several epidemiological aspects of Ptb remained unknown due to its chronic nature, variable latent to clinical manifestations in infected animals and a lack of reliable diagnostics tests, all of which have hampered the development of conclusive research evidence. Additionally, the multi-host nature of the infection and the long survival of MAP in the environment have impaired disease control. Thus, sustainable eradication of the infection has not been achieved by any country or region worldwide (OIE, 2013).

Due to the lack of successful control, Ptb continues to spread with livestock trade. Consequently, the disease is being observed in the majority of countries on every continent, commonly in domestic ruminants, particularly dairy cattle, although wildlife species can also be affected (Chiodini et al., 1984; Greig et al., 1999; Beard et al., 2001a). In New Zealand, the first case was recorded in 1912 in an imported cow (de Lisle, 2002). The first reported case in sheep was in Canterbury in 1952. In the 1980s, Ptb was confirmed in farmed deer for the first time (de Lisle et al., 1993). The recent isolation of MAP from intestinal tissue of Crohn's disease (CD) patients, a human chronic inflammatory bowel disease, has suggested a possible link between the two diseases (Harris and Barletta, 2001). It has subsequently been postulated that human cases could be caused by the consumption of MAP contaminated animal produce or contact with ruminant livestock (Hermon-Taylor, 2009). Due to a possible impact on public health, this finding has raised concerns of agricultural industries about potential barriers for live animal trade or a decrease of the consumption of animal produce.

However, conclusive evidence has not been presented, thus the issue continues to be controversially discussed by the scientific community.

The present literature review does not intend to cover all aspect of MAP infection and Ptb. Instead, the focus is on the microbiology of MAP, host genetics, pathobiology, test diagnostics, prevalence distribution, production effects, and mathematical infection models simulating the dynamics of MAP transmission, in order to address epidemiological studies in the context of the thesis. Subjects such as control measures/programmes, experimental studies, financial assessments or the association between MAP and CD were deliberately excluded from the present literature review because those aspects were beyond the scope of the present research.

2.2 Microbiology and genetic aspects of *Mycobacterium avium* subsp. *paratuberculosis*

2.2.1 Microbiology of MAP

Mycobacterium avium subsp. *paratuberculosis* belong to the *Mycobacterium avium* complex (MAC), which also includes *Mycobacterium avium* subsp. *avium*, *Mycobacterium avium* subsp. *hominissuis*, *Mycobacterium intracellulare* and the wood pigeon bacillus (*Mycobacterium silvaticum*). Members of this complex are closely related exhibiting over 90% of similarity at nucleotide level. However, these microorganisms differ substantially in their host tropisms, microbiological phenotypes, and disease pathogenicity (Motiwala et al., 2006b). In addition to the high genetic similarity of this complex, MAP in itself appears to be a relatively homogeneous

population of subtypes, exhibiting little genetic diversity compared with other bacterial pathogens (Stevenson et al., 2009). MAP is a gram positive, facultative intracellular, mycobactin J dependant, acid-fast bacterium. It is found in clumps, entangled with each other by a network of intercellular filaments (Merkal et al., 1973). Some characteristics that distinguish this bacterium from other members of the MAC are: its extremely slow growth under *in vitro* conditions, lack of mycobactin, and the presence (and number of copies) of the insertion element IS900 (14–18 copies within the genome) (McFadden et al., 1987; Green et al., 1989). Additionally, MAP presents a thick lipid-rich cell wall making up around 40% of the total dry weight of the organism. However, cell wall deficient (CWD) forms of MAP have been observed, where the cell wall could be totally (Protoplasts), or partiality (Spheroplasts) absent. The CWD forms of MAP can be induced under *in-vitro* conditions from the bacilli form of MAP, although also have been isolated, *in-vivo*, from human patients suffering chronic diseases such Crohn's disease and ulcerative colitis (Beran et al., 2006). Currently, it is unknown the specific role (if they play a role) that CWD forms of MAP could play in those disease. This is partially explained by the great difficulties associated to the handling of CWD-MAP samples (Rosu et al., 2013).

The solid cell wall, present in the bacilli form of MAP, increases their resistance to chemicals (Whan et al., 2001) and physical processes (Grant et al., 1996; Grant et al., 1998). Specifically, the cell wall has been associated with the ability of MAP to invade the host, and to survive and proliferate within macrophages, which are their target cells (McFadden, 1992). The cell wall presents several features of particular relevance for the host invasion by MAP (McFadden, 1992): First, embedded in the cell wall, there are active components such as Lipoarabinomannan (LAM), cord factor, macrophage

inhibitory factor, and superoxide dismutase. LAM is responsible for suppressing macrophage activation and T-cell stimulation, thus delaying host immune response (Sibley et al., 1988; Chan et al., 1991; Barrow, 1997). The other active components present in the cell wall are involved with MAP survival within the phagosome through the detoxification of reactive oxygen intermediaries (Barrow et al., 1995; Daffe and Etienne, 1999). Additionally, attached to the surface of the cell wall, there is a barrier formed by glycopeptidolipids, which may confer a physical protection of MAP against phagosome enzymes (Barrow, 1997). Finally, embedded in the cell wall, there are fibronectin attachment proteins. These proteins are also present in other members of MAC, and current knowledge indicates that they are responsible for the binding of MAP to M-cell (or microfold cells), which are found in the follicle-associated epithelium of the Peyer's patches, thus allowing MAP translocation across the epithelial barrier via Peyer's patches (Clark et al., 1998; Secott et al., 2004).

As said earlier, MAP is a multi-host pathogen, affecting mainly domestics and wild ruminants. Although, it has been recovered from species like foxes, stoats, crows, humans, and rabbits, Lagomorphs (rabbit, hares) are the only non-ruminants species that have presented evidence of clinical disease, based on the observation of gross or microscopic lesions associated with acid fast bacteria (Beard et al., 2001b). Despite of the multi-species nature of MAP, the transmission among susceptible species or the potential role of wildlife species in the MAP epidemiology is poorly understood.

2.2.2 Genetic aspect of MAP strains

Restriction fragment length polymorphism (RFLP) has been the most commonly used technique for strain typing of MAP isolates (Thibault et al., 2007). In this method,

fragments of MAP DNA are extracted by restriction enzymes. Fragments are then examined for similarities, differences, and their ability to be allocated into groups (Collins et al., 1990). However, this method presents several inconveniences, as it requires relatively large amounts of DNA, taking several months to harvest enough genetic material. Additionally, outcomes from this technique are difficult to standardize, hindering inter-laboratory comparisons (Thibault et al., 2008), and provides low discriminatory power for the differentiation of MAP isolates (Motiwala et al., 2006b). Despite of these shortcomings, RFLP technique has been able to identify two major groups of MAP strains, denominated Type I and II, originally named Type S (or ovine strain) and Type C (or bovine strain), respectively. The original denominations were coined based on the livestock species from which they were commonly sourced (Collins et al., 1990; Bauerfeind et al., 1996; Sevilla et al., 2005), assuming a strong host adaptation of MAP. A two-step method has been used for distinguishing between MAP Type I and II isolates, using polymorphic differences in the insertion sequence IS1311 (Marsh et al., 1999). The first step involves polymerase chain reaction (PCR) targeting the insertion sequence IS900 to confirm the presence of MAP, because the IS1311 is also present in other members of the MAC. After MAP has been confirmed, a second PCR assay targets the insertion sequence IS1311, whose product is then subjected to restriction endonuclease analysis (Whittington et al., 2000a). Collins et al. (2002) developed a rapid PCR assay, which directly distinguished MAP Type I and II after MAP confirmation. Some differences between these two MAP types are: Type I strains comprise a very slow-growing and predominately pigmented isolates which form smooth, uniform colonies (Dohmann et al., 2003). Instead, Type II strains include faster growing, non-pigmented isolates, which form rough, non-uniform colonies (Stevenson et al., 2002). However, recent studies have indicated that differences in growing speed

between Type I and II are related to differences in culture media requirements between them (Whittington et al., 2011). Through RFLP analysis a third MAP group has been identified denominated Type III or intermediate or I-type (Moebius et al., 2009). This group is considered a subtype of MAP Type I, and consists of non-pigmented bacterium (Stevenson et al., 2009). Type III has rarely been isolated, and available isolates were obtained from sheep samples in Canada, South Africa, Iceland, (Collins et al., 1990; de Lisle et al., 1992; de Lisle et al., 1993), and goats and bullfighting cattle samples from Spain (de Juan et al., 2005; Castellanos et al., 2009). No record of its isolation in New Zealand has been ever published.

The existence of MAP strain differences between sheep and cattle has also been proposed on the basis of epidemiological evidence from Scotland, Australia, and New Zealand (Collins et al., 1990). Whittington et al. (2000a), using 328 MAP isolates from different geographic locations of Australia, observed that almost without exception and regardless of geographic location, isolates from sheep have been classified as Type I, whereas isolates from cattle have been Type II. Similarly, the vast majority of isolates from goats and deer were classified as Type II (de Lisle et al., 2003; O'Brien et al., 2006). However, a few Type I strains were isolated from goats, deer and occasionally from cattle (de Lisle, 2002). A single case study documented the isolation of two different MAP strains from the same animal, implying the possibility of co-infection (Harris and Barletta, 2001). The observed differences between strains sourced from sheep and cattle could be related to a productive separation between this two species, rather than a true host specialization (Motiwala et al., 2006b). In this line, a comparative molecular study across seven European countries did not classify any isolates from sheep as Type I, whereas Type II was retrieved from a wide range of hosts, including

sheep, goats, and cattle (Stevenson et al., 2009). Although epidemiological evidence is not conclusive, it supports the view that MAP host specificity is not 100%, and the frequency and impact of cross-infection under natural exposure is still unclear. The Australian Johne's disease control and assurance programs assume that sheep and cattle strains of MAP cause epidemiologically distinct infections (Moloney and Whittington, 2008).

MAP strains present relative little genetic variation, thus population-based molecular analysis, using other traditional molecular techniques, beside RFLP, such as Multiplex PCR of IS900 integration loci (MPIL), amplified fragment length polymorphism (AFLP), pulsed field gel electrophoresis (PFGE) have provided moderate differentiation between MAP isolates. This limited discriminatory power of MAP strain typing techniques has precluded the study of associations between MAP strains and geographic distribution, pathogenicity or host affinity. Thus, molecular analysis of MAP strains requires the use of multiple genetic techniques to increase the discriminatory power in order to conduct meaningful epidemiological studies (Stevenson et al., 2009). The whole genome sequencing of the MAP strain K-10 (Li et al., 2005), has led to the development of new PCR-based methods for MAP strain typing (Harris et al., 2006). At present, the two most commonly used techniques are multiple short-sequence repeats (SSR) (Amonsin et al., 2004) and Mycobacterial Interspersed Repetitive Units Variable-Number Tandem Repeats (MIRU-VNTRs) (Thibault et al., 2007). These techniques were compared with traditional strain typing techniques such MPIL, AFLP and RFLP, and found to be more powerful strain typing techniques with a higher discriminatory power (Motiwala et al., 2006b; Thibault et al., 2008). They target specific loci in the MAP genome, indexing the number of copies of specific genetic polymorphic structures

(MIRU-VNTR) or simple homopolymeric tracts of single, di- or trinucleotides (SSR) (Thibault et al., 2008). Amonsin et al. (2004) recognized and evaluated 11 polymorphic SSR loci, named SSR-1 to SSR-11. Thibault et al. (2007) identified and assessed 8 polymorphic MIRU-VNTR loci (MIRU-X3, 292 and VNTR-3, 7, 10, 25, 32 and 47). The use of both techniques in tandem had an additive discriminatory property, rendering it a more powerful strain typing method (Thibault et al., 2008).

Previous studies have conducted molecular surveys of MAP using MIRU-VNTR and SSR techniques in isolation or tandem. For example, Thibault et al. (2007) divided a collection of 183 MAP isolated from 10 different countries into 21 subtypes, and Stevenson et al. (2009), using the same 8 MIRU-VNTRs markers as Thibault, classified 147 isolates from 7 European countries into 23 different subtypes. In another report, 71 isolates from Germany were differentiated into 15 subtypes, using as markers MIRU-1, 2, 3, 4 and VNTR-3, 7, 25, 32, 47, and 292 (Moebius et al., 2008). A combination of 10 markers (MIRU-1, 4, X3, 292, VNTR-25, 3, 7, 10 and 47) was used by van Hulzen et al. (2011) to analyze 52 dairy cattle isolates from the Netherlands, which were classified into 17 subtypes. Additionally, Castellanos et al. (2010) using 6 markers (MIRU-2, 3, VNTR-25, 32, 292, and 259), divided 70 isolates from Spain into 12 subtypes. In contrast, a reduced number of the 11 SSR markers proposed by Amonsin et al. (2004), have been used for MAP strain typing. Harris et al. (2006) used four SSR markers (SSR-1, 2, 8 and 9) to differentiate a collection of 211 isolates sourced from dairy cattle herds across the United States, observing 61 different subtypes. Pradhan et al. (2011), using the same four SSR markers divided 142 samples from three dairy herds in the northeast United States into 15 subtypes. In addition, two previous studies have used a combination of both techniques, using both groups of reference markers (8 MIRU-

VNTR and 11 SSR loci) for the analysis of MAP isolates. Based on 127 samples from different geographic locations and hosts, Thibault et al. (2008) identified 31 subtypes. Douarre et al. (2011) typed 38 MAP isolates of bovine origin in Ireland and described 22 different subtypes. The indexing of repeat copy numbers in the respective loci is a particularly suitable approach for inter-lab comparisons and phylogenetic studies (Allix-Beguec et al., 2008). However, the lack of an international standardization of markers still stands in the way of meaningful comparisons between studies, with different laboratories using different markers (Castellanos et al., 2012).

2.2.3 Pathogenicity differences of *Mycobacterium avium* subsp. *paratuberculosis* strains

Kunze et al. (1991) compared differences in *Mycobacterium avium* subsp. *avium* strain pathogenicity and observed that sourced strains containing the insertion sequence IS901 proliferated more vigorously in mice than two strains lacking IS901 isolated from humans. This study presents the first indication that genetic differences between strains, member of the MAC, may be linked to differences in pathogenicity. Thereafter, Verna et al. (2007) experimentally infected 28 one-month-old lambs with MAP type I or II. Lambs infected with MAP Type II strains presented a homogenous and mild gross pathology pattern, mainly located in the mesenteric lymph nodes. Conversely, lambs infected with Type I strains presented more severe lesions, which were spread through the intestinal lymphoid tissue. In another study, Mackintosh et al. (2007), experimentally infected 81 four-months-old male red deer with MAP Type I and II, concluding that Type I was less virulent in red deer than Type II. Similarly, O'Brien et al. (2006) observed a higher infection rate and cell-mediated immune response in deer experimentally infected with MAP type II, in comparison with animals infected with

Type I. Additionally, in vitro studies also have accounted for differences in pathogenicity or virulence between MAP strains. Secott et al. (2001) observed variations in the fibronectin binding capacity between two MAP strains, which could be associated with differences in the bacterium ability to invade the host. Gollnick et al. (2007) using bovine macrophages, observed that MAP Type II presented a longer survival in the macrophage in comparison to Type I. In other studies, variations in host immune response have been associated with different MAP strains (Janagama et al., 2006; Motiwala et al., 2006a).

2.3 Transmission

2.3.1 Within species transmission

The transmission of MAP to susceptible animals could involve several pathways, where current knowledge indicates that the faecal-oral route is the main transmission pathway for MAP (Chiodini et al., 1984; Sweeney, 1996; Whittington et al., 2004). It is thought that a susceptible animal ingests MAP orally from faeces-contaminated soil, pasture or water (Sweeney, 1996). However, Corner et al. (2003) challenged the fecal-oral route, proposing instead the respiratory tract as the primary entry point for host invasion. The authors based their hypothesis on similarities between MAP and *M. bovis*, in addition to the low infectious dose required for respiratory compared to oral invasion in experimental studies with *M. bovis*. However, despite a well argued and plausible biological pathway of MAP invasion, no conclusive evidence was presented supporting the hypothesis. A later experiment by Sweeney et al. (2006) provided evidence in support of the intestinal mucosa as the main portal for MAP invasion.

It is assumed that newborn animals are at the greatest risk of ('pseudo-vertical') infection when consuming MAP infected milk, suckling faeces-contaminated udders of dams shedding MAP or being exposed to their immediate environment (Taylor et al., 1981; Streeter et al., 1995; Sweeney, 1996). A subclinically affected dam can shed about 10^4 CFU of MAP per gram of faeces, increasing to 10^{6-8} CFU of MAP per gram of faeces at clinical stage, representing massive amounts of bacteria shed into the environment (Whittington et al., 2000b; Schroen et al., 2003; Whitlock et al., 2005). MAP has been cultured from mammary tissue, supramammary lymph node and milk of sheep, goats and cattle (McDonald et al., 2005; Nebbia et al., 2006; Salgado et al., 2007), where it is expected that up to 50% of subclinically infected ewes and up to 35% and 12% of clinically and subclinically cows shed detectable levels of MAP in their milk (Taylor et al., 1981; Sweeney et al., 1992b; Nebbia et al., 2006).

Vertical transmission also has been studied in cattle, sheep and deer, where an infected dam potentially could transmit MAP to its lambs/calf/weaner through the placenta, becoming infected in utero (Lambeth et al., 2004; van Kooten et al., 2006; Thompson et al., 2007; Whittington and Windsor, 2009). A recent meta-analysis on vertical transmission in cattle estimated that 9% (95% CI: 6 to 14%) of foetuses from subclinically infected cows were MAP positive, increasing to 39% (95% CI: 20 to 60%) in foetuses from clinically affected cows (Whittington and Windsor, 2009). In deer, intrauterine transmission was described in wild deer (Deutz et al., 2003) and in clinically affected farmed deer (van Kooten et al., 2006). Thompson et al. (2007) reported vertical transmission in subclinically infected hinds, estimating a transmission rate of 78% (95% CI = 58 to 98%). In sheep, Lambeth et al. (2004) reported 1 infected foetus among 54 born from subclinical infected ewes and 5 of 6 foetuses born infected

from clinically affected ewes. These results indicate that intrauterine infection may be low in subclinically infected ewes, and that there is a high vertical infection rate from clinically affected ewes. However, in 4 of 5 infected foetuses from the latter study, culture positive samples were obtained from the cotyledons, which represent a mix of maternal and foetal tissues. Independently of the vertical transmission rate for any of the three species, the relative importance of this transmission pathway has not been determined. There are no published investigations about the fate of animals infected intra-uterine, indicating if they will remain subclinical carriers throughout life or progress towards clinical stage.

Based on mathematical simulation, the contribution of vertical and pseudo-vertical transmission routes to the total infection burden was relatively low in herds without disease control interventions (Mitchell et al., 2008). Moreover, MAP was detected in faeces of naturally infected calves (< 3 months old) and experimentally infected, weaned sheep (4 month old) before 14 months of age and 4 months after inoculation, respectively (Bolton et al., 2005; Kawaji et al., 2011). This evidence suggests that calf-to-calf or lamb-to-lamb transmission via faeces may be an important route of transmission. Mitchell et al. (2008), using a simulation model, suggested that infection persistence in low prevalence herds was more likely in models that incorporated dam-to-daughter transmission and calf-to-calf transmission. Finally, sexual transmission has been proposed as a transmission route, based on the culture of MAP from the semen of infected bulls. However, transmission has not being demonstrated either in the inseminated dam nor their offspring (Ayele et al., 2004).

2.3.2 *Mycobacterium avium* subsp. *paratuberculosis* survival in the environment

During pre-clinical and clinical stages of MAP infection, massive amounts of bacteria are shed into the environment. On pasture, MAP contaminated faeces may be washed out by rain and physically dispersed over pasture through the feet of grazing animals, spreading beyond the focal point of deposition. MAP's relatively thick cell wall confers protection against adverse environmental conditions. Gay and Sherman (1992) reported that MAP was able to survive up to 9, 11 and 17 months in manure pats, soil and tap water, respectively. Rowe and Grant (2006) were able to culture MAP up to 12 months after it was inoculated into soil. Whittington et al. (2004) reported survival times up to 55 weeks in fully shaded environments, decreasing survival times as MAP were exposed to solar radiation and/or changes in temperature. Moreover, 24 weeks after application of infected faecal material on shaded soil, MAP was cultured from grass that had germinated in the place of inoculation. In a recent experiment by Salgado et al. (2011), intact soil columns were placed in plastic pipes (lysimeters) under controlled conditions, mimicking different rainfall conditions and artificially contaminating them with MAP-infected slurry. In this study, MAP was recovered by culture from grass in all treatment groups, suggesting that MAP tend to stay attached to the surface of the soil, instead of migrating to lower layers of the soil, despite of heavy rainfall being simulated in some of the treatment groups. Finally, Lamont et al. (2012) provided evidence that MAP may have the capacity to form spores increasing their survival time in the environment. Results from these studies are a clear indication that MAP shed by infectious animals stay in pasture for long periods. Thus, soil surface and pasture was regarded as a reservoir and source of transmission for susceptible animals.

2.3.3 Cross-species transmission

Indirect faecal-oral transmission through contaminated soil, pasture or water is the only way that MAP can be transmitted between species (Sweeney, 1996). In New Zealand, the co-grazing of several livestock species such as sheep, beef cattle and deer is a common practice. Although this practice generates the potential risk of spreading infectious diseases across ruminant species, it has the advantage of improving pasture management and help to control noxious weed (Griffiths et al., 2006), co-grazing has been associated with a reduction in the internal parasite burden (Southcott and Barger, 1975). Probably because mixed species farming is only practised in a few countries, there is a limited number of studies addressing cross-species transmission of MAP. In Iceland, available epidemiological information suggests that MAP was introduced to the country through infected sheep, imported from Europe. MAP was then transmitted to the local cattle population, where it became endemic, and was subsequently transmitted back to naïve sheep after a depopulation and restocking program had been enforced (Palsson, 1962; Fridriksdottir et al., 2000). In a report from the Netherlands, Muskens et al. (2001) reported MAP infection of naïve sheep that grazed manure fertilized pasture from MAP infected cattle. In Australia, Moloney and Whittington (2008) followed prospectively 1,774 beef cattle located in 12 sheep and beef cattle farms with know history of MAP infection in the sheep populations. Authors studied the risk of transmission of MAP Type I from sheep to cattle due to shared use of pasture. After at least two years of exposure, all cattle were ELISA negative suggesting that the transmission risk was low, although authors conceded that it might occur sporadically. In New Zealand, two studies have addressed the relationship between mixed-species farming and clinical incidence or prevalence of abnormal visceral lymph nodes (AVLN) in deer at meat inspection: in a non-random cross sectional study of farmed deer,

Glossop et al. (2007) surveyed farmer's recall of diagnosed clinical cases and observed that clinical incidence was positively associated with the time that beef cattle grazed in deer fenced area (DFA) and negatively associated with the time that sheep grazed in DFA. In another study, Verdugo et al. (2008), using slaughter records from 346,811 deer carcasses, observed changes in the prevalence of AVLN, which are commonly associated with paratuberculosis gross pathology in deer (Hunnam et al., 2011), and the species present on farm. Slaughter lines from farms where beef cattle were also present had a higher risk of AVLN compared with deer-only farms, whereas the presence of sheep was negative associated with the AVLN risk. However, this study just used a present/absent classification of sheep and beef cattle, based on census data, not considering actual co-grazing information. Although, the two previous studies may not have presented conclusive evidence for cross-species transmission of MAP between deer, beef cattle and sheep, they hypothesise associations between disease manifestation and mixed-species farming. Thus MAP transmission under cross-species co-grazing management warrants further investigation.

Despite the isolation of MAP from ruminant and non-ruminant wildlife species, there is no conclusive information about the role of wildlife as a dead-end host. Wildlife may act as a vector or reservoir, leading to indirect transmission of MAP within and between ruminant livestock species (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). Nugent et al. (2011) conducted a survey of wildlife species on and around three highly affected deer farms in New Zealand. The authors isolated MAP from the intestines and associated lymph nodes of several wildlife species such birds, hedgehogs, rabbits, brushtail possums, and feral cats. However, only

a small proportion of the positive animals were also faecal culture positive suggesting that shedding may be limited. In other countries, Ptb research of wildlife focused on rabbits. Mokresh et al. (1989) reported shedding levels of 10^2 to 10^6 MAP CFU per gram of faeces in experimentally infected rabbits. Studies in Scotland found similar MAP strains in rabbits and clinically affected cattle. Moreover, MAP strains isolated from rabbits and inoculated to naïve calves resulted in histopathological and/or microbiological evidence of infection within 6 months post-inoculation (Greig et al., 1999; Beard et al., 2001b). In New Zealand, rabbits are ubiquitous and their role in MAP transmission warrants further research.

2.4 Pathobiology and epidemiology

2.4.1 Host invasion

Current knowledge indicates that MAP targets the mucosa and associated lymphoid tissues of the host (Lugton, 1999), preferentially of the upper gastrointestinal tract. Fibronectin attachments in the MAP cell wall were found to interact with integrins (trans-membrane receptors) present in M-cells of the epithelium of Peyer's patches at the terminal ileum, producing a bridge between them, which allows the translocation of MAP across the epithelial barrier towards the basolateral side of the intestinal epithelium and through the to Peyer's patches (Momotani et al., 1988; Stabel, 2000). MAP bacteria are subsequently phagocytosed by subepithelial and intraepithelial macrophages (Momotani et al., 1988; Fujimura and Owen, 1996; Lugton, 1999). MAP probably remains in the phagosome, where they multiply intracellularly (Kaufmann, 1993). Survival within macrophages is the MAP hallmark, although evidence indicates that simultaneous intracellular multiplication and killing of MAP occurs, reflecting an

initial T-helper 1 (Th1) cellular immune response (Zhao et al., 1999). In general, MAP invasion of a naïve host could be divided into three stages: First, a focal lesion is observed at the site where MAP invaded the intestinal mucosa. Then, MAP infiltration is present at the regional lymph nodes. Finally, the third stage is characterized by a dissemination of MAP through lymph- and blood-streams, producing lesions at distant organs such as the liver, kidney and spleen (Payne and Rankin, 1961a). Recently, Reddacliff et al. (2010) and Smith et al. (2011) were able to culture MAP from skeletal muscle tissue in sheep.

2.4.2 Replication of MAP

Primary tissues for MAP replication are the intestinal epithelium and mesenteric lymph nodes, where iron concentrations are relatively high (Kolb, 1963). MAP has the capacity to survive inside phagosomes and may escape into the cytoplasm of the macrophage. There MAP will continue reproducing until the physical rupture of the macrophage. Once released, MAP is again phagocytosed by macrophages replicating the cycle (Tessema et al., 2001). Eventually, MAP organisms present in cells of the intestinal epithelium and mesenteric lymph nodes enter an exponential growth phase, characterized by fast replication and leading to a heavy bacterial shedding, with estimates of 10^3 to 10^4 MAP CFU per gram of faeces. The bacterial accumulation phase will be followed by a stationary phase (bacteriostasis) and a posterior phase of decreasing bacteria burden due to the host immune response, which is triggered by the exponential phase. At this stage, an infected animal could be able to control the disease and remain in the stationary phase for the rest of its productive life, either being latently infected or intermittently shedding small to moderate numbers of MAP. However, some animals may enter a progressive phase, characterized again by an exponential MAP

growth followed by bacteria dissemination to distant tissues associated with (histo-)pathology. Before the first exponential phase, most infected animals will test negative by serologic tests such as serum ELISA (Chiodini, 1996).

2.4.3 Histopathological lesions

Histopathological changes, based on the severity of lesions and the degree of MAP infiltration of tissues, differentiate MAP infection into two categories, a “paucibacillary” and a “multibacillary” form (Clarke and Little, 1996). The paucibacillary or tuberculoid form is characterized by lymphocytic reaction with few or no acid-fast bacteria. Conversely, the multibacillary or lepromatous form is characterized by large numbers of macrophages and epitheloid cells together with large numbers of acid-fast bacteria (Clarke, 1997). The multibacillary form represents the most severe state and is almost always associated with clinical disease (Clarke and Little, 1996; Kurade et al., 2004; Reddacliff et al., 2006; Dennis et al., 2011). However, clinical cases have also been reported from animals with a paucibacillary form (Clarke and Little, 1996; Dennis et al., 2011). The multibacillary form is considered irreversible (Dennis et al., 2011) and associated with permanent high faecal shedding of MAP (Kurade et al., 2004; Reddacliff et al., 2006; Kawaji et al., 2011). Differences in the host immune response are associated with the different forms of MAP: a predominant cell mediated immune response is characteristically present in animals with a paucibacillary form of infection. Conversely, the multibacillary form is associated with a stronger humoral response and weaker cellular immunity (Clarke, 1997).

2.4.4 Macroscopic lesion and clinical signs

Cytokine production and the cellular immune response cause the appearance of an intestinal granuloma (Clarke, 1994; Cocito et al., 1994; Lugton, 1999). This inflammatory process leads to the typical manifestations of a corrugated intestinal epithelium and characteristic malnutrition syndrome associated to cPtB (Harris and Barletta, 2001). In cattle, the inflammatory process in clinically affected animals, involves the lymph nodes draining the intestine, leading to the characteristic thickened corrugated intestine, in addition to enlarged and oedematous mesenteric lymph nodes and dilated serosal lymphatic vessels (Clarke, 1997). In sheep, gross pathology seen at post-mortem inspection, tend to be milder than in cattle, with the thickening of the intestinal wall not always being present (Taylor, 1945). In deer, gross pathology is characterized by the presence of caseous necrosis within the jejunal and ileo-caecal or retropharyngeal lymph nodes of subclinically and clinically infected/affected animals. This feature commonly interferes with routine meat inspection at slaughter plants due to the gross and histopathological similarities with tuberculosis. This extensive necrosis of lymph nodes is not present in cattle or sheep infected with MAP (de Lisle et al., 2003). Additionally, deer may not present a visible thickening of the ileum, but thickened lymphatic drainage vessels and enlarged mesenteric lymph nodes are typical features of MAP infection (Mackintosh et al., 2004). Clinical paratuberculosis signs include loss of body weight and body condition, usually developing a terminal diarrhoea, ill-thrift, and muscle wasting. Clinical signs of paratuberculosis are not pathognomonic. Wasting and diarrhoea are also common in animals with other diseases such as salmonellosis, renal amyloidosis, parasitism, and ruminal acidosis (Raizman et al., 2007b).

2.4.5 Patterns of disease progression

In cattle and deer, young calves were generally regarded as being highly susceptible to MAP infection, especially in calves less than 30 days of age, with susceptibility declining with increasing age. Cattle or deer over one year of age were regarded as highly resistant (Taylor, 1953; Gilmour et al., 1965; Larsen et al., 1975; Sweeney, 1996; Wells and Wagner, 2000; Mackintosh et al., 2010). However, it was assumed that resistance is incomplete and animals could still be infected as an adult if they stayed productive for a long period of years or were exposed to high challenge doses. They could potentially even develop the disease (Mackintosh et al., 2010). Reports exist about naive adult cattle being exposed to infection and developing clinical signs (Larsen et al., 1975; Wells and Wagner, 2000; Wells et al., 2012). It was suggested that once infection is established in cattle they will remain infected for life (Nielsen and Toft, 2008), although conclusive evidence supporting this suggestion has not been presented. There has been a general assumption that a similar susceptibility pattern may apply to sheep, although age-related resistance to infection in sheep appears to be less-pronounced than is assumed to be the case in cattle (Sergeant, 2005). Several experiments comparing infection rates between lambs and adults have not found significant differences between them (Reddacliff et al., 2004; Dennis et al., 2011; Delgado et al., 2012; McGregor et al., 2012). However, there are indications that animals infected as lambs are at a higher probability to progress toward clinical disease than animals infected as adults (McGregor et al., 2012). Additionally, in sheep there is evidence suggesting that a high dose challenge or a continued exposure to MAP could overcome age resistance to infection and clinical disease (Whittington and Sergeant, 2001; Fecteau et al., 2010).

In general, cPtb is characterized by a long incubation period. In sheep, clinical disease is most common in animals older than 2 years, with many being older than 4 years (Seaman and Thompson, 1984). Most clinical cases in cattle occur in 2- to 4-year-old animals (Chiodini et al., 1984) but the incubation period, assuming infection of neonates, can be up to 14 years (Whittington and Sergeant, 2001). In contrast to sheep or cattle, clinical cases in deer are commonly observed in yearling animals, representing a characteristic feature of the epidemiology of paratuberculosis in this species (de Lisle et al., 2003). Clinical disease in deer presents either as a sporadic occurrence of cases in adult animals or as outbreaks in young animals 8–15 months old (Mackintosh et al., 2004).

In an infected cattle herd, it is assumed that newly infected animals either enter a transient shedding state or remain latently infected without any evidence of shedding until adulthood. The latter are progress slower towards clinical disease than the former (Mitchell et al., 2012). In sheep, based on the study of shedding patterns of experimentally infected lambs, animals could enter to a “progressor” or to a “non-progressor” track. In both paths shedding could be detected as early as two months after challenge. However, animals belonging to the “non-progressor track” eventually will stop shedding around 16 months post challenge, whereas animals from the “progressor track” will remain shedding and eventually progressing toward clinical disease (Stewart et al., 2004). It is hypothesised that animals from the “non-progressor tract” have the capacity to either eliminate or control MAP infection below faecal culture detection level (Dennis et al., 2011). Kawaji et al. (2011) reported that the quantification of MAP DNA from faeces of experimentally infected lambs, identifying two groups with differences of 3 to 4 order of magnitude between low and high shedders. At post-

mortem inspection, the high shedder group presented a multibacillary form of infection, whereas the low shedder group presented no lesions or a paucibacillary form. In deer, an experimental infection study conducted by Mackintosh et al. (2007), observed that newly infected deer could enter a “fast-progression-track”, which was characterized by early shedding and clinical disease. Conversely, in the “slow-progression-track” infected animals entered a latent stage, which progressed to a low shedding state, and eventually some animals progressed to a high shedding stage and clinical disease. Additionally, Mackintosh et al. (2007) reported that some animals have the capacity to eliminate the infection, as a fraction of the experimentally infected deer become test negative and no evidence of the disease was observed at post-mortem. It is not yet understood why some infected animals progress to a clinical phase, while others remain subclinically infected during their entire productive life, or eventually manage to eliminate the infection. This phenomenon could be explained by animals’ genetic susceptibility, which could be an important factor in determining the fate of infection (Raizman et al., 2007a).

Several studies have suggested that there is a dose–response relationship between the exposure to MAP and the severity and time to onset of clinical disease (Sweeney et al., 1992b; Mackintosh et al., 2010; McGregor et al., 2012). The dose–response relationship between MAP challenge and the onset of clinical paratuberculosis has a strong implication on disease control programs. A successful control program will reduce infection prevalence and MAP burden, therefore it will become more difficult to detect infected animals because susceptible animals will be challenged by a reduced infectious dose, thus infected animals will seroconvert or start shedding MAP when older delaying the detection of new positives (Taylor, 1953).

2.5 Prevalence

2.5.1 Diagnostics test and surveillance

Currently available tests are mainly based on detection of antibodies on serum (e.g. ELISA or AGID) or milk (milk-ELISA), faecal culture (FC) or tissue culture, in solid or liquid media (Bactec), and PCR testing on faeces, milk or tissue samples for the detection of the unique DNA sequence IS900 (Nielsen and Toft, 2008). Those tests, in individual animals, present generally a poor performance, especially lacking sensitivity (Se), primarily due to the chronic nature of the infection and potential latency within herds and animals (Whittington and Sergeant, 2001). Truly positive animals include latently infected, shedding and/or clinically affected animals (Nielsen and Toft, 2008). The Se of diagnostic tests strongly depends on the disease stage, where infected, infectious and/or affected animals need to be differentiated. The Se for latently infected animals (no shedding/true-latent) is generally low (Nielsen and Toft, 2008). Infected herds can be misclassified as non-infected if prevalence is low (<5%), only a fraction of the herd is tested, and/or diagnostic tests with low to moderate Se for detecting infected animals are used. Non-infected herds can be misclassified as infected when tests with imperfect specificity (Sp) are used, with the exception of culture (Berghaus et al., 2006).

Three different tests are currently available for measuring antibodies against *M. paratuberculosis* in the serum of infected animals. These are the complement fixation (CF) test, the agar gel immunodiffusion (AGID) test, and ELISA (Harris and Barletta, 2001). The Se of these tests is high for animals with clinical symptoms, or for those that shed large numbers of bacteria (Nielsen and Toft, 2008). Therefore, the main limitation of these antibody tests is their inability to accurately identify animals early in the course

of an infection. In naturally infected cows, seroconversion has been shown to occur in 95–98% of animals shedding MAP (Nielsen and Ersboll, 2006). Seroconversion has been observed in 2.2 to 11.7 years old cows under field conditions and studies using fixed dosages and known age at infection have also found a great variation in the time to seroconversion (Nielsen and Toft, 2008). In cattle, Se of ELISA was 87% in clinical cases compared to 75% in subclinical heavy faecal shedders and 15% in subclinical light faecal shedders (Sweeney et al., 1995). The usual mix of animals in a subclinically infected herd renders Se of ELISA about 45% (Collins and Sockett, 1993) although further analysis by Whitlock et al. (1999) suggests that Se in cattle may be about 35%. Antibodies against MAP could also be detected in milk of lactating cows or bulk tank milk, using ELISA. This ‘milk-ELISA’ represents a cost effective tool for MAP surveillance in dairy cattle herds (Sergeant et al., 2008). Validation studies of milk-ELISA estimated a Se between 29% to 61% and a Sp in the range of 83% to 100% (Nielsen and Toft, 2008). In large, endemically infected sheep flocks the Se has been estimated to be about 25% (Whittington and Sergeant, 2001), although Hope et al. (2000) have reported Se to be between 35-54% for ELISA. The reason for the imperfect Se of serological tests in sheep, even in late stages of the disease, is the variability in the immune response of individuals. A significant proportion of clinically affected sheep, with well-developed histological lesions could have negative results in serological tests (Clarke and Little, 1996). In deer, previous studies by Griffin et al. (2003) and Rodgers et al. (2005) have shown that subclinically infected deer generally produce higher levels of antibody than previously reported in cattle (Collins et al., 2005) or sheep (Sergeant et al., 2003). Under field conditions, where deer have been infected naturally and vary in age, the estimated Se of an IgG1 ELISA (ParalisaTM) was about 67% for infected deer showing minimal pathology, if any (Griffin et al., 2005). A recent test validation using

Bayesian latent class analysis estimated a Se of 19% (95% CI 10-30%) on subclinically infected deer using the ParalisaTM (Stringer, 2010). In general, ELISA is a fast and low-cost serology test; however, it is less sensitive and specific than faecal culture (Whitlock et al., 1999).

Bacterial culture of tissues or faeces is a highly specific diagnostic test (Nielsen and Toft, 2008). However, it is costly and MAP culture requires long incubation periods, taking from 4-6 weeks, using automated liquid-culture systems (Bactec), until 12-16 weeks based on conventional growth on solid media (Berghaus et al., 2006). Additionally, in cattle, sero-conversion can be detected prior to MAP shedding when antibodies are tested in milk rather than serum (Nielsen, 2008). Culture of intestinal tissue is more sensitive than FC. It was observed that animals with repeated negative FC samples while being alive had positive culture results from intestinal tissues at abattoir (Whittington and Sergeant, 2001). The Sp of FC is considered to be almost 100%, if the isolates obtained at culture are confirmed to be MAP by molecular methods such as IS900-PCR. Although the potential pass-through phenomenon (Sweeney et al., 1992a) could cause non-infected animals testing FC-positive on contaminated premises, leading to false-positive reactions (Nielsen and Toft, 2008). However, Pradhan et al. (2011) in a longitudinal study on three dairy cattle farms in USA, reported that 80% of tested animals with at least a single positive faecal culture were also tissue culture positive, indicating that they were truly infected. Interestingly, it has been observed that MAP can be unevenly distributed within the faeces, contributing to possible false negative results due to intermittent shedding (Whittington and Sergeant, 2001). One way to overcome the FC costs of individual animals is to pool faecal samples. Currently, this is the most cost-effective option with acceptable Se for herd testing (Benedictus et al.,

1999; Kalis et al., 2000; van Schaik et al., 2003; Weber et al., 2004). Pooled culture has been used for identification of infected-herds, but could also be used for certification of low-risk herds and flocks (van Schaik et al., 2003). A study conducted by Van Schaik et al. (2007) determined that pools of 10 cows were the preferred option to determine the herd infection status, maximizing Se and minimizing costs, with no significant difference in Se between pools of five or ten cows. Faecal pooling has also been recommended to assess the MAP herd-level prevalence (Wells et al., 2002; Raizman et al., 2004). In sheep, pool FC with sizes of 10, 30 and 50 samples have been used to estimate animal level prevalence, reporting Se of 91%, 85% and 77%, respectively (Dhand et al., 2007). In deer, pool size of 10 samples has been used for vaccine assessment in naturally infected animals (Stringer et al., 2011) and for the herd level evaluation of risk factors for clinical disease (Glossop et al., 2007).

Molecular analysis of nucleic acids using polymerase chain reaction (PCR) assays have been applied for MAP detection in faecal, milk and tissue samples (Fang et al., 2002; O'Mahony and Hill, 2004; Bogli-Stubler et al., 2005; Stabel and Bannantine, 2005), commonly targeting the IS900 insertion element sequence (Vansnick et al., 2004; Ravva and Stanker, 2005; Rowe and Grant, 2006). These assays have demonstrated to be sensitive and specific, and have reduced the detection time (Bogli-Stubler et al., 2005; Stabel and Bannantine, 2005). However, PCR has not achieved the same Se as culture when applied directly to tissues or faeces (Collins et al., 1993). This lack of Se of the PCR test was explained by the presence of PCR inhibitors which are difficult to remove from faecal samples (Harris and Barletta, 2001). In recent years, new diagnostic protocols have removed PCR inhibitors from faecal samples, allowing the direct application of quantitative, real time PCR to faeces samples (Kawaji et al., 2007).

Current evidence indicates a greater diagnostic Se of this tool in comparison with tissues culture (Kawaji et al., 2011).

Surveillance programs have been designed to account for the relationship between test Se and disease stage. For example, only animals >3 years of age were sampled in a US programme, and >2 years for the initial test and >4 years in subsequent tests in an Australian programme (Sergeant et al., 2008). Additionally, abattoir surveillance has been implemented by the Australian sheep industry and by the New Zealand deer industry. It involves visual examination of viscera and their regional lymph nodes to identify gross pathological lesions attributable to MAP infection. Suspect lines were followed by a histological assessment (Abbott and Whittington, 2003) and possible follow-up by Bactec culture and/or PCR (Glossop et al., 2005). Abattoir surveillance has the advantage of a wide cross-section sample, where several regions can be inspected at relatively low cost, compared to expensive farm-based surveillance methods (Abbott and Whittington, 2003). However, gross lesions of lymph nodes are not specific for paratuberculosis in sheep, and such lesions are not always present in all infected animals (Fodstad and Gunnarsson, 1979; Hope et al., 2000; Abbott and Whittington, 2003). Moreover, an unknown proportion of animals affected by paratuberculosis on-farm are not submitted for slaughter. Uncertainty therefore exists about the ability of this surveillance method to detect the infection in flocks, particularly those with a low prevalence. Abbott and Whittington (2003), using a mathematical simulation model, estimated an abattoir HSe of 75% for sheep surveillance in Australia, assuming an intra-flock TP of 2%. Moreover, Bradley and Cannon (2005), using 1,200 sheep sourced from known highly infected farms in Australia, estimated an inspector level Se between 53 to 87% and a Sp between 97 to 100%. Conversely, In New Zealand

Hunnam (2011) estimated a meat inspector Se for deer lines, of only 13.3% although a high Sp of 99.9% was reported. Despite of both studied used histology to compare meat inspectors assessment, Hunnan (2011) cover a greater number of inspectors and abattoirs, and used normal animal submission as test subjects, in contrast to the known farms status from animals sourced in the Australian study, which could have biased their results. However, differences in estimates could also be attributed to pathological differences of disease manifestation between those two species.

2.5.2 Paratuberculosis prevalence and incidence

The primary objective of prevalences studies is to assess the extent of a given disease in the target population (Greiner and Gardner, 2000). Typically, such data are required for the development of control and surveillance programmes. Financial and/or logistic constraints would rule out a population census, thus prevalence estimation relies on a finite sample from the target population. The sample size is calculated using an assumed proportion of infected animals (or herds) and an acceptable error (Noordhuizen, 2001). Cross-sectional studies are commonly used to obtain the required samples, which should be designed following a random methodology in order to obtain a representative sample of the target population (Greiner and Gardner, 2000). Animal populations are not homogeneous aggregations of individuals, rather they are structured in heterogeneous strata (e.g. farms, regions, productive systems), thus stratified sampling is commonly used and survey results have to be adjusted by their sampling fraction in order to obtain an unbiased population estimate (Wang, 2002). In particular, prevalence estimation uses as a proxy, the frequency of positive results from an imperfect test to obtain the true proportion of infected animals (Greiner and Gardner, 2000). Rogan and Gladen (1978) proposed a method for the estimation of the true prevalence (TP), based on the apparent

prevalence (AP) (test results), adjusting for imperfect Se and Sp. This method is well established and recognized by the scientific community, and represents the first election tool to obtain the TP (Nielsen and Toft, 2009). However, it assumes that the Se and Sp of a test are known, which is not always the case, especially when multiple tests are used in complex testing designs. Additionally, a point estimate of test performance is often assumed, not considering uncertainty about it, which could bias TP estimates (van Schaik et al., 2003). Latent class Bayesian methods represent an alternative to the traditional Rogan and Gladen approach. This methodology combines prior information with available data to obtain and update posterior inference about TP (Branscum et al., 2004). Additionally it has the advantage that variability for Se and Sp is considered, using a flexible probabilistic framework that allows the modelling of complex testing designs as they are often used in large prevalence estimation studies.

The majority of the prevalence studies available for MAP infection report AP, not considering any type of adjustment for imperfect Se and Sp. A comprehensive review of MAP prevalences across Europe was published by Nielsen and Toft, (2009). Authors provided animal and herd level AP results for cattle, sheep, goat, deer and lamas. HAP of cattle ranged from 3% to 68%, and two sheep studies in Switzerland and Spain reported HAP of 24% and 29%, respectively. Additionally, authors attempted to provide TP and HTP estimates for all species reviewed, however incomplete or incorrect information in the source studies, precluded the estimation of this parameter in all species. A selection of prevalence studies from New Zealand and elsewhere is presented below in order to describe the extent of the disease.

For the USA dairy sector, a HAP of 21.6% was estimated in the general population, increasing to 39.7% for herds of over 300 animals (Wells and Wagner, 2000). In another study, Boelaert (2000) estimated HAP to be 18% at national level in Belgian cattle. Muskens et al. (2001) reported from a sample of 378 dairy herds in the Netherlands, that 55% tested positive. In Canada, HAP ranged 37 to 74% in dairy herds located in seven different provinces (Tiwari et al., 2006). In recent years, several studies have used a Bayesian approach to estimate the HTP in dairy herds. Lombard et al. (2013), using environmental samples from 534 herds, estimated a HTP of 91% in the USA. In two regions of Northern Italy, a HTP of 70% was estimated in commercial herds. In another study in the UK, three simultaneous diagnostics test were used, reporting that 35% of herds milking over 20 cows, were truly infected (DEFRA, 2010). Nielsen et al. (2000), using bulk tank milk ELISA, estimated a HTP of 70% in Danish dairy cattle herds. In New Zealand, no estimate of dairy herd prevalence based on a structured survey is available to date. It was reported that 12% of dairy herds were 'positive' based on diagnostic laboratory records, a figure stated likely to be an underestimate of HTP (Burton, 2002).

In sheep flocks, Sergeant and Baldock (2002), using abattoir surveillance data and Bayesian modelling, estimated a HTP between 2.4 to 4.4% Australia-wide and between 6 to 10% in New South Wales. In New Zealand, it is known that MAP is endemic in the sheep population. However, prevalence has not been estimated. Only a non-peer-reviewed economic assessment is available, which assumed in a HTP of 60-70% (Brett, 1998). Conversely, two studies are available for the New Zealand farmed deer population. In a non-random sampling survey of 115 deer herd, 44% were PFC positive (Glossop et al., 2006). Stringer et al. (2009) used the traditional Rogan and Gladen

method to estimate the HTP based on tissue culture of randomly selected lymph nodes from abattoir surveillance, and reported a national herd prevalence of 59% and animal prevalence of 45%.

In comparison with the relatively high infection prevalences previously described, the clinical Ptb tends to be rare. Cetinkaya et al. (1997) reported an annual clinical incidence (ACI) between 1.6 and 2.3 per 100 cows for the period 1993-94 in England. A survey conducted among farm managers from clinically affected dairy herds in USA (n=748), reported that 75% observed an ACI below 5 per 100 cows (USDA, 2005). In New Zealand, Norton et al. (2009) reported an ACI of 0.32 per 100 cow, using recalls inquired from farmers by mail questionnaire. There are two longitudinal studies reporting clinical incidence in sheep. Bush et al. (2006), reported an ACI between 2.1% to 17.5% in 12 infected farms in Australia. In New Zealand, a longitudinal study by Morris et al. (2006), reported ACI 1.1%, based on post-mortem histopathology diagnosis.

2.6 Production effects

The chronic gut inflammation caused by MAP infection is assumed to lead to a reduction in the intestinal capacity to absorb nutrients, mainly proteins due their larger molecular size, progressing to a protein-losing enteropathy. The negative energy/protein ratio balance could have an effect on an animal's capacity to produce milk, and grow wool or muscle fibre. Additionally, MAP infection could have an indirect effect on reproductive performance (Harris and Barletta, 2001). Thus, MAP infected livestock could be culled before the end of their productive cycle due to the clinical manifestation

of the disease, or sub-clinical effects such as decreased milk production, reduced growth rates, or failure to conceive or rear an offspring. However, not all infected animals will experience detectable production loss during their productive life (Nielsen and Toft, 2008).

Being able to identify and quantify production loss are key elements for the design and planning of disease control measures (de Lisle, 2002). In New Zealand, the only available systematic economic evaluation of Ptb concluded that “Johne’s disease does not currently cause large economic losses to the New Zealand livestock industries, relative to the value of the industries” (Brett, 1998). Under the current lack of information about prevalence and incidence in the major livestock industries in New Zealand, it is important to think about the validity of that conclusion, after more than a decade, especially if we incorporate possible indirect effects associated with Ptb, such as trade barriers or a decrease in the consumption of animal products due public health concerns. Production studies conducted in the USA, New Zealand, and Australia have pointed out a large variability of production effects between infected flocks/herds. Nordlund et al. (1996) observed that two of 23 dairy herds presented a significant reduction in milk production. Similarly, Norton (2007) followed four infected dairy herds during for three milking seasons and reported that only one of them presented a significant difference in milk production. In sheep, Dhand et al. (2007) observed a large variability in the clinical incidence and related production loss between 92 infected sheep flocks. The variability associated with production effects attributable to MAP infection implies that a large number of flocks/herds must be enrolled in cross-sectional or longitudinal studies in order to estimate a production effect at population level. For this reason the major part of studies on MAP production effects have chosen farms with

known infection status and a history of clinical incidence, generally reporting production effect at animal level. A selection of MAP production effect studies is described below, involving cattle, sheep, and deer.

MAP associated production effects have mainly been studied in dairy systems, assessing the effect of MAP infection on milk production. Effects on culling rates or reproduction performance have not been extensively investigated (Smith et al., 2010). In a study of infected animals culled at the end of lactation, on 18 infected dairy farms in the Netherlands, was recorded a decrease in milk production of 6% and 16% when they were compared with their preceding two lactations, respectively (Benedictus et al., 1987). In a longitudinal study conducted in two Minnesota (USA) dairy herds (n = 1,297 cows), the milk production of subclinically infected, FC positive cows was 11% lower than FC negative herd-mates. Infected cows also had lower reproduction performance and were removed on average 124 days earlier. In the same study, clinically affected cows produced 1,500 kg less milk and were removed from the herds on average 202 days earlier than FC negative cows (Raizman et al., 2007b). Ott et al. (1999) estimated a reduction in herd average milk production of 4% when comparing ELISA positive with negative herds, based on a population of 974 dairy cattle herds in USA. Similarly, Nordlund et al. (1996) reported a difference of 4% in milk production between ELISA positive and negative dairy cattle herds using samples from 23 herds in USA and Hendrick et al. (2005) reported a difference of 2-6% in milk production between positive and negative dairy cattle herds, based on a combination of ELISA and FC tests, using samples from 23 herds in Canada. Other studies, using different test protocols such ELISA or combinations of ELISA and FC, have found similar results at herd level, reporting a decrease in milk production of 0.8 to 17% between positive vs.

negative herds (Sweeney et al., 1995; Johnson et al., 2001; Lombard et al., 2005; Norton, 2007). The effect of MAP infection on milk components is unclear: three studies found no association between infection and milk components (Nordlund et al., 1996; Johnson et al., 2001; Lombard et al., 2005), whereas two studies found an association between MAP infection and decreased fat and protein yield (Benedictus et al., 1987; Gonda et al., 2007). Gonda et al. (2007) using a sample of 4,375 cows ELISA and FC tested from 232 US Holsteins herds, found an average decrease of 11.46 kg milk fat and 9.49 kg milk protein per lactation associated with MAP infected cows. No association between somatic cell score and infection status was found in this study.

With regard to the effect of MAP infection on culling rates, Ott et al. (1999) reported no significant differences between ELISA positive and negative herds in relation to the number of replacement animals purchased or cows slaughtered. Conversely, five independent studies have reported increased culling rates associated with MAP infection (Tiwari et al., 2002; Hendrick et al., 2005; Gonda et al., 2007; Norton, 2007; Raizman et al., 2007b; Smith et al., 2010). In New Zealand, Norton (2007) using a combination of ELISA and FC tests to categorize positive and negative cows, observed that in one out of four dairy herds, longitudinally monitored for three reproductive season, presented significant culling rates differences, where positive cows were 4.7 and 1.4 times higher risk of culling in cows older than 5 years and younger than 5 years, respectively. Smith et al. (2010) conducted a longitudinal study on 6 US dairy herds (n=2,818 cows), stratifying animals in three groups: FC- and ELISA-negative, low-positive (low-shedding or ELISA-positive only), and high-shedding. Authors reported that low positive and high-shedding cows were culled significantly earlier than test negative cows, and no differences were observed between the two positives groups. Additionally,

calving rates of negative cows were slightly lower than of ELISA positive cows, and substantially higher than those of high-shedding herd mates. Similarly, Lombard et al. (2005) and Marce et al. (2009) observed that ELISA positive cows achieved higher calving rates than their negative herd-mates. However, Marce et al. (2009) in a large retrospective study, based on data from 1,069 French dairy herds (n = 48,914 cows), noticed that the effect of higher calving rates in ELISA positive cows, decreased with the age until being significantly lower than negative herd mates. These findings are indicative that adverse MAP effects on reproduction performance may be associated with more advanced stages of infection.

In sheep, the longitudinal study conducted by Morris et al. (2006) evaluated the disease status of 3,633 Romney, Merino and Merino x Romney-cross ewes, using post-mortem histopathology. In an 8 years period, confirmed cases presented an annual clinical incidence of 1.1% of the ewes present at mating. Clinically affected ewes were disposed off earlier than non-clinically affected sheep (3.41 and 5.03 years, respectively). Additionally, clinical disease was associated with a significant 10.5% reduction in live weight of the flock and in greasy fleece weight (reduced by 0.54kg). Bush et al. (2006), based on 12 farms located in New South Wales Australia, reported a reduction in farm gross margin of 2.2% to 15.4% per year associated with sheep mortality due to cPtb. A study of the subclinical MAP infection effect on lambing was conducted by Kostoulas et al. (2006), using dairy ewes and goats from four flocks in Greece (n = 369). Authors observed that positive animals (ELISA or FC) of parity 1-3 presented higher lambing rates than their negative flock mates of the same age. However, this effect vanished in positive animals of higher parities, being consistent with the findings reported by Marce et al. (2009) and Smith et al. (2010) in dairy cattle.

No peer-reviewed study has been published about the production effect of MAP sub-clinical infection in commercially farmed deer. Economic effects of Ptb on deer may be observed on-farm and at slaughter. Outbreaks of clinical cases in yearling deer (1-2 years), the most commonly affected age group (Glossop et al., 2008), and interference with bovine tuberculosis surveillance in live or slaughtered stock may have the greatest economic impact (Mackintosh et al., 2004; Stringer, 2010). It was also suggested that subclinical disease may cause production loss, such as failure to conceive, failure to rear progeny, reduced growth rate, early culling and failure to achieve potential velvet antler production (Mackintosh and Wilson, 2003). However, no conclusive data has been presented. In particular, Thompson et al. (2007) using ParalisaTM as diagnostic test, observed that subclinically infected hinds had a pregnancy rate of 69%, which was much lower than the 85-90% commonly reported from the farms from which hinds were sourced. Nevertheless, this study involved only 35 hinds and it was not specifically designed to evaluate reproduction rates in deer.

2.7 Simulation models

The chronic nature of MAP infection, in addition to the lack of reliable diagnostics test and variable production effects, preclude the development of large studies due to high cost and logistics constraints. Mathematical simulation models have become a popular tool to MAP research. Simulation models allow gathering available knowledge in a systematic fashion, and studying the implications of disease dynamics associated with different control measures under various scenarios. Most available models are based on our basic understanding of the epidemiology of Ptb (Sergeant, 2005) and despite

uncertainties about a number of key input parameters, their outputs can add useful information to the design of control and certification schemes (Guitian and Pfeiffer, 2006).

The effects of different control measures on MAP infection dynamics have been simulated for all susceptible livestock species such as: cattle in general (Pouillot et al., 2004; Ezanno et al., 2005), dairy cattle (Collins and Morgan, 1991; Groenendaal et al., 2002; Kudahl et al., 2007; Mitchell et al., 2008; Marce et al., 2011), beef cattle (Humphry et al., 2006; Bennett et al., 2012), sheep (Sergeant and Whittington, 2000; Marquetoux et al., 2012), and deer (Heuer et al., 2012). Additionally, available models have been used for a number of different purposes, including: *i*) to investigate the epidemiology and dynamics of the disease in infected herds (Pouillot et al., 2004; Mitchell et al., 2008; Marce et al., 2011); *ii*) to evaluate alternative strategies for management and control of Ptb in infected herds (Groenendaal et al., 2002; Van Schaik et al., 2002; Dorshorst et al., 2006; Bennett et al., 2012; Heuer et al., 2012; Marquetoux et al., 2012); *iii*) to evaluate national or industry-wide strategies for cost-effective testing, herd-certification or disease management (Weber et al., 2004; Tavoranpanich et al., 2006; Tavoranpanich et al., 2008); and *iv*) to predict flock Se for sheep abattoir surveillance (Abbott and Whittington, 2003). Moreover, two comprehensive reviews of simulation model on paratuberculosis have been published, focusing on dairy cattle models for the within herd transmission of MAP (Marce et al., 2010; Nielsen et al., 2011). Some selected simulation models are discussed below.

Collins and Morgan (1991) developed a probabilistic model based on a modified Reed-Frost approach (Abbey, 1952). Their model simulated MAP transmission in an open

dairy cattle herd using deterministic equations with four different disease stages (susceptible, non-infected, infected, and culled). Authors assumed that young animals remained susceptible up to 1 year of age, when uninfected animals moved to a resistant category and infected animals became latent (subclinical and non-infectious). Calves from infected dams had the same risk of becoming infected as all other calves, and at 2 years old, latent animals become infectious for the remaining time in the herd. Additionally, the model was able to simulate control measures such as a test-and-cull program and improvements in hygienic conditions. A more complex model, called 'JohneSSim' was developed by Groenendaal et al. (2001; 2002; 2003) to support the development of a national Ptb control program for The Netherlands. This model used stochastic simulation to estimate the prevalence, incorporating the uncertainty associated with several model parameters. The model considered six different transmission routes, three adult-shedding categories (low, high and clinical), an exponential decay in susceptibility of calves up to 1 year of age when they were assumed to be fully resistant; the probability of a successful infectious contact with a susceptible calf increased as infected cows moved from low-shedding to high-shedding, and then to clinical status. In these two simulation models, shedding status was only assigned to infected adult animals and the probability of a new infection was based on the number of infectious adult animals. Whereas the two models resulted in a similar prevalence under no-control scenarios (50%), a plateau of constant prevalence was reached after 20 years in 'JohneSSim' and after 40 years in the model of Collins and Morgan (1991). In contrast to results of Collins and Morgan (1991), the 'JohneSSim' model indicated that test-and-cull programs were not economically viable. Although a significant drop in MAP prevalence could be achieved through an improvement of hygienic conditions.

Kudahl et al. (2007) presented 'PTB-Simherd', which was another stochastic and mechanistic simulation model for the study of MAP infection dynamics in a dairy herd. The main novelty of this model is the merging of an infection component with a Danish simulation model ('Simherd III') that allowed the incorporation of complex feedback mechanisms between replacement, culling and feeding (Ostergaard et al., 2000). The feedback mechanisms responded to the disease effects and the implementation of different control measures. 'JohneSSim' and 'PTB-Simherd' were relatively complex and therefore difficult to replicate. Mitchell et al. (2008) on the other hand, developed a transparent and easy to replicate model. This one was a frequency dependant simulation model whereas the previous ones were density dependant. The Mitchell- model incorporated updated knowledge of MAP epidemiology, exploring three new possible states with potential impact on transmission dynamics: *i*) a high-shedding state that reflected a much greater difference in shedding levels than previously assumed; *ii*) allowing all infected adults to produce infected calves (not just restricting this transmission to high-shedding adults); *iii*) and considering that young infected calves may be shedding MAP, rather than being latent by default, leading to calf-to-calf transmission. Results from this model, suggested that the low but steady prevalence observed in herds under control programs were better explained by the incorporation of calf-to-calf transmission (van Roermund et al., 2007), in addition to dam-to-calf transmission. Interestingly, a model developed later for beef cattle (Bennett et al., 2012), did not consider calf-to-calf of transmission.

The four models described above focussed on different modes for direct MAP transmission (animal to animal), a fair assumption for dairy farming systems in the

United States or Europe, where animals are commonly confined. However direct transmission is unlikely to be the main route in New Zealand pastoral systems where MAP is mainly ingested through contaminated pasture. Humphry et al. (2006) simulated MAP transmission in a beef cattle herd under pastoral conditions, assuming that contaminated environment was the primary source of infection. However, the model did not explicitly simulate the MAP burden and survival on pasture. It rather used an expected density of MAP in the environment. Two recent models, described by Marce et al. (2011) and Heuer et al. (2012) simulated MAP survival on contaminated pasture or in shared pens. These models considered indirect transmission through a contaminated environment as the main source of infection.

There are few published simulation models in other species than cattle. Two non-peer-reviewed models are available for sheep: Sergeant and Whittington (2000) described a modified Reed-Frost model, in which sheep progressed through susceptible, infected (incubating, light and heavy faecal-shedding, to clinical cases) and recovered states. All age groups were susceptible to infection, and the risk of infection was estimated by the total number of cases, adjusted for the level of shedding as infection progressed. A more complex sheep model was presented by Marquetoux et al. (2012). This model did not assume an age resistance to infection, and infected animals followed two progression branches. In the ‘progressor track’, newly infected animals were initially in a paucibacillary shedding state, which then progressed to a multibacillary shedding state which gave rise to clinical disease, and death or removal. The alternative branch (‘non-progressor track’), also started with a paucibacillary state, but infected animals were able to control the disease and entered a latent (non-shedding) state. Moreover, Marquetoux et al. (2012) simulated the typical seasonal lambing, slaughter, culling and

replacement system of New Zealand sheep farms. In deer, Heuer et al. (2012) described a novel model which incorporated the possibility of herd infection with two different MAP strains, considering different pathogenicity. Additionally, infected deer could follow two possible branches, a 'fast track' leading to early shedding and clinical disease, and a 'slow track' with a long period of latency with clinical disease only occurring during adulthood.

Bayesian estimation of flock/herd-level true *Mycobacterium avium* subspecies paratuberculosis infection prevalence on sheep, beef cattle and deer farms in New Zealand

C Verdugo, G Jones, WO Johnson, PR Wilson, LA Stringer, C. Heuer

3.1 Abstract

The study aimed to estimate the flock/herd-level true prevalence (HTP) of infection with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in pastoral farmed sheep, beef and deer in New Zealand. A stratified-random sample of 238 single- or multi-species farms was selected from a postal surveyed population of 1,940 farms in seven strata of livestock species on farm, single or in combination. The sample included 162 sheep flocks, 116 beef cattle herds and 99 deer herds from seven of 11 geographical regions. Twenty animals from each species present on farm were randomly selected for blood and faecal sampling. Pooled faecal culture involved a single pool from sheep flocks (20 samples/pool), and two pools from beef cattle or deer herds (10 samples/pool). To increase flock/herd sensitivity, sera from all 20 animals from culture negative flocks/herds were tested by Pourquier[®] ELISA (sheep and cattle) or Paralisa[™] (deer). Results were adjusted for sensitivity and specificity of diagnostic tests using Bayesian latent class statistical modelling. Model outcomes were additionally adjusted

by sampling fractions to obtain HTP estimates at population level. Posterior probabilities (POPR) that HTP values were different between strata were obtained.

Across all species, 69% of farms tested positive (i.e. had at least one test positive pool or animal). The highest HTP estimate for sheep flocks (76%, posterior probability interval (PPI) 70-81%), followed by deer (46%, PPI 38-55%) and beef herds (42%, PPI 35-50%). Farms with two or more species had a higher risk of being infected than single species farms. Flock/herd-level true prevalence differences were observed between the two main islands of New Zealand: sheep and beef cattle farms in the North Island had a higher HTP (80% vs. 70% (POPR = 0.96) for sheep, and 44% vs. 38% for beef cattle, POPR = 0.80), whereas the deer HTP was higher in the South Island (33% vs. 54%, POPR = 0.99). Infection with MAP is endemic at high prevalence on sheep, beef cattle and deer farms across New Zealand.

3.2 Introduction

Clinical paratuberculosis (cPtb), caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is a chronic granulomatous enteric disease, which occurs worldwide and affects domestic ruminant species including deer, sheep, and cattle. The causative agent also has been isolated from wildlife species like rabbits, foxes and bison (Chiodini et al., 1984; Greig et al., 1999; Beard et al., 2001a; Harris and Barletta, 2001; Whittington and Sergeant, 2001; de Lisle, 2005). The first cPtb case in New Zealand was recorded in 1912 in an imported cow (de Lisle, 2002). The disease was first reported in New Zealand sheep in 1952, and in the 1980's cPtb was confirmed in farmed deer (de Lisle et al., 1993). Clinical Paratuberculosis is characterized by weight loss and diarrhoea not responding to treatment, leading to emaciation and death, while sub-clinical infection may reduce productivity (Berghaus et al., 2005) or have no

measurable effect at all (Nielsen and Toft, 2008). While MAP infection can be widespread in flocks/herds and animals (Morris et al., 2006; Nielsen and Toft, 2009; Stringer et al., 2009), cPtb incidence in infected flocks/herds is typically around or below 1% (Morris et al., 2006; Glossop et al., 2008; Norton et al., 2009). Clinical outbreaks are commonly reported in deer less than 2-years-old (Glossop et al., 2008) in contrast to sheep and cattle, in which clinical disease is more commonly observed in animals older than 2-3 years (Harris and Barletta, 2001). Isolation of MAP from intestinal tissue of human patients with Crohn's disease (CD), a chronic inflammatory bowel disease, has suggested a possible link between the two diseases, where MAP contaminated animal produce could be the source of CD in humans (Mishina et al., 1996; Feller et al., 2007). The apparent association between MAP in animals and humans has raised concerns within the agricultural industry about the public health relevance of MAP, and a potential effect of high MAP prevalence on trade or consumption of milk and meat.

Infection prevalence estimation is the starting point to assess the impact of a disease, and informs the strategy and design of control programmes. No population-based estimates of MAP infection prevalence or disease incidence are currently available for sheep, beef or dairy cattle in New Zealand. Conversely in deer, a recent cross-sectional study, randomly selected lymph nodes for culture from abattoir surveillance, estimated a national herd-level true prevalence (HTP) of 59% (Stringer et al., 2009) and a previous non-random designed study, using pooled faecal culture (PFC) from 115 deer herds, found 44% of them to be positive (Glossop et al., 2006). In dairy cattle, based on diagnostic laboratory records, 12% of herds were regarded as infected, but this was considered to be an underestimate of the HTP (Burton, 2002). MAP infection is thought

to be endemic in the New Zealand sheep population, although prevalence has not been estimated. A non-peer-reviewed economic evaluation of MAP infection in New Zealand assumed a sheep HTP of 60-70% (Brett, 1998).

In New Zealand, domestic ruminants are commonly farmed in multi-species pastoral systems, where sheep, beef cattle and/or deer are often grazed on the same pastures, either concurrently or successively. Paratuberculosis research in New Zealand therefore addresses all species in an overarching strategy in order to develop an integrated approach to cPtb control (JDRC, 2011). MAP prevalence has been previously estimated in other countries using Bayesian latent-class modelling (Sergeant and Baldock, 2002; Nielsen et al., 2007; Dhand et al., 2010a; Okura et al., 2010). This methodology has the advantage that adjustments can be made for sensitivity and specificity of diagnostic tests in a flexible probabilistic framework that allows researchers to model complex testing designs in order to obtain true prevalence estimates. The objective of this study was to estimate HTP of MAP infection in single- or mixed-species sheep, beef cattle and deer farms in New Zealand.

3.3 Material and Methods

3.3.1 Selection of farms

A detailed description of farm selection and sampling protocols is presented in the Annex A. In general, farms were selected in several stages: from December 2008 to March 2009, a survey (Annex B) was mailed out to 7,998 client farmers of 28 large animal veterinary practices in four administrative regions in the North Island ((NI), Waikato, Wairarapa, Hawkes Bay, Manawatu-Wanganui), and three in the South Island ((SI), Marlborough, Canterbury, Southland), targeting commercial sheep, deer, beef and

dairy cattle operations. The survey gathered retrospective information about animal demographics, reproduction performance, cPtb incidence (up to the last four years), and grazing management information (co-grazing, concurrently or successively) of all ruminant species present, at farm level. The incidence of cPtb reported by farmers was categorised for each species as absent, suspected or confirmed (laboratory or veterinarian). Results of this study are reported elsewhere (Verdugo et al., 2010). A total of 1,940 (24.3%) correctly filled-out questionnaires were returned, constituting the sampling frame (reference population) for the second stage of the study, reported here.

Farms were eligible for sampling if they had a “commercial” operation comprising a minimum of 40 deer, 400 sheep, and/or 40 beef cattle. They were then allocated to single- or multi-species farm designation in the following seven farm type strata (FTS): sheep only (SHP), beef cattle only (BEE), deer only (DEE), sheep and beef cattle (S&B), beef cattle and deer (B&D), sheep and deer (S&D), and sheep, beef cattle and deer (SBD). Dairy farms were excluded from this second stage because the particular study of this farm type stratum was assigned to another research laboratory by the funding organization.

The objective sample size ($n=300$) was powered assuming a 50% prevalence of MAP infection at the farm-level in each stratum, using a conventional frequentist approach. However, that sample size may result in an even greater likelihood of statistically important results for Bayesian latent class analysis, due to the incorporation of valid scientific input through the priors. For each stratum, an equal number of farms were randomly selected from the sampling frame. A frequency comparison of single- and mixed-species farms, based on livestock figures, was conducted between the sampled

population, the reference population and New Zealand farm statistics (Agribase™) (AsureQuality Ltd., 2010).

3.3.2 Sampling protocol and laboratory testing

Sampling was conducted by contracted veterinary practitioners from June 2009 to July 2010. Twenty animals from each species present on farm were randomly selected. Paired faeces and serum samples were collected from sheep (ewes, 2 years and older), beef cattle (cows, 2 years and older) and deer (yearlings, 12-24 months, either sex). In addition to the 20 randomly selected animals, up to five animals with signs resembling cPtb (wasting, diarrhoea) were also sampled (clinical suspect animals) if seen in the flock/herd at the sampling date. Samples from clinical suspects were cultured in separate pools from randomly selected animals. A single-pool was prepared from sheep faeces (20 samples/pool), and two pools were prepared from beef cattle or deer (10 samples/pool) from each farm. Cultures were performed by the Wallaceville Animal Health Laboratory, Upper Hutt, using BACTEC 12B liquid culture medium containing egg yolk and mycobactin, after a decontamination step with cetylpyridinium chloride, as described by Whittington et al. (1999). If a PFC from random or suspect animals was positive, the entire flock/herd was classified as being culture positive. Individual blood serum samples from culture negative flocks/herds were tested by an ELISA test: Pourquier® ELISA in sheep and cattle (Institut Pourquier, Montpellier, France), and Paralisa™ in deer (Griffin et al., 2005). A flock/herd was defined as apparently infected for each species if any PFC or ELISA was positive (cut off = 1+ve animal or pool) in normal or clinical suspect samples for that species.

3.3.3 Statistical analysis

3.3.3.1 Model

Species-specific Bayesian latent class models were developed to estimate HTP, at national, island, and FTS level. The HTP was defined as the percentage of flocks/herds, where at least one MAP infected-animal was present. The model consider the flock/herd level results from the two tests protocol previously described, their test performance characteristics, and TP estimates, to obtain a prediction of the HTP in each level.

Considering a sample (I) of n animals, $n = k \times r$ for r pools of size k , from a given flock/herd (H). A sample which contain at least one infected animal (I^+) only can be drawn from an infected flock/herd (H^+), whereas a sample containing only non-infected animals (I^-) could be drawn from both, a non-infected flock/herd (H^-) or an H^+ . Let T_1 denote the binary flock/herd level outcome of the PFC test and T_2 the binary flock/herd level outcome of the ELISA test, with (+) denoting a positive flock/herd, and (-) a negative flock/herd. Then, for a given species in a given island (i) belonging to a given FTS (j), the testing protocol generated three possible test outcomes ($T_1^+ _ ; T_1^- T_2^+ ; T_1^- T_2^-$), where counts (y_{ij}) of those outcomes were assumed to being multinomial distributed:

$$y_{ij} = \text{multinomial}(\text{TestProb}_{ij}, nh_{ij})$$

where TestProb_{ij} is a vector of probabilities of observing the three possible test outcomes and nh_{ij} is the number of flocks/herds sampled. To model TestProb_{ij} , we condition on the status of the I sample, assuming conditional independence between PFC and ELISA outcomes, given I . Thus, the multinomial cell probabilities for the TestProb_{ij} vector are given by:

$$Pr(T_1^+ _) = Pr(I^-) \times Pr(T_1^+ | I^-) + Pr(I^+) \times Pr(T_1^+ | I^+)$$

$$Pr(T_1^- T_2^+) = Pr(I^-) \times Pr(T_1^- | I^-) \times Pr(T_2^+ | I^-) + Pr(I^+) \times Pr(T_1^- | I^+) \times Pr(T_2^+ | I^+)$$

$$Pr(T_1^- T_2^-) = 1 - Pr(T_1^+ _) - Pr(T_1^- T_2^+)$$

and based on the law of total probability:

$$Pr(T_1^+ | I^+) = \frac{Pr(T_1^+) - Pr(T_1^+ | I^-) \times Pr(I^-)}{Pr(I^+)}$$

$$Pr(T_2^+ | I^+) = 1 - \left(\frac{Pr(T_2^- | H^+) - Pr(T_2^- | I^- | H^+) \times Pr(I^- | H^+)}{Pr(I^+ | H^+)} \right)$$

The conditional probabilities are defined by: *i*) herd level true prevalence (HTP_{ij}) in a given island and strata, *ii*) the true animal level prevalence (TP), in an average infected flock/herd, *iii*) the herd level sensitivity (Hse) and specificity (Hsp) of PFC test, and *iv*) the animal level Se and Sp of the ELISA test, being modeled such that:

$$Pr(I^-) = (1 - HTP_{ij}) + HTP_{ij} \times (1 - TP)^n$$

$$Pr(T_1^+ | I^-) = 1 - Hsp$$

$$Pr(I^+) = 1 - Pr(I^-)$$

$$Pr(T_1^+) = HTP_{ij} \times Hse + (1 - HTP_{ij}) \times (1 - Hsp)$$

$$Pr(T_1^- | I^-) = Hsp$$

$$Pr(T_2^+ | I^-) = 1 - (sp)^n$$

$$Pr(T_1^- | I^+) = 1 - Pr(T_1^+ | I^+)$$

$$Pr(T_2^- | H^+) = [(1 - se) \times TP + sp \times (1 - TP)]^n$$

$$Pr(T_2^- | I^- | H^+) = Pr(T_2^- | I^-) = sp^n$$

$$Pr(I^- | H^+) = (1 - TP)^n$$

$$Pr(I^+ | H^+) = 1 - Pr(I^- | H^+)$$

and

$$Hse = 1 - [1 - (1 - TP)^k \times (1 - Pse) + (1 - TP)^k \times Psp]^r$$

$$Hsp = Psp^r$$

where Pse & Psp are the pool level sensitivity and specificity of the PFC test. For the two-test scenario.

$$\begin{aligned}
 Hsej &= Pr(I^-|H^+) \times [Pr(T_1^+|I^-) + (1 - Pr(T_1^+|I^-)) \times Pr(T_2^+|I^-)] \\
 &\quad + (1 - Pr(I^-|H^+)) \\
 &\quad \times [Pr(T_1^+|I^+) + (1 - Pr(T_1^+|I^+)) \times Pr(T_2^+|I^+)] \\
 Hspj &= Pr(T_1^-|I^-) \times Pr(T_2^-|I^-)
 \end{aligned}$$

Model based output for HTP_{ij} , for each species, were corrected by the sampling fraction using weighted averages to obtain a population estimation of these parameters, with weights being the proportion of flocks/herds, in each island/strata from which animals were sampled among all flocks/herds present in the reference population. These proportions were multiplied with the HTP_{ij} for each island/strata, and the products were added to derive the weighted strata, island and national estimate. Population adjusted HTP_{ij} were modelled such that:

$$HTP_{adj[j]} \sim (HTP_{1j} \times wni_j + HTP_{2j} \times wsi_j)$$

$$HTP_{adj[i]} \sim (HTP_{i1} \times wa_{i1} + HTP_{i2} \times wa_{i2} + HTP_{i3} \times wa_{i3} + HTP_{i4} \times wa_{i4})$$

$$HTP_{adj[NAT]} \sim (HTP_{i=1} \times iwa_1 + HTP_{i=2} \times iwa_2)$$

where $HTP_{adj[j]}$ is the adjusted flock/herd true FTS prevalence, while $HTP_{adj[i]}$ and $HTP_{adj[NAT]}$ are the adjusted island and national flock/herd level true prevalence for a given species, where wni_j & wsi_j are the NI and SI weights for a given stratum, $wa_{[ij]}$ are the FTS weights in a given farm type/island combination of a species, and $iwa_{[i]}$ are the NI and SI weights for the national adjustment. An example of the model code is presented in Annex C.

Comparison of $HTP_{adj[ij]}$ distributions between NI and SI, and among FTS were done by computation of Bayesian posterior probabilities (POPR); testing the hypothesis that

i) NI has higher $HTP_{adj[i]}$ than SI, and ii) one farm type stratum has higher $HTP_{adj[j]}$ than another. All possible FTS combinations were assessed for each species. POPR is approximated by the proportion of MC samples where the hypothesis tested was true (Okura et al., 2010). POPR values close to 1 (or 0) represent the probability that the difference between the two prevalences is positive (or negative) meaning that the first stratum has a higher (or lower) $HTP_{adj[ij]}$ than the second one. POPR values around 0.5 imply that the two prevalences under comparison are similar. A difference was regarded as being unlikely due to chance, or statistically different, when POPR was either smaller than 0.1 or greater than 0.9. For example if we were 99% sure that that one prevalence was larger than another, after seeing the data, the prevalences are regarded as highly likely to be different.

3.3.3.2 Test characteristics and predictive values

Computations of PFC Hse and Hsp are previously described. In the two-test scenario, joint herd-level sensitivity ($Hsej$), specificity ($Hspj$), and the joint herd-level predictive value positive ($HPVj.pos$) and negative ($HPVj.neg$) were derived from the equations presented above, resulting in:

$$Hsej = Pr(I^-|H^+) \times [Pr(T_1^+|I^-) + (1 - Pr(T_1^+|I^-)) \times Pr(T_2^+|I^-)] \\ + (1 - Pr(I^-|H^+)) \\ \times [Pr(T_1^+|I^+) + (1 - Pr(T_1^+|I^+)) \times Pr(T_2^+|I^+)]$$

$$Hspj = Pr(T_1^-|I^-) \times Pr(T_2^-|I^-)$$

$$HPVj.pos = HTP_{adj[NAT]} \times Hsej / (HTP_{adj[NAT]} \times Hsej + (1 - HTP_{adj[NAT]}) \times (1 - Hspj))$$

$$HPVj.neg = (1 - HTP_{adj[NAT]}) \times Hspj / ((1 - HTP_{adj[NAT]}) \times Hspj + HTP_{adj[NAT]} \times (1 - Hsej))$$

Positive and negative predictive values were adapted from Su et al. (2007) with $Hsej$ and $Hspj$ are replacing Hse and Hsp .

3.3.3.3 Priors

Models use six prior parameters: TP_{ij} , HTP_{ij} , Pse , Psp , se , and sp , where scientific input about them is modelled with independent beta distributions. Available TP_{ij} and HTP_{ij} information for sheep and beef cattle flocks/herds in New Zealand is sparse and estimates from other countries are not appropriate for New Zealand's farming conditions. To overcome the lack of published information for these two parameters, MAP infection test results from a separate data set of 67 single and mixed species farms, belonging to a large farming corporation, were used to form prior distributions. These farms included 63 sheep flocks and 49 beef cattle herds (2,274 animals) from which test results were not included in the prevalence estimation analysis reported in the present study, contributing exclusively to the construction of model priors. TP_{ij} and HTP_{ij} were assumed to be constant across strata for each species, and priors for these two parameters were obtained based on individual ELISA results using the Bayesian latent class model proposed by Branscum et al. (2004), assuming independent beta priors for TP_{ij} and HTP_{ij} . Conversely, for deer herds, prior information was obtained based on a recent study conducted by Stringer et al. (2009), which estimated TP_{ij} and HTP_{ij} in New Zealand's North and South Islands. ELISA and PFC tests characteristics were obtained from peer-reviewed literature. Beta parameters (a, b) were obtained using the free software BetaBuster available at <http://www.epi.ucdavis.edu/diagnostictests/>. Inputs parameters values, beta distributions, and references are presented in Table 3.1.

3.4 Results

A total of 238 farms were sampled, representing 162 sheep flocks, 116 beef cattle herds and 99 deer herds (7,579 animals). The target sample of 300 farms was not realised for

reasons such as animal crushes not being available (n=6), species mobs being inaccessible (n=17), or losses to follow-up following initial farm enrolment (n=39). Figure 3.1 shows the spatial distribution of the sampled farms. The main regions represented in the study were Manawatu-Wanganui in the NI, and Canterbury and Southland in the SI. The distribution of the reference population (this study) and Agribase™ data is presented in Figure 3.2 by FTS. Descriptive statistics of flocks/herds size by NI and SI are presented in Table 3.2. Sampled farms and flock/herd level apparent prevalence (HAP) were cross-tabulated by species, island, and FTS (Table 3.3). Mixed-species farms with both sheep and beef cattle were the largest stratum in the sample (39.1%), consistent with the current distribution of New Zealand farms, where this category represents 46.6% of all farms (AsureQuality Ltd., 2010). At farm level, 68.9% presented at least 1 flock/herd testing positive, and an overall HAP of 71.0%, 29.3%, and 56.6% was observed for sheep, beef cattle and deer flocks/herds, respectively.

3.4.1 Sheep flocks

Table 3.4 presents the posterior median and 95% PPI of $HTP_{adj[ij]}$ for each species and FTS. Sheep flocks had the highest $HTP_{adj[NAT]}$ (75.3% (95% PPI 68.1-81.7%)) among the species under study. In this species, estimated $HTP_{adj[i]}$ was higher in the NI than SI (79.2% and 69.7%, respectively), with an associated POPR of 0.91, representing a large difference in prevalence between islands. Moreover, an average infected flock present a TP between 2.3 to 46.3% (95% PPI), with a median of 8.2%.

Among the farms with sheep present, S&B and SBD strata had the highest $HTP_{adj[j]}$ although POPRs for differences were low to moderate with a maximum of 0.72 for the contrast between S&B vs. S&D, and 0.68 (inverse value (INV), 1 minus POPR value)

for SHP vs. S&B. All other comparisons had a POPR of around 0.5, representing no evidence of difference (Table 3.5). The comparison between prior and posterior HTP distributions is presented in Figure 3.3 and the uncertainty associated with each $HTP_{adj[j]}$ is illustrated in Figure 3.4. The use of the ELISA test increased the Hse from 0.51 when PFC alone was used, to 0.86, while decreasing Hsp from 0.99 to 0.50 (Table 3.6).

Table 3.1: Prior parameters, mode and 95% percentile of Pool sensitivity (Pse) and Pool specificity (Psp) of pooled faecal culture of MAP in liquid media (BACTEC). Sensitivity (se) and specificity (sp) of ELISA and Paralisa™ tests for MAP, and animals (TP) and flock/herd (HTP) level true prevalence for sheep, beef cattle and deer flocks/herds in New Zealand

	Mode	5%/95% Percentile	a	b	Reference
Sheep					
Pse*	0.36	0.63	3.62	6.08	(Dhand et al., 2010b)
Psp	0.99	0.999	84.89	1.17	(Dhand et al., 2010b)
ELISA se	0.415	0.483	60.56	85.23	(Sergeant et al., 2003)
ELISA sp	0.95	0.934	570.03	30.32	(Sergeant et al., 2003)
TP	0.13	0.50	0.84	3.93	Calculated
HTP	0.78	0.45	4.81	1.58	Calculated
Beef cattle					
Pse**	0.471	0.511	32.08	44.72	(Tavornpanich et al., 2004)
Psp	0.994	0.998	504.18	3.37	(Tavornpanich et al., 2004)
ELISA se	0.09	0.13	13.47	136.46	(McKenna et al., 2005)
ELISA sp	0.98	0.99	439.62	11.13	(McKenna et al., 2005)
TP	0.06	0.44	0.46	3.39	Calculated
HTP	0.40	0.20	5.42	7.97	Calculated
Deer					
Pse	0.77	0.92	9.9	3.18	(Stringer, 2011)
Psp	0.99	0.98	425.89	4.63	(Stringer, 2011)
Paralisa™ se	0.19	0.28	12.12	50.59	(Stringer, 2011)
Paralisa™ sp	0.94	0.93	1581.05	101.23	(Stringer, 2011)
TP: -NI***	0.29	0.45	7.56	18.04	(Stringer, 2011)
-SI	0.51	0.66	14.76	14.19	(Stringer, 2011)
HTP: -NI	0.44	0.64	7.48	9.42	(Stringer, 2011)
-SI	0.67	0.85	9.17	4.68	(Stringer, 2011)

*Pse was calculated as a weighed average assuming a 20% of multibacillary sheep (Abbott et al., 2004) among the infected animals in a given positive flock.

**Pse was calculated as a weighed average assuming a 10% of high shedders cows (Marce et al., 2011) among the infected animals in a given positive herd.

*** NI: New Zealand's North Island, SI: New Zealand's South Island

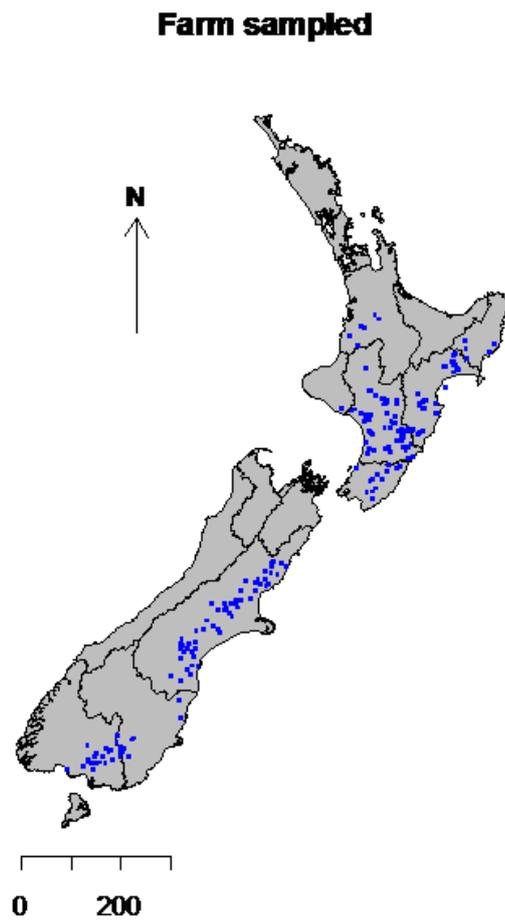


Figure 3.1: Spatial distribution of sampled farms (N = 238)

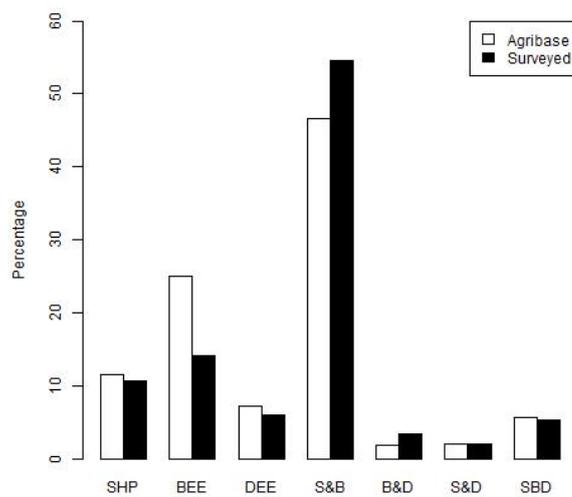


Figure 3.2: Distribution of farm type strata for those with ≥ 400 sheep, ≥ 40 beef cattle and ≥ 40 deer, sampled in the present study compared with New Zealand farm statistics (AgribaseTM)

Table 3.2: Number, mean, median, and range of flocks/herds size by Island

Flock/herd size							
Species	Island	No. flocks/herds	Mean	SD	Median	Range	P-value*
Sheep	NI	96	3,979.3	4,170.6	2,925.0	30,174	<0.01
	SI	66	3,301.1	2,648.6	2,485.5	14,253	
	Both	162	3,703.0	3,634.2	2,776.5	30,174	
Beef	NI	86	460.3	406.4	351.5	2,301	<0.01
	SI	30	289.1	239.3	238.0	907	
	Both	116	416.1	377.1	312.0	2,301	
Deer	NI	41	511.8	588.5	338.0	3,600	<0.01
	SI	58	873.9	1,100.4	530.0	6,960	
	Both	99	723.9	936.9	438.0	6,960	

*F-test for the null hypothesis that flock/herd size population in NI vs. SI have the same variance

Table 3.3: Sampled population frequency and MAP flock/herd-level apparent prevalence (HAP) at national level (NAT), North Island (NI), South Island (SI), and farm type strata to which each species belong: only sheep (SHP), only beef cattle (BEE), only deer (DEE), sheep & beef (S&B), sheep & deer (S&D), beef cattle & deer (B&D), and the three species (SBD)

Sheep flocks			Beef cattle herds			Deer herds		
Strata	N	HAP % (95% CI*)	Strata	N	HAP % (95% CI)	Strata	N	HAP % (95% CI)
NAT	162	71 (67 – 75)	NAT	116	29 (25 – 34)	NAT	99	57 (52 – 62)
NI	96	76 (72 – 80)	NI	86	31 (26 – 36)	NI	41	49 (41 – 57)
SI	66	64 (58 – 70)	SI	30	23 (15 – 31)	SI	58	62 (56 – 68)
SHP	23	65 (55 – 75)	BEE	13	15 (5 – 26)	DEE	29	52 (42 – 61)
S&B	80	71 (66 – 76)	S&B	69	36 (30 – 42)	S&D	14	57 (43 – 71)
S&D	18	72 (61 – 83)	B&D	8	25 (9 – 41)	B&D	18	67 (55 – 78)
SBD	41	73 (66 – 80)	SBD	26	19 (11 – 27)	SBD	38	55 (47 – 63)

CI = Confidence interval

3.4.2 Beef cattle herds

A $HTP_{adj[NAT]}$ of 42.5% (95% PPI 34.6-51.4%) was estimated for beef cattle herds, being the species recorded with the lowest $HTP_{adj[NAT]}$. An average infected herd presented a TP of 6.5% (95% PPI, 1.0 – 45.5%) (Table 3.4). There was a moderate to

low trend for a difference between islands of 43.9% vs. 38.4% for NI and SI, respectively (POPR = 0.78). Farms with sheep and beef ($HTP_{adjljl} = 43.3\%$) were more likely to be infected with MAP than were beef cattle in isolation ($HTP_{adjljl} = 37.9\%$), however this difference was low (POPR = 0.67 (IVN) (Table 3.5). Figure 3.3 displays the contrast between prior and posterior HTP distributions, while the uncertainty around the prevalence estimates for each beef cattle category is presented in Figure 3.5. Similar to the pattern observed in sheep flocks, the inclusion of ELISA testing of PFC negative herds increased Hse from 0.39 to 0.56 and decreased Hsp from 0.99 to 0.80 (Table 3.6).

Table 3.4: Posterior median and 95% posterior probability interval (PPI) for the true flock/herd-level MAP prevalence of sheep, beef cattle and deer flocks/herds, at national level (NAT), North Island (NI), South Island (SI), and farm type strata: only sheep (SHP), only beef cattle (BEE), only deer(DEE), sheep & beef (S&B), sheep & deer (S&D), beef cattle & deer (B&D), and the three species (SBD)

Sheep flocks			Beef cattle herds			Deer herds		
Strata	Posterior median %	95% PPI	Strata	Posterior median %	95% PPI	Strata	Posterior median %	95% PPI
NAT	75	68 – 82	NAT	43	35 – 51	NAT	46	39 – 54
NI	79	69 – 88	NI	44	34 – 55	NI	32	23 – 43
SI	70	61 – 78	SI	38	30 – 48	SI	56	46 – 67
SHP	73	60 – 84	BEE	38	19 – 60	DEE	45	32 – 59
- NI	79	61 – 92	- NI	38	18 – 62	- NI	33	16 – 53
- SI	69	50 – 85	- SI	39	24 – 56	- SI	51	34 – 70
S&B	77	64 – 88	S&B	43	32 – 57	S&D	55	40 – 70
- NI	80	63 – 92	- NI	44	31 – 61	- NI	41	21 – 63
- SI	71	53 – 86	- SI	38	23 – 54	- SI	61	40 – 80
S&D	72	58 – 84	B&D	41	30 – 53	B&D	51	37 – 66
- NI	80	61 – 92	- NI	47	31 – 63	- NI	43	24 – 63
- SI	69	50 – 85	- SI	38	23 – 55	- SI	58	37 – 78
SBD	75	63 – 85	SBD	42	31 – 54	SBD	41	30 – 54
- NI	79	62 – 92	- NI	45	29 – 62	- NI	25	12 – 43
- SI	70	52 – 86	- SI	38	24 – 55	- SI	58	40 – 75
TP	8	2 – 46	TP	7	1 – 46	TP	39	23 – 58

Table 3.5: Posterior probabilities (POPR) for true flock/herd-level prevalence comparison among the farm type strata of each specie

Sheep flocks		Beef cattle herds		Deer herds	
Farm type comparison	POPR	Farm type comparison	POPR	Farm type comparison	POPR
[SHP] - [S&B]	0.32	[BEE] - [S&B]	0.33	[DEE] - [S&D]	0.16
[SHP] - [S&D]	0.55	[BEE] - [B&D]	0.39	[DEE] - [B&D]	0.27
[SHP] - [SBD]	0.42	[BEE] - [SBD]	0.36	[DEE] - [SBD]	0.65
[S&B] - [S&D]	0.72	[S&B] - [B&D]	0.59	[S&D] - [B&D]	0.64
[S&B] - [SBD]	0.60	[S&B] - [SBD]	0.55	[S&D] - [SBD]	0.91
[S&D] - [SBD]	0.37	[B&D] - [SBD]	0.46	[B&D] - [SBD]	0.84

POPR values close to 1 (or 0) represent the probability that the difference between the two estimates is positive (or negative) meaning that the first stratum has a higher (or lower) $HTP_{adj[ij]}$ than the second one. POPR values around 0.5 imply that the two prevalence under comparison are similar.

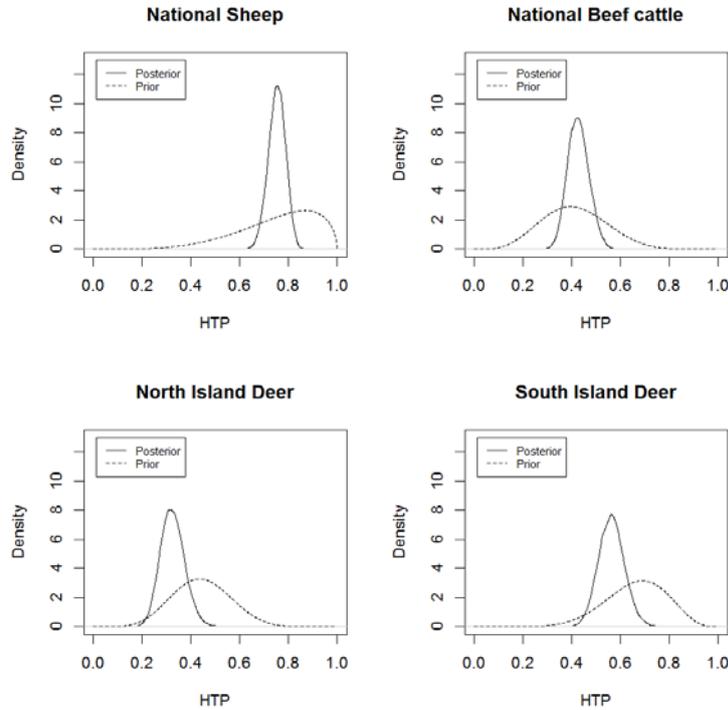


Figure 3.3: Prior and posterior distributions for flock/herd-level true prevalence (HTP) of MAP infection in New Zealand at national (sheep and beef) and island level (deer)

3.4.3 Deer herds

Deer herds had a $HTP_{adj[NAT]}$ of 46.2% (95% PPI 39.2-54.3%) and the average infected herd presented a TP between 22.8 to 58.1% (95% PPI) with a median of 38.5%. The HTP posterior median and 95% PPI for all deer strata are presented in Table 3.4. The

comparison between HTP prior and posterior distributions is presented in Figure 3.3, and uncertainty about $HTP_{adj[j]}$ estimates is shown in Figure 3.6. Herds in the NI had lower $HTP_{adj[i]}$ than the SI, 32.2% vs. 56.1%, with a POPR of 0.01 indicating a high probability that the prevalence in the two islands are different. Farms grazing deer and sheep or beef cattle had the highest $HTP_{adj[j]}$ (55.4% and 51.3% respectively) of all deer FTS. Contrast between DEE vs. S&D and B&D vs. SBD resulted in observed POPR values (0.84 and 0.84, respectively), indicating moderate evidence of $HTP_{adj[j]}$ difference between these strata. Although, the maximum difference was observed between S&D vs. SBD with a POPR of 0.91, indicating an important difference between those strata (Table 3.5). Inclusion of ParalisaTM in PFC negative herds resulted in an increase in Hse from 90% to 99%, with a corresponding decrease in Hsp from 0.98 to 0.39 when the ParalisaTM test was applied (Table 3.6).

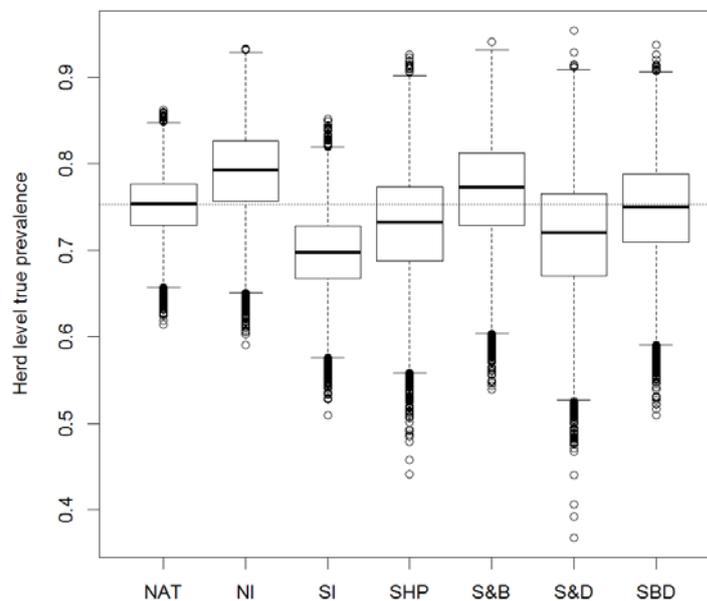


Figure 3.4: Box-plot of the posterior distribution for the true flock/herd-level MAP prevalence of sheep flocks, nationally (NAT), in the North Island (NI) and South Island (SI), and four farm type strata: sheep only (SHP), sheep & beef (S&B), sheep & deer (S&D), and all three species (SBD)

Table 3.6: Herd level sensitivities, specificities and predicted values of the PFC test (Hse, Hsp, HPVpos, and HPVneg) and PFC + ELISA joint test (Hsej, Hspj, HPVposj, and HPVnegj) of sheep, beef cattle and deer flocks/herds

	Sheep			Beef cattle			Deer		
	Posterior Median	95% PPI		Posterior Median	95% PPI		Posterior median	95% PPI	
Hse	0.51	0.41	0.62	0.39	0.25	0.55	0.90	0.69	0.99
Hsej	0.86	0.79	0.92	0.56	0.42	0.71	0.99	0.93	1.00
Hsp	0.99	0.95	1.00	0.99	0.97	1.00	0.98	0.96	0.99
Hspj	0.50	0.40	0.60	0.80	0.72	0.87	0.39	0.33	0.45
HPVpos	0.99	0.96	1.00	0.93	0.81	0.98	0.97	0.93	0.99
HPVj.pos	0.84	0.78	0.88	0.65	0.55	0.75	0.57	0.50	0.64
HPVneg	0.40	0.28	0.53	0.69	0.57	0.79	0.92	0.73	0.99
HPVj.neg	0.56	0.38	0.76	0.72	0.59	0.83	0.97	0.82	1.00

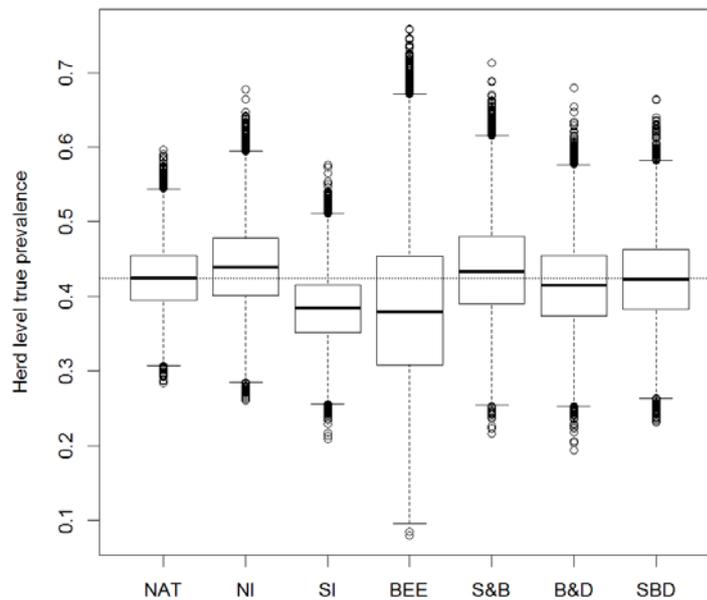


Figure 3.5: Box-plot of the posterior distributions for the true flock/herd-level MAP prevalence of beef cattle herds, nationally (NAT), in the North Island (NI) and South Island (SI), and four farm type strata: beef cattle only (BEE), sheep & beef (S&B), beef cattle & deer (B&D), and all three species (SBD)

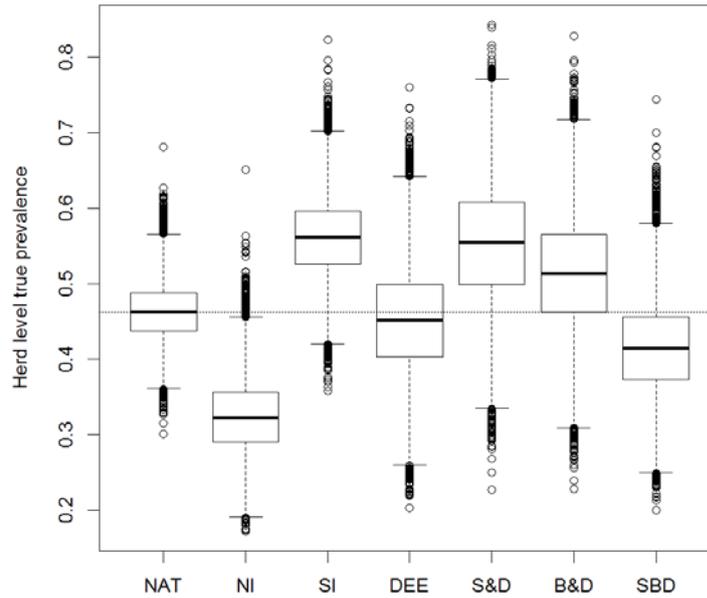


Figure 3.6: Box-plot of the posterior distributions for the true flock/herd-level MAP prevalence of deer herds, nationally (NAT), in the North Island (NI) and South Island (SI), and four farm type strata: deer only (DEE), sheep & deer (S&D), beef cattle & deer (B&D), and all three species (SBD)

3.5 Discussion

Mycobacterium avium subspecies *paratuberculosis* infection has been diagnosed in all ruminant livestock species in New Zealand. However, no study based on random selection and sampling has been conducted to quantify its population distribution at field level. Hence, this study presents the first robust prevalence estimate of MAP infection in multi-species livestock farms in New Zealand. The study is in contrast to most previous research worldwide, in which MAP prevalence has been studied in isolated species, often not taking into account the interaction with other co-habiting ruminants.

This study was based on a stratified random sample to take into consideration the complexity of the New Zealand pastoral farming system, and model HTP outputs were additionally adjusted by their sampling fraction to obtain population level estimates. Results support that MAP is an endemic, well established and widely spread infection in New Zealand, with a high proportion of sheep flocks and deer and beef cattle herds infected (75.3%, 42.5% and 46.2%, respectively).

The present research was designed to estimate prevalence of flocks/herds infected with MAP. This target condition potentially includes latently infected, shedding and/or clinically affected animals (Nielsen and Toft, 2008). The PFC test needs viable MAP organisms to render a positive result, being able to identify animals in the infectious or affected stage, as well as passive shedders. Conversely, ELISA tests identify an immune-response (sero-conversion) to MAP infection, being able to detect animals latently infected, shedding and/or clinically affected. Despite that the PFC test has a higher sensitivity than individual ELISA test, sero-conversion can be detected in milk prior to MAP shedding (Nielsen and Toft, 2008) giving the chance of identifying infected animals earlier than achievable by PFC testing. In general, it is assumed that faecal culture has a perfect Sp at flock/herd level due to the irrelevance of the pass-through phenomena present at animal level diagnosis (Evan Seargent, personal communication), however in the present research non perfect priors were chosen for PFC sheep and cattle flocks/herds, leaving a margin of 1% to account possible errors, representing a reasonable assumption.

Flock/herd level prevalence in the species researched have been investigated elsewhere, although the number of studies are limited, and comparison are difficult to draw due to

differences in the target population, farming systems, sampling methods or lack of adjustment for imperfect test characteristics. Relevant MAP infection prevalence studies, from overseas and New Zealand are discussed below.

In a national wide study, HTP was estimated in dairy cattle herds in the UK (DEFRA, 2010). Authors used a Bayesian latent class model to adjust for test characteristics of three simultaneous diagnostic tests, reporting a MAP HTP of 34.7% in dairy cattle farms milking over 20 cows in the UK. In another study, Nielsen et al (2000), reported a HTP of 70% in Danish dairy cattle farms based on bulk tank milk ELISA testing. Herd level apparent prevalence of MAP infection on US dairy farms was estimated to be at least 21.6% in the general population, and at least 39.7% for herds of over 300 animals, using ELISA testing (Wells and Wagner, 2000). In a systematic review of MAP prevalence in several European countries (Nielsen and Toft, 2009), the authors found the HAP country prevalence ranging from 3% to 68% in cattle. Due to study design, lack of information, or errors in test interpretation, the authors were unable to obtain HTP directly from the reviewed studies and could only report an approximate HTP estimate of over 50%.

A similar situation was described for deer by Nielsen and Toft (2009), in the Czech Republic. However, due to scarcity of available information the authors did not provide any HTP estimate for this species. In sheep flocks, HAP of 24% and 29% were reported in Switzerland and Spain, respectively. However, HTP estimates are not available (Reviriego et al., 2000; Muehlherr et al., 2003). Sergeant and Baldock (2002) estimated sheep HTP in Australia, based on abattoir surveillance data, their estimates ranging from 2.4 to 4.4% Australia-wide and 6 to 10% of flocks in New South Wales.

In New Zealand, two previous studies have estimated HTP in farmed deer: a non random PFC study estimated a HTP of 44% (Glossop et al., 2006), and a recent random cross-sectional abattoir study using lymph nodes culture, found a national HTP of 59%, after controlling for sampling fraction and imperfect test characteristics (Stringer et al., 2009), showing comparable results with our study.

The comparison of HTP¹ showed differences between the North and South Island in all species. However, the degree and direction of this difference was species-specific, with POPR of 0.91, 0.78, and 0.01 for sheep, beef cattle, and deer, respectively. A POPR of 0.91 in sheep flocks indicated that 91% of 25,000 model iterations had a greater HTP in the NI than SI. Conversely, a POPR of 0.01 in deer herds represents that only 1% of the posterior distribution had greater HTP in the NI than SI.

The HTP of all sheep FTS showed a higher prevalence in the NI than SI. There is a difference in sheep flocks distribution between islands, which increased the relative weight of flocks in the NI (sampling fractions), adjusting the island estimate towards a greater prevalence in the NI. Similar to the pattern for sheep flocks, the HTP of beef cattle herds was also higher in the NI. However, the contrast with the SI was lower because not all beef FTS in the NI had a higher HTP than in the SI. The HTP of beef cattle herds were also highest in the S&B stratum of the NI with a weight of 0.83 compared to a weight of 0.62 for the SI. Again this difference-adjusted MAP infection prevalence of beef cattle herds, toward a higher prevalence in the NI than SI. In contrast to sheep and beef, the HTP of deer was greater in the SI than the NI (POPR = 0.01). Stringer et al (2009) found a similar island difference among deer herds in cultured

¹ All HTP references from this section forward are sampling fraction adjusted

lymph nodes of randomly selected slaughterhouse carcasses, with HTP of 44% and 67% for NI and SI, respectively. A difference between islands, as observed in this study, has not been previously known or suspected for sheep or beef cattle. Risk factors explaining the observed differences between NI and SI have not been investigated for any of the three species, and the study of these factors was beyond the scope of this research. Studies overseas have indicated flock/herd size as a consistent risk factor for MAP infection (Jakobsen et al., 2000; Wells and Wagner, 2000; Daniels et al., 2002; Hirst et al., 2004; Woodbine et al., 2009). The comparison of the flock/herd size between islands from the sampled farms (Table 3.2), indicate that beef cattle herds and sheep flocks in the NI, and deer herds in the SI are significantly larger than in the other island. In all species, larger flock/herd size correlated with the HTP differences between islands. Larger flocks/herds may be expected to harbour one or more shedding animals and introduce susceptible young stock more consistently over time than small flocks/herds (Norton, 2007). Soil characteristics have also been proposed as risk factors (Ward and Perez, 2004; Dhand et al., 2009), affecting the ability of MAP to survive in the environment, and soil types differ between islands in New Zealand. Additionally, a recent molecular survey in New Zealand, using VNTR and SSR techniques in tandem, observed significant differences in the dominant MAP strains isolated from the NI vs. SI (Chapter 6). Hence, the difference between island specific prevalence estimates may be explained by the interaction between soil type, flock/herd size, topography, and differences in MAP strains between islands. Such factors were not considered in our study and may therefore deserve future research attention.

Mycobacterium avium subspecies *paratuberculosis* isolates have been characterized by a variety of molecular methods. Two major groups or strain types were described, Type

I (ovine, sheep or s-strain) and Type II (bovine, cattle or c-strain) (Collins et al., 1990; Bauerfeind et al., 1996; Sevilla et al., 2005). S-strain has been mainly isolated from sheep, whereas c-strain has been isolated from cattle and several wildlife species (Motiwala et al., 2006b), indicating a possible MAP-host adaptation. However, s-strain has also been isolated from clinically affected cattle in Australia and Iceland (Whittington et al., 2001), and c-strain has been isolated from sheep and non-ruminant hosts (Stevenson et al., 2002), suggesting that host species are susceptible to different MAP-types. Thus MAP may be transmitted between host species and host-pathogen adaptation is not absolute, although, Moloney and Whittington (2008) classified transmission of MAP from sheep to cattle as a sporadic event, based on field data. However, if s- and c-strains were as host specific in New Zealand sheep and beef cattle as these studies suggest, and the expected cross species transmission was low, the infection prevalence for each species would be similar on single and mixed species farms indicated by POPR values around 0.50. Since both MAP strain types were found in each species (Chapter 6) and the data suggest that the presence of multiple species on a farm increases the risk of infection for each species, our findings indicate that sheep, cattle and deer are all susceptible for both MAP types and transmission between species was likely.

For sheep, HTP was higher for the S&B and SBD strata than for the SHP stratum, although the difference was moderate to low (POPR values of 0.32 and 0.42, respectively). On the other hand, SHP and S&D strata had similar HTP with a POPR of 0.55 indicating almost no difference between these two categories. Hence, the presence of beef cattle was associated with a trend to higher HTP in sheep whereas the presence of only deer was not, suggesting that sheep were more readily infected by cattle than by

deer. Similar to sheep flocks, the HTP of beef cattle herds in multi species farms was higher than in beef-only farms. Beef cattle herds were prone to being infected when sheep were also present (POPR = 0.33), where it has been observed that 80% of beef cattle herds are infected with MAP type I (Chapter 6). Such tendency for a greater infection risk was also present in deer, where S&D farms presented a higher prevalence than only deer farms (POPR = 0.16). The HTP differences between single and mixed deer farms were moderate to low, similarly to that observed for the other two species (POPR of 0.27, 0.65 for B&D, SBD, respectively). However, inferences about these associations for deer herds were limited due to a considerably smaller sample size and, consequently, wider 95% HTP probability intervals. From the observed HTP differences between single vs. mixed species farms, we infer evidence of MAP transmission between species, where the presence of beef cattle increased the HTP of sheep and *vice versa*. Notwithstanding other above mentioned factors, these observations indicate that across-species transmission is common under New Zealand farming conditions.

Whether the observed HTP differences between species were consequences of true transmission due to grazing the same contaminated pasture or caused by more stress due to competition for access to feed, negative social interaction, a mismatch of stocking density and dry matter availability, or separating animals during mustering was not evident from the data. Moreover, multi-species farms could be exposed to a higher number of contacts with other farms through live animal movements, increasing their chances of infection by transmission between farms. Such factors may therefore be the subject of future investigations. The findings of the study underpin the understanding that research should address MAP infection and its clinical manifestation in a multi-species context. This includes the consideration of variations in grazing management

and the need to differentiate MAP strains when describing the epidemiology and consider control options.

Our farm type classification was based on a minimum number of sheep (≥ 400), beef cattle (≥ 40) and deer (≥ 40). While under this classification the chance of contact between species could be high, the presence alone does not necessarily infer that species were co-grazed. It was possible that on some farms, species were kept in different paddocks all year without grazing the same pasture jointly or in succession, thus preventing direct cross-species transmission, although indirect transmission through water run-off, wildlife, or contaminate machinery could not be ruled out. If species were effectively isolated in some of the multi-species farms, the POPR estimates would be biased towards 0.5.

Sampling fractions were applied to adjust the prevalence estimates, so that conclusions could be drawn at the population level, in this case the 1,940 farms of the immediately preceding postal survey, representing the reference population. The FTS distribution of this reference population was similar to the data in the national agriculture database (AgribaseTM) in strata of SHP, DEE, B&D, S&D, and SBD. The S&B stratum was over-represented and the BEE stratum was under-represented in the sampling frame. However, the classification of a beef farm in AgribaseTM is based on the presence of any beef cattle, whereas we only included beef breeding farms, excluding beef finishing farms, which could explain the relatively small BEE stratum in the postal survey population. We therefore decided to rely on the latter instead of AgribaseTM as a reference for obtaining sampling weights. The response rate to the postal survey was 24.3%, which might suggest that the survey dataset was biased towards farmers

experiencing cPtB. If present at all, such bias was expected to be reduced by inclusion of the study of another disease, leptospirosis, into the questionnaire. Leptospirosis is widespread in deer, beef cattle and sheep in New Zealand (Dorjee et al., 2008; Subharat et al., 2009; Heuer et al., 2010). Another possible selection bias might have occurred at the second sampling level, the stratified random selection of farms for animal sampling from the reference population. Only farmers who were able to gather all species on farm for sampling could be included, thus excluding a number of farms with insufficient facilities or lack of interest during the sampling period. The proportion of farms excluded for these reasons was 20.7% (62/300), thus the exclusion rate, and therefore the extent of potential exclusion bias was regarded as reasonably low.

In previous MAP research, the combination of serologic and culture based tests, applied in series or in parallel, have been studied (Sergeant et al., 2002; Branscum et al., 2004; Carpenter et al., 2004; Tavoranpanich et al., 2008). The testing-protocol used in this study started with PFC followed by individual ELISA testing of serum samples from PFC negative flocks/herds. This approach was chosen based on objectives: i) to obtain MAP isolates and apply strain typing techniques in further research, ii) to minimise cost, and iii) to increase the sensitivity of herd detection (Hse) through the inclusion of a second test. The prevalence of infection within flock/herd (TP), number of animals tested, and severity of cPtB in a flock/herd are other factors also influencing Hse (Tavoranpanich et al., 2008). Our latent class analysis suggested that sequential testing by PFC then ELISA increased Hse, compared to PFC only: Hse increased from 0.51 to 0.86, from 0.39 to 0.56, and from 0.90 to 0.99 in sheep flocks, beef cattle and deer herds, respectively. However, the inclusion of the ELISA decreased the specificity of the herd diagnosis (Hsp), most notably for deer herds with a substantial decrease from

0.98 to 0.39. The decrease was attributable to a relatively poor test specificity of 94% when testing clinically unaffected yearling deer by Paralisa™ (Stringer, 2011). In sheep, the negative effect of serial testing on Hsp was not as severe but still remarkable, decreasing the Hsp from 0.99 to 0.50. Conversely, in beef cattle the decrease in Hsp was discrete, from 0.99 to 0.80, respectively. Thus the inclusion of an ELISA test caused an increase in false positive flocks/herds. Nevertheless, the main diagnostic challenge for testing flocks/herds for MAP infection was the low sensitivity of individual or pooled faecal culture, hence the initial intention to remove false negative flocks/herds through additional ELISA testing. In hindsight, the gain in Hse was more than offset by the loss of Hsp. However, if it is desired to maximise the detection of MAP infection at flock/herd level while a false-positive flock/herd status is of no concern, the additional use of an ELISA on individual animals appears to be an appropriate approach. On the other hand, when the true MAP infection status of a flock/herd is the prime target parameter, such as for flock/herd certification, a false-positive diagnosis would be harmful, thus ruling out the use of an ELISA test as a sole diagnostic strategy, especially for deer herds.

The traditional method to estimate true prevalence from the results of imperfect tests, the Rogan-Gladen estimator, has recently been used in MAP research to estimate TP and HTP (Stringer et al., 2009; Mercier et al., 2010). This method has the advantage of being well established and recognized by the scientific community and recommended as the first choice method for the adjustment of apparent prevalence when the accuracy of tests is known (Nielsen and Toft, 2009). However, the complex sampling and testing design used in this study was not suitable for the use the Rogan-Gladen methodology as only individual samples from PFC negatives flocks/herds were ELISA tested, thus the

data set were unbalanced, being not possible to compute Hse and Hsp distributions representing the entire data set (for each species), as this methodology requires. To overcome these limitations a Bayesian approach was adopted. This technique combines prior knowledge about prevalence and test performance with survey data to produce updated posterior estimates. Prior parameters used in this study were gathered from different sources: test characteristics of PFC and ELISA tests were obtained directly from previous peer-review studies, as were the TP and HTP estimates for deer herds. In the case of sheep and beef cattle, test results from 112 flocks/herds, collected from another study, were used to estimate prior TP and HTP distributions. We regard these 112 flocks/herds to be typical for New Zealand livestock farming systems, and using this approach, we consider that priors parameters are reasonably well informed.

The present research also provided estimates of TP in an average infected flock/herd, those estimates should be taken with caution because they did not represent a primary target during the study design, thus the TP results were derived from only 20 animals sampled per flock/herd, which represented a very low number of individuals for an accurate estimate of TP values. Therefore, model outputs are importantly influenced by the TP prior distribution chosen for each species. In this line, sheep and beef cattle present wide PPI, conversely in deer, where more accurate information was available during the prior elicitation process, a narrow PPI interval was observed.

Considering the high national HTP observed in the present study. In addition to the annual clinical incidence recorded, among infected sheep, beef cattle and deer flocks/herds, which range from 0.04% to 0.32%, where beef cattle herds present the lowest incidence and deer herds the highest mortality/culling (Chapter 5). In New

Zealand there is a scenario of high infection prevalence but a very low clinical incidence. Hence as others have suggested, the objectives of a control program may rather be the stop of MAP spread to naïve flocks/herds and to minimize the impact of cPtb on infected farms (Norton, 2007; Stringer, 2011), providing the best nutrition and welfare possible to reduce stress and its related onset of clinical cases. This study provides evidence that MAP infection of herds and flocks is related to the presence of more than one ruminant species, suggesting that MAP is likely to be transmitted between ruminant, increasing their risk of infection. However, recent evidence suggests that the effect over clinical incidence varies depending of the species co-grazed, where has been observed that the co-grazing of beef cattle with sheep or deer increased the risk of clinical cases in these species. Conversely, the co-grazing of sheep and deer was associated with a decrease in the risk of clinical cases in both species (Chapter 4). These findings may be part of a control program aiming to the reduction of clinical cases in know infected farm, through forced or avoided contact between species grazing the same pasture in parallel or successively. Additionally, the prevalence estimates obtained in the present study could be used as a baseline for future monitoring of HTP following the implementation of a control programme, which aims to stop MAP transmission to naïve flocks/herds. The estimates may also be used as input parameters for modelling the economic effects of MAP infection, or for analysing risk factors for MAP infection in further research.

3.6 Conclusion

MAP infection is widely spread in New Zealand with a high national HTP, especially among sheep farms, with an estimated national flock prevalence of 75%. Infection

prevalence of deer and beef cattle herds is also relatively high at 46% and 43%, respectively. Prevalence differences were observed between islands for the three species. Sheep and beef cattle flock/herd-level prevalence estimates were higher in the North than South Island, while deer herds located in the SI were at greater herd-level prevalence than those in the NI. This study provides evidence for cross species transmission, inferred from different HTP estimates in the presence or absence of sheep, beef cattle or deer at farm level.

3.7 Acknowledgments

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A Bayesian assessment of the dependence of flock/herd level infection risk and clinical incidence of paratuberculosis on joint grazing of sheep, beef cattle and deer in New Zealand pastoral systems

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4.1 Abstract

The objective of this study was to assess the extent to which the joint use of pasture determines the flock/herd MAP infection status and incidence of clinical paratuberculosis (cPtB) on mixed-species farms. A total of 238 farms with 162 sheep flocks, 116 beef cattle herds and 99 deer herds (7,579 animals in total) were tested by pooled faecal culture and serum ELISA. Clinical incidence was based on the number of farmer-observed cPtB cases during four preceding years. Farmers were asked whether they grazed different species, and if so, whether they were grazed on separate or shared pasture blocks, the latter being either concurrent or successive co-grazing. These management options were lumped into a single dichotomous co-grazing risk factor variable (yes/no). Regression models were developed for assessing the co-grazing effect on flock/herd MAP infection status (logistic) and clinical incidence (Poisson), adjusting for lack of sensitivity and specificity of diagnostic tests and reporting of cPtB. The shared use of pasture was associated with paratuberculosis flock/herd infection status

and incidence. When beef cattle and sheep were co-grazed, the risk of infection was 3-4 times as high in both species than when grazing either of these species in isolation. Co-grazing beef cattle with deer was associated with a 3-fold increased likelihood of herd infection in deer. Co-grazing beef cattle with sheep, or beef cattle with deer, also was associated with increased clinical incidence in these species, compared to single species farms. Conversely, the shared use of pasture by sheep and deer was associated with a lower cPtb incidence in both species than in sheep or deer grazed in isolation. The associations concur with previous independent observational studies but require validation by studies at individual animal level to control for possible confounding effects of farm management.

4.2 Introduction

Clinical paratuberculosis (cPtb) is a bacterial disease caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) that mainly affects domestic ruminant including sheep, cattle and deer. The clinical stage is characterized by the shedding of large numbers of bacteria to the environment, chronic diarrhoea, which does not respond to treatment, leading to emaciation and finally to culling or death (Harris and Barletta, 2001). The incubation period for cPtb in sheep and cattle may be years (Sweeney, 2011) whereas it is typically only 6 months in 1-2 year old deer (Mackintosh et al., 2004). In all species, the subclinical stage is characterised by intermittent shedding, faecal excretion of variable number of bacteria, and potential production decline (Benedictus et al., 1987; Kostoulas et al., 2006; Kudahl et al., 2007; Raizman et al., 2007b). New Zealand pastoral systems are commonly mixed-species where sheep, beef cattle and/or deer jointly use the pasture at the same time or successively. In such a situation,

frequent direct or indirect contact between susceptible species is likely and this potentially increases the risk for MAP transmission across species.

Molecular analysis of MAP isolates has identified two major groups, namely Type I (sheep or S-strain) and Type II (cattle or C-strain). Previous research found that MAP Type I was commonly isolated from sheep and Type II from cattle, and that both types were isolated from deer (Collins et al., 1990; de Lisle et al., 1993; Sevilla et al., 2005; Motiwala et al., 2006b). In contrast, some studies found sheep infected with MAP Type II and cattle infected with MAP Type I (Pavlik et al., 1995; Whittington et al., 2001; Motiwala et al., 2006b; Stevenson et al., 2009), suggesting that MAP strains may have little host specificity, being capable of infecting any host given sufficient opportunity of contact (Motiwala et al., 2006b; Stevenson et al., 2009). Few studies address transmission of MAP between ruminant species and subsequent infection status and clinical incidence. Moloney & Whittington (2008) studied the risk of MAP transmission from known infected sheep flocks to cattle in Australia, concluding that transmission between these two species may occur but was a rare event. In a non-random cross sectional study, Glossop et al (2007) observed that clinical disease in deer was inversely related to the time that deer co-grazed with sheep and directly related to the time they co-grazed with beef cattle. Additionally, the prevalence of MAP-infected, abnormal visceral lymph nodes was lower among deer herds on properties that also farmed sheep, and higher in deer herds that also farmed beef cattle than in deer-only herds (Verdugo et al., 2008). The objective of this study was to assess the extent to which the joint use of pasture, on mixed-species farms, influences the flock/herd MAP infection status of MAP infection and incidence of cPtb, in sheep, beef cattle and deer on New Zealand pastoral farms.

4.3 Material and Methods

4.3.1 Farm selection and sampling protocol

Data collection was conducted in several stages, which are detailed in Annex A. In general, farms were drawn based on the results of a postal survey mailed to 7,998 clients of 28 farm animal veterinary practices, covering both North and South Island of New Zealand. In particular, the Waikato, Wairarapa, Hawkes Bay, Manawatu-Wanganui (North Island (NI)), Marlborough, Canterbury, and Southland (South Island (SI)) regions were surveyed. The questionnaire was delivered in December 2008 and received back until March 2009. The survey gathered epidemiological information of all ruminant species present at farm level, including sheep, beef cattle and deer, specifically livestock demographics, reproduction performance, paratuberculosis history and clinical incidence (up to the last four years), and grazing management information. A total of 1,940 (24.3%) correctly completed questionnaires were received, constituting the sampling frame for the second phase of the study, which involved the collection of faeces and blood samples at field level.

Sample sizes used in this study (between and within farms) were calculated to estimate the herd-level prevalence of paratuberculosis in New Zealand pastoral livestock, details of the assumptions used to estimate sample sizes are described in Chapter 3. Between June 2009 and July 2010, a stratified random selection and sampling of 238 farms from the 1,940 respondents was conducted by contracted veterinary practitioners. Strata were based on farm species composition and previous paratuberculosis history (present/absent). Selected farms had to possess a minimum flock/herd size of 400 sheep, 40 beef cattle or 40 deer. Twenty animals from each species flock/herd present on farm were randomly sampled. Paired faeces and serum samples were collected from mixed

age ewes (≥ 2 years old), mixed age beef cows and yearling deer (12-24 months, either sex). In addition, up to five animals with signs of cPtb such as wasting and/or diarrhoea (clinical suspect) were also sampled, if such animals were observed by the practitioner during sampling.

Pooled faecal culture (PFC) was conducted using BACTEC 12B liquid culture medium containing egg yolk and mycobactin after a decontamination step with cetylpyridinium chloride, as described by Whittington et al. (1999). A single faecal pool was prepared from sheep samples (20 animals/pool), and double-pools from beef cattle or deer (10 animals/pool). Faecal samples from clinical suspect animals were also pooled and cultured separately. If all available PFCs from a given flock/herd were negative, all 20 sera were subjected to individual ELISA (plus any sera from clinical suspect animals). Pourquier[®] ELISA was used in sheep and cattle samples (Institut Pourquier, Montpellier, France), and Paralisa[™] in deer samples (Griffin et al., 2005) using cut-offs as recommended by the manufacturers. A flock/herd was categorised as apparently infected by MAP if either PFC or ELISA were positive (cut off = 1+ve animal or pool), in samples from normal or from clinical suspect animals.

4.3.2 Grazing management and risk factor definitions

At sampling date, a second questionnaire (similar to the postal survey) was applied to update farm information. The joint use of pasture by more than one of the species under study was classified as concurrent co-grazing (same paddock, same time) or successive co-grazing (same paddock, different time). Grazing management information was extracted from the second survey. The co-grazing effect (concurrent or successive) over a given species grazed with a second and/or third species was assessed. Both co-grazing managements were lumped into a single risk factor variable (co-grazing), describing if

either management was present or not. For example, when MAP in sheep flocks was the outcome of interest, joint grazing with beef cattle was considered as a risk factor (= 1) if sheep and beef cattle grazed the same pasture concurrently, or sheep grazed a pasture block after it had been grazed by beef cattle (successively). Risk factors were classified as absent (= 0) in single species operations or mixed-species farms where the species flocks/herds were kept in different paddocks all year round, hence there was no direct or indirect contact to cross-contaminated pasture.

4.3.3 Statistical analysis

Regression models were developed for assessing a co-grazing effect on flock/herd MAP infection status (logistic) and cPtB incidence (Poisson), adjusting for lack of sensitivity and specificity of diagnostic tests results and reporting of cPtB. Model coefficients were estimated using Bayesian inference. For comparison purposes, unadjusted versions of those models were assessed using classical statistics logistic and Poisson Generalized Linear Models (GLM).

4.3.3.1 Herd/flock MAP infection status models

The logistic regression model proposed by McInturff et al (2004) was designed to handle binary outcomes that are subjected to error. This model is used here. It considers that for a herd level response, y_i taking values is the observed herd level binary test outcome for the i th flock/herd. The probability of a positive test result (herd level apparent infection status) using the j th testing protocol is Bernoulli (Bern) distributed with probability q_i , expressed as:

$$y_i \sim \text{Bern}(q_i) \tag{1}$$

$$q_i = z_i * Hse_j + (1 - z_i) * (1 - Hsp_j) \tag{2}$$

$$z_i \sim \text{Bern}(\tau_i) \quad (3)$$

where z_i was the true infection status of the i th flock/herd, which also has a Bernoulli distribution but with probability τ_i , representing the probability that a given flock/herd was truly infected. Hse_j and Hsp_j are the herd level test sensitivity and specificity of the j th testing protocol. A logistic regression model was used for the probability that a flock/herd with a given set of covariates was truly infected, namely

$$\text{logit}(\tau_i) = \beta_1 + \beta_2_{SP1i} + \beta_3_{SP2i} + \beta_4_{ISi} + \beta_5_{SHSi} + \beta_6_{ISi*SP1} + \beta_7_{ISi*SP2} \quad (4)$$

where $SP1_i$ and $SP2_i$ are dummy variables (0,1) for one or two co-grazing ruminant species, IS_i indicated New Zealand's North (NI=0) or South (SI=1) islands and SHS_i was the standardized flock/herd size (HS). For example, when modeling the risk of true infection in sheep flocks, the factors $SP1_i$ and $SP2_i$ represented co-grazing with beef cattle ($SP1_i$) and deer ($SP2_i$), and SHS_i represents a scaling factor to a standard population size that was calculated as:

$$SHS_i = \frac{(HS_i - \text{mean}(HS_i))}{sd(HS_i)} \quad (5)$$

Reduced versions of the full model were assessed using the Deviance Information Criterion (DIC) method (Spiegelhalter et al., 2002), to obtain the best available model. Odds Ratio (OR) and the associated 95% posterior probability interval (PPI) were computed from the final model. The association between co-variables and the herd level true MAP infection status was assessed through the computation of the posterior probability of the covariate OR being greater than one. In the case that 90% or more of the OR posterior distribution was greater than one, the covariate was considered a statistically important risk factor. Conversely, when 90% or more of the OR posterior distribution was lower than one, the covariate was considered a statistically important protective factor. Results from the error-adjusted models, previously described, were

compared with outcomes from logistic GLMs (unadjusted models), using a Maximum Likelihood Estimation method (Kleinbaum, 2007). The unadjusted models assume perfect Hse and Hsp and the classical statistic term (CST) ‘significant’ was used to describe GLMs outcomes presenting a p-value ≤ 0.05 .

4.3.3.2 Clinical incidence risk models

The number of clinical cases (X_i) reported by farmers in the i th flock/herd were assumed to be Poisson (Pois) distributed with mean λ_i , modelled as:

$$X_i \sim Pois(\lambda_i) \quad (6)$$

$$\lambda_i = HS_i * z_i * Fse_i * \exp(\delta_i) + (1 - z_i) * 0.1 \quad (7)$$

where HS_i is the population size in i th flock/herd, z_i is the herd level true infection status, defined by equations (1)-(3). Fse_i is the farmer sensitivity, defined as the probability to identify a true cPtb case when it is present, and $\exp(\delta_i)$ is the rate of occurrence of cPtb cases per animal in the i th flock/herd modelled as:

$$\delta_i = \gamma_1 + \gamma_2_{SP1i} + \gamma_3_{SP2i} + \gamma_4_{ISi} + \gamma_5_{ISi*SP1} + \gamma_6_{ISi*SP2} \quad (8)$$

where $SP1_i$, $SP2_i$, and IS_i are equivalent to the logistic regression model counterparts. Finally, the value of 0.1 is used instead of 0.0, which is the rate when $z_i = 0$, to solve a technical problem that using 0.0 will result in an error when running the model in the software package since a zero rate is not allowed for a Poisson variable. The value of 0.1 is somewhat arbitrary, but the main point is that when $z_i = 0$, there is no contribution to the likelihood function from this part of the model and so there is no impact on the analysis whatsoever.

Reduced versions of the full model were assessed using the same methodology used in the logistic adjusted models. Relative risk (RR) and its 95% PPI were calculated for all

co-variables, from the final model. Associations between the annual clinical incidence and each co-variable were assessed using similar criteria than section 4.3.3.1. A second model set assuming perfect Hse and Hse (unadjusted Poisson GLMs) were run for comparison with the error adjusted models for count data. The Poisson GLMs used similar methodology and assessment as previously described for logistic GLMs.

4.3.3.3 Adjusted model priors

Uncertainty about regression coefficients (β) was modelled as independent, normal distributions [$N(\mu, \sigma)$] for both models, with a mean equal to 0 and a variance equals to 1. This particular choice has been found by Christensen et al (2011) to induce reasonably diffuse priors on probabilities of infection; using a large variance (small precision) will induce priors on probabilities of infection that are concentrated at zero and one, which would rarely reflect anyone's subjective belief much less a prior that was meant to have little effect on the posterior, sometimes called a reference prior. Prior information about Hse_j and Hsp_j was assumed to be independent and follow a beta distribution [beta (a, b)]. In the model for count data, uncertainty about herd level true prevalences was modelled with independent beta distributions and was based on recent New Zealand data (Chapter 3). Prior parameters (a, b) were obtained using the software BetaBuster². Prior distributions and references are presented in Table 4.1. Currently, no information is available on which to specify priors for Fse_i , so the ability of farmers to correctly identify a clinical case was initially considered as perfect ($Fse_i = 1$). However, this assumption was evaluated in a sensitivity analysis by decreasing this variable, until model predictions (under the original scenario) were modified. Since no data are

² <http://www.epi.ucdavis.edu/diagnostictests>

available on animals that farmers determined to be disease free, our analysis is based on an assumption that $Fsp_i = 1$ as well, namely that there are no false negatives.

4.3.3.4 Adjusted and unadjusted models implementation and convergence assessment

In the error-adjusted models, three parallel chains with different starting values were run, using the software WinBUGS (Spiegelhalter et al., 1996). This software uses a Markov Chain Monte Carlo (MCMC) sampling algorithm to obtain a Monte Carlo (MC) sample from the posterior distribution. Models were run for 25,000 iterations, after discarding as burn-in period the first 5,000 MC samples. Model inferences were the mean of the posterior distribution and its 95% PPI for the regression coefficients, and the posterior distribution median and associated 95% PPI for ORs and RRs. Model convergence of the three chains, was assessed by visual inspection of Gelman-Rubin-Brooks plots and the Gelman-Rubin statistic was computed using the R package CODA (Plummer et al., 2006). An example of the programming code for adjusted model is presented in the Annex D. For the unadjusted models, logistic and Poisson GLMs were computed using R software, version 2.12.2 (R Core Team, 2012).

4.4 Results

A total of 238 farms were sampled including 162 sheep flocks, 116 beef cattle herds and 99 deer herds, and a total number of 7,579 sampled animals. On 68.9% of the sampled farms at least 1 flock/herd tested positive to PFC or individual ELISA test. At flock/herd level, 71.0%, 29.3%, and 56.6% of sheep flocks, beef cattle and deer herds, respectively, were test positive (see Table 4.2 for classical summary statistics). Table 4.3 describes the co-grazing patterns (concurrent or successive) on those farms with

multiple species. It shows that co-grazing was a common farming practice in mixed-species farms in New Zealand, especially among sheep and beef cattle farms. For example, 84.2% of the sampled sheep flocks were co-grazed with beef or deer and 89.3% of all sampled beef cattle herds were co-grazed with sheep or deer. Concurrent co-grazing was more common than successive co-grazing, the latter appearing to be only a marginal practice.

Table 4.1: Prior parameters, mode, and 5 or 95 percentile of prior distribution for herd level sensitivity (Hse) and specificity (Hsp) of protocols** [1,2], and flock/herd-level true prevalence (HTP) for New Zealand's North (NI) and South (SI) Island sheep, beef cattle and deer flocks/herds. Priors are based on data from Chapter 3

	Mode	95 (5*) percentile	a	b
Sheep				
Hse[1]	0.51	0.62	27.89	26.8
Hse[2]	0.86	0.92	57.13	9.58
Hsp[1]	0.99	0.95*	51.28	0.81
Hsp[2]	0.50	0.60	33.41	33.41
HTP:				
-NI	0.79	0.88	34.68	9.35
-SI	0.70	0.78	47.19	20.7
Beef cattle				
Hse[1]	0.39	0.55	10.52	16.27
Hse[2]	0.56	0.71	15.24	12.04
Hsp[1]	0.99	0.97*	141.91	1.75
Hsp[2]	0.80	0.87	56.95	14.49
HTP:				
-NI	0.44	0.55	23.04	29.36
-SI	0.38	0.48	28.12	44.91
Deer				
Hse[1]	0.90	0.99	7.79	1.14
Hse[2]	0.99	0.93*	29.06	0.57
Hsp[1]	0.98	0.96*	207.34	4.55
Hsp[2]	0.39	0.45	72.06	112.52
HTP:				
-NI	0.32	0.43	19.41	40.51
-SI	0.56	0.67	30.97	24.31

Table 4.2: Apparent prevalence (HAP) of MAP infected herds and flocks in the North and South Islands of New Zealand by species strata

Strata	North Island			South Island		
	N	HAP	(95% CI)*	N	mean	(95% CI)
Sheep (S)						
S only	10	0.70	(0.40 - 0.99)	13	0.62	(0.34 - 0.89)
S&B	58	0.76	(0.65 - 0.87)	22	0.59	(0.38 - 0.80)
S&D	5	0.80	nc	13	0.69	(0.43 - 0.95)
SBD	23	0.78	(0.61 - 0.95)	18	0.67	(0.44 - 0.89)
Overall	96	0.76	(0.72 - 0.80)	66	0.64	(0.58 - 0.70)
Beef (B)						
B only	12	0.08	nc	1	nc	Nc
S&B	56	0.38	(0.25 - 0.50)	13	0.31	(0.05 - 0.57)
B&D	3	0.67	nc	5	0.00	Nc
SBD	15	0.20	nc	11	0.18	Nc
Overall	86	0.31	(0.26 - 0.36)	30	0.23	(0.15 - 0.31)
Deer (D)						
D only	10	0.40	(0.08 - 0.72)	19	0.58	(0.35 - 0.81)
S&D	4	0.50	nc	10	0.60	(0.28 - 0.92)
B&D	8	0.63	(0.27 - 0.98)	10	0.70	(0.40 - 0.99)
SBD	19	0.47	(0.24 - 0.70)	19	0.63	(0.41 - 0.85)
Overall	41	0.49	(0.41 - 0.57)	58	0.62	(0.56 - 0.68)

*Confidence interval (CI) were calculates using the formula:

$$Test_{pos}/Test_{total} \pm 1.96 \times (SD_{Test}/\sqrt{Test_{Total}}).$$

nc: no computable

4.4.1 Association between co-grazing and infection status

After correction for misclassification of test results (adjusted models), MAP infection of a flock or herd was statistically associated with the joint use of pasture. Herd-level results for logistic models of infection risk are summarised in Table 4.4. In the three species, a reduced version of the full model, containing three covariates (SPI_i , $SP2_i$, and IS_i), was chosen as the best model based on DIC value comparison. Co-grazing sheep with beef cattle increased the estimated risk of sheep flock infection almost 4-fold (OR=3.9; 95% PPI 0.9 – 18.9), with a probability of OR being greater than one of 0.96, indicating that only 4% of the posterior distribution of the OR was below 1, hence was a statistically important result. A similar relationship was observed for infection of beef

cattle herds co-grazing with sheep (OR=2.9; 95% PPI 0.7 – 15.0), with a posterior probability of 0.92 for the OR being greater than one. Co-grazing deer and beef cattle was not statistically important, although a trend to increase the risk of infection in deer herds was observed (OR=2.2; 95% PPI 0.5 – 12.1; with probability OR>1 of 0.84). Deer herd infection risk was higher in the South than in the North Island (OR=2.7; 95% PPI 0.6 – 13.5; with probability 0.90 for OR>1).

Table 4.3: Number (and %) of herds/flocks from mixed-species farms which did (concurrently or successively) or did not co-graze with each with other species

Reference Flock/Herd (bold) and Co-grazed species	Co-grazing			Total
	None*	Concurrent	Successive	
Sheep				
- beef cattle	5 (6.3)	69 (86.3)	6 (7.5)	80
- deer	8 (44.4)	8 (44.4)	2 (11.1)	18
- beef & deer	9 (22.0)	29 (70.7)	3 (7.3)	41
- Overall	22 (15.8)	106 (76.3)	11 (7.9)	139
Beef cattle				
- sheep	5 (7.2)	63 (91.3)	1 (1.4)	69
- deer	2 (25.0)	5 (62.5)	1 (12.5)	8
- sheep & deer	4 (15.4)	20 (76.9)	2 (7.7)	26
- Overall	11 (10.7)	88 (85.4)	4 (3.9)	103
Deer				
- sheep	5 (35.7)	5 (35.7)	4 (28.6)	14
- beef cattle	6 (33.3)	11 (61.1)	1 (5.6)	18
- sheep & beef	6 (15.8)	29 (76.3)	3 (7.9)	38
- Overall	17 (24.3)	45 (64.3)	8 (11.4)	70

*Species grazed in isolation

Results from unadjusted logistic GLMs for apparent infection risk, hence assuming perfect herd sensitivity/specificity, did not render any of the risk covariates as significant (CST) in sheep, beef cattle, or deer models. The results are different from those based on the model that adjusts for possible errors in the response.

4.4.2 Association between co-grazing and clinical disease incidence

Model outputs indicated that annual cPtb incidence was associated with direct or indirect contact through co-grazing with other livestock species (Table 4.5). For all three species, the best available models, based on the DIC criterion, were reduced versions of the full models, excluding the interaction terms. When error in test outcomes was considered, the sheep model identified co-grazing with beef cattle as a statistically important risk factor (RR=1.2; 95% PPI 1.1 – 1.3; probability 0.99 for RR>1) for cPtb in sheep. Additionally, the two other covariates were statistically associated as protective factors for cPtb in sheep: co-grazing with deer (RR=0.5; 95% PPI 0.5 – 0.6; probability 0.01 for RR>1); and a lower incidence of cPtb in the South vs. North Island (RR=0.6; 95% PPI 0.6 – 0.7; probability 0.01 for RR>1).

For beef cattle herds, models did not detect any statistically important associations (probability ≥ 0.90 for RR $\neq 1$) between clinical incidence and co-grazing contact with other species or island. The deer model identified two risk and one protective factors (Table 4.5): co-grazing with beef cattle (RR=2.2; 95% PPI 1.7 – 2.8; probability 0.99 for RR>1); deer farming in the South Island (RR=9.1; 95% PPI 6.5 – 11.8; probability 0.99 for RR>1); and co-grazing with sheep (RR=0.7; 95% PPI 0.6 – 1.0; probability 0.03 for RR>1). When *Hse* and *Hsp* were assumed to be perfect (unadjusted Poisson GLMs), results and inferences were different from those observed in the error-adjusted models, described above. In the Poisson GLMs for sheep and deer, non-significant (CST) associations were observed for all covariates. Conversely, in the beef cattle model co-grazing with sheep was significantly (CST) associated to a greater cPtb incidence (p-value = 0.01). Finally, the sensitivity analysis indicated that a reduction of

Table 4.4: Inferences for regression coefficients β_s , posterior mean (PM) and standard deviation (PSD), posterior odds ratio (POR, median and 95% posterior probability interval (PPI)), and posterior probability (Pr(POR > 1)) of PORs being greater than one for error-adjusted logistic regression models; On the right side, OR, 95% confidence intervals and p-values for ordinary logistic Generalized Linear Models (GLM) for flock/herd MAP infection status

Regression coefficient	Description	Error-adjusted logistic regression model						Error-unadjusted logistic GLM			
		B_s		POR	95% PPI	Pr(POR>1)	OR	95% CI	P-value		
		PM	PSD								
Sheep											
β_1	Intercept	2.15	0.66								
β_2	co-grazing with beef	1.37	0.77	3.88	0.89	18.91	0.96	1.21	0.57	2.55	0.61
β_3	co-grazing with deer	0.39	0.87	1.45	0.27	8.50	0.67	0.85	0.38	2.00	0.71
β_4	Island	0.52	0.84	1.67	0.34	9.07	0.73	0.58	0.28	1.19	0.14
Beef cattle											
β_1	Intercept	1.41	0.76								
β_2	co-grazing with sheep	1.10	0.81	2.94	0.66	15.01	0.92	1.49	0.58	4.19	0.43
β_3	co-grazing with deer	0.24	0.92	1.26	0.22	8.13	0.60	0.81	0.29	2.10	0.67
β_4	Island	0.04	0.94	1.03	0.17	6.64	0.51	0.75	0.26	1.96	0.56
Deer											
β_1	Intercept	1.65	0.78								
β_2	co-grazing with sheep	0.31	0.90	1.35	0.25	8.21	0.63	0.64	0.27	1.52	0.32
β_3	co-grazing with beef	0.82	0.83	2.24	0.46	12.05	0.84	1.92	0.81	4.66	0.14
β_4	Island	1.01	0.80	2.74	0.59	13.46	0.90	1.73	0.75	4.06	0.20

Table 4.5: Inferences for regression coefficients γ_s , posterior mean (PM) and standard deviation (PSD), posterior relative risk (PRR, median and 95% posterior probability interval (PPI)), and posterior probability ($\text{Pr}(\text{PRR} > 1)$) of PRRs being greater than one for error-adjusted Poisson regression models; On the right side, RR, 95% confidence intervals and p-values for Poisson Generalized Linear Models (GLM) for annual clinical incidence

Regression coefficient	Description	Error-adjusted Poisson regression model					Error-unadjusted Poisson GLM				
		Γ_s PM	PSD	PRR	95% PPI	$\text{Pr}(\text{PRR}>1)$	RR	RR 95% CI	P-value		
Sheep											
γ_1	Intercept	-4.05	0.05								
γ_2	co-grazing with beef	0.16	0.06	1.17	1.05	1.30	0.99	0.81	0.43	1.54	0.52
γ_3	co-grazing with deer	-0.63	0.05	0.53	0.48	0.58	0.01	0.65	0.31	1.33	0.24
γ_4	Island	-0.46	0.05	0.63	0.57	0.69	0.01	0.6	0.31	1.13	0.11
Beef cattle											
γ_1	Intercept	-6.30	0.57								
γ_2	co-grazing with sheep	-0.40	0.55	0.70	0.20	1.75	0.22	5.59	1.46	21.36	0.01
γ_3	co-grazing with deer	-0.46	0.62	0.64	0.19	2.07	0.23	0.96	0.37	2.48	0.94
γ_4	Island	0.05	0.61	1.07	0.30	3.28	0.54	1.29	0.54	3.09	0.57
Deer											
γ_1	Intercept	-5.04	0.10								
γ_2	co-grazing with sheep	-0.31	0.16	0.73	0.55	1.04	0.03	1.56	0.51	4.74	0.43
γ_3	co-grazing with beef	0.77	0.14	2.16	1.64	2.80	0.99	1.42	0.54	3.78	0.48
γ_4	Island	2.20	0.16	9.06	6.38	11.80	0.99	0.74	0.42	4.29	0.62

farmer observed clinical sensitivity from 1.00 up to 0.50, in the error-adjusted Poisson models, did not result in any detectable change in model outputs for any of the three species, indicating that model results are highly insensitive to this parameter.

4.5 Discussion

The main purpose of this study was to investigate relationships between herd-level MAP infection and annual cPtb incidence, and contact with other livestock species through shared pasture. The results indicated that joint grazing of sheep and beef cattle was associated with increased infection risk for both species. Likewise, joint grazing of beef cattle and deer was associated with a trend for increased infection risk between deer herds. Conversely, the co-grazing of sheep and deer was associated with decreased annual clinical incidence in both species. Grazing contact with beef cattle constituted a risk factor for cPtb in sheep and deer. Thus, although beef cattle herds present the lowest herd-level infection prevalence itself (Chapter 3), co-grazing with beef cattle represents a potential risk factor for infection and clinical disease on sheep and deer flocks/herds.

There is evidence that the most important infection source for sheep, deer and cattle is MAP from pasture. Faecal-oral transmission is regarded as the main horizontal transmission route and the only way that MAP can be transmitted between species (Sweeney, 1996). Other routes involved in within species transmission are the vertical and pseudo-vertical. In those routes, an infected dam could transmit MAP to its lambs/calf/weaner through the placenta, becoming infected in utero (Lambeth et al., 2004; van Kooten et al., 2006; Thompson et al., 2007; Whittington and Windsor, 2009), through the ingestion of contaminated colostrum, milk or through close contact with

faecal-contaminated udders (Taylor et al., 1981; Streeter et al., 1995; Sweeney, 1996). Although it has been suggested that vertical and pseudo-vertical transmission are able to sustain infection, evidence indicates that their contribution to the total infection burden is relatively low in herds without any control interventions (Mitchell et al., 2008). Additionally, sexual transmission has been proposed since MAP has been isolated from bull semen, although there has been no demonstration of MAP or cPtb in the offspring of infected bulls (Ayele et al., 2004).

The between-species transmission cycle may start with animals in pre-clinical or clinical stages, shedding massive amounts of bacteria into the environment (Harris and Barletta, 2001). Faeces contaminated by MAP may be washed out by rain and physically dispersed over pasture through the feet of grazing animals. In a recent randomized experiment (Salgado et al., 2011), soils were artificially contaminated with MAP-infected slurry and sprinkled with different amounts of water mimicking rainfall, MAP was recovered in grass by culture from all treatment groups. This finding indicates that MAP tends to stay attached to the soil surface instead of migrating to lower layers of the soil (Salgado et al., 2011), being available to expose susceptible animals through growing grass. Additionally, MAP has been isolated up to 12 months after its inoculation in soil (Rowe and Grant, 2006) and has recently been found to form spores and survive for possibly even longer periods in soil (Lamont et al., 2012). Hence soil may be a reservoir for MAP and the source of prolonged exposure for grazing livestock. It is however, not known how long sufficient infectivity can be retained as MAP spores or if sufficient MAP concentration is reached on grass growing through such infected soil to be infective.

In this study, grazing contact between species mostly occurred concurrently although in some instances, by successive use of the same pasture, one species after another, in expected short time intervals. Due to the characteristic long survival of MAP in the environment, authors considered that MAP decay in soil would not play an important role in the reduction of MAP cross-species transmission, as could be the case under a predominantly successive grazing management. The following discussion refers to any contact between species, concurrently or successive co-grazing, which potentially gave rise to horizontal transmission as 'joint grazing'. For farms reporting that different livestock species were grazed on separate pasture blocks all year, cross pasture contamination through water run off could not be ruled out. This consideration may have biased the results, but we believe such bias was small because the proportion of farms where this could have occurred was small (11 to 24% of mixed-species farms, Table 4.3).

Two major MAP types have been identified using molecular techniques such as pulse field gel electrophoresis (PFGE) and restriction fragment length polymorphism (RFLP). They are commonly denominated Type I (sheep or S-strain) and Type II (cattle or C-strain). Additionally, there is a third MAP type denominated Type III (intermediate or I type) (Moebius et al., 2009), which is considered a subtype of Type I (Stevenson et al., 2009). However, it has never been reported in New Zealand. Currently, MAP Type I and II are differentiated using a PCR-assay targeting the insertion sequence IS1311 in the MAP genome after MAP-confirmation using another PCR-assay targeting the insertion sequence IS900, because IS1311 is also present in other *Mycobacteria* species (Motiwala et al., 2006b). Based on previous molecular epidemiological data from Australia and New Zealand, a strong preference of these MAP types for specific hosts

has been suggested (Collins et al., 1990; Sevilla et al., 2005): MAP Type I was predominantly isolated from sheep, and Type II from cattle and a range of wildlife species such as fox, stoat, weasel, crow, rook, jackdaw, rat, wood mouse, hare, and badger (Beard et al., 2001a; Stevenson et al., 2002). However, epidemiological studies in Europe isolated MAP Type II from a wide range of hosts, including sheep, goats, and cattle (Stevenson et al., 2009), indicating that the former postulated host-specificity for sheep and cattle, could be due to a lack of contact between these hosts rather than a genetic host specialization of the pathogen (Motiwala et al., 2006b).

In New Zealand, both MAP types have been isolated from deer, and both types were associated with cPtb in this species (de Lisle et al., 1993). Type II has been isolated more often from deer than Type I (de Lisle et al., 2006). However, this finding was based only on samples from suspected cases (live or slaughtered animals) submitted to a diagnostic laboratory. A recent randomised survey of the New Zealand population found MAP Type II being predominant in deer and dairy cattle, but Type I being the predominant type in beef cattle and sheep (Chapter 6). The latter finding could be explained by a transmission of MAP Type I from sheep to cattle on sheep-and-beef farms, the predominant farming system in New Zealand. Clinical Ptb cases have been reported in cattle infected with MAP Type I in Australia and Iceland (Whittington et al., 2001). In spite of the isolation of MAP from different hosts, there are a limited number of studies suggesting MAP cross-species transmission in countries other than New Zealand. Epidemiological information gathered in Iceland indicated that MAP was introduced to the country through infected sheep, imported from Europe, then spread to the local cattle population and back to sheep after a depopulation and restocking program (Palsson, 1962; Fridriksdottir et al., 2000). In the Netherlands, Muskens et al

(2001) showed MAP infection in sheep that grazed on pasture previously fertilized with manure from MAP infected cattle suggesting an indirect transmission. Moloney & Whittington (2008) studied the risk of transmission of MAP Type I from sheep to cattle on farms where cattle shared pasture with known infected sheep. The authors concluded that there was a risk of transmission but the risk was low and transmission might only occur sporadically. Hence, current evidenced suggests that MAP can be transmitted across species given sufficient host density and contact between species.

Differences in MAP strain types have also been associated with differences in host immunopathological response to infection. Verna et al. (2007), experimentally infected lambs with MAP Type I and II, demonstrating that MAP Type I generated a more severe pathology in sheep than Type II. Gollnick et al. (2007), in an *in vitro* study of bovine macrophages, found differences in MAP survival and phagocytosis between MAP Type I and Type II strains, suggesting a greater virulence in MAP Type II for bovine cells. Variations in host immune response were also associated with different MAP strain types (Janagama et al., 2006; Motiwala et al., 2006a). O'Brien et al. (2006) concluded that MAP Type II was more pathogenic than Type I for deer, based on the infection rate and the cell-mediated immune response in experimentally infected young red deer.

In a non-random cross sectional study of mixed-species pastoral farms in New Zealand, Glossop et al. (2007) observed that farmer-diagnosed clinical cases were positively associated with the time that deer co-grazed with beef cattle and negatively associated to the time that deer co-grazed with sheep. Similarly, an analysis of deer abattoir surveillance records in New Zealand indicated a higher risk of the presence of abnormal visceral lymph nodes (associated with paratuberculosis gross pathology) in deer slaughter lines from farms that also had beef cattle than from deer-only farms (Verdugo

et al., 2008). When deer lines were from farms with sheep, the prevalence of abnormal visceral lymph nodes was lower than on deer-only farms. However, no detailed information about grazing management was available for that study. To our knowledge, there are no reports about similar associations between mixed-species farming and paratuberculosis manifestation in sheep or beef cattle. Although some preliminary evidence suggests that pathogenicity differences between MAP types may exist, epidemiological associations between strains and host species have not yet been conclusively shown.

The results from the present study suggest cross species transmission, where the co-grazing of sheep with beef cattle was associated with an increase on the infection risk and clinical incidence in sheep flocks. This effect could be explained by beef cattle mainly harbouring MAP type I strains, which commonly are associated with sheep (Chapter 6). Thus, susceptible sheep will be exposed to MAP strains akin to them, from fellow infected sheep and as well from infected beef cattle. The extra MAP challenge would have an effect on the infection status and clinical incidence. When co-grazed with cattle shedding MAP on pasture, sheep may be challenged by higher infectious doses due to a greater faecal excretion, and hence be at a greater risk to progress to clinical disease (Kurade et al., 2004; Delgado et al., 2012).

Similar to the increased risk of infection in sheep, co-grazing beef cattle with sheep increased the risk of infection in beef cattle herds. However, the presence of sheep was not associated with an increase of clinical disease incidence. Moreover, beef cattle presented the lowest clinical incidence of all the three species studied (Chapter 5). The finding that beef cattle were mainly infected with MAP Type I strains and rarely

developed clinical disease, supports the hypothesis that MAP Type I may be less pathogenic in beef cattle than in sheep, as discussed above. Hence, transmission of ovine MAP from sheep to beef cattle would explain our observation of an increased risk of infection but generally low incidence of cPtB in beef cattle in this country. In New Zealand, sheep and beef cattle are more often farmed together than in isolation. Cows with their calves and ewes with their lambs are commonly co-grazed for three months, sometime longer, starting close to seasonal calving/lambing in August/September. Such conditions favour the transmission of MAP between these species.

Similar to the associations observed between sheep and beef cattle, the presence of beef cattle increased the risk of infection (trend) and clinical disease incidence in deer. Although beef cattle are mainly infected with MAP Type I strains, the opposite was observed in farms where deer and beef cattle were co-grazed: here, beef and deer commonly shared MAP Type II strains (Chapter 6). These observations suggest that deer were more susceptible to bovine strains than beef cattle.

Conversely, the co-grazing of sheep and deer had an apparently protective effect on the clinical disease incidence in both species. A possible biological explanation is related to sheep and deer harbouring different MAP strains (Chapter 6) with different host-specific virulence. Such cross species exposure to less pathogenic strains could potentially induce an immune response, resulting in a further decrease of clinical disease incidence. Another protective effect of co-grazing could be explained by more efficient use of feed energy from pasture or synergistic effects related to parasite control (Southcott and Barger, 1975). Additionally, a lower stocking density of sheep could be expected when sheep are co-grazed with deer and *vice versa*, reducing the risk of

within-species transmission. Apparent protective effects of sheep against clinical disease and pathological lesions at slaughter in deer has been described (Glossop et al., 2007; Verdugo et al., 2008). Conversely, the apparent protective effect of deer on sheep flocks has not been previously reported, hence this finding should be taken with care due to the limited number of sheep flocks co-grazed with deer in the data set.

Previous research showed differences in prevalence estimates for all species under study between NI and SI (Hunnam, 2011; Stringer, 2011). Thus, the island effect could have biased the associations between infection status, incidence and co-grazing. Therefore, this co-variable was included as a confounding factor in both adjusted and unadjusted models. The misclassification error using pooled faecal testing and ELISA was substantial, depending on species tested, with median herd level sensitivity (Hse) ranging from 0.39 to 0.99 and median herd level specificity (Hsp) ranging from 0.39 to 0.99 (Chapter 3). For both types of data (infection status/incidence), two models were compared, one accounting for response error, and the other not. At the comparison between adjusted- and unadjusted-models, differences in regression output were observed. Almost all unadjusted-models did not record any significant (CST) association with flock/herd MAP infection status or incidence. Conversely, after test results misclassification was accounted for in the adjusted-model, risk and apparent protective effects of cross-species co-grazing were observed. The only exception was the unadjusted Poisson model for cPtb incidence in beef cattle, which suggested that co-grazing with sheep was a significant (CST) risk factor (P-value=0.01), where none of 22 beef cattle herds in single species farms reported clinical cases, whereas 15 of 66 beef cattle herds located in sheep & beef farms reported at least one clinical disease case. However, such association is not present in the error-adjusted counterpart, where no

statistically important associations were observed. This difference could be explained by a possible bias present in the incidence data reported by farmers. In New Zealand, the number of sheep flocks truly infected and reporting clinical cases are the double than beef cattle herds (Chapters 3 & 5). Thus, disease awareness by the farmer in one species (sheep) could bias the report in another (beef cattle). Therefore, when the count of cases reported by the farmer was adjusted by the true infection status of the herd, such association was no longer present. Moreover, at the comparison of adjusted and unadjusted model outcomes, adjusted models had substantially smaller credible intervals around OR and RR estimates than the confidence intervals estimated from the unadjusted models. Based on the performance between the two modelling approaches, the authors consider the adjusted approach a more robust analytical technique when the lack of Hse and Hsp could induce substantial error in the sanitary classification of animals or herds, as has been observed in paratuberculosis (Nielsen and Toft, 2008).

Since clinical incidence was based on farmer-diagnosed cases over the past four years, interview bias due to poor and variable recall of past events could not be ruled out. Moreover, cases could have been missed by the farmer due to the challenge of monitoring accurate stock numbers in this extensive, whole-year outdoor grazing system. However, the sensitivity analysis showed that the coefficients of the adjusted Poisson models remained relatively stable when farmer-reported sensitivity was reduced from 100% up to 50%, indicating that cases escaping the observation and recording by farmers had little impact on the inferences. Beside of recall bias, farmer could potentially confuse cases with other diseases with a similar clinical manifestation. However, the adjusted Poisson model considers cases only reported from truly infected herds or flocks, minimizing such possible bias effect.

4.6 Conclusion

Joint grazing of sheep, beef and deer, a common farming practice in New Zealand, appears to have an important impact on the flock/herd MAP infection status and the incidence of cPtb. A higher infection risk when sheep and beef shared the same pasture as compared to grazing in isolation, and a similar effect, although less important, in deer and beef cattle, suggest that MAP is transmitted between these species. We therefore conclude that sheep flocks, deer and beef cattle herds are at higher risk of MAP infection when they share pasture. In contrast however, the data suggested a strong protective effect against cPtb incidence in sheep when grazed with deer and lower cPtb incidence in deer when grazed with sheep. By contrast, there was an increased cPtb incidence in deer when grazed with cattle. These findings warrant further investigation to confirm that they exist at animal level. Such interactions may present new opportunities for the herd control of cPtb.

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Association between *Mycobacterium avium* subspecies paratuberculosis infection and production performance in New Zealand pastoral livestock

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5.1 Abstract

Clinical paratuberculosis (cPtB) is a disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). In domestic ruminants MAP infection is largely sub-clinical, but can result in chronic diarrhoea leading to emaciation and death. Sub-clinical infection has been associated with reduced carcass value, decreased milk production and impaired fertility. This study evaluated flock/herd level associations between MAP infected/affected livestock and production performance, on single or mixed-species (sheep, beef cattle and deer) farms in New Zealand. Production was measured in term of clinical incidence, culling, pregnancy, and lambing/calving/tailing rates. Twenty faeces and blood samples for pooled MAP culture and individual animal ELISA if cultures were negative, respectively, production data, and cPtB case histories were obtained from each species on 238 farms (162 sheep, 116 beef cattle and 99 deer flocks/herds), randomly selected from respondents to a postal survey in seven regions of New Zealand. Generalized linear models were used to test for association between infection-status and production performance. Flocks/herds were assigned to three infection-status

categories: i) non-infected ('reference'), ii) test positive but no clinical cases ('infected') and iii) test positive with clinical cases ('affected').

MAP 'infected' status was significantly ($p = 0.03$) associated with reduced calving rates in beef cattle herds and lower culling rates in deer herds and sheep flocks. Moreover, in sheep flocks and deer herds, significant and a marginally significant ($p = 0.05$ and 0.09 , respectively) association was observed between 'affected' flocks/herds and lower tailing rates in sheep and weaning rates in deer, respectively. While these results show significant association between lower reproductive outcomes and MAP infection in herds/flocks, inference about causation cannot be made since it is biologically plausible that factors contributing to lower reproduction performance *per se* may be the same as those contributing to infection and/or disease attributable to MAP.

5.2 Introduction

Clinical paratuberculosis (cPtB), caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is a chronic, debilitating disease that affects ruminants around the world. Clinical disease is characterised by weight loss and diarrhoea not responding to treatment, leading to emaciation and death. The incidence risk of cPtB is rarely higher than one percent per year in infected flocks/herds (Morris et al., 2006; Glossop et al., 2008; Norton et al., 2009) whereas non-apparent infections could be present in more than 50% of animals in infected herds of dairy cattle, beef cattle, deer, or in sheep flocks (Nielsen and Toft, 2009). Sub-clinical production effects may be more significant economically than clinical disease. The main production effects of cPtB reported in animals are reduced milk yield (Benedictus et al., 1987; Kudahl et al., 2007), premature culling, and reduced slaughter value (Benedictus et al., 1987). However, not all infected

animals will experience detectable production loss during their productive life (Nielsen and Toft, 2008).

Most sub-clinical and cPtb production effects studies have been carried out mainly in dairy cattle. Those observed at the individual animal level, in dairy cattle have been related to milk production decrease, premature culling (Norton, 2007; Raizman et al., 2007b), and reduction in calving rates compared with MAP negative herd-mates (Smith et al., 2010). In sheep, studies have focused largely on the clinical effects of the disease, showing how cPtb is associated with an increase in mortality, premature culling, and reductions in flock and greasy fleece weight (Bush et al., 2006; Morris et al., 2006). At the sub-clinical level, MAP infection in sheep has been associated with reduced reproduction performance in dairy ewes (Kostoulas et al., 2006). In sheep, the incidence of clinical disease and associated production loss has been shown to vary substantially among infected flocks (Dhand et al., 2007). As a result, observational studies of the sub-clinical effects of MAP infection require relatively large numbers of flocks to detect biologically important effects. To the best of our knowledge no peer-reviewed published information is available about the production effect of cPtb or MAP infection in commercially farmed deer. Since incubation periods of cPtb appear to be shorter in deer than in cattle or sheep (Mackintosh et al., 2004), it is assumed that the economic impact of cPtb in deer is mainly due to mortality between 1-2 years of age (Glossop et al., 2008). Other proposed but unproven effects include reduced growth rates, decreased reproductive performance (Thompson et al., 2007), an interference with bovine tuberculosis surveillance in live or slaughter stock (Mackintosh et al., 2004; Stringer, 2011), and decreased velvet antler production (Mackintosh and Wilson, 2003).

A systematic economic evaluation of MAP infection and cPtb in New Zealand, carried out by Brett (1998) concluded that ‘paratuberculosis does not currently cause large economic losses to the New Zealand livestock industries, relative to the value of the industries’. It is important to review the validity of that conclusion, after more than a decade, particularly when considering the potential indirect effects associated with trade restrictions or consumption decrease due to possible public health concerns. Moreover, insufficient information was available about MAP prevalence for Brett’s study, a precondition for sound inferences to be made at the national level. Production loss identification and quantification are key elements of any cost-benefit analysis, and fundamental for further design and planning of control strategies. The objective of this study was to assess association between flock/herd level infection-status (‘infected’ or ‘affected’), and production performance, measured in terms of clinical incidence, reproductive parameters and culling rates, in sheep, beef cattle and deer flocks/herds across New Zealand.

5.3 Material and methods

5.3.1 Data collection and laboratory analysis

A detailed description of the stages involved in farm selection and sampling is presented in the Annex A. In general, this was a cross-sectional study of commercial sheep, deer and beef cattle enterprises in New Zealand. To be eligible for the study at least 40 deer, 400 sheep, or 40 cattle were required to be present on single or mixed-species farms. A total of 28 veterinary practices were enrolled in 6 selected regions representing all major agro-climatic zones. An agreement of understanding was signed with each practice. As part of the agreement, practices shared their client list (‘surveyed population’) and agreed to sample selected farms (‘sampled population’). From December 2008 to March

2009, a survey was mailed out to 7,998 commercial farm managers accessed through the client database of the veterinary practices in New Zealand's North Island (NI) regions of Waikato, Wairarapa, Hawkes Bay, Manawatu-Wanganui, and South Island (SI) regions of Marlborough, Canterbury and Southland. The survey gathered information about livestock demographics, reproductive performance, and farm manager-observed cPtb cases in the last four years, in sheep, deer, beef or dairy cattle. The questionnaire included a detailed case definition of cPtb symptoms for each species, and a question on whether suspected cPtb cases were diagnosed by a veterinarian, or confirmed by laboratory testing. Additionally, the questionnaire enquired about the presence of leptospirosis which is a widespread endemic disease in New Zealand's sheep, deer, and cattle flocks/herds (Dorjee et al., 2008; Subharat et al., 2009; Heuer et al., 2010). Questions about this disease were included to take advantage of the national scope of the survey, and to reduce potential bias by encouraging the replies from not only farm managers with experience with cPtb, who would be more likely to respond to an invitation to a questionnaire of this type. A total of 1,940 (24%) valid surveys were returned. Those who responded to the survey comprised the sampling frame (the 'surveyed population') for the second stage of the study.

In the sampling phase, a total of 300 farms were selected in a stratified-random fashion ('sampled population'), sampling a similar number of farms from each stratum. For this purpose, the 1,940 responding farms were allocated to seven farm type strata (FTS), based on the livestock species composition: sheep only (SHP), deer only (DEE), beef cattle only (BEE), sheep and beef (S&B), beef and deer (B&D), sheep and deer (S&D), and sheep, deer and beef (SBD). Dairy cattle farms were not included in the second phase of the study. Blood and faecal samples were collected by farm veterinarians from

June 2009 to July 2010 to establish species and farm MAP status. At the time of sampling, veterinarians used a second questionnaire, identical to the first one as described above, to update the demographic and production information collected from the initial postal survey of the previous year. These farms are referred to as the ‘sampled population’.

Twenty clinically normal animals, from each species present on farm, were randomly selected for blood and faecal sampling in the following age groups: mixed age ewes (two years and older), mixed-age beef cows, and 12 - 24 month-old deer (either sex). In addition to these 20 clinically normal animals, up to five additional animals per species were sampled if the veterinarian suspected they may be affected with cPtb (‘suspect cPtb’). To obtain a random sample of animals, each targeted age group was rounded up and yarded. Animals for sampling were selected using a systematic random sampling procedure as they were released from the yard. Animals selected for sampling were then re-yarded for faecal sampling. Veterinarians were instructed to use a new plastic glove for each faecal sample and then to transfer each sample into an individual 50 mL screw-cap bottle. Samples were placed in cooler boxes and submitted to Massey University by postal courier the same day. In order to encourage the fulfilment of sampling protocols by veterinarians, the agreement with the contracted practices included a clause allowing access to the test results of their clients, subject to previous authorization of the respective farm managers. After arrival at Massey University, a single pool of 20 faecal samples from sheep submissions, and two pools of 10 samples each from deer and cattle, were prepared prior to submission to a specialized laboratory³ for testing, using a liquid culture medium system (BACTEC 12B), containing egg yolk and mycobactin,

³ AgResearch Laboratories, 66 Ward Street, Wallaceville, Upper Hutt, New Zealand.

following previous sample decontamination with cetylpyridinium chloride as described by Whittington et al. (1999). Samples from 'suspect cPtb' animals were also pooled if more than one was collected from a species mob, and they were cultured separately from pools from clinically normal animals. Serum was separated from blood either immediately upon arrival at Massey University or for some consignments the following day after storage at 4 °C. Serum was stored at -20 °C until testing. All sera from culture negative species on any farm were tested by a Pourquier[®] ELISA (sheep and cattle) (Institut Pourquier, Montpellier, France) or Paralisa[™] (deer) (Griffin et al., 2005b) according to standard protocols. A species was considered infected if either one or more pooled faecal cultures (PFC) or one or more sera were test positive.

5.3.2 Statistical analysis

5.3.2.1 Descriptive statistics and annual clinical incidence estimation

Production parameters of interest, gathered through the postal survey and updated at sampling, are described in Table 5.1. They included calving, weaning, tailing, pregnancy, and culling rates. All data management and statistical analyses were carried out using R version 2.12.2 (R Core Team, 2012). Descriptive statistics were calculated for all production parameters of interest from the 'sampled population', stratified by species and age group (pregnancy rate only). Descriptive statistics are presented along with New Zealand livestock production statistics⁴ for comparison. The annual clinical incidence risk of cPtb (ACI) was calculated in both 'surveyed' and 'sampled' populations. This parameter was estimated as the average number of cases reported during four years divided by the average size of the population at risk in those four years. To avoid the inclusion of non-specific cases with similar clinical signs, but due to

⁴<http://www.beeflambnz.com/information/> and <http://www.deernz.org/>

Table 5.1: Definition of dependant variables used for analyses and modelling, by species

Species	Parameters of interest	Definition
Beef cattle	Calving % across age groups	Number calved 2009 (08) / Number pregnant 2008 (07)
	Pregnancy %, Age =1 (15mo heifers)	Number 15mo heifers scanned pregnant in 2009 (08) / Number 15mo heifers mated in 2009 (08)
	Pregnancy %, Age =2 (27mo heifers)	Number 27mo heifers scanned pregnant in 2009 (08) / Number 27mo heifers mated in 2009 (08)
	Pregnancy %, Age =3 (MA cows)	Number MA cows scanned pregnant in 2009 (08) / Number MA cows mated in 2009 (08)
	Culling % Weaning %	Number sold or culled / Number of animals in flock at time of sampling Weaners in 2009 (08) / Hinds at calving 2008 (07)
Deer	Pregnancy %, Age =1 (R2YO hinds)	Number R2YO hinds scanned pregnant in 2009 (08) / Number R2YO hinds mated in 2009 (08)
	Pregnancy %, Age =2 (MA hinds)	Number MA hinds scanned pregnant in 2009 (08) / Number MA hinds mated in 2009 (08)
Sheep	Culling %	Number sold or culled / Number of animals in flock at time of sampling
	Tailing %	Lambs at tailing in 2009 (08) / Number pregnant ewes wintered in 2008 (07)
	Pregnancy %	Number scanned ewes pregnant in 2009 (08) / Number ewes mated in 2009 (08)
	Culling %	Number sold or culled / Number of animals in flock at time of sampling

mo = months old, MA= mixed age, R2YO= rising two-year-old.

conditions other than cPtB, these were only considered as cases if farm managers declared confirmed cPtB (by veterinarian or laboratory) in the ‘surveyed population’, or flocks/herds were test positive in the ‘sampled population’.

5.3.2.2 Production outcomes and infection-status analysis

The association between production outcomes and MAP infection-status were analysed in ‘the sampled population’. Flock/herd level infection-status classification was derived from the cross tabulation of MAP test status and the observation of clinical cases (suspected or confirmed) during the preceding four years, based on merged data from both questionnaires (postal survey; interview with the veterinarian at sampling). This classification resulted in three herd/flock infection-status categories: i) test negative and no clinical cases (‘non-infected’ or ‘reference’); ii) test positive and no clinical cases (‘infected’); and iii) test positive and clinical cases (‘affected’). If a species was test negative but the farm manager reported suspected cPtB cases, these flocks/herds were excluded as the test results could have been false-negatives or an infection in previous years could have cleared up prior to the time of sampling. For each species flock/herd, the reproductive rates were analysed as the number of successes divided by the population at risk (for example, the number MA cows pregnant divided by the total number of MA cows mated). This outcome was assumed to follow a binomial distribution, thus logistic regression was used to evaluate the production outcome among ‘infected’ and ‘affected’ flocks/herds compared to the ‘reference’ category. Model over-dispersion was estimated using the ratio of Pearson residuals divided by residual degrees of freedom to evaluate the assumption that flocks/herds were independent. If the ratio was greater than 1.5, robust standard errors were calculated for the adjustment of standard errors using the so called ‘sandwich estimator’ (Diggle et al.,

2002). For sheep tailing rates, which commonly assume values over 1, a Poisson regression model was used, where the response variable was the count of lambs at tailing. The log of the flock size was used as offset to scale the outcome to an annual incidence. Assessment and adjustment of model over-dispersion were conducted using the same methodology previously described for the logistic regression models. Full models included infection-status, as the main effects of interest, and the potential confounding effects of island (North Island and South Island), population at risk and FTS. Interactions between infection-status and age class (pregnancy rates only) or infection-status and FTS were included. The pregnancy rate models included up to three age-classes per farm (Table 5.1), and each were adjusted for the length of the mating period. Likelihood ratio tests were used to identify significant variables where $p < 0.05$. The following full models were evaluated:

Cal(Wea,Cul) / PAR ~ disease-status + FTS + island + PAR + (disease-status x FTS)

Tai ~ disease-status + FTS + island + PAR + (disease-status x FTS)

Preg ~ disease-status + FTS + island + PAR + age class + mating period + (disease-status x age-class)

where Cal = calving rate, Wea = weaning rate, Tai = count of lambs at tailing, Cul = culling rate, Preg = pregnancy rate, and PAR = population at risk. Only significant variables were retained to estimate the odds ratios (OR) of disease-status.

5.4 Results

A total of 238 farms were sampled including 162 sheep flocks (3,257 animals), 116 beef cattle herds (2,332 animals) and 99 deer herds (1,990 animals). Sixty-nine *percent* of the 238 farms had at least 1 flock/herd testing positive to PFC or ELISA tests. An overall flock/herd level apparent MAP infection prevalence (HAP) of 71% (95% CI 67% to 75%), 29% (95 CI 25% to 34%), and 57% (95% CI 52% to 62%) was observed for sheep, beef cattle and deer flocks/herds, respectively. Depending on the outcome parameter, up to 9 sheep flocks, 13 beef cattle, and 19 deer herds were excluded from the analysis due to missing information or extreme values, very unlikely to be valid (above/below three standard deviations from the population mean).

5.4.1. Associations with production in sheep flocks

Pregnancy rate data were excluded from the production effect analyses due to their inconsistency. Around 50% of the respondents provided the overall number of foetuses scanned over the number ewes mated instead of the number ewes pregnant over the number ewes mated. Valid information for tailing and culling rates were provided by 159 and 153 farm managers, respectively, and used to estimate the observed mean and 95% CI for these parameters (Table 5.2). Additionally, 18 and 16 flocks were excluded from the analysis of tailing and culling rates, due to the report of clinical cases in test negative flocks.

Table 5.2: Mean and 95% confidence interval of tailing and culling rates in sheep flocks, stratified by MAP infection and clinical paratuberculosis (cPtB) status

Sheep flocks	Tailing rate (n=159)	Culling rate (n=153)
Non-infected	1.36 (1.28 - 1.45)	15.5 (12.1 - 18.9)
Infected	1.32 (1.28 - 1.37)	11.5 (9.0 - 13.9)
Affected	1.30 (1.26 - 1.34)	12.7 (10.8 - 14.6)
All categories	1.32 (1.29 - 1.35)	12.8 (11.4 - 14.2)

In the 'surveyed population', cPtB cases confirmed by veterinarian-assessment or based on laboratory results were reported in 5.4% of flocks, an ACI of 3 (95% CI 1 to 5) cases per 1,000 head of stock per year. In the 'sampled population', occurrences of clinical cases were reported from 55% of test positive flocks, with an ACI of 2 (95% CI 1 to 3) cases per 1,000 head of stock per year (Table 5.3). Significant variables retained in the regression analyses are presented in Table 5.4. All fitted models were over-dispersed, so robust standard errors were used to adjust the best fitted model for correlation of production outcomes within flocks. MAP 'infected' flocks had significantly ($p=0.01$) lower culling rates than 'reference' flocks (OR=0.58; 95% CI 0.38 – 0.88). Additionally, 'affected' flocks had significantly lower ($p=0.05$) count of lambs at tailing (RR=0.80; 95%CI 0.63 – 0.99). No other significant differences were observed between the occurrence of 'affected' or 'infected' flocks and production outcomes.

5.4.2 Association with production in beef cattle herds

The observed mean and 95% CI of production parameters are presented in Table 5.5, based on information from 107, 110, and 103 beef cattle herds, for pregnancy, calving, and culling rates, respectively. Additionally, 13 herds were excluded from production effect modelling, due to the conflict of reporting cPtB cases while testing negative for infection. In the 'surveyed population', 1.7% of the beef cattle herds reported confirmed clinical cases, estimating an ACI of 2 (95% CI 1 to 3) cases per 1,000 head of stock per year. In the 'sampled population', 23.5% of tested positive herds reported clinical cases compatible with cPtB, presenting an ACI of 4 (95% CI 1 to 8) cases per 10,000 head of stock per year (Table 5.3). Significant variables retained and model outputs are presented in Table 5.6. Robust standard errors were used to adjust the best fitted models

for correlation in the production outcomes within herds. No significant differences were observed in pregnancy rates or culling rates between ‘infected’ or ‘affected’ herds, in comparison to the ‘reference’ category. Conversely, ‘infected’ herd had significantly ($p=0.03$) lower calving rates compared to the ‘reference’ herds, presenting an OR=0.70 (95% CI 0.50 – 0.97). No significant differences were observed between calving rates in ‘affected’ herds and the ‘reference’ herds.

Table 5.3: Descriptive statistics of clinical incidence in confirmed infected flocks/herds from survey and sampled farms

Source	Variables	Beef cattle	Deer	Sheep
Survey farms	Number of flocks/herds reporting confirmed cPtB over total number (and %)	21/1,265 (1.7)	43/237 (18.1)	68/1257 (5.4)
	Mean annual cPtB case rate (%) on confirmed positive farms (and 95% CI)	0.21 (0.07 - 0.34)	0.42 (0.16 – 0.65)	0.27 (0.08 - 0.45)
	Minimum (%)	0.00	0.00	0.00
	Maximum (%)	0.89	2.17	2.49
	Median (%)	0.12	0.2	0.05
Sampled farms	Number of flocks/herds reporting clinical cases in positive flocks/herds over total number (and %)	8/34 (23.5)	31/56 (55.4)	63/115 (54.8)
	Mean mortality rate (+ve farms) (%) and 95% CI	0.04 (0.01 - 0.08)	0.32 (0.05 – 0.60)	0.16 (0.09 - 0.24)
	Minimum (%)	0.00	0.00	0.00
	Maximum (%)	0.40	6.60	3.57
	Median (%)	0.00	0.03	0.02

CI=confidence interval

Table 5.4: Poisson model coefficient for tailing data and Logistic model coefficients for culling data in sheep flocks

Variable	Tailing rate (n=141)			Culling rate (n=137)		
	Estimate	S.E.	p-value	Estimate	S.E.	p-value
(Intercept)	-0.9765	0.2220	<0.0001	-1.9456	0.3057	<0.0001
Non-infected	Ref.			Ref.		
Infected	-0.0604	0.1247	0.6281	-0.5255	0.2114	0.0129
Affected	-0.2344	0.1193	0.0495	0.0357	0.2119	0.8664
Sheep Only	Ref.			Ref.		
Sheep and beef	-0.0976	0.2051	0.6342	0.0267	0.2853	0.9254
Sheep and deer	-0.1704	0.2147	0.4274	0.6155	0.2644	0.0199
Sheep, beef and deer	-0.0454	0.2003	0.8208	0.1736	0.2689	0.5186
Island (reference=NI)	0.4166	0.1040	0.0001	0.0637	0.1685	0.7053
Flock size	0.0000	0.0000	0.0059			

5.4.3 Association with production in deer herds

Production parameters observed, mean and 95% CI were based on valid information from 84, 80, and 81 herds, corresponding to pregnancy, weaning, and culling rates data, respectively (Table 5.7). Furthermore, 13, 14 and 14 herds were excluded from production models assessment, for pregnancy, weaning and culling rates, respectively, due to clinical cases reported by farm managers in test negative herds. In the ‘surveyed population’, 18.1% of deer herds had confirmed cPtb cases, with an ACI of 4 (95% CI 2 to 7) cases per 1,000 head of stock per year. In the sampled population, 55% of test positive herds reported clinical cases, with an estimated ACI of 3 (95% CI 1 to 6) cases per 1,000 head of stock per year (Table 5.3). The best fitting models estimates are presented in Table 5.8. As for sheep and beef cattle analyses, models were over-dispersed and robust standard errors were used to adjust data correlation within herds. ‘Infected’ herds had significantly ($p=0.009$) lower culling rates (OR= 0.44; 95% CI 0.25 – 0.75) compared with the ‘reference’ herds. There was a trend for weaning rates to be lower among ‘affected’ herds ($p=0.09$) compared to the ‘reference’ herds, with an

OR=0.86 (95% CI 0.71 – 1.03). No significant differences were observed between ‘infected’ or ‘affected’ herds and the ‘reference’ herds for pregnancy and calving rates.

Table 5.5: Mean and 95% confidence interval of reproduction parameters of beef cattle herds, stratified by MAP infection and herd status for clinical paratuberculosis (cPtB)

Beef cattle herds	Pregnancy rate Age class (n=107)				Calving rate (n=110)	Culling rate (n=103)
	15mo	27mo	MA	All		
Non-infected	86.7 (81.7 - 91.6)	91.8 (89.4 - 94.2)	90.3 (88.5 - 92.1)	90.0 (88.4 - 91.6)	91.6 (90.1 - 93.1)	13.5 (11.0 - 15.9)
Infected	85.2 (77.6 - 92.7)	87.5 (80.5 - 94.5)	88.5 (85.2 - 91.8)	87.6 (84.4 - 90.7)	88.0 (84.8 - 91.1)	15.2 (10.3 - 20.0)
Affected	89.2 (75.5 - 99.9)	84.2 (74.9 - 93.4)	87.8 (81.6 - 93.9)	87.0 (82.1 - 91.9)	93.7 (89.6 - 97.9)	15.2 (8.9 - 21.5)
All categories	86.6 (82.7 - 90.5)	90.1 (87.5 - 92.6)	89.6 (88.1 - 91.2)	89.1 (87.7 - 90.5)	90.9 (89.5 - 92.2)	14.0 (11.9 - 16.1)

mo = months old, MA= mixed age.

5.5 Discussion

This study assessed the relationship between flock/herd-level infection-status and some production outcomes of interest, namely pregnancy, calving/weaning/tailing and culling rates, in three different livestock species. The main findings were significantly lower calving rates in ‘infected’ beef cattle herds, significantly lower count of lambs at tailing in “affected” sheep flocks, and marginally significant trend for lower weaning rates in “affected” deer herds. In addition, culling rates were significantly lower among ‘infected’ sheep flocks and deer herds when compared to uninfected flocks/herds.

This research represents the first attempt to estimate the production effects of MAP infection and clinical disease, at a population level, in New Zealand pastoral systems. Two longitudinal studies are available for dairy cows (Norton, 2007) and sheep (Morris

et al., 2006). Although those studies were appropriate for describing accurate relationships between the disease and productivity at the individual animal level, they were limited to a small number of herds and flocks, and so the results could not be generalised to the national level.

This study used stratified random sampling methodology in order to observe MAP effects under a range of production systems. The first step involved a postal survey to clients of 28 farm animal veterinary practices in seven administrative regions of New Zealand, targeting single- or mixed-species commercial sheep, deer, beef and dairy cattle operations. This step established a sampling frame of 1,940 farms that correctly replied to the survey. From this pool, farms were selected for faecal and blood sampling in the second step, aiming to sample an equal number of farms from seven FTS, based on the presence/absence of sheep, beef cattle, and deer. Since FTS were covariates of all analytical models, the associations between production outcomes and species infection-status were averaged over stratum categories, so are valid for the sampling frame, and by inference, for the population. Comparisons of FTS frequencies between the 'surveyed population' and New Zealand farm statistics were presented elsewhere, showing a similar distribution of the seven strata among those three populations (Chapter 3).

The 24% response rate of the postal survey might indicate a possible bias in the data towards farms experiencing clinical Johne's disease. Such bias can not be ruled out, but if present at all, the authors expected to have reduced it by enquiring simultaneously about leptospirosis. A possible selection bias could have occurred at the animal sampling stage, where the target of 300 farms was not achieved because a number of

Table 5.6: Logistic regression coefficients for production parameters in beef cattle herds

Variable	Pregnancy rate (n=94)			Calving rate (n=97)			Culling rate (n=90)		
	Estimate	S.E.	p-value	Estimate	S.E.	p-value	Estimate	S.E.	p-value
(Intercept)	2.3682	0.3345	<0.0001	2.8829	0.3871	<0.0001	-1.9421	0.2467	<0.0001
Non-infected	Ref.			Ref.			Ref.		
Infected	-0.1049	0.1469	0.4752	-0.3599	0.1668	0.0309	-0.0807	0.2510	0.7479
Affected	-0.3056	0.2329	0.1896	0.1152	0.3599	0.7489	0.0793	0.3194	0.8039
Mating period (days)	-0.0003	0.0028	0.9104						
Island (reference=NI)	0.3652	0.1538	0.0176	0.5446	0.1820	0.0028	-0.5117	0.1621	0.0016
Age 1 = 15mo)	Ref.								
Age 2 = 27mo	0.6669	0.2084	0.0013						
Age 3 = MA	0.5352	0.1749	0.0022						
Beef only	Ref.			Ref.			Ref.		
Sheep and beef	-0.6901	0.2588	0.0077	-0.7318	0.4081	0.0729	0.4063	0.3117	0.1924
Deer and beef	-0.2407	0.4145	0.5615	0.1171	0.5138	0.8198	0.5317	0.3164	0.0929
Sheep, deer and beef	-0.4506	0.3059	0.1408	-0.8836	0.4249	0.0376	0.3388	0.3096	0.2737
Herd size	-0.0004	0.0002	0.0197						

mo = months old, MA= mixed age, NI=North Island.

Table 5.7: Mean and 95% confidence interval of production parameters of deer herds, stratified by MAP infection and herd status of clinical paratuberculosis (cPtb)

Herd status	Pregnancy rate (n=84)			Weaning rate (n=80)	Culling rate (n=81)
	R2YO	MA	All		
Non-infected	84.2 (77.6 - 90.8)	93.9 (91.2 – 96.5)	89.8 (86.3 - 93.3)	87.0 (85.1 - 88.9)	13.0 (9.3 – 16.8)
Infected	89.2 (77.4 - 92.6)	95.4 (84.9 – 96.2)	92.4 (83.6 - 92.8)	88.6 (86.0 - 91.2)	10.6 (6.5 – 14.7)
Affected	89.2 (85.6 - 92.8)	95.4 (93.7 – 97.0)	92.4 (90.3 - 94.5)	86.3 (83.0 - 89.7)	9.9 (7.2 - 12.5)
All categories	86.5 (83.3 - 89.8)	93.4 (91.3 – 95.5)	90.4 (88.4 - 92.3)	87.2 (85.6 - 88.8)	11.2 (9.2 – 13.1)

R2YO=rising two years old, MA=mixed age, All=all age groups.

Table 5.8: Logistic regression coefficients for production parameters in deer herds

Variable	Pregnancy rate (n=71)			Weaning rate (n=66)			Culling rate (n=67)		
	Estimate	S.E.	p-value	Estimate	S.E.	p-value	Estimate	S.E.	p-value
(Intercept)	1.8375	0.4335	<0.0001	1.7701	0.1179	<0.0001	-1.5737	0.2894	<0.0001
Non-infected	Ref.			Ref.			Ref.		
Infected	-0.1019	0.2746	0.7105	0.0835	0.1186	0.4813	-0.8129	0.2702	0.0026
Affected	0.1783	0.2442	0.4655	-0.1562	0.0930	0.0931	-0.2419	0.1839	0.1886
Mating period (days)	-0.0012	0.0028	0.6638						
Island (reference=NI)	0.8309	0.1596	<0.0001	0.3286	0.0896	0.0003	-0.0707	0.1977	0.7208
Age 1 = R2YO	Ref.								
Age 2 = MA	0.7814	0.1660	<0.0001						
Deer only	Ref.			Ref.			Ref.		
Deer and sheep	-0.8879	0.3085	0.0040	-0.2002	0.1543	0.1945	0.1636	0.3264	0.6162
Deer and beef	-0.6872	0.2547	0.0069	0.1957	0.1485	0.1875	0.1194	0.2862	0.6766
Deer, sheep and beef	-0.2543	0.2879	0.3772	-0.0273	0.1077	0.7998	-0.3161	0.2481	0.2028
Herd size	0.0001	<0.0001	0.0852						

R2YO=rising two years old, MA=mixed age.

farms were not accessed due to lack of sufficient facilities for sampling or were not able to gather all animal species present on farm, or were not able to be sampled during the study period. This highlights the logistical difficulty of undertaking such research on commercial pastoral farms in New Zealand. The effect of such bias, if present at all, potentially could have excluded poorly managed farms (likely in farms with lack of proper handling facilities), which might be prone to experiencing greater cPtb effects, biasing the results toward no-difference. However, the proportion of farms excluded for all described reasons was 21% (62/300), thus the exclusion rate, and therefore the extent of potential bias was considered by the authors to be reasonably low.

In the 'sampled population', infection-status classification combined laboratory test results information with clinical cases observed by farm managers in the previous four years. This approach allowed the flock/herd level classification to be 'non-infected' (test negative and no cases, 'reference'), 'Infected' (test positive and no clinical cases), and 'affected' (test positive and presence of cases). MAP infection status assessment is constrained by low sensitivity (Se) and imperfect specificity (Sp) of available tests in live animals. With the progression of the infection, test characteristics at the individual animal level improve, achieving acceptably accurate test results only in the pre-clinical and clinical stages of the disease (Nielsen and Toft, 2008). A low Se potentially leads to an increasing number of false negative results. However, MAP status classification in this study was made at the flock/herd level of 20 animals per herd and was based on the combination of two tests used in series. This increased the probability of detecting MAP-infected-flocks/herds. The relevant Se measure was thus herd level sensitivity (HSe). Previous analysis of the data set estimated a HSe of 0.90, 0.69, and 0.98 for MAP detection in sheep, beef cattle, and deer flocks/herds, respectively (Chapter 3).

The sampling design had the disadvantage of reducing herd-level specificity (HSp), with estimates of 0.78, 0.94 and 0.38, for sheep beef cattle, and deer flocks/herds, respectively. The lower HSp would have increased the number of false positive flock/herds.

Clinical cases were based on farm manager records or recalls. However, data assessing farm manager's diagnostic ability is sparse. One study conducted by Glossop et al. (2008) reported a misclassification rate of 60% for the herd level diagnosis of cPtb, based on the assessment of 174 deer farm managers across New Zealand. The sampling strategy used in the present study was not designed to evaluate farm manager diagnosis, making it difficult to assess the quality of the clinical data provided by them. Nevertheless, we attempted to minimise the potential confounding effect of non-specific case reports by farm managers by the inclusion of a precise case description for cPtb in the survey and the classification as 'clinical cases' of only those previously confirmed or being from test positive flocks/herds. We believe these measures reduced but may not have eliminated the inclusion of false positive flocks/herd in the data analysis.

We acknowledge however, that evaluating associations between MAP status and reproduction performance at flock/herd level only assumes that such associations also hold at animal level. This may not be a valid assumption considering that both low reproductive outcomes for example, and MAP infection or cPtb may have a common cause such as 'poor management' with nutritional stress being the most likely, given the nature of pastoral farming in New Zealand. Such individual animal level inference from group level data is known as ecological fallacy (Dohoo et al., 2003). Moreover, in an infected flock/herd only a small proportion of animals will be in more advanced stages of disease (Sweeney, 2011), thus the majority of animals will not present any detectable

clinical signs with little effect on productive performance. This would bias the effect of MAP infection or cPtb on flock/herd level effects towards the null and require a much higher sample than feasible for this study. Nevertheless, this study was able to evaluate the association between infection-status and production outcomes in the three livestock species, representing initial information to be consolidated by further study research.

In general, MAP infection causes a reduction of intestinal nutrient absorption, especially proteins, leading to a negative energy/protein balance which could have an indirect effect on reproduction performance (Harris and Barletta, 2001). Although MAP has been isolated from fetuses of infected deer (van Kooten et al., 2006), it has not been studied to our knowledge, whether infected offspring from infected dams was readily progressing to clinical disease to the same extent as horizontally infected animals. Livestock infected with MAP could be culled prematurely due to the clinical manifestation of the disease, reduced growth rates, decreased milk production, failure to conceive or wean a calf/lamb, or due to the implementation of a test and cull control program. Yet our finding was of lower culling rates in 'infected' or 'affected' sheep flocks and deer herds. This may be explained by a longer productive lifetime giving rise to a longer period at risk for the development of disease and clinical signs. This explanation however, presents low culling intensity as a risk for the occurrence of infection, not the other way round.

Other studies carried out by Gonda et al. (2007) in 232 dairy herds ($n = 4,375$ cows) in USA, by Raizman et al. (2007b) and Smith et al. (2010), found that MAP was associated with higher culling rates in dairy cattle. Production effects have been mainly studied in dairy systems, principally focusing on on the relationship with milk

production. However, its effects over reproduction performance or culling rates have not been extensively studied (Smith et al., 2010). A longitudinal study involving two dairy herds ($n = 1,297$ cows) in Minnesota USA found that clinically normal, faecal culture positive cows produced approximately 11% less milk, and were removed on average 124 days earlier than negative herd-mates (Raizman et al., 2007b). Reproductive performance of culture positive cows was also lower than of negative cows. Similar effects were observed in that study for clinically affected cows, being removed on average 202 days earlier, and having a lower lifetime production of around 1.5 tons milk compared to herd-mates. Other studies, using different tests and definitions for MAP infection, have found similar results: a decrease in milk production between 0.8 and 17% in test positive cows compared to negative herd-mates (Benedictus et al., 1987; Sweeney et al., 1994; Nordlund et al., 1996; Ott et al., 1999; Johnson et al., 2001; Lombard et al., 2005; Norton, 2007). In another longitudinal study in the USA, conducted by Smith et al. (2010) on 6 commercial dairy farms ($n = 2,818$ cows), observed that low positive cows (low-shedding or ELISA-positive) and high-shedding cows were culled earlier than test negative cows. Additionally, calving rates of test-negative cows were slightly higher than those of low positive herd-mates, and substantially higher than those of high-shedding cows.

Although findings from the above studies could potentially be applied to beef cattle, the management practices between these two types of herds differ substantially, especially in New Zealand where livestock farming is based on whole year grazing. In beef cattle herds, this study observed lower calving rates in 'Infected' than non-infected herds. Previous studies conducted in dairy cattle have shown contradictory results, where ELISA positive cows achieved higher calving rates than their negative herd-mates

(Lombard et al., 2005; Marce et al., 2009; Smith et al., 2010). However, in a retrospective study using data from 1,069 dairy herds ($n = 48,914$ cows) in France (Marce et al., 2009) observed that the higher calving rates effect in positive animals decreased as cows parity increased. Smith et al. (2010) found that animals in high shedding stage of infection achieved lower calving rates than non-infected animals. These observations suggest that negative MAP effects on reproduction could become manifest in advanced stages of infection. In our results, being affected by Ptb (i.e. clinical cases in infected herds) had no significant effect on calving rates of beef cattle herds. However, few beef cattle herds were classified as 'affected', decreasing the power of finding an effect in this category.

A study in deer conducted in New Zealand by Thompson et al. (2007) observed that MAP infected hinds achieved lower pregnancy rates (69%), which was lower than the 85–90% commonly reported in the herds from where infected hinds were sourced. Nevertheless, their study was not designed to evaluate the reproduction performance of MAP infected hinds, or mimic normal production conditions. In addition, the limited number of animals used in the study ($n = 35$) challenges the validity of their inferences about reproductive effects. The present study did not detect lower pregnancy rates among 'infected' or 'affected' deer herds compared with non-infected herds, but a trend ($p=0.09$) of lower weaning rates in 'affected' herds, similar to the finding in beef cattle suggesting increased foetal loss, stillbirth or mortality among young stock.

A longitudinal study of New Zealand sheep by Morris et al. (2006), using post-mortem histopathology for cPtb diagnosis ($n = 3,633$ ewes), evaluated production effects associated with cPtb in Romney, Merino and Merino x Romney-cross ewes. The authors

reported a significant difference in the mean age at disposal between clinical and no-clinical sheep (3.41 and 5.03 years, respectively), in addition to a significant 10.5% difference in live weight and a reduction by 0.54kg of greasy fleece weight between clinical and no-clinical animals (Morris et al., 2006). In another study conducted on 12 infected farms in New South Wales Australia, sheep mortality ranged from 2.1% to 17.5%, resulting in an estimated decrease in farm gross margin due to cPtb of 2.2% to 15.4% (Bush et al., 2006). In a study conducted by Kostoulas et al. (2006) assessing the effect of sub-clinical MAP infection in four flocks of dairy ewes and goats in Greece ($n = 369$), positive (ELISA or culture) animals of parity <4 had higher lambing rates than their negative flock mates. But this effect was not present in positive animals of higher parities. This suggests a similar effect as reported in cattle, where MAP effects on reproduction performance were observed in older animals attributable to more advanced stages of the disease (Marce et al., 2009; Smith et al., 2010).

In the present study however, pregnancy/fertility data provided by sheep farm managers were inconsistently reported, thus we have been unable to assess this previously observed animal level effect (Kostoulas et al., 2006), at flock level. The inconsistency of the pregnancy data, despite a clear definition being supplied, reflects current monitoring practices of commercial sheep farming, where tailing rate is the most common parameter used by farm managers for assessing reproduction performance and scanning is not standard practice. Although it was not possible to assess pregnancy data of sheep, lamb tailing rates were significantly lower in 'affected' flocks. This observation was similar to the association of cPtb on calving rates in beef cattle and weaning rates in deer.

5.6 Conclusions

Data from this study suggest that MAP infection was associated to reduced calving rates in beef cattle herds by 3.6%. Lower tailing rates of lambs and a trend for lower weaning rates of deer were observed in ‘affected’ compared to non-infected mobs. Lower culling rates were found in ‘infected’ sheep and deer flocks/herds. Given that the incidence of reported cPtB was generally very low (<1%), the results of this study indicate that sub-clinical production loss may be higher than due to cPtB. However, while the results are fairly representative for the national population of livestock farms, the described associations at flock and herd level need to be confirmed at animal level to exclude potential confounding due to ecological bias.

5.7 Acknowledgements

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Molecular epidemiology of *Mycobacterium avium* subsp. *paratuberculosis* isolated from sheep, cattle and deer on New Zealand pastoral farms

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6.1 Abstract

The present study aimed to describe the diversity and epidemiological associations of subtypes of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) from single and mixed-species pastoral farms in the North and South Islands of New Zealand. A total of 206 independent MAP isolates (15 beef cattle, 89 dairy cattle, 35 deer, 67 sheep) were sourced from 172 species-mobs (15 beef cattle, 66 dairy cattle, 31 deer, 60 sheep). Seventeen subtypes were identified, using a combination of 5 loci characterised using the variable number of tandem repeats (VNTR) method and 1 locus was characterised using the short sequence repeat (SSR) method. Rarefaction analysis, analysis of molecular variance (AMOVA), F_{st} pairwise comparisons and proportional similarity index (PSI) were used to describe subtype population richness, genetic structure and potential associations between geographical location and livestock sectors.

The rarefaction analysis suggests a significantly higher subtype richness in dairy cattle herds when compared to the other livestock sectors. AMOVA results indicate that the main source of subtype variation is attributable to the livestock sector from which samples were sourced indicating that subtypes are generally sector-specific. Additionally, there is a relatively high degree of subtypes circulation between the two islands, since a small proportion (9%) of the total variance was found at this level. The pairwise F_{st} results were similar, with low F_{st} values for island differences within a livestock sector when compared to between sector analyses, representing a low subtype differentiation between islands. However, for a given island, potential associations were seen between dominant subtypes and specific livestock sectors. Three subtypes accounted for 76% of the isolates. The most common of these was isolated from sheep and beef cattle in the North Island, the second most frequent subtype was mainly isolated from dairy cattle (located on either island), whilst the third most common subtype was associated with deer farmed in the South Island. The PSI analysis suggests similarities in subtypes sourced from sheep and beef cattle. This contrasted with the isolates sourced from other livestock sectors, which tended to present sector-specific subtypes. Sheep and beef cattle were mainly infected with MAP Type I (ovine or type S), while dairy cattle and deer were almost exclusively infected with MAP Type II (bovine or type C). However, when beef cattle and deer were both present at farm level, they harboured similar subtypes.

This study supports that cross-species transmission of MAP occurs on New Zealand farms although close contact between species appears to be required, as for sheep and beef cattle which are commonly grazed together in New Zealand.

6.2 Introduction

Mycobacterium avium subsp. *paratuberculosis* (MAP), an intracellular pathogen, is the causative agent of clinical paratuberculosis (cPtB), a debilitating disease that mainly affects domestic ruminants worldwide. During its clinical manifestation, it is commonly characterized by chronic diarrhoea that does not respond to treatment, leading to emaciation and eventually to death or culling. Clinical PtB has a complex epidemiology, with a long incubation period, that often involves several years before the onset of clinical signs (Sweeney, 2011). However, most infected animals do not experience detectable production losses or reach a clinical stage of disease during their productive lifetime, but remain sub-clinically infected (Nielsen and Toft, 2008). Moreover, it has been observed that some infected sheep and deer have the capacity to eliminate the infection (Gilmour et al., 1978; Stewart et al., 2004; Mackintosh et al., 2007; Dennis et al., 2011; Kawaji et al., 2011). It is not yet fully understood why some infected animals progress to a clinical phase, while others never show any clinical signs and eventually manage to eliminate the infection. One possibility is that disease outcomes are influenced by MAP strain differences. Gollnick et al. (2007) observed that the survival of MAP in bovine macrophages was influenced by strain type. Similarly, studies conducted by Janagama et al. (2006) and Motiwala et al. (2006a) suggest that different MAP strains trigger different host immune reactions. Furthermore, experimental infections in deer and sheep have indicated variations in disease virulence associated with different MAP strain types (O'Brien et al., 2006; Mackintosh et al., 2007; Verna et al., 2007).

Population-based molecular analysis of MAP strains has been hindered by the high genetic similarity of this species. Traditional molecular techniques such as Multiplex

PCR of IS900 integration loci (MPIL), amplified fragment length polymorphism (AFLP), pulsed field gel electrophoresis (PFGE) and restriction fragment length polymorphism (RFLP) have been used to the study of MAP. In particular, analyses using RFLP and PFGE techniques have identified two major strain groups (or types), named Type I (ovine or type S) and Type II (bovine or type C) (Collins et al., 1990; Bauerfeind et al., 1996; Stevenson et al., 2002). Previous studies using samples from New Zealand, Australia and Spain showed that Type II strains were commonly isolated from cattle, deer and goats (Collins et al., 1990; Sevilla et al., 2005). Conversely, Type I strains were mainly isolated from sheep (Collins et al., 1990; Sevilla et al., 2005; Motiwala et al., 2006b). However, opposing outcomes in strain segregation have been observed recently in a multi-host molecular study of MAP isolates across Europe (Stevenson et al., 2009). In that study, no Type I strains were isolated from sheep or goat field samples and Type II was retrieved from a broad range of host species. Thus, the crude separation of MAP strains into types I and II has not yielded conclusive indication of MAP host specificity and it has been difficult to obtain further meaningful epidemiological classification of MAP isolates with these methods.

Mycobacterium avium subsp. *paratuberculosis* exhibits little diversity compared with other bacterial pathogens, thus molecular analysis requires the use of multiple molecular techniques to increase discriminatory power, in order to conduct meaningful epidemiological studies (Stevenson et al., 2009). The whole genome sequencing of the MAP strain K-10 (Li et al., 2005) has allowed the development of PCR-based methods for the study of MAP strain diversity (Harris and Barletta, 2001). These methods represent more powerful typing techniques than MPIL, AFLP and RFLP for the analysis of MAP isolates (Motiwalala et al., 2006b), and they have allowed the differentiation of

MAP types I and II into several subtypes. The two PCR-based methods that are most frequently used for the analysis of MAP isolates are Mycobacterial Interspersed Repetitive Units, Variable-Number Tandem Repeats (MIRU-VNTRs) (Thibault et al., 2007) and multiple short-sequence repeats (SSR) (Amonsin et al., 2004). These techniques target elements in the MAP genome, indexing the number of copies of specific genetic polymorphic structures (MIRU-VNTR) or simple homopolymeric tracts of single, di- or trinucleotides (SSR) (Thibault et al., 2008). The indexing of repeat copy numbers in the respective loci is a particularly suitable approach for inter-lab comparisons and phylogenetic studies (Allix-Beguec et al., 2008). To date, most studies employed either MIRU-VNTR or SSR typing (Amonsin et al., 2004; Harris et al., 2006; Thibault et al., 2007; Moebius et al., 2008; Stevenson et al., 2009; van Hulzen et al., 2011). However, the use of MIRU-VNTR and SSR methods in tandem offers an improved genotyping approach for high-resolution typing of MAP isolates, due to their additive discriminatory power (Thibault et al., 2008). Increased ability to distinguish MAP isolates will undoubtedly provide a better understanding of the mechanisms involved in the geographic distribution of MAP, host-specificity, and disease severity (Motiwala et al., 2006b; Stevenson et al., 2009; van Hulzen et al., 2011).

In New Zealand, domestic ruminants are commonly farmed in multi-species pastoral systems, where sheep, beef cattle (dairy to a lesser extent) and deer are often grazed on the same pastures. This management practice may generate opportunities for cross-species transmission of MAP strains. Thus, in order to understand the epidemiology of this pathogen, molecular studies of MAP should address all relevant susceptible livestock sectors in an overarching strategy. The objective of this study was to characterize the geographic distribution and molecular diversity of MAP isolates

obtained from sheep, cattle (beef and dairy) and deer, located on single or mixed-species pastoral farms in New Zealand, using a combination of MIRU-VNTR and SSR assays for MAP strain-typing.

6.3 Materials and Methods

6.3.1 Source of *Mycobacterium avium* subsp. *paratuberculosis* isolates

Mycobacterium avium subsp. *paratuberculosis* isolates were obtained from three sources. The first, (S1) was from faecal samples, collected by contracted veterinary practitioners between June 2009 to July 2010 as part of the New Zealand Johne's Disease Research Consortium initiative (JDRC, 2012). That project involved a national assessment of the epidemiological situation of MAP infection and cPtb in New Zealand in which 7,579 animals were sampled on 238 single or mixed-species farms, randomly selected from a population of 1,940 commercial properties, previously surveyed as described by Verdugo et al. (2010). The sampled farms were from seven of 11 administrative regions of New Zealand, involving 162 sheep flocks, 116 beef cattle herds and 99 deer herds. The second source (S2) was all productive sites belonging to a national farming corporation sampled from July to December 2010. This source comprised samples from 112 single- or mixed-species farms, representing 61 sheep flocks, 16 deer herds, 49 beef and 42 dairy cattle herds (3,510 sampled animals). For S1 and S2, twenty animals from each species flock/herd present on farm were randomly selected for sampling. Sample size calculations have been presented elsewhere (Chapter 3). In sheep flocks, mixed age ewes 2-years and older were selected, with mixed-age cows selected from beef and dairy herds and yearling deer aged 12-24 months of either sex being eligible on deer farms. In addition to the 20 randomly selected animals, up to five animals with signs of cPtb (wasting, and/or diarrhoea) were also sampled (suspect

animals), if such animals were seen in the flock/herd on the sampling date. Samples from suspect animals were pooled separately from those from randomly selected animals. One pool was prepared from sheep submissions (20 samples/pool), and two from cattle (beef and dairy) or deer samples (10 samples/pool). The collection of all S1 and S2 samples was designed and implemented by the EpiCentre, Institute of Veterinary, Animal and Biomedical Sciences, Massey University. The third source of MAP isolates (S3) was collected by the Livestock Improvement Corporation (LIC), which screened 332 dairy herds from across the country by bulk tank milk (BTM) ELISA. Milk of cows from BTM-positive herds (n=64) was then sampled, and tested by individual milk ELISA (IME). Faecal samples from IME-positive cows were collected byASUREQuality technicians under LIC direction. These were individually cultured for MAP.

A total of 1,861 pooled (S1 & S2) and 284 individual (S3) samples (11,373 animals) were cultured for up to 8 weeks, using BACTEC 12B liquid culture medium, containing egg yolk and mycobactin, after a decontamination step with cetylpyridinium chloride, as described by Whittington et al. (1999). MAP confirmation was carried out by testing mycobactin J dependency in solid media. All cultures were performed at the AgResearch laboratory, NCBID Wallaceville, Upper Hutt, New Zealand.

6.3.2 *Mycobacterium avium* subsp. *paratuberculosis* strain-typing

Confirmed positive samples were classified as MAP Type I or Type II using a PCR-based method developed by Collins et al. (2002). Isolates were strain-typed based on the combination of assays of five MIRU-VNTR markers denominated 292, 25, X3, 7, and 3 (Thibault et al., 2007) and one SSR marker denominated SSR-8 (Amonsin et al., 2004). These molecular typing assays were selected based on their ability to optimally

and reproducibly distinguish a diverse array of archived New Zealand MAP isolates (58 Type I and 65 Type II) from cattle, deer and sheep that were collected between 1985 and 1993 and were held in the AgResearch archive at Wallaceville (data not shown). DNA preparation and PCR reaction conditions were performed as described in Subharat et al. (2012). The number of repeats at each of the 6 marker sites was recorded, and the unique combination of repeats for each marker formed a specific haplotype profile for each isolate, which was used as an indicator for a given MAP subtype.

6.3.3 Data analysis

On several occasions there were two or more PCR products detected when a VNTR loci was amplified from a single sample, suggesting multiple subtypes were present. When two different products were detected at one of the five VNTR loci, the haplotype was split so that each different subtype was represented in the data set. Samples in which three products were observed in a single VNTR assay, or when two products were observed at more than one VNTR loci were excluded from the analysis. Moreover, data analysis only considered epidemiologically unrelated subtypes (independent), thus identical isolates from the same source species, in a given farm, were counted as one (Moebius et al., 2008; Castellanos et al., 2010). Subtype frequencies were cross-tabulated by New Zealand's North (NI) and South Islands (SI), livestock sector, and MAP type (I or II). The diversity index (DI) of the data set strain-typed by the combined five MIRU-VNTRs and one SSR markers was estimated using the Simpson's Index of Diversity (DI) algorithm described by Hunter and Gaston (1988).

$$DI = 1 - \left[\frac{1}{N(N-1)} \sum_{j=1}^s n_j(n_j - 1) \right]$$

where N is the total number of isolates in the typing scheme, s is the total number of distinct subtypes discriminated by the typing method, and n_j is the number of isolates belonging to the j^{th} subtype.

The subtype richness among the livestock sectors was evaluated using rarefaction analysis (Magurran, 2003), which estimated genotype richness in random sub-samples from the generated dataset. This technique allows for standardization and comparison of datasets (Gotelli and Colwell, 2001). The rarefaction analysis was implemented in R, version 2.14.1 (R Core Team, 2012), using the package VEGAN 2.0.2, function RAREFY (Oksanen et al., 2011).

The genetic structure variation of MAP subtypes was explored using an analysis of molecular variance (AMOVA); this method uses molecular data to estimate population genetic differentiation. AMOVA explicitly extends the procedures and formats used in the traditional analysis of variance, in order to estimate the degree of intra-specific genetic subdivision and allows a flexible exploration of hierarchical structures (HS) within the dataset (Excoffier et al., 1992). AMOVA was performed by partitioning the data into a HS, where MAP isolates were divided into 8 “populations” demarcated by livestock sector and island of origin. These 8 populations were then grouped into the four livestock sectors and the covariance was calculated at three levels of resultant hierarchy: within a livestock sector within an island; between the islands within a livestock sector; and between the livestock sectors regardless of island. A schematic representation of the HS used in the AMOVA analysis is presented in Figure 6.1 (HS1). The AMOVA analysis was conducted using the Arlequin software package (Excoffier et al., 2005). The matrix of Euclidian distances between all pairs of haplotypes was used

to perform the AMOVA. The variance components and Φ -statistics were tested by nonparametric randomization tests with 1,000 repetitions to obtain the probability of having more extreme variance components and Φ -statistic than the observed values by chance alone. Pairwise F_{st} -values were also computed for two HS denoted HS1 and HS2, assessing genetic differences of MAP subtypes within and between islands respectively, among the four livestock sectors. The first structure (HS1) employed the same hierarchy used for the AMOVA analysis described above. In this structure, F_{st} -values were estimated for livestock sectors within a given island, thus a total of 12 pairwise comparisons were computed. In the second structure (HS2), MAP isolates were also divided into 8 “populations” demarcated by livestock sector and island of origin as in HS1. However, the 8 “populations” were divided into two islands (NI and SI), each one comprising four livestock sectors. For HS2, F_{st} -values were estimated for livestock sectors between islands, thus a total of 16 pairwise comparisons were computed. A schematic representation of HS1 and HS2 is displayed in Figure 6.1. For any or both HS under study, F_{st} -values were estimated as genetic distances based on pairwise differences in subtypes, and the null hypothesis (no differences between the two populations being compared) was tested by 1,000 permutation simulations of haplotypes between populations with a significant p-value of 0.05. Wright (1978) suggested a qualitative assessment of F_{st} -values, where results in the range between 0.0 to 0.05, 0.05 to 0.15, 0.15 to 0.25, and >0.25 indicates little, moderate, great, and very great genetic differentiation, respectively. Pairwise F_{st} analyses were performed using the Arlequin 3.5.1.2 software (Excoffier et al., 2005).

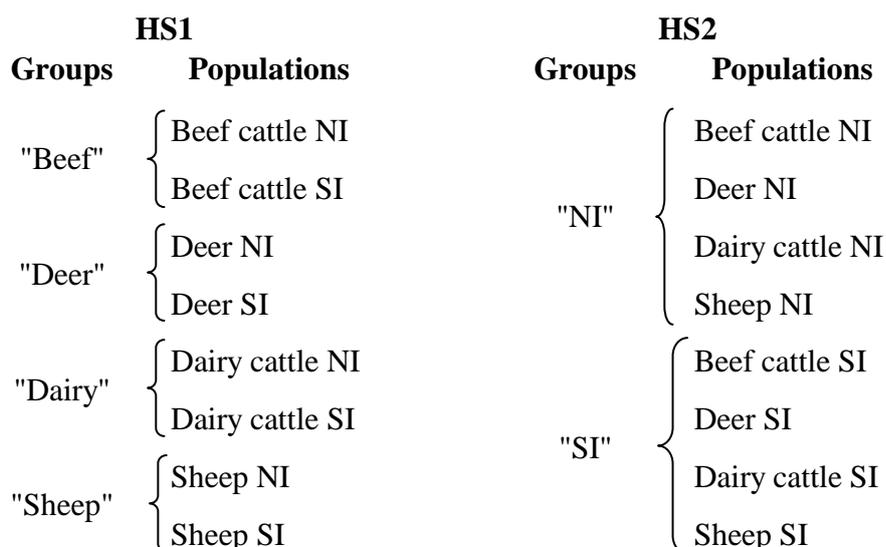


Figure 6.1: Schematic representation of the hierarchically structured data set used for AMOVA test (HS1) and for the computation of pairwise F_{st} -values (HS1 & HS2), considering New Zealand's North (NI) and South (SI) Islands, and four livestock sectors

To further examine the differences in subtypes between livestock sectors, the similarity between the frequency distributions of subtypes from the four different sector sources was assessed using the proportional similarity index (PSI) or Czekanowski index. This index represents a measure of the area of intersection between two frequency distributions (Rosef et al., 1985). The PSI was estimated such that:

$$PSI = 1 - 0.5 \sum_i |p_i - q_i|$$

where p_i and q_i represent the proportion of isolates belonging to subtype i out of all subtypes from sources p and q (Feinsinger et al., 1981; Rosef et al., 1985). The values for PSI range from zero to one, where zero indicates no common subtypes distributions between two sector sources, and 1 represents identical frequency distributions of subtypes between two sector sources. This analysis measures the tendency of two given hosts (livestock sectors) to harbour a given subtype. This tendency is influenced by host and strain physiology and other factors such as how frequently different farm species were co-grazed and thus can provide an indication of the tendency for between-host horizontal transmission of the different subtypes. Bootstrap confidence intervals for PSI

values were computed based on the methodology proposed by Garrett et al. (2007), using an algorithm developed by Muller et al. (Mullner et al., 2010). PSI analysis was implemented in R, version 2.14.1 (R Core Team, 2012).

6.4 Results

A total of 365 MAP isolates (S1=120, S2=45, S3=200) were successfully typed with a combination of MIRU-VNTR and SSR assays. This included pool samples from 5 deer herds and 11 sheep flocks from animals with clinical signs of infection (cPtb), but no clinically ill beef cattle were available during the course of this work. Although multiple subtype infection (double or triple amplification in a single VNTR marker), was not observed at herd level in beef cattle; 8 pooled sheep, 2 pooled deer and 30 individual dairy samples had multiple subtypes. A total of 159 isolates were excluded from the data analysis, due either to three products being observed in a single VNTR loci (n=19), two products being observed at more than one VNTR loci (n=4), or isolates being epidemiologically related (n=136, from 56 flocks/herds). This last exclusion group was heavily influenced by differences in sampling and testing protocols between the isolate sources (individual vs. pool faecal culture), where 102 of the epidemiologically related isolates were from S3, obtained from 34 dairy herds.

Thus, 206 isolates (S1=91, S2=38, S3=77) were identified as epidemiologically-unrelated samples. They were classified into 17 subtypes with an overall DI of 0.76, based on the haplotype frequencies generated by the combination of the 6 markers (Table 6.1). These 206 isolates were sourced from 172 species mobs (15 beef cattle, 60 sheep, 31 deer, and 66 dairy cattle), located on 162 farms across New Zealand. The 17 subtypes were arbitrarily designated letters *A* to *Q*, being exclusively assigned to a particular MAP type (I or II). In this way, subtypes *A* to *F* belong to MAP Type I group,

and subtypes *G* to *Q* belong to MAP Type II group. The number of isolates from each subtype by Island and species is presented in Table 6.2. The proportion of isolates classified as MAP-Type I vs. -Type II was 82:124 (Table 6.2). There were 113 and 93 isolates included from the NI and SI, respectively, with Type I being slightly more frequently isolated from the NI (56.6%), and Type II being the dominant MAP type in the SI (80.7%). Most (75.7%) isolates were distributed in the three most common subtypes, being *C* (30.6%), *M* (29.1%), and *O* (16.0%). Strain type *M* was more frequently isolated from the SI (NI:SI=27.4% vs. 31.2%), whereas a stronger island association was observed with *C* and *O*, with *C* mainly found in the NI (NI:SI=45.1% vs.12.9%) and *O* mainly found in the SI (NI:SI=0.9% vs. 34.4%). Conversely, seven subtypes (*B*, *F*, *H*, *J*, *K*, *N*, and *Q*) were observed only once, with five of these isolated from dairy cattle (Table 6.2). At livestock sector level, only four of the 17 subtypes (*A*, *C*, *M*, and *O*) were observed in all four sectors. Beef cattle herds and sheep flocks were mainly infected with MAP Type I (80.0% and 86.8% respectively), with *C* being the predominant subtype (73.3% and 67.2% respectively). Conversely, deer herds were mainly infected with MAP Type II (91.4%), with *O* (62.9%) being the most common subtype, which was also found in all other livestock sectors. The dairy cattle sector presented the greatest number of subtypes (n=14). Most (89.9%) dairy MAP isolates belonged to MAP Type II, with 73% of them represented in just two subtypes, *M* (56.2%) and *I* (16.9%). Strain type *M* was also isolated from all of the other livestock sectors, but *I* was observed only in the dairy sector. Subtypes *A*, *C*, *D*, *F*, *L* and *O* were isolates from pool samples from suspected sheep, while only subtypes *M* and *O* were isolates from pool samples from suspected deer. At the comparison between S1 and S2, for sheep, beef cattle and deer samples, S1 presented a greater richness with 11

subtypes, whereas S2 presented only four subtypes (*A*, *C*, *L* and *O*), which were also present in S1, being the subtypes *C* and *O* the most frequent in both populations.

Table 6.1: Number of isolates in each MAP subtype profiles, and copy numbers for the five MIRU-VNTR markers and one SSR marker used for the strain-typing

Subtype	MIRU-VNTR markers				SSR		MAP type	Total number of isolates
	292	25	X3	7	3	8		
A	3	3	1	1	1	3	I	9
B	3	3	1	2	1	3	I	1
C	4	3	1	1	1	3	I	63
D	4	3	1	2	1	3	I	5
E	5	3	1	1	1	3	I	3
F	7	3	1	1	1	3	I	1
G	3	3	2	1	2	5	II	2
H	2	3	2	2	2	5	II	1
I	3	2	2	2	2	5	II	15
J	3	2	2	3	2	5	II	1
K	3	3	2	2	2	3	II	1
L	3	3	2	2	2	4	II	7
M	3	3	2	2	2	5	II	60
N	3	3	2	3	2	5	II	1
O	4	3	2	2	2	4	II	33
P	4	3	2	2	2	5	II	2
Q	5	2	2	2	2	5	II	1
Total								206

Figure 6.2 shows the rarefaction curves for the four livestock sectors under study. The greatest number of unique subtypes was obtained from dairy cattle, whereas beef cattle and deer herds showed the least subtype richness. Rarefaction curves for the dairy sector did not show signs of flattening, indicating that further sampling of dairy herds could still provide more MAP subtypes. The 95% CI rarefaction curves in Figure 6.2 illustrate an overlap between sheep, deer and beef cattle curves, indicating that subtype richness was not significantly different between these three livestock sectors. Conversely, the dairy cattle curve clearly does not overlap at the apex of the other curves, indicating that the observed differences in subtype richness are likely to be significant.

Table 6.2: Number of each subtype by Island, livestock sector and MAP type

Subtype	New Zealand		Livestock sector				Total
	North Island	South Island	Beef cattle	Deer	Dairy cattle	Sheep	
A ¹	8	1	1	1	3	4	9
B	1	0	0	0	0	1	1
C ¹	51	12	11	2	5	45	63
D ¹	3	2	0	0	0	5	5
E	1	2	0	0	1	2	3
F ¹	0	1	0	0	0	1	1
G	0	2	0	1	1	0	2
H	0	1	0	0	1	0	1
I	13	2	0	0	15	0	15
J	1	0	0	0	1	0	1
K	0	1	0	0	1	0	1
L ¹	1	6	0	1	4	2	7
M ²	31	29	1	7	50	2	60
N	1	0	0	0	1	0	1
O ^{1,2}	1	32	2	22	4	5	33
P	0	2	0	1	1	0	2
Q	1	0	0	0	1	0	1
Total	113	93	15	35	89	67	206

MAP type							
Type I	64	18	12	3	9	58	82
Type II	49	75	3	32	80	9	124
Total	113	93	15	35	89	67	206

¹subtypes isolated from samples of clinical paratuberculosis suspected sheep.

²subtypes isolated from samples of clinical paratuberculosis suspected deer

The AMOVA results for the HS under study are presented in Table 6.3. This analysis indicated that around 40% of the total variance in subtypes occurred significantly ($p < 0.0001$) within a given livestock species on a given island. In addition to this, a small (9%) but significant ($p < 0.0001$) proportion of the variance was attributable to between island differences in subtypes obtained from a single livestock sector. However, the greatest source of variation in the dataset (51%, $p = 0.04$) was attributable

to differences between the livestock sectors irrespective of the island on which the animals were located.

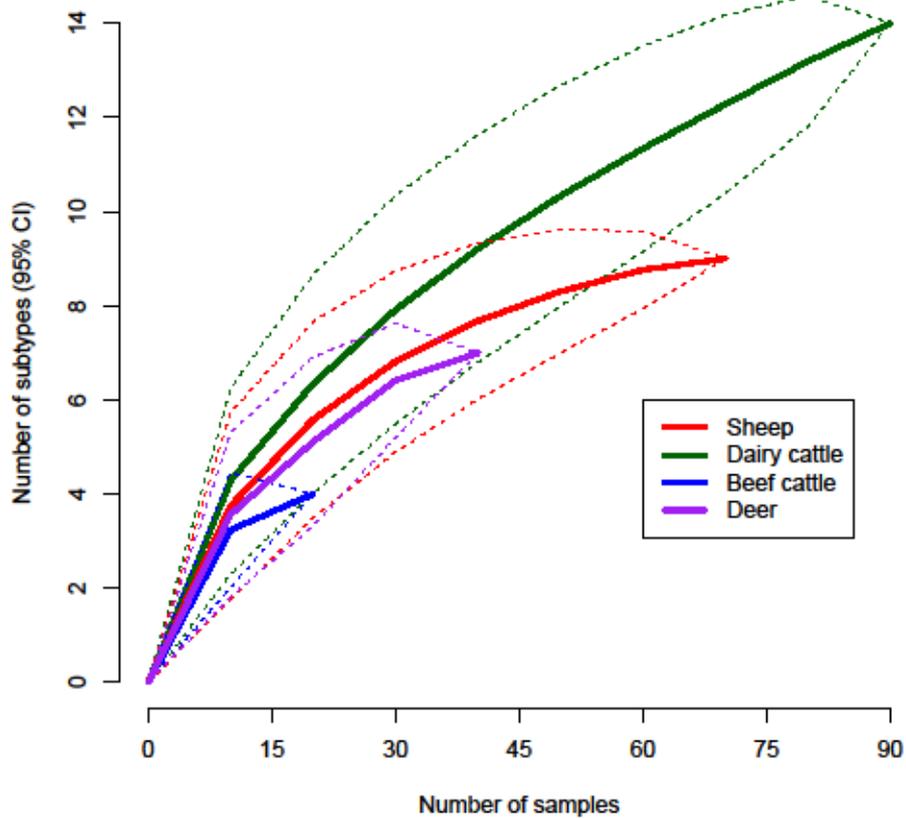


Figure 6.2: Rarefaction curves of MAP subtypes, isolated from four livestock sectors in New Zealand 2010-2011. Dotted lines represent 95% confidence intervals

Table 6.3: Analysis of Molecular Variance (AMOVA), describing results for the two hierarchical structures (HS) used to study the generic population variation associated with geography and livestock sector

Variance component	Observed partition			Φ -statistic
	Variance	% total	p-value	
Between the livestock sectors	0.87029	51.19	0.03812	$\Phi_{CT}=0.512$
Between islands but within a livestock sector	0.16028	9.43	<0.0001	$\Phi_{SC}=0.193$
Within a livestock sector within an island	0.66944	39.38	<0.0001	$\Phi_{ST}=0.606$

The F_{st} pairwise comparisons for HS1 and HS2 are presented in the Figure 6.3 and 6.4, respectively. All significant comparisons (p-values <0.05) had F_{st} -values greater than 0.25, indicating a substantial genetic differentiation between the two populations being compared (Wright, 1978). In the particular case of the comparison between isolates obtained from the NI versus SI from dairy herds, the F_{st} -value was 0.07, indicating that there is moderate genetic differentiation between the Islands and suggesting that common subtypes may be circulating between these two dairy cattle populations. Along this line, similar subtype richness was observed in the NI and SI in the dairy cattle sector, where the same numbers of isolates with the dominant subtype (*M*) were collected from both islands. The comparisons: i) between beef cattle in the NI vs. SI, ii) between beef cattle and sheep (independently of the island), and iii) between deer and dairy cattle in the NI, returned non-significant, low F_{st} -values, indicating common subtypes between these populations.

The similarity of subtypes between the different livestock sectors using the PSI analysis is presented in Table 6.4. The highest PSI value was observed for the subtype association between beef cattle and sheep (PSI=0.84), suggesting that these two species tended to have similar subtypes in relation to the overall diversity, in agreement with the results of the pairwise F_{st} analysis. The high value between these two livestock sectors appears to be related to the largest subtype (*C*) of which only 2/63 isolates were found in deer and 5/63 in dairy cattle. Low PSI ratings were observed between sheep and dairy cattle (PSI=0.21), beef and dairy cattle (PSI=0.20), and deer and sheep (PSI=0.22). These findings suggest that sheep and beef cattle tend to have similar subtypes, whereas both sheep and beef cattle are hosts of different subtypes from those found in dairy cattle. The subtype association between beef cattle and deer presented a low PSI value

in the overall data (PSI=0.29). However, in a data subset, considering isolates from farms where both deer and beef cattle were present (n=7), the PSI value increased to 0.74, providing strong evidence for a contact-dependent transmission between these two species (Table 6.4). Deer and dairy sectors presented a relatively low PSI value of 0.38. The subtypes present in deer and sheep were dissimilar, even when both species were present at farm level (PSI 0.32), indicating that these two species tend to be infected by different MAP subtypes.

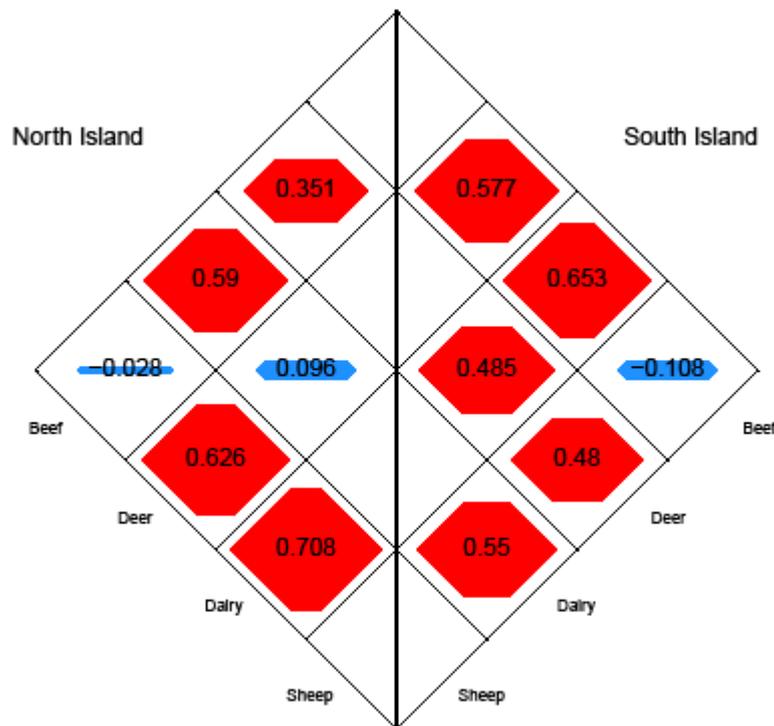


Figure 6.3: Pairwise F_{st} -values assessment for HS1. Comparison between populations located within New Zealand North Island (NI) and South Island (SI). F_{st} -values in red are significant at a p-value <0.05 . Sizes of the boxes are proportional to the F_{st} -values

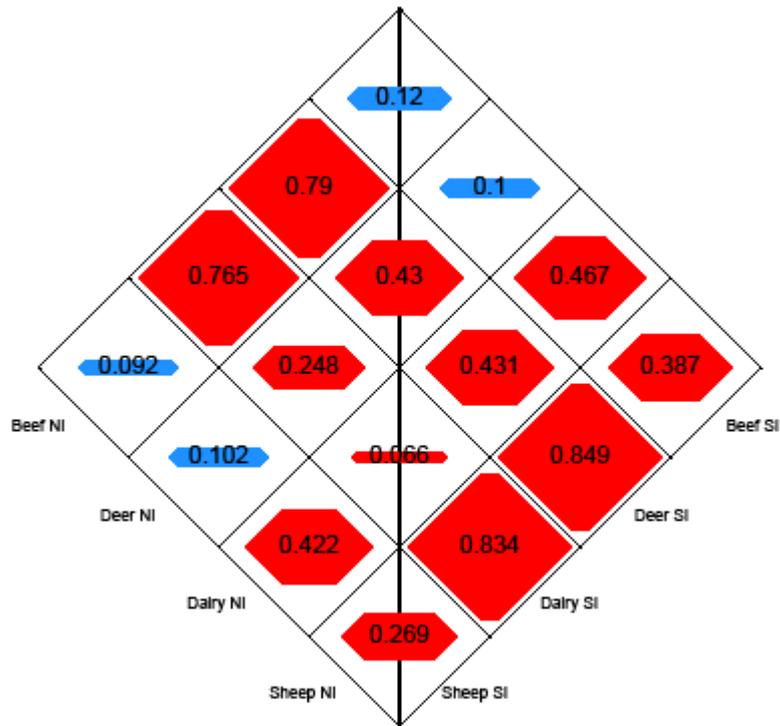


Figure 6.4: Pairwise F_{st} -values assessment for HS2. Comparison between populations located between New Zealand's North Island (NI) and South Island (SI). F_{st} -values in red are significant at a p-value <0.05 . Sizes of the boxes are proportional to the F_{st} -values

Table 6.4: Pairwise proportional similarity indices (PSI) and 95% CI comparison assessing the correlation of subtypes between species. The upper part of the table (over the diagonal line of ones) includes subtypes from all farms irrespective of direct contact, the lower part is reduced to farms where the two species were both present suggesting direct contact was likely to occur

	Beef cattle	Dairy cattle	Deer	Sheep
Beef cattle	1	0.20 (0.06 – 0.35)	0.29 (0.09 – 0.49)	0.84 (0.61 – 0.87)
Dairy cattle	No contact	1	0.38 (0.19 – 0.49)	0.21 (0.11 – 0.28)
Deer	0.74 (0.08 – 0.85)	No contact	1	0.22 (0.09 – 0.32)
Sheep	0.82 (0.59 – 0.86)	No contact	0.32 (0.06 – 0.52)	1

6.5 Discussion

This study is the first to describe the relationship between the 17 MAP subtypes observed, livestock sectors, and geographical location in New Zealand. Isolates of MAP were strain-typed using a combination of two molecular techniques. Samples involved the whole spectrum of ruminant production systems and MAP-susceptible livestock in New Zealand. Three dominant subtypes comprised 76% of isolates. There was a small difference in subtype distribution between North and South Islands. A key finding was that although there are strong differences in the MAP subtypes present between the livestock sectors, there is clear evidence of cross species MAP transmission. Sheep and beef cattle shared similar subtypes, in opposition to deer and dairy cattle that tended to present sector-specific subtypes, with the exception of deer and beef cattle when both were simultaneously present on farms.

The isolates used in this study are considered to be representative of MAP isolates across the country because they were sourced from 3 structured surveys of prevalent ruminant hosts (deer, sheep and cattle) which were conducted over a limited period of time and wide geographical area in New Zealand. Nevertheless, despite that samples from more than 11,000 animals were cultured, rarefaction analysis indicated that more extensive sampling could still yield additional subtypes not observed in the present study. Specifically, the curve for the dairy sector was still increasing steeply at 90 samples, which indicates that if more isolates were typed the subtype richness would still increase markedly. In contrast, the sheep curve is starting to plateau, indicating that increasing the sampling intensity for this livestock sector is less likely to reveal many more unique subtypes. Beef cattle and deer sector curves also appeared to plateau; however the smaller sample set available from these livestock sectors precluded a

conclusive assessment of the actual subtype richness. Nevertheless, the 17 subtypes identified were able to provide further insight into MAP epidemiology, describing associations not reported previously.

Molecular surveys of MAP elsewhere, also using MIRU-VNTR and SSR techniques in isolation or tandem, have revealed similar MAP subtype richness to that observed here with the New Zealand optimised set of MIRU-VNTR/SSR assays. Thibault et al. (2007) divided a collection of 183 MAP isolates from a variety of animal hosts from 10 countries into 21 subtypes using 8 MIRU-VNTR markers (3, 7, 10, 292, X3, 25, 47, 32), and Stevenson et al. (2009) classified 147 isolates from a wide array of animal species⁵ from 7 European countries into 23 subtypes. Additionally, Castellanos et al. (2010) divided 70 isolates from farmed animals and wildlife from Spain into 12 subtypes using 6 markers (MIRU-2, 3, VNTR-25, 32, 292, and 259). Moebius et al. (2008) were able to sub-divide 71 isolates from cattle herds in Germany into 15 subtypes, using as markers MIRU-1, 2, 3, 4 and VNTR-3, 7, 25, 32, 47, and 292. A combination of 10 markers (MIRU-1, 4, X3, 292, VNTR-25, 3, 7, 10 and 47) was used by van Hulzen et al. (2011) to analyze 52 dairy cattle isolates from the Netherlands, which were classified into 17 subtypes.

A sub-set of the 11 SSR markers proposed by Amonsin et al. (2004), have been used to strain-typing MAP isolates. Using four of these SSR markers (SSR-1, 2, 8 and 9), Harris et al. (2006) divided 211 isolates sourced from dairy cattle herds across United States into 61 different subtypes, and Pradhan et al. (2011) divided 142 samples, sourced from three dairy herds in the northeast United States into 15 subtypes. Two other studies have used a combination of the different types of reference markers (8

⁵ Badger, cow, crow, fox, fallow deer, goat, hare, jackdaw, moufflon, rabbit, red deer, rook, sheep, stoat, weasel, and wood mouse.

MIRU-VNTR and 11 SSR loci) for the analysis of MAP isolates; Thibault et al. (2008) identified 31 subtypes in 127 samples from different geographic (10 countries) and host sources and Douarre et al. (2011) recognized 22 different subtypes in a group of 38 MAP bovine isolates from Ireland.

Although the indexing of repeat copy numbers in the respective loci could facilitate the comparison of subtypes across regions, it is often too costly to be practical to assay all samples at all the possible loci, especially when some of the markers do little to distinguish isolates from a particular region. Unfortunately the lack of an international standardization of markers hinders the possibility of meaningful comparisons between studies (Castellanos et al., 2012).

The data set collected in this study was analyzed using different statistical techniques. AMOVA results indicated that the largest influence on subtype variation is the livestock sector from which an isolate was obtained, although there is evidence of a small (9%) but significant ($p < 0.0001$) component of the subtype variation that was explained by island differences. The pairwise F_{st} results were similar, with relatively low F_{st} values for island differences within a livestock sector when compared to results from between-sector analyses. Additionally, some subtypes were simultaneously isolated from different livestock sectors, regardless of whether there was direct contact between livestock species at farm level. These results suggest a circulation of subtypes across livestock sectors and between the two geographic areas assessed in this study. However, the three most frequently occurring subtypes were associated with specific livestock sectors on specific islands.

Results from several recent epidemiological studies indicate that cross-species transmission may play a more important role in MAP epidemiology than previously considered. While historically, cattle were infected mainly with Type II strains (Collins et al., 1990; Sevilla et al., 2005; Motiwala et al., 2006b), typing results from the present study indicate that Type I strains are frequent in New Zealand cattle, and in beef cattle are more common than Type II. This is strong evidence for cross-species transmission. The most common subtype (*C*) was mainly isolated from sheep and beef cattle located in the NI. This further represents strong evidence for cross-species transmission, as has been suggested in other parts of the world (Stevenson et al., 2009), consistent with the close grazing association between sheep and beef cattle on most of New Zealand's sheep and beef farms. Stevenson et al. (2009), in a typing survey of European countries, observed that sheep, previously thought to mainly harbour Type I strains, were infected with MAP Type II only. In Australia, where current knowledge indicates that sheep were exclusively infected with MAP Type I, transmission to beef cattle has been assessed in known infected sheep farms that also co-graze beef cattle (Moloney and Whittington, 2008). That study failed to directly demonstrate between species transmission, and considered it as a possible but a rare event. The opposite effect was observed in Iceland, where evidence suggest that MAP-infection was transmitted from sheep to the local cattle population and then back to the sheep population after a depopulation and restocking program (Palsson, 1962; Fridriksdottir et al., 2000). The reasons why different transmission patterns are observed, even in countries with relatively similar farming practices, such as Australia and New Zealand, remains unknown. It is therefore necessary to generate local information, considering the complexities of the disease at field level, where management practices, environmental

conditions or interaction with other diseases could also influence the progress of the infection.

Verdugo et al. (Verdugo et al., 2010) estimated the true herd level prevalence of MAP infection in different livestock sectors in New Zealand. That study reported the highest prevalence of sheep flocks (76%, 95% CI = 70 – 81%) whereas beef cattle herds presented with the lowest prevalence (42%, 95% CI = 35 – 50%). In another New Zealand report, beef cattle presented with the lowest animal level cPtb incidence among all livestock sectors infected with MAP (Verdugo et al., 2011). In the light of the subtype similarity observed between these two livestock sectors (PSI = 0.84), these findings suggest that sheep are the main source of MAP infection for beef cattle, with transmission occurring due to the close contact between these two species at farm level. However, sheep strains may be less virulent for beef cattle than they are for sheep, as was observed in an *in-vitro* study where bovine monocyte-derived macrophages infected with MAP type II presented more severe cytopathic effects than those infected with MAP type I (Gollnick et al., 2007). This could also explain the low cPtb incidence observed in beef cattle, which were mainly infected with MAP Type I. The previously suggested host specialization of MAP strains between cattle and sheep populations (Collins et al., 1990; Whittington et al., 2001; Sevilla et al., 2005) could reflect a lack of interaction between these species, rather than a true adaptation to specific host populations (Sevilla et al., 2005; Motiwala et al., 2006b).

The rarefaction analysis illustrated in Figure 6.2 and the subtype distribution shown in Table 6.2 indicates that New Zealand dairy cattle tend to present a greater richness of subtypes, when compared to the other studied livestock sectors. Fourteen of the 17

subtypes observed in NZ were isolated from dairy cattle. Two subtypes (*I* (16%) and *M* (57%)) accounted for 73% of the 80 dairy isolates, while five dairy subtypes were observed only once and were never isolated from any other livestock sector. The richness of subtypes, presence of dominant subtypes, and the reports of single observed subtypes have also been shown among dairy cattle herds in other countries. Van Hulzen et al. (2011) observed that from the 17 subtypes identified, 55.8% of the isolates were represented by a single subtype, and 12 subtypes were observed once in The Netherlands. Douarre et al. (2011) found that the most prevalent MIRU-VNTR-SSR subtype observed in dairy cattle herds in Ireland represented 21% of isolates of the 22 observed subtypes, while the second most common represented a 10% of isolates. Pradhan et al. (2011) reported that from the 15 subtypes identified in a sample of 142 isolates, 66.2% belonged to two dominant subtypes. Dairy cattle in the present study were also more likely to harbour multiple subtypes than the other livestock sectors. While multiple subtypes may be expected for samples from mixed species farms from pools of 10-20 animals, they occurred at a relatively low frequency (10/154 samples, 6%). Conversely, this was not expected for samples from dairy cattle which came from single animals, yet occurred at a higher frequency (30/211, 14%). It could not be ruled out however, that the exclusion of isolates with multiple products at the same VNTR locum, that only occurred in samples from S3 but not in dairy samples from S2, accounted for a bias in this comparison. Multiple subtype infection has been observed in other molecular typing analyses (Sevilla et al., 2007; Michel et al., 2008; Romero et al., 2008; Castellanos et al., 2010).

The trend for subtype richness in dairy cattle might reflect management practices of this sector, where extensive relocation of entire dairy herds from the NI to the SI has been

observed in New Zealand over the last 20 years⁶. Thus selling or purchasing of animals is common and generates opportunities for strain sharing, competition and evolution. Dairy cattle herds tend to be managed differently from the other ruminant sectors, in which animals are commonly co-grazed on multispecies farms in New Zealand. This relative isolation could explain the more dairy-specific subtypes and lower PSI values reported in this sector. The higher apparent diversity of dairy cattle types may also reflect that the dairy MAP epidemic in New Zealand is more mature than the sheep and deer epidemics and there has been a longer time for possible strain evolution. For example, cPtb was first reported in New Zealand dairy cattle in 1912 (Stephens and Gill, 1937) but it was reported for the first time in New Zealand sheep 40 years later (Williamson and Salisbury, 1952) and in farmed deer 67 years later (Gumbrell, 1986). The deer sector was almost exclusively infected by MAP Type II strains, mainly subtype *O*. This subtype appears to be well adapted to farming conditions in the SI and to be more virulent for deer, compared with other strains. Glossop et al. (2007) reported a significantly higher cPtb incidence among deer farms located in the SI than NI. The paucity in incidences of infection of deer with Type I strains could result from lower virulence of Type I than Type II strains for deer (Mackintosh et al., 2007). If this hypothesis is confirmed, it would suggest that grazing deer with sheep may reduce the infectious burden of MAP on pasture for deer without increasing the risk of cPtb in sheep due to the reduction in clinical cases, which represent the disease stage where the greater amount of MAP colonies are shed into the environment. Additionally, available MAP on pasture will be diluted between two species where one seems not susceptible.

⁶ Ministry of primary industries (MPI), 2012 <http://www.stats.govt.nz/infoshare/Default.aspx>

The F_{st} pairwise comparison provided further insight into the molecular epidemiology of MAP in New Zealand. To our knowledge, this type of analysis has not been previously reported for MAP isolates. In general, a high F_{st} -value implies a considerable degree of genetic differentiation between two populations under comparison. The negative F_{st} -values observed in some associations should be interpreted as a lack in genetic differentiation between the two populations (Foster et al., 2006). This was the case in the subtype comparison between dairy cattle in the NI versus SI and between sheep versus beef cattle (regardless of island), reinforcing the observations from the other analyses presented in this research. An important genetic differentiation was found between MAP subtypes from the beef and dairy cattle populations, where Type I was predominant in beef cattle and Type II in dairy cattle, stressing the likely importance of close contact for effective transmission of MAP under New Zealand farming conditions. A similar situation might exist if dairy cattle and deer were co-grazed. In the NI these two livestock sectors presented a low F_{st} -value. In the absence of close direct contact, transmission might occur when dairy calves are moved to multiple-species farms to be raised for meat production or heifers graze off farms before the first service, as this could be the case in the NI. Thus, animal movements across livestock sectors and geographical areas is a subject that warrants further study in order to better understand MAP transmission.

Finally, the absence of a plateau in the rarefaction curves may be an indication of a limited sample size; thus a potential bias in the results cannot be ruled out. Nevertheless, we are confident in the results because of the large geographical area covered by the surveys, and the diverse array of susceptible livestock sectors that were sampled.

6.6 Conclusion

This research has described MAP subtype richness and provided evidence of cross-species transmission between host species that are in close contact on New Zealand farms. This was demonstrated by isolations of the same subtypes from sheep and beef cattle, which are commonly farmed together in New Zealand. Dairy cattle subtypes were similar in herds on the NI and SI but these subtypes largely differed from those found in other livestock sectors. Deer mainly harboured a specific subtype not frequently isolated from other farm sectors, but deer harboured similar subtypes to beef cattle when both were simultaneously present at farm level whereas co-grazed deer and sheep maintained their unique subtype distribution.

6.7 Acknowledgments

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A mathematical model of *Mycobacterium avium* subsp. *paratuberculosis* transmission in a pasture based sheep-beef farm in New Zealand

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7.1 Abstract

Paratuberculosis (Ptb) is a chronic enteric infection caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), affecting wild and domestic ruminants. In the New Zealand pastoral farming system, it is common practice to co-graze Ptb susceptible livestock species (sheep, beef cattle, deer) together, either concurrently or successively on the same pasture. Recent molecular strain typing data revealed that sheep and beef cattle often share the same MAP strain, indicating that MAP is transmitted across-species through pasture. Due to the chronic nature of Ptb and the poor accuracy of diagnostic tests, the costs of longitudinal field studies to investigate infection dynamics or intervention strategies are extremely high. Mathematical modelling is a low-cost alternative to such high-input field studies. The aim of this study therefore was to develop a two-host (sheep and beef cattle) model of infection dynamics under different co-grazing (CG) regimes. Secondly, effects of various disease control measures on infection prevalence and clinical incidence were evaluated. Available survey data about infection prevalence and clinical incidence in sheep flocks and beef cattle herds were used to calibrate the model. Three control scenarios were evaluated: i) direct grazing

contact between an infected and a naïve species, ii) grazing of a naïve species on MAP-contaminated pasture after spelling pasture from grazing for several months, iii) test & cull (T&C) of animals, alone or in combination with increased 'farmer surveillance' for early identification and removal of pre-clinical animals (high shedders).

Naïve beef cattle (sheep) became infected when they were co-grazed with infected sheep (beef cattle) around seasonal lambing/calving (July-September). When sheep and beef cattle were both infected, such seasonal CG increased the infection prevalence from 20% to 26% in sheep, and from 13% to 21% in beef cattle compared to grazing the two species in isolation. Extending the CG period increased the prevalence at equilibrium for both species. Extending pasture spelling periods from 9 to 15 months reduced the pasture contamination with MAP by up to 99%. However, infection of naïve animals was still possible after such long spelling times, although the resulting infection prevalence remained <1% for at least 25 years. The simultaneous application of control measures on both species was the most efficient approach to reduce the prevalence and incidence. Test & cull starting at equilibrium decreased infection prevalence of beef cattle 9-fold after 25 years, but it took 6 years in beef cattle and 14 years in sheep to reduce prevalence by one half. When T&C of beef cattle was combined with increased 'farmer surveillance', the extra reduction in prevalence was relatively marginal. Conversely, applying the combined intervention in sheep had a much larger effect than T&C alone. Species isolation in combination with an increased farmer surveillance, decreased the prevalence rapidly in sheep but slowly in beef. However, this combination was the most effective intervention in both species.

7.2 Introduction

Paratuberculosis (Ptb), caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is a chronic granulomatous enteric infection that occurs worldwide and affects wild and domestic ruminant species such as deer, sheep and cattle (Harris and Barletta, 2001; Whittington and Sergeant, 2001; de Lisle, 2005). Clinical Ptb causes weight loss and diarrhoea, does not respond to treatment, and leads to emaciation and eventually death or premature culling. The majority of infected animals remain latent or sub-clinical without ever developing evident signs of the disease or production decline (Sweeney, 2011). Despite the worldwide distribution of MAP and Ptb, relatively little is known about infection sources or transmission pathways (within and between species), especially about transmission between domestic livestock species and wild animals. The lack of information is mainly due to the poor sensitivity of available diagnostic tests to determine infection, notably serum antibody ELISA and faecal culture (Nielsen and Toft, 2008). Infection mainly occurs during early life, although clinical Ptb is usually observed in adult sheep and cattle (2-3 years). Therefore MAP is potentially carried for several years before the onset of shedding and/or clinical signs (Sweeney, 2011).

In New Zealand, sheep, deer and beef cattle (hereafter referred to as ‘beef’) are most commonly farmed jointly in pastoral systems. These pastoral farms are characterized by all year grazing and strictly seasonal calving/lambing. Beef, sheep and deer are often grazed either concurrently (same paddock, same time) or successively (same paddock, different time), and either set-stocked or rotated through pasture blocks allowing for pasture spelling and regrowth of grass. Contact through grazing the same MAP-contaminated pasture could result in transmission of MAP across species. Transmission through pasture is facilitated by the survival of MAP in the environment for several

months (Whittington et al., 2004; Rowe and Grant, 2006). Survey data from New Zealand showed that sheep co-grazed with beef have a greater risk of infection with MAP than any one of them on single-species farms (Chapter 3). In addition, the finding of similar MAP strains in sheep and beef from the same farms versus different strains on different farms is strong evidence that transmission across species occurs (Chapter 6). Moreover, MAP transmission between sheep and beef has been reported elsewhere (Palsson, 1962; Fridriksdottir et al., 2000; Muskens et al., 2001). The contrary was observed on pastoral farms in Australia, although authors concluded that the risk of transmission is low due to previous evidence of cross-species transmission in Australia (Moloney and Whittington, 2008).

It is therefore of interest to understand the consequences of MAP transmission between species on disease dynamics, production output and disease control. Since field studies targeting such interrelationships require expensive, long term investigations, mathematical modelling has emerged as an attractive, low-cost alternative. Modelling has been used to analyze the spread and control of economically important livestock diseases such as foot-and-mouth disease and classical swine fever (Bennett, 1992; Dijkhuizen and Morris, 1997; Jalvingh et al., 1999; Bates et al., 2003; Carpenter et al., 2004b). Mathematical models for Ptb in dairy cattle were reviewed recently (Marce et al., 2010; Nielsen et al., 2011). Several models also exist for Ptb in sheep and beef (Juste and Casal, 1993; Sergeant and Whittington, 2000; Humphry et al., 2006; Bennett et al., 2012; Marquetoux et al., 2012) and one for deer (Heuer et al., 2012). However, Ptb disease dynamics in a two-host setting of mixed-species farms have neither been studied nor modelled. The objective of this study was therefore to develop a sheep&beef model (SNBm) for pastoral farming, simulating MAP infection and disease dynamics

within and between species. The SNBm was then used to assess the effect of control measures on transmission dynamics under typical New Zealand farming conditions.

7.3 Material and Methods

7.3.1 Model development

Two species-specific deterministic state transition models were developed and merged into a single system. In the SNBm, MAP is shed on pasture, where it has limited survival time. Indirect transmission occurs through the consumption of MAP-contaminated pasture. The model considers two age-structured, closed populations, and two alternative (concurrent or successive) co-grazing CG schemes. It assumes that MAP on pasture was equally accessible by all animals. In addition to the ingestion of contaminated pasture, MAP is transmitted vertically to the foetus in-utero, or pseudo-vertically through the MAP-contaminated udder by faeces of infected dams. The general model structure is described in Figure 7.1.

The beef component was adapted from the model developed by Mitchell et al. (2008) for MAP transmission in dairy cattle. The adapted version considered the typical age structure and grazing management of beef in New Zealand, and included two possible progression tracks (transient shedding or latent) for newly infected animals. Beef were divided into four age categories: calves (0-6 months, subscript 1), weaners (6-12 months, subscript 2), heifers (12-24 months, subscript 3), and adult cows (>24 months, subscript 4). Even though rare occurrences of infection in adult cows has been observed (Mitchell et al., 2012), it was assumed that animals were fully resistant to infection after 12 months of age (Windsor and Whittington, 2010). Therefore, there were two

susceptible bovine states (bS_1 & bS_2) and two resistant bovine states (bR_3 & bR_4) (Figure 7.1). Susceptible calves (bS_1) could become infected at probability λ_{b1} , and

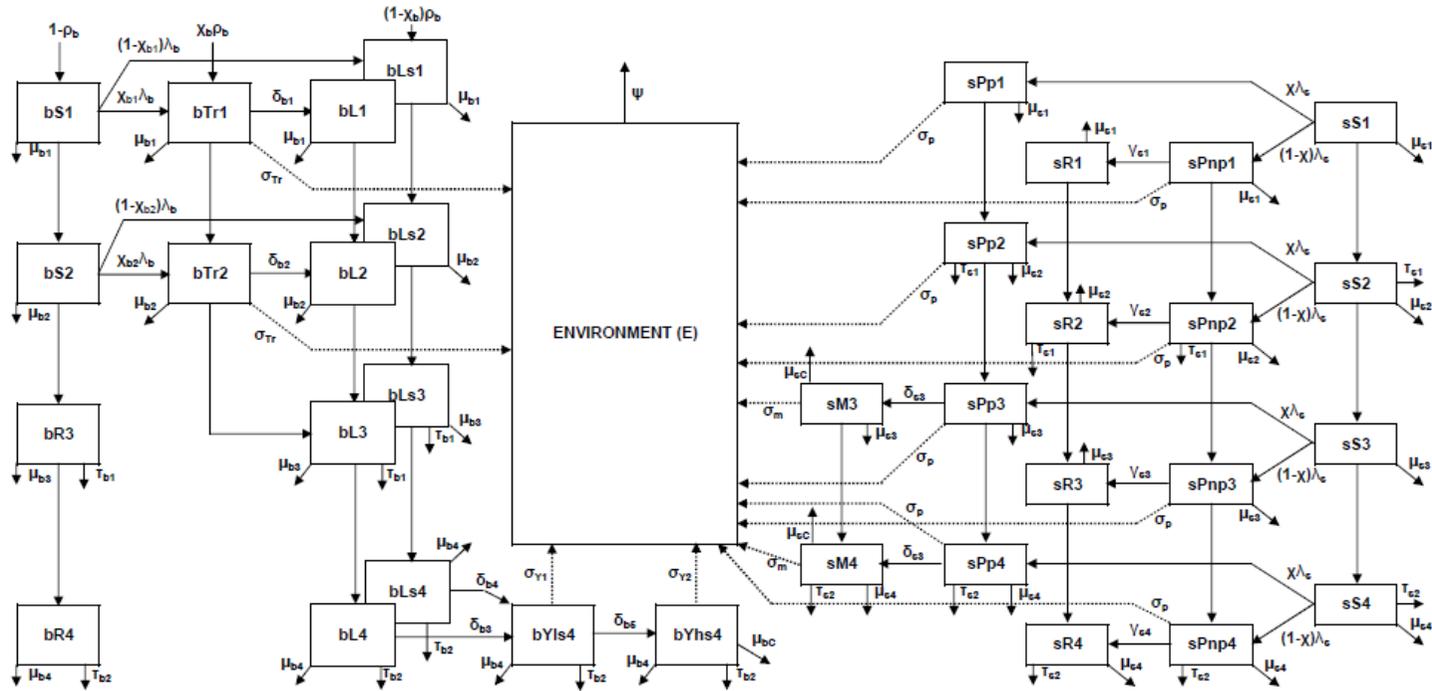


Figure 7.1: Model framework describing the compartments present in the beef components (left), environment (middle) and sheep component (right). Beef population is divided in four age categories: 1=Calves (0-6 months), 2=Weaners (6-12 months), 3=Heifers (12-24 months) and 4= Cows (>24 months). Calves born from infected dams could be born infected with a probability ρ_b or susceptible beef (bS1 & bS2) could enter to a transient shedding stage (bTr1 & bTr2) at a $\chi_{b1-2}\lambda_b$ probability, or enter directly to a slow progressing latent stage (bLs1 & bLs2) at a $(1-\chi_{b1-2})\lambda_b$ probability. Transient animals will move to a latent stage (bL1 & bL2) at a rate δ_{b1-2} . Infected animals will remain latent until adulthood when they become infectious, first at a low shedding compartment (bYls4) at rates δ_{b3-4} . Then they will become high shedder (bYhs4) at a rate δ_{b5} . It is assumed that beef older than 12 months is fully resistant to infection (bR3 & bR4). Sheep population was divided in four age categories: 1=Lambs (0-3 months), 2= hoggets (3-12 months), 3=Growers (12-24 months) and 4=Ewes (>24 months). All age categories are equally susceptible to infection. Susceptible sheep (sS1-4) could enter to a progressor subclinical shedding stage (sPp1-4) with a probability $\chi\lambda_s$, and then progress to clinical stage (sM3-4) at a rate δ_{s3-4} . Or susceptible animals could enter to a non-progressor subclinical shedding stage (sPnp1-4) with a probability $(1-\chi)\lambda_s$, and then recover from infection (sR1-4) at a rate γ_{s1-4} . The environment component account Map dynamic at pasture level, the bacteria is shed by infectious animals at rates σ_{Tr} , σ_{Y1} , σ_{Y2} , σ_P and σ_M . Map decay naturally at a rate ψ

susceptible weaners at a probability λ_{b2} (Windsor and Whittington, 2010; Mitchell et al., 2012). Additionally, calves born from an infected dam were at risk of vertical/pseudo-vertical transmission with probability ρ_b . A newly infected animal could become a transient shedder (bTr_1 & bTr_2) with probability χ_b , or enter directly to a latent and slow-progression compartment (bLs_1 & bLs_2), with probability $1-\chi_b$ (McDonald et al., 1999; Stewart et al., 2007; Mitchell et al., 2012). Transient animals stopped shedding and moved to a latent stage (bL_1 & bL_2) at rates δ_{b1} and δ_{b2} . Finally, latent (bL_4) or latent-slow progressing cows (bLs_4) started to shed during adulthood (>24 months) at rates δ_{b3} and δ_{b4} respectively, where $\delta_{b3} > \delta_{b4}$. Infectious (Y) cows were first in a low shedding (ls) compartment ($bYls_4$) and then progressed to a high shedding (hs) state ($bYhs_4$) at rate δ_{b5} . Animals in age categories 1-4 were dying of natural causes with probability μ_{b1-4} and high shedding animals additionally died of clinical disease at rate μ_{bc} . Adult animals were culled at rate τ_b for normal management purposes.

The sheep component is based on the work by Marquetoux et al. (2012). This model simulates the introduction and maintenance of MAP in a pastoral sheep flock under seasonal farming conditions in New Zealand. To make it comparable to the beef component, the seasonal pattern in the sheep flock demographics was omitted and age-specific characteristics of MAP infection dynamics were retained. Four age categories were considered: lambs (0-3 months, subscript 1), hoggets (3-12 months, subscript 2), two-tooth (12-24 months, subscript 3), and mixed age ewes (>24 months, subscript 4). All age categories were equally susceptible to infection, but rates of subsequent progression to disease versus recovery varied with the age at infection (Reddacliff et al., 2004; Dennis et al., 2011; Delgado et al., 2012; McGregor et al., 2012).

Susceptible sheep became infected with probability λ_{s1-4} representing the faecal-oral route. Vertical transmission in sheep was considered negligible (Lambeth et al., 2004), thus was not incorporated in the model. Additionally, available data suggested a quick onset of early, transient faecal shedding following infection in most or all sheep (Kawaji et al., 2011), thus the model did not incorporate a latent stage. A newly infected animal could enter either a progressor track (with probability χ_s) resulting ultimately in clinical disease and death, or a non-progressor track (with probability $1 - \chi_s$) leading to a stable recovery state (Gilmour et al., 1978; Stewart et al., 2004; Dennis et al., 2011; Kawaji et al., 2011). The progressor track started with a sub-clinical shedding compartment (sPp_{1-4}), where infected animals shed low amounts of bacteria (paucibacillary form). This stage was characterized by lymphocytic infiltration of the intestinal mucosa with the presence of few or no acid fast bacteria (Perez et al., 1999), with little or no impact on physiology. Infected animals >12 month further progressed to a pre-/clinical, high-shedding (multibacillary) stage (sM_{3-4}) at rate δ_{s3-4} . The multibacillary stage is characterized by the presence of massive amounts of acid fast bacteria, macrophages and a granulomatous reaction in the intestinal mucosa, and is highly associated with signs of clinical Ptb (Clarke and Little, 1996; Kurade et al., 2004; Dennis et al., 2011). In the non-progressor track, sheep first entered a sub-clinical, low shedding compartment ($sPnp_{1-4}$) equivalent to the sPp_{1-4} compartment in the progressor track. Further progression led to a stable, non shedding state of “recovery” (sR_{1-4}) at rate γ_{s1-4} . Sheep in the recovery state could be either latently infected or truly recovered from infection. In any case these animals would never become clinically affected and were not susceptible to new infection with MAP. The model assumed an inverse relationship between age at infection and the probability of entering the progressor track rather than the non progressor track (McGregor et al., 2012). Similar to beef, the age specific rates

of death from natural causes were μ_{s1-4} . Multibacillary animals were affected by their infection with MAP and subsequently died from clinical disease at rate μ_{sc} . Adult animals were culled at rate τ_s .

The environmental component (Env) holds MAP on pasture shed by infectious livestock for the time of survival, exposing susceptible sheep or beef to infection. Env was subdivided between eight to ten paddocks, depending on whether beef and sheep were managed in isolation or by CG (Figure 7.2). Animals were allocated to a paddock depending on species and age class. Infectious beef were shedding MAP at rates σ_{Tr} , σ_{Y1} , and σ_{Y2} , during stages bTr_{1-2} , $bY1s_4$, and $bYhs_4$ respectively. Similarly, infectious sheep were shedding MAP into the environment at rates σ_P or σ_M , depending on their state of progression (paucibacillary or multibacillary). Shedding rates in both species were adjusted by the amount of faeces (kg/months) that each age category produced. MAP bacteria in the environment decayed at rate ψ . The contamination burden of each paddock depended on the number of animals in each infectious stage, the length of the grazing period (in months), and MAP survival. For each species separately, animals were allocated to two groups, each group including two age categories (weaners+heifers; cows+calves; ewes+lamb; hoggets+two-tooths). Two paddocks per group were grazed in a rotational fashion for periods of two months (Figure 7.2). In this manner, beef weaners and heifers were allocated to paddocks E1 and E5, cows and calves (until weaning) to paddocks E2 and E6, ewes and lambs (until weaning) to paddocks E3 and E7, and hoggets and two-tooth to paddocks E4 and E8. To simulate cross species transmission, CG (either concurrently or successively) was allowed between ewes/lamb and cows/calves, by merging paddocks E2 and E3 into E9 and paddocks E6 and E7 into E10, where E9 and E10 were rotationally grazed, using the

same time intervals as for single species grazing during the CG period (Figure 7.2). In these extra paddocks, calves and lambs were seasonally present since calving/lambing until weaning. The CG of dam-offspring pairs of beef and sheep from pre-calving/lambing until weaning is a common practice in New Zealand sheep&beef farms. A detailed description of the model differential equations is presented in the Annex E.

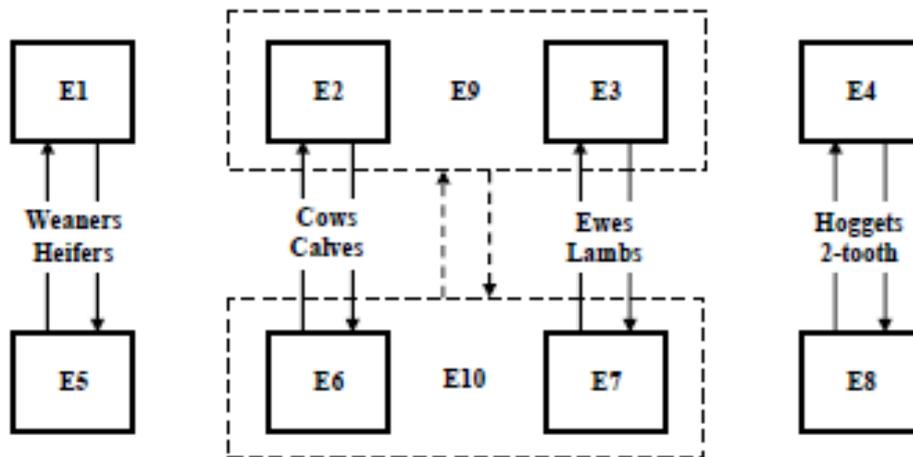


Figure 7.2: Distribution of age groups in pairs of paddock grazed in a rotational fashion. Dashed lines indicate paddocks that were merged during co-grazing

7.3.2 Force of infection equations

In beef the force of infection (λ_{b1-2}) was modelled as:

$$\lambda_{b1-2} = 1 - \exp(-\beta_b * DA_{b1-2})$$

where β_b was the transmission parameter, and DA_{b1-2} represented the number of ‘infectious doses’ available to susceptible calves (1) and weaners (2) from the environment due to the presence of shedding animals in the same paddock (Annex E). Hence, λ_{b1} estimated the infection probability in susceptible calves and λ_{b2} the infection probability in susceptible weaners. For susceptible weaners, β_b was adjusted by a susceptibility (Sus_b) factor (Annex E), to account for the lower susceptibility to infection in older animals (Windsor and Whittington, 2010; Marce et al., 2011). When

cross-species transmission was simulated, MAP shed by CG ewes and lambs was added directly to DA_{b1} , thus the total dose of MAP present on co-grazed pasture contributed to the force of infection for susceptible calves, irrespectively of the species source of MAP. The parameter ρ_b comprises vertical and pseudo-vertical transmissions, while β_b represented horizontal transmission due to the consumption of contaminated pasture.

Unlike the beef model, the sheep component presented two different force of infection equations (λ_{s1} & λ_{s2-4}), to estimate MAP transmission in age categories 1 and 2 to 4, respectively. In lambs (age 1), the force of infection was divided into two components to account for both pseudo-vertical and horizontal (within and between species) transmission of MAP. The probability of infection λ_{s1} was thus modelled as:

$$\lambda_{s1} = (1 - \exp(-\beta_{s1} * sBL)) + (1 - \exp(-\beta_{s2} * bBL * I_3))$$

where, β_{s1} and β_{s2} were the transmission parameters, sBL was the bacterial load (infectious dose) shed by ewes and lambs, bBL was the bacterial load shed by cows and calves, and I_3 was a CG indicator (equal to 1 during CG periods, 0 otherwise). The first part of the equation represented the challenge occurring for new born susceptible lambs due to pseudo-vertical transmission of MAP via close contact with infectious dams, particularly while suckling, in addition to horizontal transmission due to contaminated pasture shed by fellow infectious lambs and ewes. The transmission risk of lambs from contaminated pasture could not be neglected since lambs can graze substantial amounts of grass very early after birth, and thus would not only be exposed to the faeces of their dams via close contact but also to pasture. The corresponding β_{s1} was calibrated based on an experimental infection study in lambs kept on contaminated pasture together with infected and non-infected ewes, where it was observed that the rate of infection between lambing and weaning was higher in lambs born from infected dams than in lambs born

from uninfected dams (Reddacliff et al., 2004). The second part of the equation represented the contribution to the force of infection from MAP shed by infectious beef present in the paddock during CG only. Thus β_{s2} represented the transmission parameter for indirect horizontal transmission, and it was also used in all other age groups beside lambs. Specifically, after weaning the force of infection λ_{s2-4} represented horizontal transmission via contaminated pasture, for ages 2 to 4 and was calculated according to the following:

$$\lambda_{s2-4} = 1 - \exp(-\beta_{s2} * DA_{s2-4})$$

where DA_{s2-4} was the number of ‘infectious doses’ available on pasture to susceptible (2) hoggets, (3) two-tooth, and (4) ewes due to the presence of infectious animals (Annex E). When CG was allowed, DA_{s4} encompassed also the shedding contribution from infectious beef present on the paddock.

Model parameters were obtained from published studies. Parameter values and references are presented in Table 7.1. The SNBm was implemented in the software Berkeley Madonna⁷, using a fourth order Runge-Kutta algorithm (RK4) to compute the ordinary differential equations described in Annex E. The continuous time model was run with monthly time steps for outputs. For each time step, the number of animals in each specific state as well as the number of MAP ‘infectious doses’ present in the environment were calculated (Figures 7.1 & 7.2). A total herd size of 100 beef and flock size of 1,000 sheep was assumed, holding stocking density constant. For each species, the model was run without infection until the distributions of animals in each age category were at a demographic equilibrium, representing the initial population structure. Two infected heifers and four infected two-tooth were then introduced to

⁷ <http://www.berkeleymadonna.com>

initiate infection. Available data on prevalence and annual incidence of clinical Ptb were used as a reference for model calibration.

7.3.3 Model calibration

In New Zealand, a recent national survey estimated a MAP herd level true infection prevalence of 72% (95% posterior probability interval (PPI): 61 – 83%) in sheep and of 36% (95% PPI: 19 – 58%) in beef, when those flocks/herds were farmed in isolation (single species farming). These herd level prevalences increased to 78% (95% PPI, 67 – 87%) and 44% (95% PPI, 33 – 56%) when sheep and beef, respectively, were both present on the same farm (Chapter 3). From that study, animal level true prevalence of MAP infection was estimated at 20% in sheep (95% PPI, 6 – 53%) and 13% in beef (95% PPI, 2 – 46%), across farm types. Annual clinical incidence (ACI) of 0.16% (95% confidence interval (CI), 0.09 - 0.24%) in sheep and 0.04% (95% CI, 0.01 - 0.08%) in beef were estimated from the recall of farm managers in a survey of sheep and beef farms (Chapter 5).

In the present study, it was assumed that sheep or beef were infected with MAP Type I strain, which is the predominant MAP type infecting both sheep and beef flocks/herds in New Zealand (Chapter 6). Individual species models were fitted in the absence of CG, targeting equilibrium prevalences of 20% and 13%, and clinical incidences of 1% and 0.5% for sheep and beef respectively. The transmission parameter β_b , corresponding to horizontal transmission of MAP to susceptible beef via pasture, was calibrated through iterative model runs to meet the observed prevalence of 13%. The progression parameter (δ_{b5}), which regulates the progression from bYls₄ to bYhs₄, was adjusted to meet the clinical Ptb incidence of 0.5%. In sheep, the transmission parameter β_{s2} was calibrated to the value that resulted in the observed survey prevalence of 20% of MAP

infection while the incidence was left to depend on published parameters for progression to clinical disease (Table 7.1).

Table 7.1: Definition of variables and parameters used in the model

Symbol	Definition	Comp.	Value	Reference
bS_{1-2}	Susceptible animals: 1=calves; 2=weaners	Beef	NA	NA
bR_{3-4}	Resistant animals: 3= heifers; 4=cows	Beef	NA	NA
bTr_{1-2}	Transient shedding animals	Beef	NA	NA
bL_{1-4}	Latent animals	Beef	NA	NA
bLs_{1-4}	Latent animals (slow progressing track)	Beef	NA	NA
$bYls_4$	Infectious low shedding cows	Beef	NA	NA
$bYhs_4$	Infectious high shedding cows	Beef	NA	NA
sS_{1-4}	Susceptible animals: 1=lambs; 2=hoggets; 3=growers; 4=ewes	Sheep	NA	NA
$sPnp_{1-4}$	Paucibacillary non-progressor infectious animals	Sheep	NA	NA
sPp_{1-4}	Paucibacillary progressor infectious animals	Sheep	NA	NA
sR_{1-4}	Recovered animals	Sheep	NA	NA
sM_{3-4}	Multibacillary infectious animals	Sheep	NA	NA
E_{1-8}	Paddock Map contamination depending species and age category grazing: E1/E5 = weaners + heifers, E2/E6= Cows + calves, E3/E7= Ewes + lambs, E4/E8 = hoggets + growers	Env	NA	NA
σ_{bTr1}	Transient calves	Beef	$4.50 \cdot 10^6$	(Marce et al., 2011)
σ_{bTr2}	Transient weaners	Beef	$4.95 \cdot 10^7$	
σ_{bYls4}	Low shedding cows	Beef	$2.34 \cdot 10^9$	
σ_{bYhs4}	High shedding cows	Beef	$9.00 \cdot 10^{13}$	
	Values have been adjusted by faeces production per age category			
W_b	Beef weaning time	Beef	6 months	
ω_b	Beef herd replacement rate	Beef	TBC	
ζ_b	Vertical & pseudo-vertical transmission for low shedding infectious animals (ζ_{b1}) and high shedding infectious animals (ζ_{b2})	Beef Beef	$\gamma_{b1} = 0.15$ $\gamma_{b2} = 0.45$	(Benedictus et al., 2008; Whittington and Windsor, 2009)
μ_{b1-4}	Expected normal average mortality rate for age categories 1 to 4.	Beef	$\mu_{b1} = 0.0125$ $\mu_{b2} = 0.00058$ $\mu_{b3} = 0.00058$ $\mu_{b4} = 0.0185833$	(Marce et al., 2011)
μ_{bC}	Expected Ptb clinical mortality	Beef	0.17	(Marce et al., 2011)

Symbol	Definition	Comp.	Value	Reference
ρ_{b1-3}	Rate of exit due to aging	Beef	$\rho_{b1} = 1/6$ $\rho_{b2} = 1/6$ $\rho_{b3} = 1/12$	NA
τ_b	Expected normal culling rate	Beef	0.1	NA
χ_b	Probability of an infected animal to enter to a transient shedding state	Beef	0.10	(McDonald et al., 1999; Stewart et al., 2007)
δ_{b1-5}	Compartment exit rate: 1=exit rate from bTr_1 , 2=exit rate from bTr_2 , 3=exit rate from bL_4 , 4=exit rate from bLs_4 , 5=exit rate $bYls_4$	Beef	$\delta_{b1} = 1/6$ $\delta_{b2} = 1/6$ $\delta_{b3} = 1/13$ $\delta_{b4} = 1/19$ $\delta_{b5} = 1/90$	(Marce et al., 2011; Mitchell et al., 2012)
v_{b1-3}	Probability to identify an infected cow: 1=latent (bL_4, bLs_4); 2=infectious low shedding ($bYls_4$); 3=infectious high shedding ($bYhs_4$)	Beef	TBC	Calculated NA
α_b	Extra culling rate due improved farmer surveillance	Beef	0.043	
θ_b	False positive probability when a test & cull program is implemented	Beef	TBC	NA
λ_{b1-2}	Force of infection equation	Beef	TBC	NA
β_b	Indirect transmission rate due bacterial intake	Beef	$7.909 * 10^{-16}$	
Sus_b	Infection susceptibility proportion of weaners compared to calves	Beef	0.09	(Marce et al., 2011)
σ_{sP}	Monthly shedding rate of an infectious ewe: P=paucibacillary,	Sheep	$\sigma_{sP} = 1.5 * 10^8$	(Whittington et al., 2000b)
σ_{sM}	M=multibacillary		$\sigma_{sM} = 3.0 * 10^{12}$	
η_{1-3}	Shedding adjustment factor for differences in the quantity of faeces produced by ages 1 to 3	Sheep	$\eta_1 = 0.2$ $\eta_2 = 0.6$ $\eta_3 = 0.8$	
W_s	Sheep weaning time	Sheep	3 months	NA
ω_s	Sheep flock replacement rate	Sheep	TBC	NA
μ_{s1-4}	Expected average mortality rate (normal)	Sheep	$\mu_{s1} = 0.026$ $\mu_{s2} = 0.0064$ $\mu_{s3} = 0.0064$ $\mu_{s4} = 0.00878$	
μ_{sC}	Expected Ptb clinical mortality	Sheep	0.18	(Dennis et al., 2011)
ρ_{s1-3}	Rate of exit due to aging	Sheep	$\rho_{s1} = 1/3$ $\rho_{s2} = 1/9$ $\rho_{s3} = 1/12$	NA
λ_{s1-4}	Force of infection equations	Sheep	TBC	NA
β_{s1}	Horizontal and pseudo-vertical transmission rate to lambs due contact with infectious ewes	Sheep	$3.074 * 10^{-15}$	NA
β_{s2}	Horizontal transmission rate due to MAP contaminated pasture	Sheep	$3.373 * 10^{-16}$	NA
τ_s	Expected normal culling rate	Sheep	0.1	NA

Symbol	Definition	Comp.	Value	Reference
χ_{s1-4}	Probability of an infected animal to enter to a paucibacillary progressor state	Sheep	$\chi_{s1} = 0.5$ $\chi_{s2} = 0.35$ $\chi_{s3} = 0.20$ $\chi_{s4} = 0.05$	(Delgado et al., 2012; McGregor et al., 2012)
δ_{s1-4}	Compartment exit rate from a sPp_{3-4} to sM_{3-4} states respectively	Sheep	1/15	(McGregor et al., 2012)
v_{s1-3}	Probability to identify an infected ewe: 1= $sPnp_4$; 2= sPp_4 ; 3= sM_4	Sheep	TBC	
A_s	Extra culling rate due improved farmer surveillance	Sheep	0.045	
Θ_s	False positive probability when a test & cull program is implemented	Sheep	TBC	
γ_s	Compartment exit rate from a $sPnp_{1-4}$ to sR_{1-4} states	Sheep	0.16	(Stewart et al., 2004)
$Se_{ELISA-s1-3}$	Sensitivity ELISA by infection state: $sPnp_{1-4}$; sPp_{1-4} sM_{1-4}	Sheep	$Se_{ELISA-s1} = 0.32$ $Se_{ELISA-s2} = 0.32$ $Se_{ELISA-s3} = 0.77$	(Nielsen and Toft, 2008)
$Sp_{ELISA-s}$	Specificity ELISA test	Sheep	0.98	(Nielsen and Toft, 2008)
$Se_{ELISA-b1-3}$	Sensitivity ELISA for cattle by infection state: bTr_{1-2} , bL_{1-4} , bLs_{1-4} , $bYls_4$, $bYhs_4$	Beef	$Se_{ELISA-b1} = 0.15$ $Se_{ELISA-b2} = 0.30$ $Se_{ELISA-b3} = 0.71$	(Nielsen and Toft, 2008)
$Sp_{ELISA-b}$	Specificity ELISA test	Beef	0.96	(Nielsen and Toft, 2008)
CGT	Co-grazing time	Env	3 months	
RT	Rotation time between paddocks	Env	1 months	
Ψ	Bacterial decay rate	Env	0.4 summer 0.2 winter	(Whittington et al., 2004)

TBC: To be calculated. Rates were calculated in a monthly base.

7.3.4 Simulation scenarios

Model calibration: Initially, individual species models were run individually in naïve populations (state 0) after the introduction of infected animals. Models were run until an apparent equilibrium prevalence was reached (state 1). Starting with the single-species equilibrium distribution of animals in age specific infection states, CG was simulated using a CG period of three months per year, until a new equilibrium state was reached

by each species (state 2). Simulations were run over long time periods (1,200 months) in order to reach equilibrium states after new CG management or control measures were introduced. Prevalence was reported at 300 months (25 years) and at the end of the simulation time.

7.3.4.1 Effect of direct contact on pasture between an infected and a naïve species (Scenario 1)

Cross-species transmission of MAP between a naïve and an infected species during concurrent CG was simulated. First, naïve beef were co-grazed with infected sheep, which were at an equilibrium prevalence of 20%. Likewise naïve sheep were co-grazed with infected beef, which were at an equilibrium prevalence of 13%. CG times of 2, 4 or 6 months per year were simulated and compared with the starting prevalence and incidence of no-CG.

7.3.4.2 Effect of grazing a naïve species on MAP-contaminated pasture (Scenario 2)

The risk of MAP transmission within and between species from contaminated pasture, after spelling times of 6, 9, 12, and 15 months was assessed. Firstly, naïve sheep (or beef) grazed paddocks which had been contaminated by the same infected species prior to pasture spelling. Secondly, naïve sheep (beef) grazed paddocks which had been contaminated by the opposite infected species [beef (sheep)]. Before the start of spelling periods, paddocks had been grazed by infected animals for the last five years, at equilibrium prevalences of 13% and 20% for beef and sheep, respectively. The model accounted for differences in the MAP decay rate (ψ) between summer and winter months (Whittington et al., 2004). Pasture spelling periods started in early summer

(January). The objective of this simulation was to estimate the necessary spelling time to stop MAP transmission cycle through contaminated pasture.

7.3.4.3 Effect of controlling MAP infection in flocks/herds under CG managements (Scenario 3)

Changes in prevalence and disease incidence were studied after control measures were applied in only one or in both infected species and where both species were co-grazed for 3 months per year (state 2). Specific control measures were: i) test & cull (T&C) once a year using an ELISA test, ii) increased 'farmer surveillance' of clinical animals, reducing the time that high shedders survived in the farm by 50%. This feature was simulated by increasing the values of μ_{bC} and μ_{sC} by a 50%. Finally, iii) joined use of T&C and 'farmer surveillance'. The objective was to compare the efficacy of control when another infected species was left without control, with the efficacy of control involving both species simultaneously. Additionally, increased 'farmer surveillance' was simulated in the absence of CG. Simulations of testing strategies were adjusted for lack of sensitivity and specificity, considering a variable ELISA performance for each disease stage (Table 7.1).

7.4 Results

The model simulated demographic dynamics and MAP transmission in age groups of single or mixed-species typical for New Zealand sheep&beef farms. Transmission parameters that could not be estimated from existing data were calibrated to achieve model outputs reflecting observed prevalence and incidence from field studies of single- and mixed-species farms with and without CG.

In beef, the single species system levelled to an equilibrium infection prevalence of 13.1% with an annual Ptb clinical incidence of 0.5% (Figure 7.3A & 7.3B). At this endemic state, there were 17.7% susceptible, 69.1% resistant, 0.1% transient, 8.8% latent, 4.0% low shedding, and 0.2% high shedding animals. The equilibrium infection

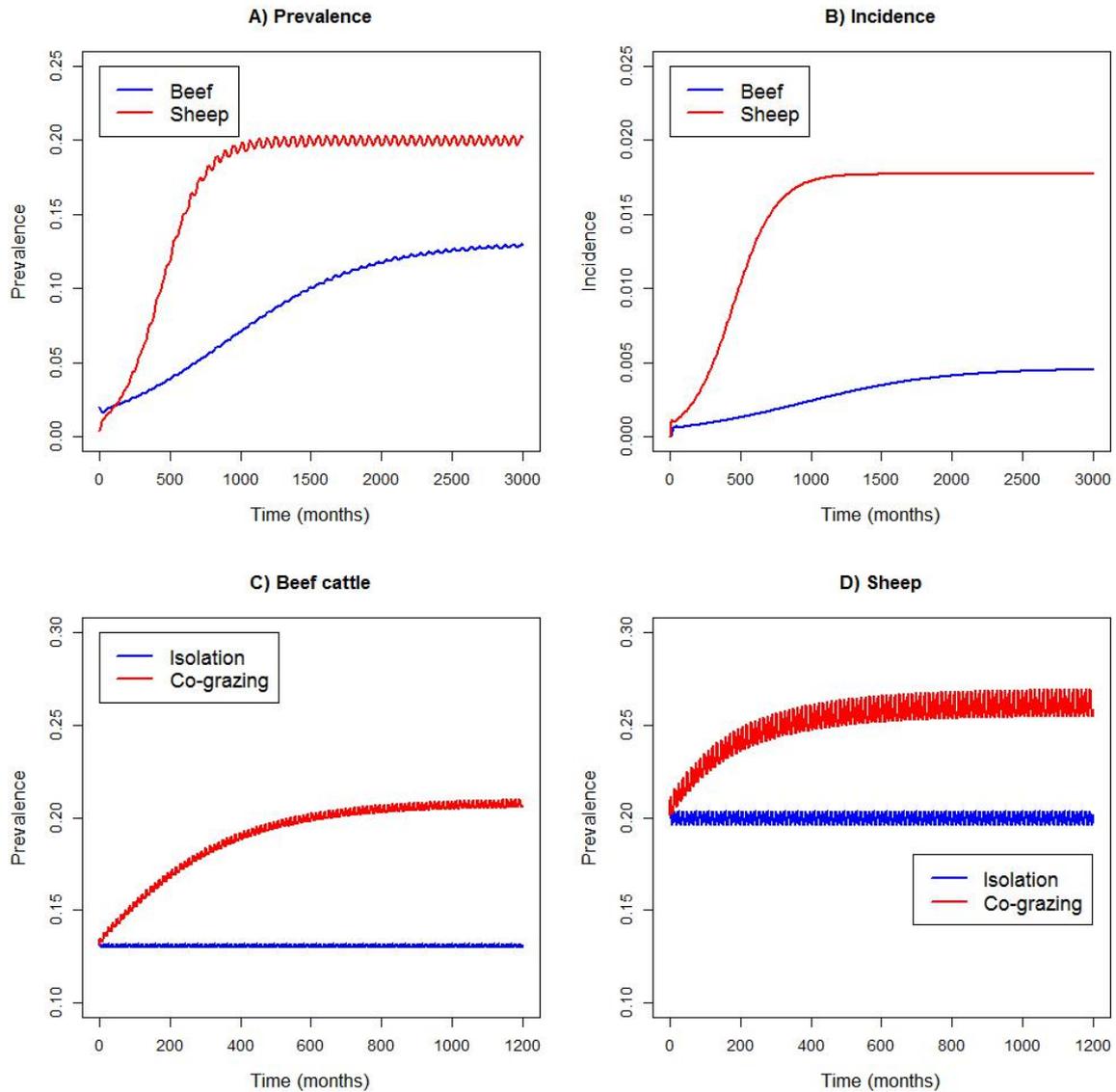


Figure 7.3: Model calibration: Prevalence (A) and incidence (B) after introducing two latently infected heifers to naïve beef and four paucibacillary-progressing, weaned sheep to naïve sheep without contact between species (no co-grazing). Prevalence of beef (C) starting from equilibrium after co-grazing with infected sheep for 3 months per year. Prevalence of sheep (D) starting from equilibrium after co-grazing with infected beef for 3 months per year

prevalence for the default single species situation of sheep was 19.9% with an annual Ptb clinical incidence of 1.7% (Figure 7.3A & 7.3B), and a population distribution of

79.8% susceptible, 5.9% paucibacillary (progressor), 3.3% paucibacillary (non-progressor), 10.2% resistant, and 0.8% multibacillary animals. When both species were infected on mixed-species farms and co-grazed for 3 months per year, infection prevalence in beef increased from 13.1 to 20.7% (Figure 7.3C). Similarly, infection prevalence also increased in sheep, by a similar amount, from 19.9 to 26.0% (Figure 7.3D). When the herd/flock was initially naïve, the model predicted that it may last about 40 years for prevalence to reach 3-10%, and that it continued to increase further for over 100 years. The introduction of 3 months per year CG sheep with beef increased the endemic level by 4% for beef within about 16 years (192 months). During the same time, CG increased the prevalence of sheep by a similar amount. Table 7.2 compares prevalence and incidence of survey data and model outputs.

Table 7.2: Comparison of prevalence and incidence from survey data and model outputs of sheep and beef in single ('only') or mixed-species farms (S&B)

Farm types	Observed		Simulated	
	Prevalence (%)	Incidence (%)	Prevalence (%)	Incidence (%)
Sheep only	20	0.16	19.9	1.7
Beef only	13	0.04	13.1	0.5
Sheep (S&B)	21	NA	26.0	2.3
Beef (S&B)	17	NA	20.7	0.7

NA = Not available

7.4.1 Effect of direct contact on pasture between an infected and a naïve species (Scenario 1)

Transmission of MAP across species was predicted to occur when naïve beef or sheep were concurrently co-grazed with their infected counterpart for periods of 2 to 6 months per year. However, the effect was not the same for both species: transmission was faster from infected beef to naïve sheep than *vice-versa* (Figures 7.4A & 7.4B). Twenty five years (300 months) after a naïve species was co-grazed with infected animals of the

other species, the prevalence was 15.3% and 24.7% in sheep and 6.7% and 14.7% in beef, for CG periods of 2 to 6 months per year, respectively. After another 600 months, equilibrium was reached for sheep at 24.1% and 30.8% for CG periods of 4 and 6 months, respectively. In beef, reach an equilibrium state took almost the double of the time than sheep, levelling at 17.7% and 25.4%, for CG periods of 4 and 6 months, respectively. These results suggest that transmission cross-species occurs through pasture and that its impact on prevalence depends of the duration of the CG periods.

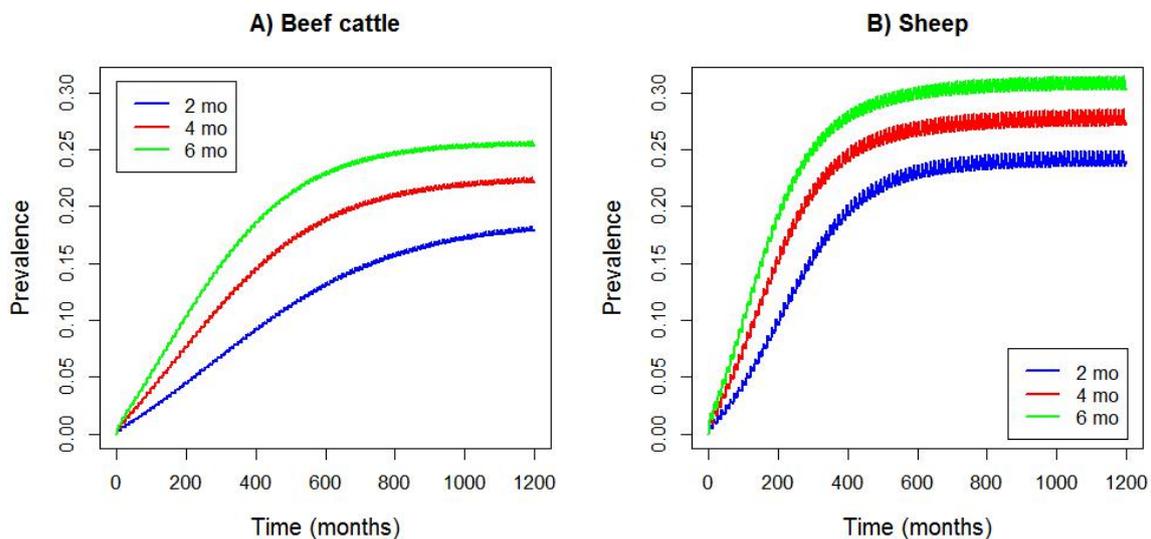


Figure 7.4 (Scenario 1): Epidemic curves after naïve beef were co-grazed with infected sheep (A) or naïve sheep with infected beef (B) for 2, 4 or 6 months (mo) per year

7.4.2 Effect of grazing a naïve species on MAP-contaminated pasture (Scenario 2)

Spelling pasture for periods of 6 to 15 months decreased MAP contamination by 91 – 99% of the initial bacteria load but the remaining 1-9% was sufficient to transmit the infection to a naïve population and establish a stable prevalence. Independently of MAP source (sheep or beef), epidemic curves indicated that sheep were infected by grazing contaminated pasture at a rate 2-45 times as high as beef depending on the spelling

period (Figure 7.5). When pasture had previously been grazed by infected beef, the prevalence of a new herd of initially naïve beef grazing the contaminated pasture ranged from 0.01% - 0.07%, 25 years after exposure, increasing to 0.04% - 0.41% at the end of the simulation time (homologous exposure; Figure 7.5A). The large range in prevalence

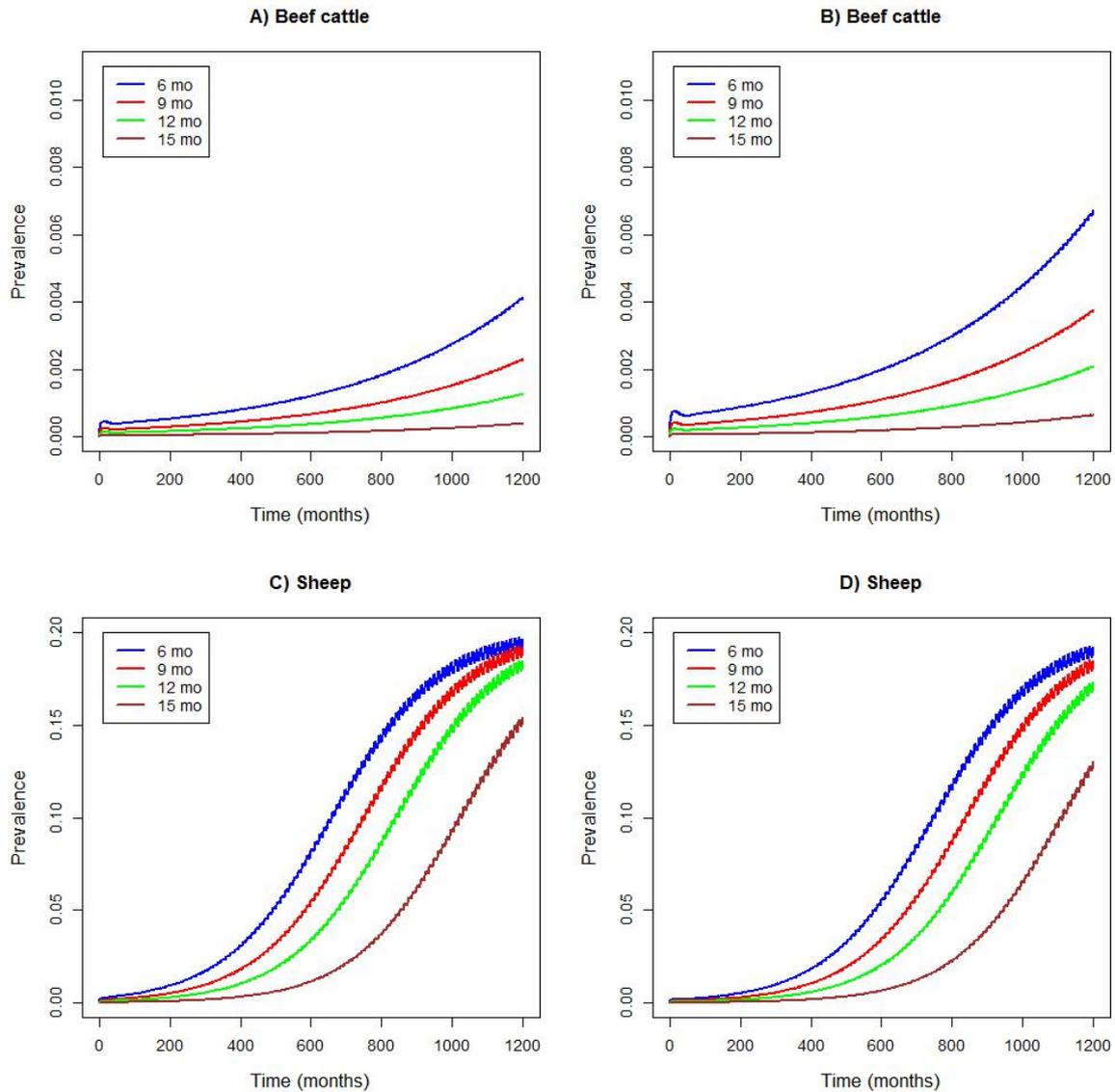


Figure 7.5 (Scenario 2): Prevalence of initially naïve beef (A) or sheep (C) after they were rotationally grazed on paddocks that were previously grazed by the same infected species (beef or sheep, resp. after spelling times of 6, 9, 12, and 15 months (mo)). Subfigures B and D show results of the same scenario when the naïve species was grazed on paddocks that were previously grazed by the opposite infected species

depended on the time that pasture was spelled before the naïve herd started grazing (6, 9, 12, and 15 months). In the same scenario, the prevalence in sheep was 0.17% -

1.73%, increasing to 15.44% - 19.63% at the end of the simulation time (Figure 7.5C). At heterologous exposure (naïve beef exposed to pasture previously contaminated by infected sheep), infection prevalence of beef tended to be higher compared to homologous exposure, regardless of spelling times (Figures 7.5B). Conversely, sheep presented lower prevalence under the heterologous exposure in comparison to the homologous scenario (Figure 7.5D). Simulation results suggested that spelling times longer than 9 months lead to a very low risk of transmission via contaminated pastures to beef: their final prevalence was below 0.38% after 100 years of exposure (Figure 7.5A & 7.5C).

7.4.3 Effect of controlling MAP infection in flocks/herds under CG managements (Scenario 3)

Changes in the equilibrium prevalences were observed in both species, in response to the implementation of control measures reducing prevalence to varying extents (Figure 7.6). When control was only applied to beef, annual T&C was an effective control measure, with a drop in prevalence from 20.7% to 3.6% in 25 years and reaching an equilibrium prevalence of 2.9%. Farmer surveillance without T&C reduced prevalence to 10.4% in 25 years, reaching an equilibrium prevalence of 7.8% (Figure 7.6A). The marginal benefit of both measures combined was small compared with the use of T&C alone, with a drop in prevalence to 2.7% in 25 years, and equilibrium at 2.4%. Alternatively, the simultaneous implementation of control measures in both species was more effective. When T&C and 'farmer surveillance' were jointly applied, the prevalence in beef dropped to 0.4% in 25 years (equilibrium at 0.01%). The contribution of 'farmer surveillance' under simultaneous control in both species had a greater impact than in the single species control scenario, since T&C alone caused a reduction in

prevalence to 2.3% in 25 years (equilibrium at 0.54%). When ‘farmer surveillance’ was the only measure applied in both species, prevalence in beef dropped to 7.19% in 25 years (equilibrium at 0.75%). Interestingly, the isolation of both species (no CG), in addition to an improved ‘farmer surveillance’, reached similar equilibrium prevalences as the ones observed in the control methods using T&C. However, the drop in prevalence was slower, reducing prevalence to 5.9% in 25 years (Figure 7.6B).

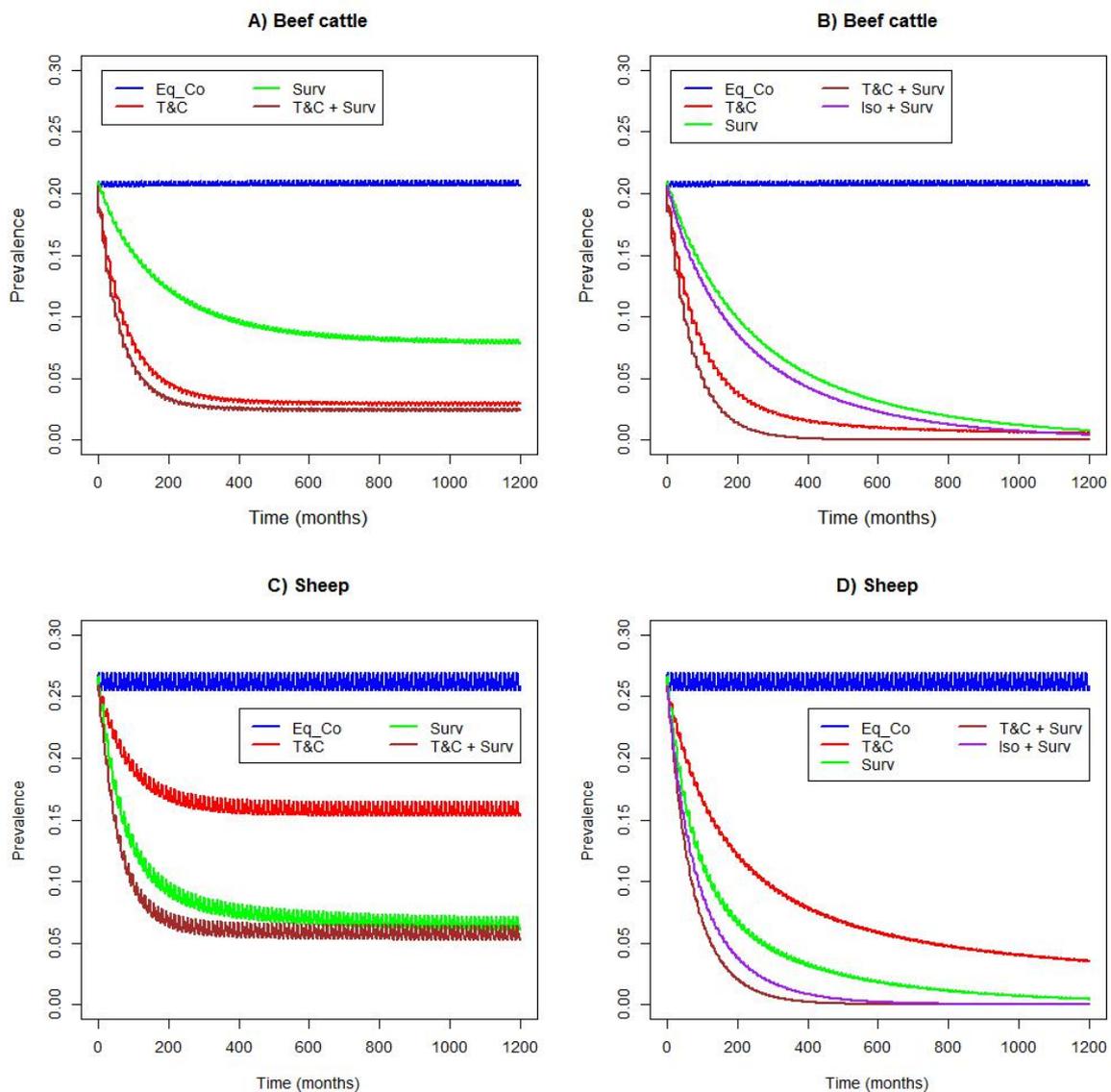


Figure 7.6 (Scenario 3): Prevalence change due to test and cull (T&C) and/or increased surveillance in only beef (A) or only sheep (C) under a co-grazing regime of 3 months per year (Eq_co= prevalence without control; T&C= annual test and cull using an ELISA, Surv= increased animal surveillance reducing the time to removal of clinical animals by 25%).

Prevalence change in beef (B) and sheep (D) when control measures were applied to both species simultaneously

In sheep, the control measures also reduced the prevalence of infection. However, the decrease due to T&C was smaller in sheep than in beef (Figure 7.6C & 7.6D): whereas prevalence in beef reduced 6 to 9 fold 25 years after the implementation of T&C, it only decreased 2 to 3 fold in sheep. When applied in sheep only, 'farmer surveillance' was more effective than T&C, dropping prevalence to 7.6% in 25 years, whereas T&C reduced the prevalence to 15.9% during the same period. A combination of the two measures caused a reduction of 26.0% to 5.6% in 25 years (equilibrium at 5.3%; Figure 7.6C). Alternatively, when control measures were applied simultaneously in both species, the use of T&C and 'farmer surveillance' in tandem produced the fastest prevalence reduction in sheep from 26.0% to 0.7% in 25 years (equilibrium at 0.01%). Although, the isolation of sheep and beef, in tandem with an improved 'farmer surveillance' generated the second fastest drop in prevalence to 1.8% in 25 years, decreasing until reach the same equilibrium prevalence than the combination of T&C and 'farmer surveillance' after 50 years. Similarly to the results from the single species control scenario, the contribution of 'farmer surveillance' was greater than T&C for Ptb control in sheep, where the application of T&C alone allowed a prevalence reduction to 9.7% in 25 years (equilibrium at 3.6%), whereas 'farmer surveillance' alone to 4.3% in 25 years (equilibrium at 0.4%; Figure 7.6D).

Figure 7.7 illustrates the decrease of the clinical disease incidence associated with each control strategy. Under no control and CG periods of 3 months per season, beef levelled at an ACI of 7 cases per 1,000 heads of stock (0.7%) and sheep at 23 cases per 1,000 heads of stock (2.3%). In all scenarios, the clinical incidence followed similar trends as

the prevalence. However, due to increased ‘farmer surveillance’ the number of cases increased initially for a short period and then decreased rapidly and consistently. In beef, T&C or the joint use of T&C and ‘farmer surveillance’ reduced the ACI to <1 case per 1,000 heads of stock (Figures 7.7A & 7.7B).

In sheep, T&C (only) reduced ACI less effectively than in beef. Only when control measures were applied simultaneously in both species, did the ACI in sheep drop below 1 case per 1,000 heads of stock. Combining T&C with improved ‘farmer surveillance’ caused the fastest reduction of clinical incidence. The separation of sheep and beef together with an increased ‘farmer surveillance’ had a similar effect on ACI as T&C and ‘farmer surveillance’ (Figures 7.7C & 7.7D).

7.5 Discussion

This study aimed to model MAP dynamics of infection in pastoral based sheep&beef farms, where the two species had indirect contact via the shared use of pasture. New Zealand pastoral production systems are characterized by whole year outdoor grazing and seasonal production. The results contributed to improve our understanding of the epidemiology of Ptb, of the conditions for MAP cross-species transmission and of the effect of control measures on prevalence and clinical disease incidence. Infected sheep or beef were able to transmit MAP to their naïve counterpart species during seasonal CG, and the length of the CG period correlated with the prevalence of infection. Moreover, control measures reduced the infection prevalence and clinical incidence. The application of control measures in both species was the most efficient approach, where the combined use of T&C and increased ‘farmer surveillance’ or species isolation (no CG) and ‘farmer surveillance’ achieved the greatest reduction in prevalence and clinical incidence.

It is common practice in sheep&beef farms in New Zealand to co-graze these species together for 2-6 months starting about one month before calving/lambing in August-September and continuing until weaning. The practice is called set-stocking. Another common form of pasture use is rotational grazing where pasture blocks are grazed for 1-2 months by sheep and/or beef, and then set aside (spelled) to allow the re-growth of grass. The model outlined in the present study evaluated the impact of these farming practices with and without disease control measures on the dynamics of infection with MAP in sheep and beef.

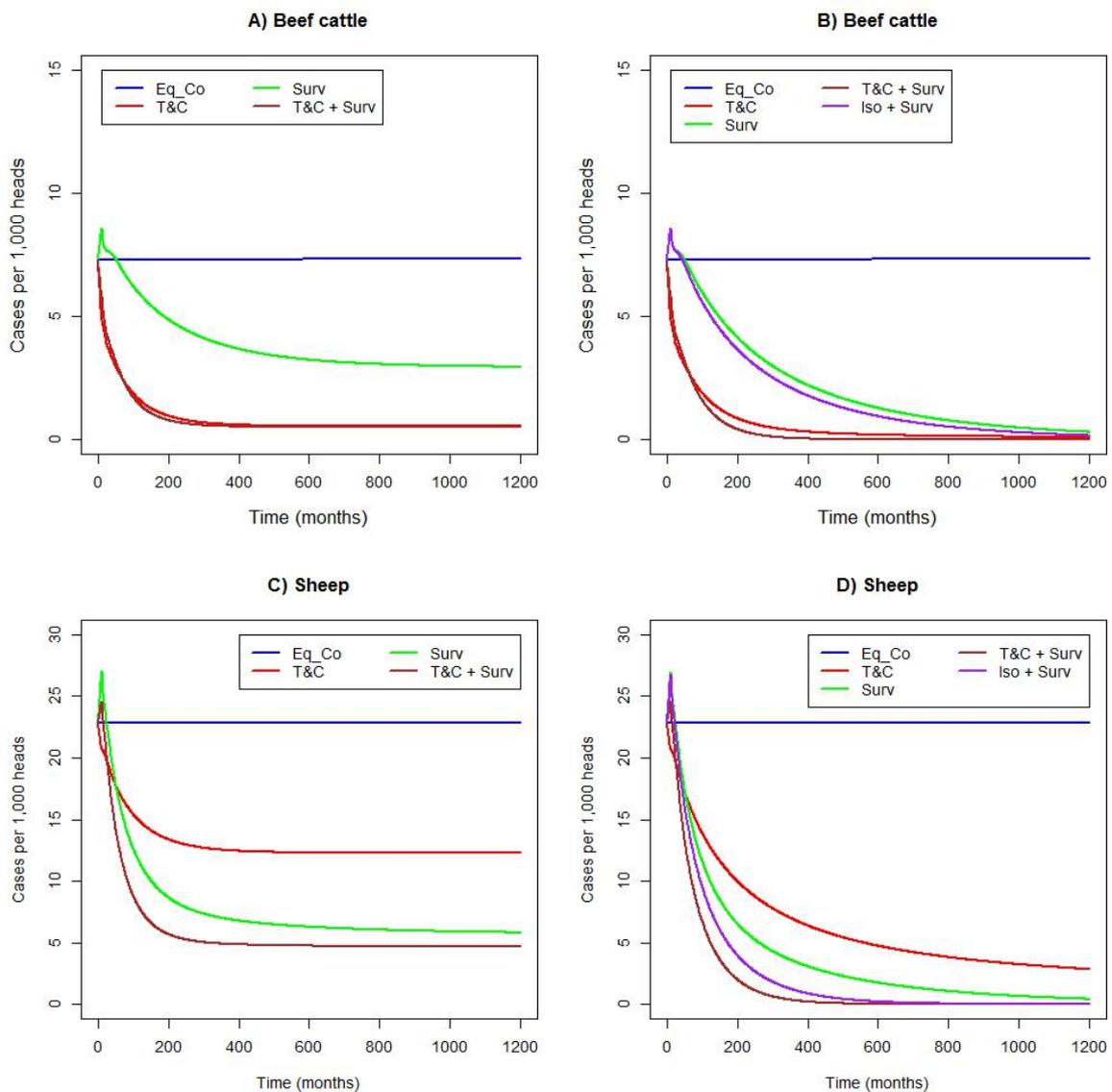


Figure 7.7 (Scenario 3): Annual clinical incidence (cases per 1,000 heads of stock) change due to test and cull (T&C) and/or increased surveillance in only beef (A) or only sheep (C) under a co-grazing regime of 3 months per year (Eq_co= prevalence without control; T&C= annual test and cull using an ELISA, Surv= increased animal surveillance reducing the time to removal of clinical animals by 25%). Annual clinical incidence change in beef (B) and sheep (D) when control measures were applied to both species simultaneously

The two-host model presented here was based on two pre-existing simulation models for sheep (Marquetoux et al., 2012) and dairy cattle (Mitchell et al., 2008), which were adapted to represent a sheep&beef farm, allowing for MAP circulation between species to simulate cross-species transmission. Pasture was the environmental component included as the vector for MAP transmission within and between species. MAP transmission within species was also allowed through vertical transmission in utero (only beef), and pseudo-vertical transmission due to the suckling of faeces-contaminated udders by lambs and calves. Except for horizontal transmission and progression to clinical disease in beef, all model parameters were based on or derived from published information. Horizontal transmission parameters were calibrated by choosing values that delivered model outputs equivalent to recent survey data about Ptb prevalence and clinical incidence in sheep and beef in single- and mixed-species pastoral farming systems in New Zealand (Chapter 3). These data indicated higher prevalences (at animal and herd level) in sheep&beef farms than in single species farms. The simulated scenarios represented different kind of common pasture management practices, long spelling times to evaluate whether and when MAP transmission would become insufficient to sustain infection in the herd or flock, and control measures such as T&C, earlier removal of clinical cases and preventing cross-species transmission by separation of infectious species. Since mixed species pastoral farming is uncommon in most other countries with intensive agriculture systems, most published reports about MAP infection or clinical disease involved a single host. Moreover, livestock in production systems of the Northern hemisphere are commonly kept under confined

conditions. Thus, to our knowledge, this is the first two-host pasture based model for the study of MAP transmission. Nevertheless, transmission parameters and calibration data for the sheep model were based on a number of studies from similar systems in Australia (Lambeth et al., 2004; Dennis et al., 2011; Kawaji et al., 2011; McGregor et al., 2012). Our model was informed by single-host simulation models of a generalised cattle system (Pouillot et al., 2004; Ezanno et al., 2005), dairy cattle (Collins and Morgan, 1991; Groenendaal et al., 2002; Kudahl et al., 2007; Mitchell et al., 2008; Marce et al., 2011), beef (Humphry et al., 2006; Bennett et al., 2012), sheep (Juste and Casal, 1993; Sergeant and Whittington, 2000; Marquetoux et al., 2012), and deer (Heuer et al., 2012).

Most existing models about the dynamics of MAP in livestock use various forms of direct transmission (animal to animal). Direct transmission may be applicable to production systems in the United States or Europe, where animals are kept indoors for most parts of the year and summer grazing may occur for only short periods. In New Zealand pastoral systems however, indoor housing is inexistent and livestock graze outdoor all year round. Humphry et al. (2006) simulated the spread of MAP in a beef herd under pastoral conditions, assuming the environment was the primary source of infection. Their approach accounted for the density of the infectious agent in the environment without, however, considering MAP survival on pasture. Models proposed by Marce et al. (2011) and Heuer et al. (2012) were the first to simulate MAP survival in the environment and consider indirect transmission through contaminated pasture or shared pens. Modelling indirect transmission between animals via contaminated pasture and MAP survival in the environment were key features of the New Zealand system of sheep&beef farming.

The environmental component in the model was divided into eight paddocks, four per species. Pairs of paddocks facilitated rotational grazing by age specific mobs. In order to simulate CG, two extra paddocks were created by merging existing ones holding ewes and cows around the time of seasonal lambing/calving together with their offspring until weaning, thus allowing the CG of four species/age groups. On a typical New Zealand sheep&beef farm, weaners and heifers or hoggets and two-tooth are grazed separately from the adult/offspring mob. This was realised by sub-dividing species mobs in different paddocks according to age. MAP survival on pasture was different in summer and winter months, following observations of the effect of seasonal variation in temperature and moisture on the viability of the bacteria in the environment (Whittington et al., 2004). This resulted in oscillating prevalence curves.

The beef component was a modified version of the model developed by Mitchell et al. (2008) that studied the Ptb epidemiology in dairy cattle under housed conditions. Our modifications of that model included the presence of four age groups instead of three as well as the indirect transmission of MAP from contaminated pasture. In the original model, infected calves progressed linearly from a transient shedding state to a latent state until adulthood and later progressed to permanent shedding states and ultimately clinical disease. However, a recent meta-analysis of experimental infection studies in cattle (Mitchell et al., 2012) provided evidence that transient shedding in calves occurs only in about 10% of calves, while the remainder enter directly into a latent state of infection. Thus, the model was adapted to accommodate for the possibility of two progression pathways for newly infected cattle, a proportion (χ_b) entering a phase of transient shedding, and $1-\chi_b$ entering a slow progressing latent stage. Not all recently

published cattle models include a transient state (Bennett et al., 2012) where calves can infect other calves (van Roermund et al., 2007) and where infected calves have the capacity to maintain low levels of infection in a herd (Mitchell et al., 2008).

The sheep component was based on a novel model proposed by Marquetoux et al. (2012). Two models of MAP infection dynamics have been described for sheep (Juste and Casal, 1993; Sergeant and Whittington, 2000). The model of Marquetoux et al. (2012) was developed to study Ptb epidemiology, production loss due to Ptb, and cost-benefits of disease control in sheep flocks in New Zealand. We implemented minimal modifications of this model by omitting seasonal production outputs because they were beyond the scope of the objectives of this study.

In the two species model, infection probability equations were modified to allow MAP transmission between sheep and beef, accounting for MAP being shed simultaneously by both species under CG management. All simulations assumed that sheep and/or beef were infected with the same MAP Type I strain. Historically, the MAP Type I was commonly associated with infection in sheep worldwide but rarely isolated from cattle (Collins et al., 1990; Sevilla et al., 2005). However, a recent molecular typing survey of MAP in New Zealand showed that sheep and beef were both most commonly infected with MAP type I (Chapter 6). Results suggested that infection with multiple strains within the same herd or flock were rare. The molecular analysis performed in that study also provided strong evidence for the similarity of the strains found in beef and sheep, suggesting that transmission between these two host species does occur in New Zealand pastoral systems. Conversely, an Australian study investigating the risk of MAP transmission to naïve beef co-grazed with known infected sheep, concluded that the risk was low and transmission occurred rarely (Moloney and Whittington, 2008). Thus, there appear to be differences in pastoral farming systems in New Zealand and Australia

which may impact MAP transmission dynamics. This could possibly be attributed to higher stocking densities in the New Zealand system leading to an increase in contact frequency between species which may be conducive for cross-species transmission.

Transmission parameters in each species model were calibrated to reflect observed animal level MAP infection prevalences of 13% and 20% for beef and sheep respectively. Conversely, prevalences as high as 50% have been reported in herds/flocks without any control measures in other simulation models (Collins and Morgan, 1991; Sergeant and Whittington, 2000; Groenendaal et al., 2002). In New Zealand, Ptb is known to be present in cattle since 1912 and in sheep since 1952 (de Lisle, 2002) and the majority of infected sheep or beef flocks/herds were not under any active control program. Despite that, a prevalence of 50% was rarely reported in these two species (Chapter 3). Thus, equilibrium prevalences to which the models were calibrated were lower than previous models.

Simulated equilibrium prevalences for single species models were similar to surveyed prevalences in sheep (19.9% vs. 20%) and beef (13.1% vs. 13%).

In a situation of CG for 3 months per year, the simulated prevalence in beef increased by 7.6% and in sheep by 6.1%. These results were higher than those from our survey data. Hence, simulated prevalence under CG showed a stronger increase than observed in the field but was still well within limits of uncertainty of the data. Clinical incidence in beef of 0.5% was based on the calibration of the rate of progression from low to high shedding (δ_{b5}). Data collected in New Zealand based on faecal culture results (Chapter 3), indicate that despite the possibility for beef to be infected and shedding MAP, these animals rarely progressed to clinical disease (Chapter 5). The low clinical incidence may be explained by the finding that beef were predominantly infected with MAP Type I, which appears to be less pathogenic in this species than Type II (Janagama et al.,

2006; Gollnick et al., 2007). Current evidence indicates that cattle infected with MAP Type I are able to tolerate MAP infection with rare occurrences of clinical disease (Stewart et al., 2007). Additionally, beef presented 8-fold lower ACI than dairy cattle in New Zealand (Norton et al., 2009). Therefore, a lower progression parameter (δ_{b5}) was used than reported in dairy cattle (Mitchell et al., 2008; Marce et al., 2011) in order to meet a clinical Ptb incidence of 0.5%. In sheep, an ACI of 1.7% per season was simulated, based on parameters extracted from literature only, being relatively close to the expected 1%.

Model results indicated that under pastoral conditions in mixed-species farms, sheep were able to transmit MAP to beef and vice versa. Co-grazing for periods of two to six months (Scenario 1) were sufficient to transmit and sustain the infection from an infected to a naïve species. When both species were infected with MAP under CG conditions, the model predicted an increase of the level of pasture contamination resulting in a greater prevalence of infection (at equilibrium) in both species compared to a single-species farming system. These findings concur with our findings from population surveys (Chapters 3 & 5).

Simulation outcomes suggested a faster spread of infection in sheep than in beef, when latently infected animals were introduced in each population under no CG. Infection prevalence in sheep reached a plateau after half the time required by beef. The faster spread of MAP infection among sheep could be explained by the difference in susceptible age groups: in sheep all ages could become infected, whereas in cattle only animals under 12 months old were susceptible to infection. When naïve beef were exposed to infected sheep, prevalence increased faster than when individual, infectious

beef cattle were introduced to a naïve beef herd. For example in the CG-scenario 1, a prevalence of 10% was reached after 15 to 38 years for CG periods of 2 to 6 months per season (Figure 7.4A), whereas such prevalence was reached after 125 years in the model calibration scenario (Figure 7.3A). The larger effect on prevalence in beef due to CG with infected sheep could be explained by differences in shedding rates and stocking density between sheep and beef. Subclinically and clinically affected ewes produced $1.5 \cdot 10^{08}$ and $3.0 \cdot 10^{12}$ MAP cfu per month respectively, whereas low and high shedding cows produced $2.3 \cdot 10^{09}$ and $9.0 \cdot 10^{13}$ MAP cfu per month respectively. However, despite a 2-fold higher shedding rate of cattle, the number of sheep per hectare was 10 times higher than for beef, which numerically resulted in 5-fold the amount of MAP being brought on pasture by sheep exposing beef than vice-versa. Moreover, the latent stage in cattle was longer than in sheep, and shedding was mainly limited to adult animals, whereas in sheep shedding was present in all age groups. Furthermore, consistent with the assumption that MAP Type I was less pathogenic in cattle than in sheep (Janagama et al., 2006; Gollnick et al., 2007), the progression to high shedding stage and clinical disease (compartment bYhs4) was limited in beef. Therefore, the proportion of high shedders in sheep was higher than in beef (0.2% vs. 0.8%). Consequently sheep contributed a higher bacterial load of MAP to pasture than beef. When naïve sheep were exposed to infectious beef cattle, prevalence in sheep increased faster than in naïve beef in the similar scenario 1, and also faster than through introducing infectious sheep to a naïve sheep flock (model calibration scenario). Although naïve sheep faced a lower challenge from infected beef than vice-versa, the faster infection spread among sheep could be explained by differences in the transmission parameters, where $\beta_{s1} > \beta_{s2} > \beta_b$. Hence the value of the parameters that were chosen to mirror field observations, reflected a greater susceptibility and

transmission of MAP (Type I) in sheep. Longer CG periods caused higher infection prevalence with a stronger response in both species being slightly higher in beef, although final prevalence was always higher in sheep. This suggests that CG increased the total bacterial load of pasture and consequently accelerated the disease dynamic in both species.

Long spelling periods were simulated to assess if they could interrupt the transmission cycle within and between species. However, even the longest spelling time of 15 months could not prevent infection of a naive population from grazing a pasture that was previously grazed by infected animals of either species. The fact that, even after long spelling periods, beef and sheep eventually returned to some endemic level of prevalence may be attributable to the deterministic nature of the model. A stochastic approach would be more biologically relevant as it would allow accidental fade out (Marce et al., 2011). Model results suggested that spelling MAP contaminated pasture for 9 to 15 months was effective for preventing Ptb in naïve beef, and to a lesser extent in sheep. During the first 10 years of exposure, the prevalence of initially naïve animals was below 0.04% in beef and below 0.31% in sheep. As simulation time increased the difference between beef and sheep gradually increased in favour of beef. After 25 years, the prevalence of beef was still below 0.06% and sheep about 1.0%. However, typical pasture spelling times in New Zealand are 1 month or less. Thus the opportunity cost of keeping paddocks depopulated for such long periods of time would probably exceed the cost of the disease. Thus implementation of such management would not be economically feasible for farmers, under model assumptions, due to the tenacity of MAP to sustain in the environment and remain virulent. In particular, the assumption that MAP is able to keep its virulence intact after months in the environment is critical.

Currently, there is a lack of research in this area and if new evidence appears showing a decreased virulence (due environmental exposure) could represent a new scenario, where pasture spelling would represent a cost-effective management for the control of the disease.

The effect of traditional control measures on infection prevalence and clinical disease incidence, under CG conditions was evaluated in the third simulation (Scenario 3; Figures 7.6 & 7.7). Results showed a decrease in prevalence associated with each control measure. The effect was substantially greater when control measures were implemented in both species simultaneously. In this scenario, the implementation of a T&C once a year in beef, reduced prevalence from 20.7% to 6.9% after 10 years, and dropping further to 2.3% after 25 years. The additional gain of combining T&C with increased 'farmer surveillance' was greater when interventions targeted both species than in the single species control scenario. The use of 'farmer surveillance' only reduced prevalence from 20.7% to 13.1% after 10 years, and dropping to 7.2% after 25 years. The significant effect of T&C in beef stands in contrast to other published findings that reported a marginal effect of T&C on prevalence reduction (Groenendaal et al., 2002). The difference could be explained by differences in the systems simulated as well as the scenarios under comparison. In the model presented by Groenendaal et al. (2002), authors simulated a dairy cattle system under housed conditions, which presents a higher animal density than a pastoral system, reporting a simulated prevalence of 50% (under no control). Although, Groenendaal et al. (2002) did not reported the clinical incidence in their model, ACI of dairy cattle under housed conditions has been reported between 2-5% in USA and England (Çetinkaya et al., 1997; USDA, 2005). Conversely, our model resulted in an ACI of 0.5% and a prevalence of 13%, hence a much lower

infection pressure. Probably therefore, T&C had a greater effect than the observed in Groenendaal et al. (2002) model.

In sheep the consistent and regular use of either T&C or 'farmer surveillance', as part of a simultaneous species control, reduced prevalence by 10-16% in 10 years. Thus when both were used in tandem a larger reduction in infection prevalence was observed. Conversely to the effect of T&C in beef, this control measure was the less effective in sheep out of all simulated intervention options. This indicated that once the disease had been established, T&C was less effective in sheep than in beef. Additionally, if T&C was stopped in any of the species, it would be expected that the overall prevalence would return towards the initial equilibrium. The simulated control measures neither considered the cost of control nor the production losses due to sub-/clinical disease. Although, in sheep and beef the simultaneous use of an increased 'farmer surveillance' (only or jointly with species isolation) had a slower prevalence reduction than options using T&C, the use of such measures produced an important prevalence reduction which drifted toward zero in the long run. Thus, an early identification and removal of animals with signs of clinical Ptb would minimize the environmental contamination from such animals, which would decrease the infection pressure, and consequently, prevalence. A cost-benefit simulation of control strategies against paratuberculosis in New Zealand dairy cattle concluded that regardless what measures were applied and despite strong reduction of prevalence, the cost were higher than the benefits of control (Norton, 2007), rendering disease control financially unattractive. The prevalence survey (Chapter 3) concluded that MAP infection was widely spread and endemic in New Zealand mixed-species pastoral systems. Thus the gain of reducing infection prevalence by means of grazing sheep and beef separately all year round

would most likely be small in the general population. However, it could be a contribution towards reducing prevalence in heavily infected farms. Farmers indeed derive benefits from CG beef and sheep, through more efficient use of pasture enhancing the profitability per hectare. Co-grazing of ovine and bovine species is also a valuable management tool to control noxious weed on pasture (Griffiths et al., 2006) and parasitic diseases in both species, in a pastoral environment like New Zealand (Southcott and Barger, 1975). Thus the deleterious effects of Ptb may well be more than offset by beneficial effects of CG on other production and health traits.

Simulation results also illustrated variations in clinical disease incidence associated to different control measures. Although it is expected that clinical incidence follows the prevalence in a general trend, currently the specific determinants of clinical disease are unknown. Moreover, a recent field study in deer herds has observed the presence of herds with high prevalence (~50%) and almost no clinical disease, whereas other herds presenting lower prevalence than the previous ones, had a much higher clinical incidence (Stringer, 2010), suggesting a variable relationship between prevalence and incidence in different herds. Thus, it is necessary further studies to validate the clinical incidence results from the present study.

7.6 Conclusion

We present the first two-host model to study cross-species transmission of MAP under pastoral farm management. Sheep and beef infected each other through the shared use of contaminated pasture during seasonal CG periods. An increase of the infection prevalence in both species was associated with this practice. However, the increase was different in the two species: sheep responded to the extra challenge from infected beef with a higher prevalence than beef exposed to infected sheep. As the length of the CG

period increased, prevalence increased in both species. In a rotational grazing system, typical periods of pasture spelling were inefficient for controlling Ptb. The prevalence of infection could be reduced substantially in beef when a T&C program was implemented, but was the less effective measure in sheep. The results suggested that infection was more difficult to control using T&C in sheep than in beef under pastoral conditions.

In sheep, the combination of an increased ‘farmer surveillance’ and species isolation had a similar performance than join application of ‘farmer surveillance’ and T&C. In beef, such control measures combination reduced the prevalence to <1%, in the long run. Those results suggest that the simultaneous application of species isolation in tandem with an increased ‘farmer surveillance’ could be an efficient approach for prevalence reduction in sheep&beef farms.

7.7 Acknowledgements

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General discussion

8.1 An overview of the research carried out in this thesis

The present research was implemented under the New Zealand Johne's Disease Research Consortium (JDRC), a partnership between livestock industries, research centres and government for the research and control of paratuberculosis (PtB). The focus was on multi-species farms where sheep, beef cattle and deer are frequently co-grazed, providing opportunities for cross-species transmission of the causal agent *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Thus, JDRC has addressed all susceptible livestock species (except dairy cattle) in an overarching strategy, with a general aim of developing an integrated approach for PtB research. The main objective of this thesis was to gain epidemiological insight to MAP infection dynamics and clinical paratuberculosis (cPtB) patterns in New Zealand commercial sheep, beef cattle and deer populations.

In order to fulfill this main objective, several secondary objectives were defined. These secondary objectives were addressed by individual epidemiological studies involving (i) a national farm-level quantification of MAP infection prevalence, (ii) estimation of the annual cPtB incidence, (iii) association between infected/affected flocks/herds and production, in terms of reproductive performance and culling rates in the three species under research, (iv) the association of cross-species co-grazing (CG) with infection prevalence and clinical disease, and (v) a description of identifiable MAP strains in the

national livestock population. Molecular strain typing data of MAP were analyzed to make inferences about likely transmission routes. In addition, (vi) cross-species infection dynamics of MAP transmission were assessed through the development of a mathematical sheep/beef simulation model.

Work leading to this thesis was prone to the ambition to generate valid epidemiological inferences, at national level, in three livestock species simultaneously. Thus, data collection started with a large national postal survey followed by the collection of blood and faeces from commercial sheep, beef and/or deer farms. Sampling, sample manipulation, and testing required the coordination of farm managers, veterinary practices, postal services, external laboratories and in-house personnel to successfully generate accurate data for analysis. In Chapter 3 a two-stage sample collection was described and herd-level infection prevalence estimates presented. Firstly, a postal survey was mailed out to 7,998 clients of 28 farm animal veterinary practices in seven administrative regions of New Zealand. From these, 1,940 (24.3%) correctly filled-out questionnaires were returned. Secondly, 300 farms were randomly selected from strata of single and multi-species farms of these 1,940 survey returns. They were approached for blood and faecal sampling, returning samples from a total of 238 farms and 7,579 animals. The questionnaire gathered data regarding livestock demographics, production parameters, cPtb incidence, and grazing management information of all ruminant species present. Data analyses for mixed-species grazing and production performance associations from these surveys were presented in Chapters 4 and 5. During the collection of data from those commercial farms, Landcorp Ltd., the largest New Zealand farming corporation, requested the MAP infection status assessment from all livestock species, including dairy cattle, on their farming sites across New Zealand. This involved the sampling of 3,510 animals in 112 single or mixed species farms. The data

generated in this second sampling activity was used to inform prior distributions used in the Bayesian model described in Chapter 3. In Chapter 6 MAP isolates from both surveys were differentiated by molecular strain-typing, a technique that was simultaneously developed by collaborators at AgResearch, Wallaceville. In addition, we obtained strain types from 77 MAP isolates of dairy cattle, courteously provided by Livestock Improvement Corporation (LIC) as part of a JDRC collaboration agreement. The combined data sets used in the present thesis represent a unique data resource for drawing inferences about paratuberculosis epidemiology in several species at national level.

Together, the findings from these chapters have established baseline estimates, at national level, for infection prevalence, clinical incidence, culling rates, and reproductive performance on infected and affected flocks/herds. Moreover, it has provided new insights about epidemiological features of MAP infection in New Zealand, including strong evidence of cross-species transmission. For example, a key finding was that most isolates from beef cattle were MAP Type I (ovine or S-type). This thesis has also addressed questions stated in previous research in New Zealand by Norton (2007), Stringer (2010), and Hunnam (2011), such as need for better prevalence and incidences estimates, quantification of the association between sub/clinical MAP infection with reproductive performance, and the study of geographical and host distribution of MAP strain types in New Zealand. Additionally, this research has generated a sample bank of serum and faeces from 11,089 animals, stored at -80C and available for further research.

8.2 Review of the individual epidemiological studies

8.2.1 National farm-level quantification of MAP infection prevalence

Chapter 3 mainly focuses on estimating the true national flock/herd level prevalence (HTP) of MAP infection of commercial sheep, beef cattle and deer flocks/herds. Sampled species presented median population sizes of 2,777, 312, and 438 animals, for sheep, beef cattle and deer respectively. The data set collected (n=238 farms) involves typical single or mixed-species commercial farms, with flock/herd sizes ranging from 83 to 31,880 animals, with and without previous cPtb history, and covering the New Zealand administrative regions of Waikato, Wairarapa, Hawkes Bay, Manawatu-Wanganui, Marlborough, Canterbury, and Southland. Therefore, inferences derived from the present data set were obtained from a large area of New Zealand and are representative of the infection prevalence at national level. Prevalence estimates were adjusted by their sampling fraction in order to generate valid population-based inferences. The complex sampling protocol used in this study precluded the use of the method developed by Rogan and Gladen (1978), which is the standard technique to obtain true prevalence estimates from biased test results (Nielsen and Toft, 2009). Thus, a Bayesian approach was chosen instead using latent class modeling, which has become a popular and well established method to assess test performance and estimate prevalence, when test characteristics are unknown (Alinovi et al., 2009; Kostoulas et al., 2009; Dhand et al., 2010a; Norton et al., 2010; Okura et al., 2010; Liapi et al., 2011; Pozzato et al., 2011; Raizman et al., 2011; Wang et al., 2011; Menten et al., 2013). Currently, this methodology is recognized by the Animal Health Organization as a tool to assess and validate diagnostics test (OIE, 2012).

The study results demonstrate that MAP is an endemic and wide-spread infection, with national HTP estimates of 75% sheep flocks, 43% beef and 46% deer herds. Prevalence

comparisons between countries are difficult to draw due to differences in the target population, farming systems and test performance adjustment. However, our prevalence estimates were similarly high as those reported in Denmark and USA where production conditions are vastly different (Nielsen et al., 2000; Wells and Wagner, 2000; Nielsen and Toft, 2009). This might either be coincidence or indicate that there is a maximum threshold at the high end of endemic prevalence that is largely independent of environmental conditions. Conversely, in New South Wales (Australia), Sergeant and Baldock (2002) reported lower HTP estimates of 6 to 10% in sheep flocks despite similar pasture based production systems in Australia as in New Zealand. The differences in HTP estimates between Australia and New Zealand could be in part explained by differences in livestock density, in that the higher animal density in New Zealand would favor MAP transmission. Additionally, MAP was first reported in Australian sheep on early 1980s, thirty years after it was first reported in New Zealand sheep, thus presenting a shorter time for infection spread (Abbott et al., 2002; de Lisle, 2002). Moreover, New Zealand it is characterized by a temperate and rainy weather, conversely Australia sheep country is mostly hot and dry, presenting climatic conditions that limited the survival of MAP in comparison to New Zealand (Whittington et al., 2004).

Interestingly, there were the differences in HTP between New Zealand' North (NI) and South Islands (SI), notably a much higher HTP for deer (56 vs. 32%) and lower prevalence for sheep and beef cattle in the SI. Such differences have not being previously reported in sheep or beef cattle and confirm previously observed differences in deer (Stringer, 2010; Hunnam, 2011). While, higher HTP by island followed the trend of larger herd or flock size, there is little other biological evidence explaining this

island differential for MAP infection. The study of the risk factors leading to those differences was beyond the scope of Chapter 3. In the absence of data, it might be hypothesized that island differences might be attributable to climatic differences, soil types, flock/herd sizes, topography, animal movement, and/or MAP strains (Jakobsen et al., 2000; Wells and Wagner, 2000; Daniels et al., 2002; Hirst et al., 2004; Ward and Perez, 2004; Dhand et al., 2009; Woodbine et al., 2009). Evidence for MAP strain circulation between islands was presented in Chapter 6. However, dominant strains cluster in specific hosts in a specific island, where the dominant strain isolated from sheep and beef cattle flocks/herds was mainly located in the NI, whereas the dominant strain (most frequent) retrieved from deer herds was mainly located in the SI. This pattern correlates with prevalence differences between islands reported in Chapter 3, and could be one of the factors leading to this phenomenon. If MAP virulence was associated with molecular strain type, this might contribute to the prevalence difference. Hence, the virulence hypothesis warrants future research.

The Chapter 3 also provides animal level true prevalence (TP) estimates. In the case of sheep and beef cattle, the Bayesian latent class model used TP prior estimates obtained from ELISA test results from 63 sheep flocks and 49 beef cattle herds belonging to Landcorp Ltd. Conversely, prior TP estimates for deer were obtained from a published study (Stringer, 2010). In any case, TP priors used to parameterize the model were independently sourced from the present data set. True prevalence estimates presented in this chapter provide information not available previously for sheep and beef cattle in New Zealand, and represent updated knowledge of TP in deer. The study was not originally designed to estimate this parameter. Thus the 20 animals sampled per flock/herd might be not enough to see the whole spectrum of MAP prevalence

distribution among infected flocks/herds. Thus, further validation of TP estimates is required. Nevertheless, the baseline prevalence (animal and herd level) and clinical incidence (Chapter 5) data will be used to inform economic analysis research, currently under development at the Massey University JD research group. Additionally, these results could be used to monitor the performance of a control program tending to stop the infection of naïve flocks/herds.

8.2.2 Evidence of MAP cross-species transmission and molecular analysis

Herd level true prevalence was higher for sheep, beef cattle or deer in mixed-species farms compared to herds or flocks on single-species farms (i.e. without contact to another species). At the level of herd/flock infection status, this alone represents evidence for MAP transmission between species, a conclusion supported by strain type data in Chapter 6. Mixed-species interactions were further explored in Chapter 4 where the risk of MAP infection and clinical disease of a species was associated with the presence or absence of co-grazing with other species. Co-grazing effects were adjusted for possible confounding factors such as island and flock or herd size. This research represents a further contribution for the study of the association between CG with MAP infection prevalence and clinical disease in sheep, deer or beef cattle. Results indicated that contact with beef cattle was associated with an increase of the infection risk and cPtb incidence for sheep and deer (trend). Similarly, sheep was associated to a larger risk of infection for beef cattle. Conversely however, co-grazing sheep with deer was associated with a reduction in the clinical incidence in both species. The latter finding could be explained by MAP strains having variable host-specificity and virulence, as already postulated when examining prevalence differences between islands. Moreover,

identical molecular MAP strain types were found more often in sheep and beef or deer and beef when these species were on the same farm than when they were on different farms, as the PSI estimates indicated (Chapter 6).

Specifically, with the aim to generate more robust evidence about cross-species transmission of MAP, Chapter 6 describes the distribution of MAP strains among PFC positive sheep, deer, beef and dairy cattle from our dry stock survey (Chapter 3) and from purposively sampled dairy farms undertaken by LIC. Associations between MAP strains, host species and farm locations were presented. The study was a collaborative project with AgResearch at Wallaceville (Drs. D. Collins, G. de Lisle, and M.A. Price-Carter) and represents an updated molecular description of MAP strains in New Zealand ruminant livestock based on national wide surveys. Previous strain typing studies used samples passively submitted to the AgResearch laboratory at Wallaceville for MAP strain-typing (Collins et al., 1990; Collins et al., 2002; de Lisle et al., 2006). These studies described the development of the strain-typing technology used in this study and compared strain types collected between 1985 and 1993 (Collins et al., 2012). The laboratory work involved two molecular techniques used in tandem, in order to increase their discriminatory power, which has been recommended for the study of highly homogenous organism like MAP (Motiwala et al., 2006b). The results provide strong evidence of cross-species transmission, supporting findings in Chapters 3 and 4. They revealed that sheep and beef cattle carried similar MAP strains, even when they were located in different farms. In contrast, deer and beef cattle harbored the same strains only when they were located on the same farm. While 80% of beef cattle isolates were MAP Type I, they carried MAP Type II when they were on farms that also kept deer, the latter being predominantly also infected with MAP Type II. These results challenged previous beliefs in New Zealand and Australia that sheep were infected with MAP Type

I and beef cattle with MAP Type II, and that there was little if any transmission between these species (Collins et al., 1990; Whittington et al., 2001; Sevilla et al., 2005; Moloney and Whittington, 2008). Thus, MAP transmission may occur at close contact between species, as it is common in New Zealand livestock farming systems. However, only 15 MAP isolates were retrieved and strain-typed from beef cattle herds (Chapter 6), which represent a reduced pool of samples to conclusively establish the dominance of MAP Type I in beef cattle, thus further studies are required for a final conclusion.

The rarefaction analysis results, presented in Chapter 6, revealed that MAP isolates from dairy cattle presented the greatest MAP strain richness of all host species studied. However, the lack of plateau in Dairy rarefaction curve was indicative that more intensive sampling should yield extra strain types not observed in the present research. The dominant MAP strain from dairy cattle was equally frequent in both islands. High strain richness in dairy cattle herds has been reported before (Harris et al., 2006; Douarre et al., 2011; Pradhan et al., 2011). Those observed patterns may reflect a degree of production separation with the others hosts and a greater exchange of animals between dairy farms. The homogeneity of dairy strains between islands may be a consequence of extensive relocation movements of entire dairy herds from the North to the South island as one of the main developments of structural agricultural change in New Zealand over the last 20 years⁸.

The structured surveys used in chapter 6 involved the sampling of 11,373 animals to obtain 206 MAP isolates. This implied a huge monetary investment, as well being time and labor consuming, to obtain and test a relatively low number of isolates. However,

⁸ Ministry of primary industries (MPI), 2012 <http://www.stats.govt.nz/infoshare/Default.aspx>

the number of MAP isolates provided sufficient statistical power, and thanks to a stratified random survey design generated strong and valid inferences at national level. The number of MAP isolates obtained for this study precluded the performing of more complex data analysis, as could be the relation between MAP strains and clinical disease, thus a greater sample size is required in order to generate more robust inferences.

Together, results from Chapters 3, 4, and 6 represent strong evidence that MAP was transmitted across species, being frequent between sheep and beef cattle on mixed-species farms. However, our findings are in contrast to results from a previous study in Australia, which suggest that MAP transmission between sheep and beef cattle was a rare event in pastoral based systems (Moloney and Whittington, 2008). Additionally, the findings of Chapter 4 and 6 lead to suggest that co-grazing may offer options for controlling cPtb incidence. The results observed in deer herds co-grazed with sheep or beef cattle animals, agree with previous research in New Zealand, which observed similar positive and negative associations, respectively between CG and clinical incidence (Glossop et al., 2008; Verdugo et al., 2008). These observed effects could be related to differences in pathogenicity associated to MAP strains. Mackintosh et al. (2007), observed in experimentally infected red deer that MAP Type I was less virulent than Type II. Similarly, O'Brien et al. (2006) observed a lower infection rate and cell-mediated immune response in deer experimentally infected with MAP Type I.

8.2.3 Novel Bayesian models

The Bayesian mathematical model described in Chapter 3 was developed to handle multiple tests applied in series, in this case combining pooled faecal culture PFC (1 or 2

pools per 20 animals) with testing 20 individual animals by ELISA if PFCs were negative. The two advantages of this approach were that the model estimated test accuracy, adjusted for it and delivered unbiased estimates of so called ‘true’ herd or flock prevalence. The model developed in that chapter is a novel analytical technique that could be used in further epidemiological research as it allows more flexible sampling designs. Results generated by this Bayesian model were used to code analytical models in Chapter 4 and for parameterising the simulation model in Chapter 7. Specifically, HTP, TP, and herd level test performance (Hse and Hsp) information were included in the data analysis outlined in the Chapter 4, and TP estimates were used to calibrate transmission parameters in the two species mathematical simulation model described in Chapter 7.

Statistical analysis performed in Chapter 4 employed error-adjusted logistic and Poisson regression models using Bayesian inference. The Bayesian approach was chosen because it represents a flexible framework which allowed the consideration of lack of test accuracy into the analysis. Thus models performed in Chapter 4 included test results adjusted by Hse and Hsp estimates reported in Chapter 3. Those test performance estimates indicated that substantial bias in test results existed, which would have important distorting effects on regression model outputs if no adjustments had been made. This was evident when results from the adjusted regression models were compared with equivalent but unadjusted classical statistics regression models. Furthermore, the error-adjusted Poisson regression model outlined in Chapter 4 is also a novel analytical technique that could be easily applied to other diseases, increasing the available set of tools for epidemiological research. The novelty of this model is that beside the adjustment of test results, the count of cases reported could be adjusted by the

true infection status of the flock/herd, considering the true infection status of flocks/herds. Additionally, the model adjusts the count of cases by the ability of the farm manager to detect clinical cases in their respective flocks/herds (farmer diagnosis sensitivity).

In particular, data analysis in Chapter 4 considered successive co-grazing (same paddock different time) and concurrent co-grazing (same paddock same time) as equal risk factors, hence being merged into a single co-grazing category. This may be acceptable due to the long survival of MAP on soil (Rowe and Grant, 2006), thus presenting both co-grazing managements as a similar risk of MAP transmission. On the other hand, a point that could be improved for future analysis of co-grazing risk studies was the plain use of co-grazing information as a binary exposure variable. The models could be adapted to incorporate the MAP infection status of the co-grazed species. Earlier versions of the model presented in this chapter tried to incorporate such information. However, the amount of available data, hence statistical power, was insufficient for demonstrating meaningful patterns. The reason was that not all species mobs on the sampled farms were tested for Ptb. Thus exposure was more crudely categorized to retain a maximum of species mobs for analysis.

8.2.4 Association between MAP and production performance

The Chapter 5 described the association between flock/herd level disease status and production, measured in terms of clinical incidence, pregnancy, calving/weaning/tailing and culling rates. For data analysis purposes, flocks/herds were allocated to three categories depending of their disease status: non-infected, infected, and affected. This approach was chosen to differentiate sub-clinical and clinical disease effects on

productivity. In test positive flocks/herds, clinical cases compatible with clinical paratuberculosis (cPtB) were reported from 24% to 55% of flocks/herds with an annual clinical incidence (ACI) between 0.04 to 0.32 cases/100 animals, where beef cattle presented the lowest ACI and deer the highest. The combination of this information with the prevalence data gathered in Chapter 3 is clear evidence of a high MAP infection prevalence but a low clinical disease incidence. Hence, cPtB appears to be a relatively minor cause of wastage in these species, especially in beef cattle herds. Norton et al. (2009) reported an ACI of 0.31 cases/100 cow years in New Zealand dairy cows, being comparable with the ACI observed in deer. In the latter species, Glossop et al. (2008) reported an ACI around 1% in New Zealand. However, the herds in that study were not randomly selected; instead a voluntary enrollment was used leading to a likely bias towards herds with a relatively high clinical disease incidence.

Sub-clinical infection in beef cattle herds was significantly ($p < 0.05$) associated with lower calving rates than non-infected herds. Moreover, clinically affected sheep flocks presented with a significantly lower count of lambs at tailing ($p=0.05$), and deer herds presented with a trend to lower weaning rates ($p=0.09$). Lower calving rates have been reported previously in MAP infected dairy cattle herds (Marce et al., 2009; Smith et al., 2010). Conversely, associations between clinically affected flocks/herds and lower tailing or weaning rates, respectively, have not been previously reported in sheep or deer. A concern about the data in Chapter 5 is that production performance was measured at flock/herd level whereas their association with disease was assumed to be valid at animal level. However, if both production and disease had a common antecedent, for example 'poor management', low production as well as cPtB could have been the consequence of such antecedent cause and be independent of each other. Thus

these associations need to be confirmed at animal level through longitudinal monitoring if causation is to be concluded. As long as animal-level studies from pastoral livestock are not available, our current results may be used to inform economic models evaluating the effect of sub-clinical and cPtb in New Zealand sheep, beef cattle and deer but clearly stating the limitations of available data.

8.2.5 Sampling bias

Data analyzed in Chapters 3, 4, and 5 was collected in multiples stages as previously mentioned. The 24.3% response rate of postal survey could be considered low, thus a selection bias in the subsequent sample collection stage could not be ruled out. Specifically, the low response rate could bias the data set toward farms experiencing higher losses associated to cPtb, which probably were more prone to reply the survey. However, the questionnaire enquired simultaneously about Leptospirosis, which is widespread infection in New Zealand (Dorjee et al., 2008; Subharat et al., 2009; Heuer et al., 2010). Thus if such bias was present at all, it is expected to have been reduced by the inclusion of another disease in the questionnaire. Additionally, clinical cases were based on farm manager reports, so interview could be subject to potential recall bias and case misclassification because clinical symptoms of cPtb are similar to chronic wastage attributable to other causes such as parasite infestation and trace element deficiency. In the Poisson model, if bias was present, it was controlled by adjusting cases considering the truly infection stats of flocks or herds, in addition to assess the effect of different farmer sensitivities in model outputs. Similarly in Chapter 5, cPtb incidence estimation only included cases from farms that reported confirmed Ptb or those that were PFC or ELISA positive. It is expected that the potential misclassification bias present in the data set would have been reduced by such measures.

8.2.6 Mathematical simulation modeling

The Chapter 7 was the next step towards utilizing information generated by Chapters 3, 4 and 6 to answer questions about species interactions with regard to the spread of MAP between sheep and beef on mixed-species farms. Mathematical simulation of disease dynamics was used to address these questions adopting two published models, one for dairy cattle developed in the US (Mitchell et al., 2008) and a sheep model developed by our group in collaboration with Cornell University, USA (Marquetoux et al., 2012). The sheep model was based on New Zealand pastoral farming systems and parameterized from published transmission studies in Australia (Kurade et al., 2004; Dennis et al., 2011; Kawaji et al., 2011; McGregor et al., 2012). The dairy model was modified to fit New Zealand pastoral beef conditions. Transmission parameters for beef cattle were calibrated to render prevalence and incidence rates from the survey described in Chapters 3 and 5. The two models were then merged to allow the indirect transmission of MAP through joint grazing of the same pasture. This supported by current knowledge suggesting that pasture contamination is the primary source of MAP transmission within and between species (Sweeney, 2011). The majority of published mathematical Ptb models have assumed a direct (animal to animal) transmission (Collins and Morgan, 1991; Sergeant and Whittington, 2000; Groenendaal et al., 2002; Pouillot et al., 2004; Ezanno et al., 2005; Kudahl et al., 2007; Mitchell et al., 2008; Bennett et al., 2012). This may be a fair assumption under housed management, as typical of USA or Europe. Conversely, we believe the indirect transmission through pasture used in this model suits the typical New Zealand pastoral farming system.

The interest in Chapter 7 was to evaluate the conditions under which MAP would or would not be transmitted between species. Additionally, the effect of different CG times

and pasture contamination levels on prevalence and clinical disease were assessed. It is believed the model mimics MAP transmission in a typical sheep & beef farm in New Zealand because MAP infection prevalence and cPtb incidence was comparable to survey results of Chapters 3 and 5. To the author's knowledge, MAP transmission has previously not been simulated in two species simultaneously. Results indicated that when both species were negative, infection spread faster among sheep, reaching a higher prevalence than cattle, after the introduction of infected animals in the respective flock/herd. Moreover, co-grazing periods of two months per year were sufficient to transmit MAP from an infected to a naïve species resulting in a stable and lasting prevalence in the target species. Naïve sheep appeared to be more sensitive to MAP exposure from infected cattle than *vice versa*, agreeing with model assumptions based on field data. Co-grazing two infected species also had more profound effects on prevalence in cattle than sheep, where cattle experience a slightly higher prevalence increase than sheep, although the final equilibrium prevalence was always higher in sheep.

Common pasture spelling times (periods during which pasture was not grazed and allowed to re-grow) of one month was the default management in the baseline model. Spelling periods between 9 to 15 months were able to reduce the transmission risk to a naïve species. However, such long spelling periods are not economically feasible under New Zealand conditions, hence the model suggests that pasture spelling is not a recommendable measure for controlling Ptb. The objective of such simulation was to know the necessary resting time to stop the transmission cycle rather than to propose a control measure, as part of valid scientific curiosity. On the other hand, controlling Ptb in one of both species did not sufficiently reduce prevalence in the other, thus Ptb would re-emerge making single-species control ineffective. Hence, the results highlighted the

need to involve all susceptible species in a control program rather than the one testing positive or being clinically affected at a given time. The model can now be used to simulate several scenarios of pasture and animal management with or without controlling Ptb. Hence, a tool is available that could support the development of control strategies for Ptb in sheep and beef cattle, such as vaccination. For cost-benefit evaluation, the model could be extended to include production effects of Ptb and control cost to assess whether benefits from control exceed the cost associated with the disease.

8.3 Implications of thesis findings for control recommendations

In the light of the high HTP reported in this thesis, the pursuit of national eradication of Ptb is unlikely to be achievable. The lack of reliable diagnostics tools, multiple routes of transmission, long incubation periods, the interaction of multiple susceptible species, long survival of MAP at pasture level, and the potential maintenance and transmission of MAP by wildlife species present challenges for effective control. This view is supported by experience elsewhere. In a control program in Australia for example, 41 sheep farms were depopulated for 15 to 21 months and restocked with low risk animals. Three years after restocking, 28/41 flocks were MAP positive again (Taylor and Webster, 2005). In the Netherlands, despite of a long-term control campaign aiming to reduce prevalence in dairy herds, which began in 1952 with the introduction of a compensation subsidy for the culling of clinically suspected animals, and in 1983 a vaccination program was implemented, almost no reduction in prevalence has been achieved (Benedictus et al., 2000).

Considering the reported failures when eradication was the goal, it sounds more reasonable to focus on more achievable goals, such as the reduction of annual clinical disease incidence (ACI), which is considered the main source of economic losses associated with Ptb in sheep, deer, and beef cattle (Harris and Barletta, 2001; Bush et al., 2006; Glossop et al., 2008). However, the ACI appears to be extremely low in general, even though we might have underestimated it. It must be considered that only a part of infected herds were able to observe or reported clinical cases. This finding suggests that cPtb may not be a major contributor to animal wastage compared to other conditions like infertility or lameness. Thus, in the majority of Ptb infected farms, the cost of traditional control measures such as test-and-cull would likely be higher than the economic loss due to sub-clinical or cPtb. Moreover, robust studies linking risk factors and cPtb incidence are lacking. Differences in breed susceptibility or differential immune responses have been suggested as factors related to cPtb (Kurade et al., 2004; Morris et al., 2006; Norton, 2007). Immunosuppressive stress factors such as transport, social pressure or inadequate feeding management could also affect the ability of a susceptible or infected animal to resist or control the infection (Griffin and Thomson, 1998). However, no quantitative data has been presented assessing the link between those stress factors and clinical disease. Additionally, associations with more pathogenic MAP strains have been suggested on the grounds of experimental studies (Janagama et al., 2006; O'Brien et al., 2006; Gollnick et al., 2007).

Molecular strain-typing technology is relatively expensive, being prohibitive for its widespread or indiscriminate direct application on commercial farms. However, the continue development of this technology could represent an interesting tool in the future, where the identification of the specific MAP strain(s) involved at farm level infection, could be used as a predictor of the likely disease outcome at that specific

farm. Other molecular technique, such as PCR could be a rapid technique for the identification of high shedders, removing them quickly from the herd/flock, minimizing their contribution to MAP burden (Heuer et al., 2012). On the other hand, infectious dose is one of the currently known cPtb risk factors. Delgado et al. (2012) observed that the clinical fate of infected lambs was related to the infection dose, where animals exposed to higher doses were at greater risk to develop clinical disease. Furthermore, the present research has observed an association between CG and clinical disease. Such findings warrant further research and if validated as causative, could play a role in the control of clinical disease incidence.

The low disease impact perceived by most farm managers (Norton, 2007) could explain the reduced enrollment in voluntary control programs elsewhere (Raizman et al., 2006), and a similar response could be expected on New Zealand sheep, beef or deer farms, where an ACI below 0.4% was common for most farms reporting cases.

Nevertheless, a change in attitude towards Ptb control may in the future arise from public health concerns with regard to Crohn's disease. In recent years a growing number of publications addressed the association between MAP and Crohn's Disease (Gitlin et al., 2012; Robertson et al., 2012). However, a causal association has not yet been conclusively shown. But even in the absence of scientifically conclusive evidence, producers may have to consider that public health perceived concerns may lead to market access problems. The circulation of semi-scientific, alarming information through the mass media could trigger public concerns sufficient to lead to a decrease of the consumption of milk and meat or the demand for products to be 'MAP-free' certified. Thus, it may be advisable that producer associations are prepared for such scenarios and continue to promote further research of MAP, cPtb and their possible

consequences for public health. Two recent studies have been able to culture MAP from sheep skeletal muscle tissue (Reddacliff et al., 2010; Smith et al., 2011). However, there are not studies available, assessing the current level of animal-produce contamination or addressing the risk of human consumption of MAP from different animal products.

It was already pointed out, that despite probably low economic disease impacts in the population, there is a small number of farms experiencing high incidence cases and substantial economic loss. For this group, research addressing factor that trigger heavy economic losses is required, in addition to the development of specific control measures that are effective in such situations. Hence control must be farm specific, considering information like clinical incidence, increased premature culling associated with signs of therapy resistant wastage, and MAP strains, where more virulent strains could be predictor of future heavy losses. Currently available control measures in heavily affected farms are further discussed.

A control strategy should consider all susceptible species present at farm level, in a coordinated fashion because one species could act as a reservoir, being a constant source of infection, in spite of not being clinically affected, as the data suggest in the case of beef cattle, when co-grazed with sheep or/and deer. In particular, larger infectious doses are associated with higher cPtb incidence, thus the reduction of MAP challenge is a key element. Common recommendations like improved hygienic conditions or early calf separation from dam were proposed for intensive dairy farms, under housed conditions. The application of such measures is prohibitive (or impossible) under the seasonal pastoral management used in New Zealand. Additionally, MAP burden is commonly associated with general hygienic conditions of animal barns. However, this can not be applied to the New Zealand pastoral farming

situation. On the other hand, during pre-clinical and clinical stages a massive number of MAP organisms are shed daily into the environment. Simulation results (Chapter 7) suggest that the separation of species on mixed-species farms, together with an increased farmer surveillance to reduce the time that clinical animals stay in the herd/flock had a significant effect, reducing prevalence toward zero in the long term. Therefore, it is recommended in known infected farms, the immediate culling of animals presenting signs of wasting and diarrhoea, decreasing the bacterial burden. However, managers should be aware that other disease like parasitism or mineral deficiencies could trigger similar signs, thus a well planned sanitary and nutritional program are preconditions for the application of such control measure. In case that managers have doubts of the real condition of the suspected animals, they could be placed in species paddocks, where it could be used an inexpensive rapid serologic tests, such as ELISA, which presents reasonable sensitivity and specificity in clinical animals (Nielsen and Toft, 2008). Thus, suspected clinically affected animals could be rapidly identified and removed from the flock/herd.

Alternatively, evidence indicates that vaccination of susceptible animals is an effective control measure to reduce the onset of clinical cases, although MAP shedding is still present, albeit commonly reduced (Benedictus et al., 2000; Reddacliff et al., 2006; Stringer et al., 2011). A recent study suggest that the long-term use of vaccination as control measure, significantly reduce infection prevalence (Dhand et al., 2012). However, still too little is currently known about the cost-effectiveness of such a control measure, especially in the light of the long-term application and variable results observed (Dhand et al., 2012).

8.4 Future Work

Clinical paratuberculosis was originally described in 1894 by H. A. Johne and L. Frothingham (Harris and Barletta, 2001). Despite more than 100 years of cumulative research during which important advances have been achieved, there remain knowledge gaps in MAP epidemiology that preclude the assessment and design of sound control plans to effectively tackle MAP infection and/or clinical incidence. The following list outlines key areas where further epidemiological insight is required:

- **Better estimates of animal level prevalence** of latent infection, sub-clinical infection, pauci- and multi-bacillary shedding among infected pastoral flocks and herds in New Zealand. While some crude estimates of overall infection were available from Chapter 3, that study was designed to estimate group level, not animal level prevalence. Such information would be required for disease simulation and economic assessments, improving our understanding of disease dynamics and the financial feasibility of control. The sampling and testing activities undertaken in this thesis have provided a list of infected flocks/herds, which could be included in further studies.
- **Role of wildlife species in the epidemiology of MAP infection.** MAP has been isolated from wildlife species around clinically affected farms in New Zealand (Nugent et al., 2011). Hence, wildlife may be a reservoir for the contamination of the environment and could lead to (re-)infection of domestic livestock. However, it has not been demonstrated that wildlife are able to effectively transmit MAP to livestock. One way to explore the transmission hypothesis is to compare MAP strains isolated from the two populations. Additionally, a specific point that should be address is if the amount of MAP shed by wildlife species could constitute an infectious dose or not. A dataset is available from Chapter 6

that includes one or more MAP strains from sheep, deer, beef and/or dairy cattle of 162 survey farms. Wildlife present in a sample of these farms could be captured and sampled, and MAP isolates strain-typed and compared with the dataset. It is recommended to focus on high prevalence or heavily affected farms in order to increase the chances to obtain positive samples from the wildlife species present.

- **MAP strain specific virulence.** A number of studies including Chapter 4 found evidence about associations between phenotypic Ptb expression and host-pathogen interactions. These findings generated a virulence hypothesis of MAP strains within and between hosts which should be explored. MAP virulence could explain why some farms experience high incidence of clinical disease and others, with a similar level of infection, do not. It might also explain why beef cattle that were readily infected with MAP Type I (Chapters 6 and 7) experience almost no cPtb even though calf and dam spend up to six months together on pasture before weaning, thus under conditions where transmissions between shedding adults and susceptible young stock is highly likely. If a virulence difference existed among MAP strains, strain typing might constitute a new tool for informing control. Therefore, a comparison of MAP strains collected from latently infected, sub-clinically affected and clinically affected animals is a recommended approach to evaluate a virulence hypothesis.
- **Intervention threshold in affected farms.** Considering current available control tools, the incidence level at which intervention is economically justified has not been described. Where combination of control measures could have different economic thresholds. Such information could be investigated through mathematical modeling (Chapter 7) with the additional consideration of

economic effects. Results would be likely to support the development of a farm-by-farm approach.

- **Progression of MAP infection.** Only a small subset of infected animals develops clinical disease. The determinants for progression are currently unknown. They might include host, pathogen and environmental factors. Longitudinal studies over several years would be required to find those determinants, where some selected MAP infected farm could be followed during the study time, recording management practices and productive outputs, in addition to the serial collection of faecal and serum samples for the isolation of MAP and diagnosis of animals and their progression. Ideally, mixed-species farm could be targeted to be able to observe the effect of species interaction on infection/disease progression. Despite the relatively high cost and time requirements, the knowledge of disease determinants is crucial for developing effective tools and strategies for control.
- **Intervention studies for effective reduction of prevalence and incidence.** As proposed in Chapter 7, (co-)grazing management, test-and-cull and vaccination are options for control in heavily infected/affected farms, which have not been evaluated at a cost-benefit scale. This possible control option could be included in the longitudinal study proposed in the previous point.

8.5 Conclusion

This thesis has provided epidemiological insight on MAP infection and cPtb incidence on mixed-species farms, including sheep, beef cattle and/or deer, under seasonal pastoral management in New Zealand. The research has described epidemiological inferences not previously reported and has produced novel analytical tools, which can

be adopted for future research. MAP infection was widely spread at national level evidenced by high HTP estimates in sheep, deer and beef cattle. In contrast, cPtb incidence was very low. Hence a large proportion of infected flocks/herds experience minimal or non-detectable economic loss due to infection or disease, precluding the commitment to a national control program. Thus, control should depend on a farm specific risk assessment of heavily affected farms where intervention might be cost effective.

The co-grazing of two or more different species was associated with an increased risk of MAP infection. However, this increased infection risk was not always associated with a rise in clinical incidence. The co-grazing of sheep and deer was associated with a reduction of cPtb incidence in both species. Thus, co-grazing management appears to have potential merit for reducing clinical disease. Further research is therefore warranted to validate this finding. Notably, sheep and beef cattle and deer and beef cattle often shared the same strains when they were co-grazed. This was strong evidence for cross-species transmission. Conversely, sheep and deer harbored different MAP strains, suggesting that MAP transmission between them was unlikely or uncommon.

The simulation of MAP transmission between sheep and beef cattle, under different co-grazing regimes, supported the notion to involve all susceptible species in a farm level control program. The model described in Chapter 7 can be modified to include production effects and intervention cost in order to assess the cost-effectiveness of various disease and control scenarios. Thesis results contribute to decisions around justification of control strategies, and if justified, the development of control strategies, and indicate areas where further research is needed.

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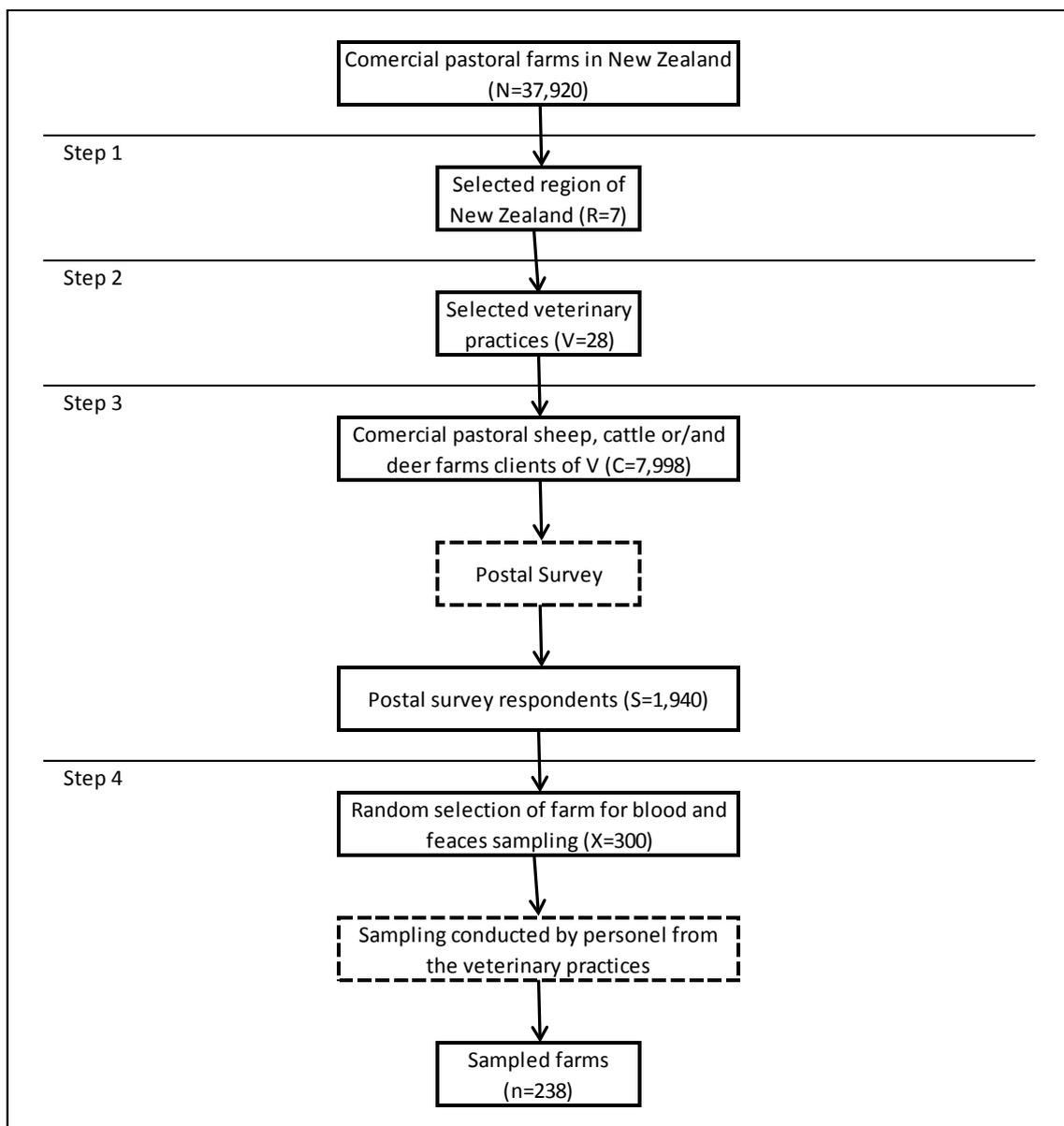
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Study design:

Farm selection and sampling protocols



The sampling protocol followed a multi-stage design involving a total of four main steps, which are schematically presented in the figure above. In the first step, priority New Zealand regions were selected, four in the North Island and three in the South Island (7 out of 11). In particular, the regions of Waikato, Wairarapa, Hawkes Bay, Manawatu-Wanganui (NI), Marlborough, Canterbury, and Southland (SI) were chosen on the basis of their concentration of ruminant livestock, in addition to represent different agro-ecological zones typical of New Zealand. It was assumed that the selected regions are representative of the entire country.

In each region, the biggest (in terms of number of clients and area serviced) large animal veterinary practices ($V=28$) were identified. All identified veterinary practices agreed to participate in the present study. An agreement of understanding was signed, where the participation of each party in the present study was defined. In particular, the veterinary practices handled their client databases and accepted to perform sampling activities to a preferential rate. In retribution, laboratory test results were compiled and submitted to their respective veterinary practice, previous written acceptance from the farm manager. The third step involved the cleaning and screening of the databases provided by the veterinary practice. Duplicated client entries were deleted and farms having less than 40 cattle/deer or less than 400 sheep were excluded from the study. The population size cut-off was included in order to focus on commercial farms, excluding lifestyle farms that potentially could also seek veterinary services. This process resulted in the selection of 7,998 farms, which represented the surveyed population (C). To each farm present in this population, a postal survey was sent enquiring information about: demographics, grazing management, production outputs, and previous history of paratuberculosis and leptospirosis in each of the ruminant species present. The questionnaires were posted in December 2008, and they were received back until March 2009.

A total of 1,940 correctly filled surveys were received back (24.3% response rate), and this population represented the sampling frame (S) for the next step. A total of 300 farms (X) were randomly selected for blood and faeces sampling. An equal number of farms, across seven farm type categories, were targeted. In particular, the seven categories represent all possible combinations of the three livestock species under study, being: sheep only (SHP), beef only (BEE), deer only (DEE), sheep & beef (S&B), sheep and deer (S&D), beef and deer (B&D), and sheep, beef and deer (SBD).

Finally, the last step involved the sampling of the randomly selected farms. Sampling activities were directly conducted by the veterinary practices. A standard sampling protocol was followed, where 20 animals were randomly selected from each species mob present, targeting mixed age ewes (two years and older), mixed-age beef cows, and 12 - 24 month-old deer (either sex). The randomness was achieved through racing the mob across the paddock, in order to mix them, and then, the first 20 animals were selected. From each animal, pair of blood and faeces samples were obtained and submitted to Massey University for processing. The processing activities involved the development of serum and faeces bank, where collected samples are currently stored at -80C. Additionally, faecal samples were pooled, a single pool from sheep flocks (20 samples/pool), and two pools from beef cattle or deer herds (10 samples/pool), and submitted for culture to a specialized laboratory (AgResearch, Wallaceville). If pool faecal culture rendered a negative test results, individual serum samples were submitted to New Zealand Vet Pathology lab in Palmerton North for ELISA testing (sheep and beef) or to the Disease Research Laboratory (DRL) at Otago University for Paralisa™ testing (deer). In addition to the 20 normal animals sampled, up to five extra animals (suspected animals), per each species, were sampled if signs of paratuberculosis (diarrhoea, emaciation, etc) were observed in them by the veterinarian, at the sampling

date. Samples from suspected animals were analysed separated from the sampled collected from normal animals but tested following the same protocol previously described.

A total of 238 farms were sampled (n), the remaining 62 farms were not sampled for several reason, such as animal crushes not being available (6), species mobs being inaccessible (17), or losses to follow-up, during sampling period, following initial farm enrolment (39). The sampled farms represent 162 sheep flocks, 116 beef cattle herds and 99 deer herds, comprehending 7,579 animals.

ANNEX B

**Research into Johne's Disease and Leptospirosis
in New Zealand**

Farm health and production Survey

Research into Johne's Disease and Leptospirosis in New Zealand

Farm health and production Survey



“Help to control Johne's disease and Leptospirosis, and reduce the risk of Leptospirosis among farmers, their families and workers”

Your Support is Vital for Research into Johne's Disease and Leptospirosis in New Zealand

Please complete and return this questionnaire - your information will help understanding of Johne's disease and Leptospirosis, development of control methods to improve animal health and farm productivity, and reduce the risk of Leptospirosis in farmers, their families, workers and others. We appreciate that you are probably busy and that it can be hard to find the time to complete a survey. However, no matter how busy we might be, most of us still stop for a tea break now and again. I'd like you to have your next tea break on us, and I hope that while you're enjoying this, you might find time to complete and return this questionnaire.

Confidentiality

All information will be held strictly confidential by the researchers. No information will be released that is in any way identifiable to individual farms, owners or personnel.

This survey

This questionnaire requests information about health and productive performance of livestock. It also asks questions specifically about Johne's disease and Leptospirosis. The questionnaire has been sent to all farmers registered as clients of veterinary practices in Wairarapa, Hawkes Bay, Manawatu-Wanganui, Canterbury and Southland. The funding contribution by the C. Alma Baker Trust is acknowledged.

ALL RECIPIENTS ARE ASKED TO COMPLETE THIS QUESTIONNAIRE.

CONSENT REQUEST

We also seek participants for further research starting in 2009 involving collection of more detailed information along with faecal and blood samples from about 20 animals of each species on selected farms to get a better understanding of JD (funded by Livestock Industries and FRST via the Johne's Disease Research Consortium), and Leptospirosis (funded by Rural Women NZ and Industry).

Note: Full information will be provided before final commitment. Participation will include permitting access to information from the **Animal Health Board** (cattle and deer), and **Johne's Management Limited** and **Disease Research Laboratory** databases (deer only).

All sampling and testing will be free of charge and results will be provided.

I am prepared to consider participation in further research (circle): YES NO

Contacts: A/Prof Cord Heuer, Epicentre, (06) 350 5948 (Email: c.heuer@massey.ac.nz); Prof Peter Wilson (Email: p.r.wilson@massey.ac.nz), Institute of Veterinary, Animal & Biomedical Sciences, Massey University, PB 11222, Palmerston North. Ph (06) 3569099 ext 7619

PLEASE RETURN (in the reply paid envelope) TO: Cristobal Verdugo, Epicentre, (06) 350 5903 (Email: c.verdugo@massey.ac.nz) Private Bag 11 222, IVABS, Massey University, Palmerston North.

The survey has 10 parts:

- 1 - 5: General information on your farm.
- 6 - 9: Specific information about deer, sheep, and cattle; please fill those that apply to your farm.
- 10: Source of data.

1. Contact details

- 1.1 Property name: _____
- 1.2 AHB (Animal Health Board) number: _____ Dairy No.: _____
- 1.3 Contact person: _____
The contact person is (please circle): *Owner / Manager / Both*
- 1.4 Veterinary or vet-practice commonly used (name): _____
- 1.5 Postal address: _____
- 1.6 Farm address (if different): _____
- 1.7 District: _____ Region: _____
- 1.8 Phone (home): _____ Phone (business, if different): _____
- 1.9 Fax: _____ Mobile: _____
- 1.10 E-mail: _____

2. Livestock

- 2.1 Please describe your **farm type** (Tick more than one as appropriate) and the number of **livestock wintered in June 2008.**

Species	Farm activity (tick as many apply)	Class	Number wintered
Deer	<input type="checkbox"/> Breeding	Calves/weaners (0-12 months)	
	<input type="checkbox"/> Finishing	Yearlings (12-24 months)	
	<input type="checkbox"/> Velvet	Adults hinds (24+ months)	
		Adults stags (24+ months)	
Sheep	<input type="checkbox"/> Breeding	Hoggets	
	<input type="checkbox"/> Finishing	Ewe 2-tooths	
	<input type="checkbox"/> Wool	Ewes (Mixed age)	
		Rams	
Beef Cattle	<input type="checkbox"/> Breeding	Calves (0-6 months, both sexes)	
	<input type="checkbox"/> Finishing	Yearlings (6-24 months)	
	<input type="checkbox"/> Stud farm	Cows (24+ months)	
		Bulls/steers (24+ months)	
Dairy Cattle	<input type="checkbox"/> Milking	Calves (0-6 months)	
	<input type="checkbox"/> Heifer rearing/grazing	Heifers (6-24 months)	
	<input type="checkbox"/> Dry stock	Cows (24+ months)	
		Bulls/steers (24+ months)	
Goat		Any age/sex	
Alpaca/llama		Any age/sex	
Pig		Any age/sex	

- 2.2 Please estimate the average number of workers in **regular contact with livestock on-farm**, in 2008. Include farmer, family and employed workers. _____ (Include part-timers, e.g. 3½).

3. Land use and topography

- 3.1 Total farm area _____ acres / ha (please circle)
- 3.2 Effective farm area* _____ acres / ha (please circle)
* Grazable by livestock incl. fodder crops
- 3.3 How much of your effective area do you use for cash crops? _____ acres / ha (please circle)
- 3.4 Please **rank the net cash surplus** for each enterprise, **"1" being the most profitable**. If an enterprise is not present, please enter "0".

Deer	Sheep	Beef cattle	Dairy cattle	Other
Example: 2	0	1	0	3

4. Leptospirosis and Johne's disease

- 4.1 Have you heard of the disease called "Johne's disease"?
- YES NO
- 4.2 Have you heard of the disease called "Leptospirosis"?
- YES NO
- 4.3 Has Leptospirosis ever been diagnosed or suspected in yourself, family members or farm workers?
- YES NO

If YES, please tick and complete the appropriate boxes in the table below:

Leptospirosis cases	Number of cases	Doctor or laboratory confirmed	Suspected* (but not confirmed)	Year
You personally				
Family members				
Workers				

*Signs of Leptospirosis are severe flu-like, but without nasal discharge or cough. They include fever, headache, sensitivity against light, chills, muscle aches, vomiting, and may include dark urine, red eyes, abdominal pain, diarrhoea, and/or a rash.

- 4.4 Have your farm or pet dogs been vaccinated against Leptospirosis in 2008?
- YES NO Don't know
- 4.5 How many dogs were on this property 2008 (including farm and pet)? _____

5. Grazing pasture management

5.1 Have different livestock species shared pasture, either co-grazed (same paddock, same time) or alternately grazed (same paddock, different time) in the last 12 months?

YES

NO

5.2 If YES, please complete the table below (see examples).

	Grazing pattern	Deer	Sheep	Beef cattle	Dairy
Example 1 Deer	Co-grazed		Deer yearlings with MA ewes		
	Alternately grazed			Deer yearlings follow cows	
Example 2 Beef cattle	Co-grazed		calves with hoggets		
	Alternately grazed	Beef yearlings follow adult hinds			
Deer	Co-grazed				
	Alternately grazed				
Sheep	Co-grazed				
	Alternately grazed				
Beef cattle	Co-grazed				
	Alternately grazed				
Dairy Cattle	Co-grazed				
	Alternately grazed				

The next sections relate to each species: please complete as appropriate.

6.8 Have any of your deer **been diagnosed with Leptospirosis** by your veterinarian and/or through laboratory testing of blood, over the past **3 years**?

YES NO

6.9 If **YES**, or you have **suspected cases of Leptospirosis**, please list your observations in the table below: *Suspected = you believed it was Leptospirosis based on blood stained urine (red water), multiple sudden deaths, often with jaundice, but not confirmed by veterinarian or laboratory.*

Month(s)	Year (2005-8 only)	Age range	Number of cases	Briefly describe the cases	Confirmed by Veterinarian or Lab.-Test? (tick as many apply)		
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
Example June	2005	< 0.5 yrs	7	7 weaners found dead, no signs of disease seen prior	No <input type="checkbox"/>	Vet <input checked="" type="checkbox"/>	Lab <input checked="" type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>

6.10 Were any deer vaccinated against **Leptospirosis** in 2008?

YES NO

If "YES", please provide information about the vaccination protocol.

Deer class		Vaccine name (if known)*	Number of times vaccinated	If more than once, give time interval
Example:		Leptavoid® 3	2	4-6 weeks.
Weaners	Hinds			
	Stags			
Yearlings	Hinds			
	Stags			
Adults	Hinds			
	Stags			

*Note: The 7 in 1 vaccine includes Leptospirosis.

7 Sheep (If you do not have sheep, go to section 8).

7.1 Which is the predominant sheep breed present on your farm? _____

7.2 Please report reproduction results for 2008

Ewe Stock Class	Number of ewes mated	Rams dates		Number scanned pregnant		Number of pregnant ewes wintered	Number of lambs at docking / tailing
		"IN"	"OUT"	Single	Twins / Triplets		
Hogget							
2-Tooth							
Mixed age							

7.3 Have you observed or suspected any ewes' abortion since mating? Yes / No

If **Yes**, how many aborted? _____ Diagnosis? _____ Vet confirmation Yes / No

7.4 Please report the number of sheep that had each fate since mating (2008) until December 15, 2008 (If not known exactly, please give your best estimate).

Stock Class	Number sold/culled		Number dead or lost		Number purchased	
	Wet	Dry	Wet	Dry	Wet	Dry
Hogget						
2-Tooth						
Mixed age (MA)						

7.5 Please enter the number of **sheep that died on farm** (any reason) in the last 12 months, or if not known, give an estimate of mortality % (see detail under the table below).

	Lambs*	Hogget**	2-Tooth**	Mixed age**	
				Ewes	Rams
Number dead					
Estimated mortality %					

*Number dead lambs (on farm)/ original number born in the last season.

**Number of dead in the last 12 months / the original number 12 months ago.

7.6 Were any sheep vaccinated against **Johne's disease** in the last 12 months? Yes / No

If **"YES"**, please provide information about the vaccination protocol.

Sheep class	Vaccine name (if known)*	Number of times vaccinated	If more than once, give time interval
Example:	Gudair®	1	NA
Lambs			
Hoggets			
Ewe 2-tooths			
Ewes (Mixed age)			
Rams			

7.7 Have any of your sheep **been diagnosed with Johne's disease** by your veterinarian and/or through laboratory testing of blood/faeces/tissue, over the past **3 years**? Yes / No

7.8 If **YES**, or you have **suspected cases of Johne's disease**, please list your observations in the table: *Suspected = you believe it was JD based on severe wasting in animals that does not respond to treatment, sometimes wool break/poor fleece, ending in death and/or a small, distinct "tail" to the mob, but not confirmed by veterinarian or laboratory.*

Month(s)	Year (2005-8 only)	Age range	Number of cases	Confirmed by Veterinarian or Lab.-Test? (tick as many apply)		
Example: July	2007	3-4 yrs	3	No <input type="checkbox"/>	Vet <input checked="" type="checkbox"/>	Lab <input checked="" type="checkbox"/>
				No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
				No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
				No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
				No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
				No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
				No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>

7.9 Have any of your sheep **been diagnosed with Leptospirosis** by your veterinarian and/or through laboratory testing of blood, over the past **3 years**? Yes / No

7.10 If **YES** or you have **suspected cases of Leptospirosis**, please list your observations in the table: *Suspected = you believed it was Leptospirosis based on blood stained urine (red water) or multiple sudden deaths often with jaundice but not confirmed by veterinarian or laboratory.*

Month(s)	Year (2005-8 only)	Age range	Number of cases	Briefly describe the cases	Confirmed by Veterinarian or Lab.-Test? (tick as many apply)		
Example October	2007	2-3 yrs	20	Ewes presented abortion and red water, 10 ewes died.	No <input type="checkbox"/>	Vet <input checked="" type="checkbox"/>	Lab <input checked="" type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>

7.11 Were any sheep vaccinated against **Leptospirosis** in 2008? Yes / No

If "YES", please provide information about the vaccination protocol.

Sheep class	Vaccine name (if known)*	Number of times vaccinated	If more than once, give time interval?
Example:	Leptavoid® 2	2	4 to 6 weeks.
Lambs			
Hoggets			
Ewe 2-tooths			
Ewes (Mixed age)			
Rams			

*Note: The 7 in 1 vaccine includes Leptospirosis.

8 Beef cattle (if you do not have beef cattle, go to section 9)

8.1 Please report reproduction results for 2008.

Stock Class	Number mated (2007 – 08)	Bull dates (2007-08)		Number scanned or vetted pregnant	Number pregnant wintered (2008)	Number of pregnant calving on-farm	Number of progeny born (spring 2008)
		IN	OUT				
15mo heifers							
27mo heifers							
MA cows							

8.2 If accurate numbers are not available, what was the estimated calving rate (%) across age groups this year (no.calved /no.pregnant)? _____

8.3 Have you observed or suspected any heifer/cow aborting? Yes / No
If **Yes**, how many aborted? _____ diagnosis? _____ Vet confirmation Yes / No

8.4 What was the number of calves marked from this year's (2008) calving season? _____
Not available yet

8.5 Please report the number of beef cattle that had each fate since mating (2007-08) until December 15, 2008 (If not known exactly, please give your best estimate).

Stock Class	Number sold / culled		Number dead / lost		Number purchased	
	Wet	Dry	Wet	Dry	Wet	Dry
Calves						
15 month heifers						
27 months heifers						
Mixed age cows						

8.6 Please enter the number of **beef cattle that died on farm** (any reason) in the last 12 months, or if not known, give an estimate of mortality % (see detail under the table below).

	15 month heifers*	27 months heifers*	Mixed age*	
			Cows	Bulls
Number dead				
Estimated mortality %				

*It is number dead (on farm)/original number present 12 months ago.

8.7 Were any beef cattle vaccinated against **Johne's disease** in the last 12 months? Yes / No
If **"YES"**, please provide information about the vaccination protocol.

Beef cattle class	Vaccine name (if known)	Number of times vaccinated	If more than once, give time interval?
Example:	Gudair ®	1	NA.
Calves			
Yearlings			
Cows			
Bulls/steers			

8.8 Have any of your beef cattle **been diagnosed with Johne's disease** by your veterinarian and/or through laboratory testing of blood/faeces/tissue, over the past **3 years**? Yes / No

8.9 If **YES**, or you have **suspected cases of Johne's disease**, please list your observations below: *Suspected = you believe it was JD based on chronic diarrhoea and weight loss that does not respond to treatment, sometimes a 'bottle jaw and not confirmed by veterinarian or laboratory.*

Month(s)	Year (2005-08 only)	Age range	Number of cases	Confirmed by Veterinarian or Lab.-Test? (tick as many apply)		
Example: October	2006	3-4 yrs	2	No <input checked="" type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
				No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
				No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
				No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
				No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
				No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
				No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>

8.10 Have any of your beef cattle **been diagnosed with Leptospirosis** by your veterinarian and/or through laboratory testing of blood, over the past **3 years**? Yes / No

8.11 If **YES** or you have **suspected cases of Leptospirosis**, please list your observations below: *Suspected = you believed it was Leptospirosis based on blood stained urine (red water) or multiple sudden deaths often with jaundice but not confirmed by veterinarian or laboratory.*

Month(s)	Year (2005-8 only)	Age range	Number of cases	Briefly describe the cases	Confirmed by Veterinarian or Lab.-Test? (tick as many apply)		
Example June	2007	0.5-1 yr	5	Red water was observed in five yearlings	No <input type="checkbox"/>	Vet <input checked="" type="checkbox"/>	Lab <input type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>
					No <input type="checkbox"/>	Vet <input type="checkbox"/>	Lab <input type="checkbox"/>

8.12 Were any beef cattle vaccinated against **Leptospirosis** in 2008? Yes / No

If "YES", please provide information about the vaccination protocol.

Beef cattle class	Vaccine name (if known)	Number of times vaccinated	If more than once, give time interval?
Example	7-in-1	1	NA
Calves			
Yearlings			
Cows			
Bulls/steers			

9 Dairy cattle including heifer rearing/grazing (If you do not have dairy cattle, go to section 10)

9.1 Please report 2007-2008 reproduction results

Stock Class	Number mated (spring 2007)	Number diagnosed pregnant (2007-08)	Empty rate (%)	Number diagnosed pregnant wintered (2008)	Number diagnosed pregnant that calved (2008)
Heifers					
Cows					

9.2 Please report the number of dairy cattle that had each fate since mating until December 15, 2008 (If not known exactly, please give your best estimate).

Stock Class	Number sold / culled		Number dead / lost		Number purchased	
	Wet	Dry	Wet	Dry	Wet	Dry
Heifers						
Cows						

9.3 Please state the fate of calves born in 2008.

	Reared heifers	Reared bulls	Bobbed	Died	Sold/culled	Unknown
Number						

9.4 Please enter the number of **dairy cattle that died on farm** (any reason) in the last 12 months, or if not known, give an estimate of mortality % (see detail under the table below).

	15 month heifers*	27 months heifers*	Mixed age*	
			Cows	Bulls
Number dead				
Estimated mortality %				

*It is number of dead (on farm)/original number present 12 months ago.

9.5 If you also rear/graze heifers commercially, please provide the following:

Number of heifers reared during 2008	
Number of client farms <u>from</u> which heifers were received during 2008	

9.6 Were any dairy cattle vaccinated against Johne's disease in the last 12 months? Yes / No

If "YES", please provide information about the vaccination protocol.

Dairy cattle class	Vaccine name (if known)	Number of times vaccinated	If more than once, give time interval?
Example	Gudair ®	1	NA.
Calves			
Yearlings			
Cows			
Bulls/steers			

9.11 Were any dairy cattle vaccinated against **Leptospirosis** in 2008?

Yes / No

If **“YES”**, please provide information about the vaccination protocol.

Dairy cattle class	Vaccine name (if known)	Number of times vaccinated	If more than once, give time interval?
Calves			
Yearlings			
Cows			
Bulls/steers			
Example:	7-in-1	2	6 weeks after first dose.

10 The information provided **in this questionnaire** was based on (just tick one):

- Written records of farm data
- Memory
- Mostly memory + a few recorded data
- Mostly recorded data + memory

Thank you for your time !

Comments (Especially about this survey, JD or Leptospirosis):

WinBUGS code:

Model for the estimation of herd level true prevalence

Prevalence estimation in a sequential design, using pool faecal culture (PFC) test 1), and ELISA testing (test 2) for Paratuberculosis diagnosis.

NOTE: a positive herd is PFC or ELISA positive, and a negative herd is PFC negative AND ELISA test negative.

Probability estimation

p[1] probability of first test positive

p[2] probability of first test negative AND second test positive

p[3] probability of first test negative AND second test NEGATIVE

where: PFC = test 1 and ELISA = test 2. tau is between herd prevalence (proportion of infected herd in the population), and pi is the within herd prevalence (individual level prevalence). North Island =[1], South Island =[2]

ONE POOL PER HERD:

model{

z1[1:3] ~ dmulti(p1[], n1)# Only Sheep NI

#Two states: 1. true positive sample (ps) and 2. true negative sample
#(ns):

#prob of -ve sample given +ve herd

Pns1.ph<-pow(1-pi[1], ni)

Pps1.ph<- 1-Pns1.ph

Pns1<- (1-tau[1]) +tau[1]*Pns1.ph #prob for state 1

Pps1<- 1 - Pns1 #prob for state 2

#Prob of Elisa -ve/+ve (Pne/Ppe) for each state ("." means "given"):

Pne1.ns<-pow(sp2, ni)

Ppe1.ns<- 1 - Pne1.ns

#prob of apparent Elisa -ve per #sampled individual

Pan1 <- (1-se2)*pi[1]+sp2*(1-pi[1])

#prob all sampled are Elisa -ve (given +ve herd)

Pne1 <- pow(Pan1, ni)

```

#using law of total # probability
Pne1.ps <- (Pne1 - Pne1.ns*Pns1.ph)/Pps1.ph
Ppe1.ps<-1 - Pne1.ps

#Prob of FC -ve/+ve for each state:
Pnf1.ns<-Hsp1[1]
Ppf1.ns<-1 - Hsp1[1]

Ppf1<-tau[1]*Hse1[1]+(1-tau[1])*(1-Hsp1[1])
Ppf1.ps<-(Ppf1-Ppf1.ns*Pns1)/Pps1
Pnf1.ps<-1-Ppf1.ps

#Now put it all together:
p1[1] <- Pns1*Ppf1.ns + Pps1*Ppf1.ps
p1[2] <- Pns1*Pnf1.ns*Ppe1.ns + Pps1*Pnf1.ps*Ppe1.ps
p1[3] <- 1 - p1[1] - p1[2]

#Sheep + Beef NI
z2[1:3] ~ dmulti(p2[], n2)

Pns2.ph<-pow(1-pi[2], ni) #prob of -ve sample given +ve herd
Pps2.ph<- 1-Pns2.ph
Pns2<- (1-tau[2]) +tau[2]*Pns2.ph #prob for state 1
Pps2<- 1 - Pns2 #prob for state 2

Pne2.ns<-pow(sp2, ni)
Ppe2.ns<- 1 - Pne2.ns

#prob of apparent Elisa -ve per individual
Pan2 <- (1-se2)*pi[2]+sp2*(1-pi[2])

#prob all sampled are Elisa -ve (given +ve herd)
Pne2 <- pow(Pan2, ni)

#using law of total probability
Pne2.ps <- (Pne2 - Pne2.ns*Pns2.ph)/Pps2.ph
Ppe2.ps<-1 - Pne2.ps

Pnf2.ns<-Hsp1[2]
Ppf2.ns<-1 - Hsp1[2]

Ppf2<-tau[2]*Hse1[2]+(1-tau[2])*(1-Hsp1[2])
Ppf2.ps<-(Ppf2-Ppf2.ns*Pns2)/Pps2
Pnf2.ps<-1-Ppf2.ps

p2[1] <- Pns2*Ppf2.ns + Pps2*Ppf2.ps
p2[2] <- Pns2*Pnf2.ns*Ppe2.ns + Pps2*Pnf2.ps*Ppe2.ps
p2[3] <- 1 - p2[1] - p2[2]

z3[1:3] ~ dmulti(p3[], n3)# Sheep + Deer NI

#prob of -ve sample given +ve herd
Pns3.ph<-pow(1-pi[3], ni)
Pps3.ph<- 1-Pns3.ph

Pns3<- (1-tau[3]) +tau[3]*Pns3.ph #prob for state 1
Pps3<- 1 - Pns3 #prob for state 2

```

```

Pne3.ns<-pow(sp2, ni)
Ppe3.ns<- 1 - Pne3.ns

#prob of apparent Elisa -ve per #sampled individual
Pan3 <- (1-se2)*pi[3]+sp2*(1-pi[3])

#prob all sampled are Elisa -ve (given +ve herd)
Pne3 <- pow(Pan3, ni)

#using law of total # probability
Pne3.ps <- (Pne3 - Pne3.ns*Pns3.ph)/Pps3.ph
Ppe3.ps<-1 - Pne3.ps

Pnf3.ns<-Hsp1[3]
Ppf3.ns<-1 - Hsp1[3]

Ppf3<-tau[3]*Hse1[3]+(1-tau[3])*(1-Hsp1[3])
Ppf3.ps<-(Ppf3-Ppf3.ns*Pns3)/Pps3
Pnf3.ps<-1-Ppf3.ps

#Now put it all together:
p3[1] <- Pns3*Ppf3.ns + Pps3*Ppf3.ps
p3[2] <- Pns3*Pnf3.ns*Ppe3.ns + Pps3*Pnf3.ps*Ppe3.ps
p3[3] <- 1 - p3[1] - p3[2]

#Sheep + Beef + Deer NI
z4[1:3] ~ dmulti(p4[], n4)

#prob of -ve sample given +ve herd
Pns4.ph<-pow(1-pi[4], ni)
Pps4.ph<- 1-Pns4.ph
Pns4<- (1-tau[4]) +tau[4]*Pns4.ph #prob for state 1
Pps4<- 1 - Pns4 #prob for state 2

Pne4.ns<-pow(sp2, ni)
Ppe4.ns<- 1 - Pne4.ns

#prob of apparent Elisa -ve per individual
Pan4 <- (1-se2)*pi[4]+sp2*(1-pi[4])

#prob all sampled are Elisa -ve (given +ve herd)
Pne4 <- pow(Pan4, ni)

#using law of total probability
Pne4.ps <- (Pne4 - Pne4.ns*Pns4.ph)/Pps4.ph
Ppe4.ps<-1 - Pne4.ps

Pnf4.ns<-Hsp1[4]
Ppf4.ns<-1 - Hsp1[4]

Ppf4<-tau[4]*Hse1[4]+(1-tau[4])*(1-Hsp1[4])
Ppf4.ps<-(Ppf4-Ppf4.ns*Pns4)/Pps4
Pnf4.ps<-1-Ppf4.ps

p4[1] <- Pns4*Ppf4.ns + Pps4*Ppf4.ps
p4[2] <- Pns4*Pnf4.ns*Ppe4.ns + Pps4*Pnf4.ps*Ppe4.ps
p4[3] <- 1 - p4[1] - p4[2]

```

```

z5[1:3] ~ dmulti(p5[], n5) # Only Sheep SI

#prob of -ve sample given +ve herd
Pns5.ph<-pow(1-pi[5], ni)
Pps5.ph<- 1-Pns5.ph
Pns5<- (1-tau[5]) +tau[5]*Pns5.ph      #prob for state 1
Pps5<- 1 - Pns5                        #prob for state 2

Pne5.ns<-pow(sp2, ni)
Ppe5.ns<- 1 - Pne5.ns

#prob of apparent Elisa -ve per #sampled individual
Pan5 <- (1-se2)*pi[5]+sp2*(1-pi[5])

#prob all sampled are Elisa -ve (given +ve herd)
Pne5 <- pow(Pan5, ni)

#using law of total # probability
Pne5.ps <- (Pne5 - Pns5.ph)/Pps5.ph
Ppe5.ps<-1 - Pns5.ph

#Prob of FC -ve/+ve for each state:
Pnf5.ns<-Hsp1[5]
Ppf5.ns<-1 - Hsp1[5]

Ppf5<-tau[5]*Hse1[5]+(1-tau[5])*(1-Hsp1[5])
Ppf5.ps<-(Ppf5-Ppf5.ns*Pns5)/Pps5
Pnf5.ps<-1-Ppf5.ps

#Now put it all together:
p5[1] <- Pns5*Ppf5.ns + Pps5*Ppf5.ps
p5[2] <- Pns5*Pnf5.ns*Ppe5.ns + Pps5*Pnf5.ps*Ppe5.ps
p5[3] <- 1 - p5[1] - p5[2]

#Sheep + Beef SI
z6[1:3] ~ dmulti(p6[], n6)

#prob of -ve sample given +ve herd
Pns6.ph<-pow(1-pi[6], ni)
Pps6.ph<- 1-Pns6.ph
Pns6<- (1-tau[6]) +tau[6]*Pns6.ph      #prob for state 1
Pps6<- 1 - Pns6                        #prob for state 2

Pne6.ns<-pow(sp2, ni)
Ppe6.ns<- 1 - Pne6.ns

#prob of apparent Elisa -ve per individual
Pan6 <- (1-se2)*pi[6]+sp2*(1-pi[6])

#prob all sampled are Elisa -ve (given +ve herd)
Pne6 <- pow(Pan6, ni)

#using law of total probability
Pne6.ps <- (Pne6 - Pns6.ph)/Pps6.ph
Ppe6.ps<-1 - Pns6.ph

Pnf6.ns<-Hsp1[6]
Ppf6.ns<-1 - Hsp1[6]

Ppf6<-tau[6]*Hse1[6]+(1-tau[6])*(1-Hsp1[6])

```

```

Ppf6.ps<-(Ppf6-Ppf6.ns*Pns6)/Pps6
Pnf6.ps<-1-Ppf6.ps

p6[1] <- Pns6*Ppf6.ns + Pps6*Ppf6.ps
p6[2] <- Pns6*Pnf6.ns*Ppe6.ns + Pps6*Pnf6.ps*Ppe6.ps
p6[3] <- 1 - p6[1] - p6[2]

z7[1:3] ~ dmulti(p7[], n7)# Sheep + Deer SI

#prob of -ve sample given +ve herd
Pns7.ph<-pow(1-pi[7], ni)
Pps7.ph<- 1-Pns7.ph
Pns7<- (1-tau[7]) +tau[7]*Pns7.ph #prob for state 1
Pps7<- 1 - Pns7 #prob for state 2

Pne7.ns<-pow(sp2, ni)
Ppe7.ns<- 1 - Pns7.ns

#prob of apparent Elisa -ve per #sampled individual
Pan7 <- (1-se2)*pi[7]+sp2*(1-pi[7])

#prob all sampled are Elisa -ve (given +ve herd)
Pne7 <- pow(Pan7, ni)

#using law of total # probability
Pne7.ps <- (Pne7 - Pns7.ns*Pns7.ph)/Pps7.ph
Ppe7.ps<-1 - Pns7.ps

Pnf7.ns<-Hsp1[7]
Ppf7.ns<-1 - Hsp1[7]

Ppf7<-tau[7]*Hse1[7]+(1-tau[7])*(1-Hsp1[7])
Ppf7.ps<-(Ppf7-Ppf7.ns*Pns7)/Pps7
Pnf7.ps<-1-Ppf7.ps

#Now put it all together:
p7[1] <- Pns7*Ppf7.ns + Pps7*Ppf7.ps
p7[2] <- Pns7*Pnf7.ns*Ppe7.ns + Pps7*Pnf7.ps*Ppe7.ps
p7[3] <- 1 - p7[1] - p7[2]

#Sheep + Beef + Deer NI
z8[1:3] ~ dmulti(p8[], n8)

#prob of -ve sample given +ve herd
Pns8.ph<-pow(1-pi[8], ni)
Pps8.ph<- 1-Pns8.ph

Pns8<- (1-tau[8]) +tau[8]*Pns8.ph #prob for state 1
Pps8<- 1 - Pns8 #prob for state 2

Pne8.ns<-pow(sp2, ni)
Ppe8.ns<- 1 - Pns8.ns

#prob of apparent Elisa -ve per individual
Pan8 <- (1-se2)*pi[8]+sp2*(1-pi[8])

#prob all sampled are Elisa -ve (given +ve herd)
Pne8 <- pow(Pan8, ni)

```

```

#using law of total probability
Pne8.ps <- (Pne8 - Pne8.ns*Pns8.ph)/Pps8.ph
Ppe8.ps<-1 - Pne8.ps

Pnf8.ns<-Hsp1[8]
Ppf8.ns<-1 - Hsp1[8]

Ppf8<-tau[8]*Hsel[8]+(1-tau[8])*(1-Hsp1[8])
Ppf8.ps<-(Ppf8-Ppf8.ns*Pns8)/Pps8
Pnf8.ps<-1-Ppf8.ps

p8[1] <- Pns8*Ppf8.ns + Pps8*Ppf8.ps
p8[2] <- Pns8*Pnf8.ns*Ppe8.ns + Pps8*Pnf8.ps*Ppe8.ps
p8[3] <- 1 - p8[1] - p8[2]

# HTP[2]= Adjusted NI herd level true prevalence (HTP)
# (adjusted for sampling fraction as weighted average)
# wa10 to wa40 are the weights for the 4 farm types categories in the
# NI, wa10=only SHP, wa20=S&B, wa30=S&D, wa40=ALL sp.
# HTP[2] <- ((tau[1]*wa10)+(tau[2]*wa20)+(tau[3]*wa30)+(tau[4]*wa40))

# HTP[3]= Adjusted SI herd level true prevalence (HTP)
# wa11 to wa41 are the weights for the 4 farm types categories in the
# SI, wa11=only SHP, wa21=S&B, wa31=S&D, wa41=ALL sp.

HTP[3] <- ((tau[5]*wa11)+(tau[6]*wa21)+(tau[7]*wa31)+(tau[8]*wa41))

# HTP[1]= Adjusted national level (NAT) HTP (weighted average)
# wni and wsi is the weights for NI and SI respectively

HTP[1] <- ((HTP[2]*wni)+(HTP[3]*wsi))

# HTP[4-7] are the adjusted HTP for each of the 4 farm type categories
# wni1-4 and wsi1-4 are the NI and SI weights for each of the 4
#categories
HTP[4] <- ((tau[1]*wni1)+(tau[5]*wsi1)) # SHP HTP
HTP[5] <- ((tau[2]*wni2)+(tau[6]*wsi2)) # SHP & BEE HTP
HTP[6] <- ((tau[3]*wni3)+(tau[7]*wsi3)) # SHP & DEE HTP
HTP[7] <- ((tau[4]*wni4)+(tau[8]*wsi4)) # ALL sp HTP

# Posterior distribution comparisons
p.diff[1] <- HTP[2]-HTP[3] # NI - SI
p.diff[2] <- HTP[4]-HTP[5] # SHP - SHP & BEE
p.diff[3] <- HTP[4]-HTP[6] # SHP - SHP & DEE
p.diff[4] <- HTP[4]-HTP[7] # SHP - ALL sp.
p.diff[5] <- HTP[5]-HTP[6] # SHP & BEE - SHP & DEE
p.diff[6] <- HTP[5]-HTP[7] # SHP & BEE - ALL sp.
p.diff[7] <- HTP[6]-HTP[7] # SHP & DEE - ALL sp.

# Bayesian posterior probability (POPR) Pr(>1)
o.diff[1] <- step(p.diff[1])
o.diff[2] <- step(p.diff[2])
o.diff[3] <- step(p.diff[3])
o.diff[4] <- step(p.diff[4])
o.diff[5] <- step(p.diff[5])
o.diff[6] <- step(p.diff[6])
o.diff[7] <- step(p.diff[7])

```

```

#Individual test characteristics
Hse1[1] <- 1-(pow((1-pow(1-pi[1],k))*(1-sel)+pow(1-pi[1],k)*sp1,r))
Hsp1[1] <- pow(sp1, r)
Hse1[2] <- 1-(pow((1-pow(1-pi[2],k))*(1-sel)+pow(1-pi[2],k)*sp1,r))
Hsp1[2] <- pow(sp1, r)
Hse1[3] <- 1-(pow((1-pow(1-pi[3],k))*(1-sel)+pow(1-pi[3],k)*sp1,r))
Hsp1[3] <- pow(sp1, r)
Hse1[4] <- 1-(pow((1-pow(1-pi[4],k))*(1-sel)+pow(1-pi[4],k)*sp1,r))
Hsp1[4] <- pow(sp1, r)
Hse1[5] <- 1-(pow((1-pow(1-pi[5],k))*(1-sel)+pow(1-pi[5],k)*sp1,r))
Hsp1[5] <- pow(sp1, r)
Hse1[6] <- 1-(pow((1-pow(1-pi[6],k))*(1-sel)+pow(1-pi[6],k)*sp1,r))
Hsp1[6] <- pow(sp1, r)
Hse1[7] <- 1-(pow((1-pow(1-pi[7],k))*(1-sel)+pow(1-pi[7],k)*sp1,r))
Hsp1[7] <- pow(sp1, r)
Hse1[8] <- 1-(pow((1-pow(1-pi[8],k))*(1-sel)+pow(1-pi[8],k)*sp1,r))
Hsp1[8] <- pow(sp1, r)

Hse2[1] <-1-(pow(Pan1, ni))
Hsp2[1] <- pow(sp2, ni)
Hse2[2] <-1-(pow(Pan2, ni))
Hsp2[2] <- pow(sp2, ni)
Hse2[3] <-1-(pow(Pan3, ni))
Hsp2[3] <- pow(sp2, ni)
Hse2[4] <-1-(pow(Pan4, ni))
Hsp2[4] <- pow(sp2, ni)
Hse2[5] <-1-(pow(Pan5, ni))
Hsp2[5] <- pow(sp2, ni)
Hse2[6] <-1-(pow(Pan6, ni))
Hsp2[6] <- pow(sp2, ni)
Hse2[7] <-1-(pow(Pan7, ni))
Hsp2[7] <- pow(sp2, ni)
Hse2[8] <-1-(pow(Pan8, ni))
Hsp2[8] <- pow(sp2, ni)

# Joint herd level test characteristics estimation
Hsej[1] <- Pns1.ph*(Ppf1.ns+(1-Ppf1.ns)*Ppe1.ns)+(1-
Pns1.ph)*(Ppf1.ps+(1-Ppf1.ps)*Ppe1.ps)
Hspj[1] <- Hsp1[1]*Hsp2[1]
Hsej[2] <- Pns2.ph*(Ppf2.ns+(1-Ppf2.ns)*Ppe2.ns)+(1-
Pns2.ph)*(Ppf2.ps+(1-Ppf2.ps)*Ppe2.ps)
Hspj[2] <- Hsp1[2]*Hsp2[2]
Hsej[3] <- Pns3.ph*(Ppf3.ns+(1-Ppf3.ns)*Ppe3.ns)+(1-
Pns3.ph)*(Ppf3.ps+(1-Ppf3.ps)*Ppe3.ps)
Hspj[3] <- Hsp1[3]*Hsp2[3]
Hsej[4] <- Pns4.ph*(Ppf4.ns+(1-Ppf4.ns)*Ppe4.ns)+(1-
Pns4.ph)*(Ppf4.ps+(1-Ppf4.ps)*Ppe4.ps)
Hspj[4] <- Hsp1[4]*Hsp2[4]
Hsej[5] <- Pns5.ph*(Ppf5.ns+(1-Ppf5.ns)*Ppe5.ns)+(1-
Pns5.ph)*(Ppf5.ps+(1-Ppf5.ps)*Ppe5.ps)
Hspj[5] <- Hsp1[5]*Hsp2[5]
Hsej[6] <- Pns6.ph*(Ppf6.ns+(1-Ppf6.ns)*Ppe6.ns)+(1-
Pns6.ph)*(Ppf6.ps+(1-Ppf6.ps)*Ppe6.ps)
Hspj[6] <- Hsp1[6]*Hsp2[6]
Hsej[7] <- Pns7.ph*(Ppf7.ns+(1-Ppf7.ns)*Ppe7.ns)+(1-
Pns7.ph)*(Ppf7.ps+(1-Ppf7.ps)*Ppe7.ps)
Hspj[7] <- Hsp1[7]*Hsp2[7]
Hsej[8] <- Pns8.ph*(Ppf8.ns+(1-Ppf8.ns)*Ppe8.ns)+(1-
Pns8.ph)*(Ppf8.ps+(1-Ppf8.ps)*Ppe8.ps)
Hspj[8] <- Hsp1[8]*Hsp2[8]

```

```

# Positive (HPVpos) and negative (HPVneg) predictive values
HPVpos1[1] <- tau[1]*Hse1[1] / (tau[1]*Hse1[1] + (1-tau[1])*(1-
Hsp1[1]))
HPVneg1[1] <- (1-tau[1])*Hsp1[1] / ((1-tau[1])*Hsp1[1] + tau[1]*(1-
Hse1[1]))
HPpos1[1] <- tau[1]*Hse1[1] + (1-tau[1])*(1-Hsp1[1])
HPVpos1[2] <- tau[2]*Hse1[2] / (tau[2]*Hse1[2] + (1-tau[2])*(1-
Hsp1[2]))
HPVneg1[2] <- (1-tau[2])*Hsp1[2] / ((1-tau[2])*Hsp1[2] + tau[2]*(1-
Hse1[2]))
HPpos1[2] <- tau[2]*Hse1[2] + (1-tau[2])*(1-Hsp1[2])
HPVpos1[3] <- tau[3]*Hse1[3] / (tau[3]*Hse1[3] + (1-tau[3])*(1-
Hsp1[3]))
HPVneg1[3] <- (1-tau[3])*Hsp1[3] / ((1-tau[3])*Hsp1[3] + tau[3]*(1-
Hse1[3]))
HPpos1[3] <- tau[3]*Hse1[3] + (1-tau[3])*(1-Hsp1[3])
HPVpos1[4] <- tau[4]*Hse1[4] / (tau[4]*Hse1[4] + (1-tau[4])*(1-
Hsp1[4]))
HPVneg1[4] <- (1-tau[4])*Hsp1[4] / ((1-tau[4])*Hsp1[4] + tau[4]*(1-
Hse1[4]))
HPpos1[4] <- tau[4]*Hse1[4] + (1-tau[4])*(1-Hsp1[4])
HPVpos1[5] <- tau[5]*Hse1[5] / (tau[5]*Hse1[5] + (1-tau[5])*(1-
Hsp1[5]))
HPVneg1[5] <- (1-tau[5])*Hsp1[5] / ((1-tau[5])*Hsp1[5] + tau[5]*(1-
Hse1[5]))
HPpos1[5] <- tau[5]*Hse1[5] + (1-tau[5])*(1-Hsp1[5])
HPVpos1[6] <- tau[6]*Hse1[6] / (tau[6]*Hse1[6] + (1-tau[6])*(1-
Hsp1[6]))
HPVneg1[6] <- (1-tau[6])*Hsp1[6] / ((1-tau[6])*Hsp1[6] + tau[6]*(1-
Hse1[6]))
HPpos1[6] <- tau[6]*Hse1[6] + (1-tau[6])*(1-Hsp1[6])
HPVpos1[7] <- tau[7]*Hse1[7] / (tau[7]*Hse1[7] + (1-tau[7])*(1-
Hsp1[7]))
HPVneg1[7] <- (1-tau[7])*Hsp1[7] / ((1-tau[7])*Hsp1[7] + tau[7]*(1-
Hse1[7]))
HPpos1[7] <- tau[7]*Hse1[7] + (1-tau[7])*(1-Hsp1[7])
HPVpos1[8] <- tau[8]*Hse1[8] / (tau[8]*Hse1[8] + (1-tau[8])*(1-
Hsp1[8]))
HPVneg1[8] <- (1-tau[8])*Hsp1[8] / ((1-tau[8])*Hsp1[8] + tau[8]*(1-
Hse1[8]))
HPpos1[8] <- tau[8]*Hse1[8] + (1-tau[8])*(1-Hsp1[8])

# Join Positive (HPVpos) and negative (HPVneg) predictive values
# Addapted from Su et al, (2008). I am assuming that Hsej and Hspj are
# equivalent to Hse and Hsp.
HPVpos[1] <- tau[1]*Hsej[1] / (tau[1]*Hsej[1] + (1-tau[1])*(1-
Hspj[1]))
HPVneg[1] <- (1-tau[1])*Hspj[1] / ((1-tau[1])*Hspj[1] + tau[1]*(1-
Hsej[1]))
HPpos[1] <- tau[1]*Hsej[1] + (1-tau[1])*(1-Hspj[1])
HPVpos[2] <- tau[2]*Hsej[2] / (tau[2]*Hsej[2] + (1-tau[2])*(1-
Hspj[2]))
HPVneg[2] <- (1-tau[2])*Hspj[2] / ((1-tau[2])*Hspj[2] + tau[2]*(1-
Hsej[2]))
HPpos[2] <- tau[2]*Hsej[2] + (1-tau[2])*(1-Hspj[2])
HPVpos[3] <- tau[3]*Hsej[3] / (tau[3]*Hsej[3] + (1-tau[3])*(1-
Hspj[3]))
HPVneg[3] <- (1-tau[3])*Hspj[3] / ((1-tau[3])*Hspj[3] + tau[3]*(1-
Hsej[3]))
HPpos[3] <- tau[3]*Hsej[3] + (1-tau[3])*(1-Hspj[3])

```

```

HPVpos[4] <- tau[4]*Hsej[4] / (tau[4]*Hsej[4] + (1-tau[4])*(1-
Hspj[4]))
HPVneg[4] <- (1-tau[4])*Hspj[4] / ((1-tau[4])*Hspj[4] + tau[4]*(1-
Hsej[4]))
HPpos[4] <- tau[4]*Hsej[4] + (1-tau[4])*(1-Hspj[4])
HPVpos[5] <- tau[5]*Hsej[5] / (tau[5]*Hsej[5] + (1-tau[5])*(1-
Hspj[5]))
HPVneg[5] <- (1-tau[5])*Hspj[5] / ((1-tau[5])*Hspj[5] + tau[5]*(1-
Hsej[5]))
HPpos[5] <- tau[5]*Hsej[5] + (1-tau[5])*(1-Hspj[5])
HPVpos[6] <- tau[6]*Hsej[6] / (tau[6]*Hsej[6] + (1-tau[6])*(1-
Hspj[6]))
HPVneg[6] <- (1-tau[6])*Hspj[6] / ((1-tau[6])*Hspj[6] + tau[6]*(1-
Hsej[6]))
HPpos[6] <- tau[6]*Hsej[6] + (1-tau[6])*(1-Hspj[6])
HPVpos[7] <- tau[7]*Hsej[7] / (tau[7]*Hsej[7] + (1-tau[7])*(1-
Hspj[7]))
HPVneg[7] <- (1-tau[7])*Hspj[7] / ((1-tau[7])*Hspj[7] + tau[7]*(1-
Hsej[7]))
HPpos[7] <- tau[7]*Hsej[7] + (1-tau[7])*(1-Hspj[7])
HPVpos[8] <- tau[8]*Hsej[8] / (tau[8]*Hsej[8] + (1-tau[8])*(1-
Hspj[8]))
HPVneg[8] <- (1-tau[8])*Hspj[8] / ((1-tau[8])*Hspj[8] + tau[8]*(1-
Hsej[8]))
HPpos[8] <- tau[8]*Hsej[8] + (1-tau[8])*(1-Hspj[8])

# Priors information
#Mode=0.35; 95% sure < 0.43
se2 ~ dbeta(36.7751, 67.7323)
#Mode=0.98, 95% sure > 0.98 # ELISA test se
sp2 ~ dbeta(1862.6459, 23.6111)
## Mode=0.23, 95% sure secul < 0.54
se1 ~ dbeta(2.7391, 6.8223)
## Mode=0.999, 95% sure spcul > 0.985
sp1 ~ dbeta(226.11335, 1.22534)

## Mode=0.81, 95% sure tau > 0.66(between herd prev) LC data
tau[1] ~ dbeta(21.621,5.837)
tau[2] ~ dbeta(21.621,5.837)
tau[3] ~ dbeta(21.621,5.837)
tau[4] ~ dbeta(21.621,5.837)
## Mode=0.72, 95% sure tau > 0.56 (between herd prev) LC data
tau[5] ~ dbeta(20.4703,8.5718)
tau[6] ~ dbeta(20.4703,8.5718)
tau[7] ~ dbeta(20.4703,8.5718)
tau[8] ~ dbeta(20.4703,8.5718)
## Mode=0.13, 95% sure pi1ni < 0.50 (within herd prev) LC data
pi[1] ~ dbeta(1.7271, 5.8657)
pi[2] ~ dbeta(1.7271, 5.8657)
pi[3] ~ dbeta(1.7271, 5.8657)
pi[4] ~ dbeta(1.7271, 5.8657)
pi[5] ~ dbeta(1.7271, 5.8657)
pi[6] ~ dbeta(1.7271, 5.8657)
pi[7] ~ dbeta(1.7271, 5.8657)
pi[8] ~ dbeta(1.7271, 5.8657)

}

```

WinBUGS code:**Error adjusted logistic and Poisson regression models for the identification of prevalence and clinical disease incidence risk factors****Logistic regression model:**

Risk factor assessment of grazing management over lab test MAP status, y = sheep MAP test status test = SHEEP: Test protocol, combination of PFC or ELISA test from normal or suspected animals. test[1-4] 1=PFC normal animals, 2=PFC normal & suspected animals, 3=PFC+ELISA normal animals, 4=PFC+ELISA normal & suspected animals z is the true infection MAP status of sheep. 1 = positive, 0 otherwise x6 = North Island (0), South Island (1), beeG = Beef grazed with sheep, deeG = Deer grazed with sheep, Exp = Sheep population at risk (flock size)

```

model{
for(i in 1:nh){
  z[i] ~ dbern(tau[i])
  y[i]~ dbern(q[i])
  q[i] <- z[i]*Hse[test[i]]+(1-z[i])*(1-Hsp[test[i]])

  SPR[i] <- (Exp[i]-mean(Exp[]))/sd(Exp[])

  logit(tau[i]) <- beta[1] + beta[2]*beeG[i] + beta[3]*deeG[i] +
beta[4]*x6[i] + beta[5]*(beeG[i]*x6[i]) + beta[6]*(deeG[i]*x6[i])+
beta[7]*(Exp[i]-mean(Exp[]))/sd(Exp[])
}

# Priors (Values obtained from the prevalence model)

Hse[1] ~ dbeta(27.5157, 27.5157) # mode =0.50, 95% <0.61
Hse[2] ~ dbeta(27.5157, 27.5157) # mode =0.50, 95% <0.61
Hse[3] ~ dbeta(9.6956, 1.9662) # mode =0.90, 95% >0.63
Hse[4] ~ dbeta(9.6956, 1.9662) # mode =0.90, 95% >0.63
Hsp[1] ~ dbeta(560.7246, 6.6538) # mode =0.99, 95% >0.98
Hsp[2] ~ dbeta(560.7246, 6.6538) # mode =0.99, 95% >0.98
Hsp[3] ~ dbeta(52.7296, 15.5904) # mode =0.78, 95% <0.85
Hsp[4] ~ dbeta(52.7296, 15.5904) # mode =0.78, 95% <0.85

```

```

for (j in 1:7){
  beta[j]~dnorm( 0.0, 1 )
  OR[j] <- exp( beta[j] )
  Pr0[j] <- equals(beta[j],0)
  Pr[j] <- (step(beta[j]))- Pr0[j]
}
}

```

Poisson regression model:

Risk factor assessment of grazing management over Ptb clinal cases reported by farmers, cs = number of cases in a given sheep flock, y = Sheep Ptb test status, test = Test protocol, combination of PFC or ELISA test from normal or suspected animals. test[1-4] 1=PFC normal animals, 2=PFC normal & suspected animals, 3=PFC+ELISA normal animals, 4=PFC+ELISA normal & suspected animals, deeG = Deer grazed with Sheep. Including Co-grazing (same paddock same time) and alternate grazing (same paddock, different time but sheep flock use the paddock after deer), beeG = Beef cattle grazed with sheep, x6 = North Island (0), South Island (1), Exp = Sheep flock size

```

model{
  for (i in 1:162) {
    cs[i] ~ dpois(mmu[i])

    mmu[i] <- Exp[i]*zs[i]*exp(mmmu[i])*se + (1-zs[i])*0.1
    mmmu[i] <- beta[1] + beta[2]*deeG[i] + beta[3]*beeG[i] + beta[4]*x6[i]
    + beta[5]*(deeG[i]*x6[i]) + beta[6]*(beeG[i]*x6[i])

    I[i] <- x6[i] + 1

    zs[i] ~ dbern(tau[I[i]])
    y[i]~dbern(q[i])
    q[i] <- zs[i]*Hse[test[i]]+(1-zs[i])*(1-Hsp[test[i]])
  }

  for (i in 1:2) { for (j in 1:2) { for (k in 1:2) {
    rate[i,j,k] <- exp(beta[1] + (i-1)*beta[2] + (j-1)*beta[3] + (k-
1)*beta[4]) # all possible data combinations to compare probabilities
}}}

# priors
tau[1] ~ dbeta(47.3948, 12.5987) # NI mode = .80 95% sure < .87
tau[2] ~ dbeta(67.4828, 29.4926) # SI mode = .70 95% sure < .77

Hse[1] ~ dbeta(27.5157, 27.5157) # mode =0.50, 95% <0.61
Hse[2] ~ dbeta(27.5157, 27.5157) # mode =0.50, 95% <0.61
Hse[3] ~ dbeta(9.6956, 1.9662) # mode =0.90, 95% >0.63
Hse[4] ~ dbeta(9.6956, 1.9662) # mode =0.90, 95% >0.63
Hsp[1] ~ dbeta(560.7246, 6.6538) # mode =0.99, 95% >0.98
Hsp[2] ~ dbeta(560.7246, 6.6538) # mode =0.99, 95% >0.98
Hsp[3] ~ dbeta(52.7296, 15.5904) # mode =0.78, 95% <0.85
Hsp[4] ~ dbeta(52.7296, 15.5904) # mode =0.78, 95% <0.85

```

```
for (i in 1:162) {fp[i] <-0
}
se <- 1
for (j in 1:6){
  beta[j]~dnorm( 0.0, 1 )
  RR[j] <- exp( beta[j] )
  Pr[j] <- step(beta[j])
}
}
```

Differential equations:

Sheep & beef cattle mathematical simulation model

D.1 Logical indicators

$$time = Z(1 \leq t \leq 12)$$

$$time.RT = Z(1 \leq t \leq 2)$$

$$I_1 = \text{if } \cdot time \leq W_b \cdot \text{then} \cdot 1 \cdot \text{else} \cdot 0$$

Wb = time to weaning of beef calves (usually 6 months)

$$I_2 = \text{if } \cdot time \leq W_s \cdot \text{then} \cdot 1 \cdot \text{else} \cdot 0$$

Ws = time to weaning of lambs (usually 3 months)

$$I_3 = \text{if } \cdot time \leq CGT \cdot \text{then} \cdot 1 \cdot \text{else} \cdot 0$$

CGT = length of the co-grazing time (usually 3-4 months)

$$I_4 = \text{if } \cdot time.RT \leq RT \cdot \text{then} \cdot 1 \cdot \text{else} \cdot 0$$

RT = length of the rotation time (each 1 months)

D.2 Environment

D.2.1 MAP survival dynamic equations:

Pairs of blocks are grazed in a rotational fashion; animals rotate between them at RT.

E1 = E5 is contamination of young stock pasture by weaned calves/heifers (block 1 &

5)

$$\frac{dE_1}{dt} = \sigma_{bTr_2} * bTr_2 * I_4 - \psi * E_1$$

$$\frac{dE_5}{dt} = \sigma_{bTr_2} * bTr_2 * (1 - I_4) - \psi * E_5$$

E2 = E6 is pasture contamination by adult/offspring beef (block 2 & 6)

$$\frac{dE_2}{dt} = (\sigma_{bTr_1} * bTr_1 * I_1 + \sigma_{bYls_4} * bYls_4 + \sigma_{bYhs_4} * bYhs_4) * I_4 - \psi * E_2$$

$$\frac{dE_6}{dt} = (\sigma_{bTr_1} * bTr_1 * I_1 + \sigma_{bYls_4} * bYls_4 + \sigma_{bYhs_4} * bYhs_4) * (1 - I_4) - \psi * E_6$$

E3 = E7 is pasture contamination by adult/offspring sheep (block 3 & 7)

$$\frac{dE_3}{dt} = (\sigma_{sP} * (\eta_1 * (sPp_1 + sPnp_1) * I_2 + sPp_4 + sPnp_4) + \sigma_{sM} * sM_4) * I_4 - \psi * E_3$$

$$\frac{dE_7}{dt} = (\sigma_{sP} * (\eta_1 * (sPp_1 + sPnp_1) * I_2 + sPp_4 + sPnp_4) + \sigma_{sM} * sM_4) * (1 - I_4) - \psi * E_7$$

E4 = E8 is contamination of young stock pasture by hoggets and two-tooth (block 4 &

8)

$$\frac{dE_4}{dt} = (\sigma_{sP} * (\eta_2 * (sPp_2 + sPnp_2) + \eta_3 * (sPp_3 + sPnp_3)) + \sigma_{sM} * \eta_3 * sM_3) * I_4 - \psi * E_4$$

$$\frac{dE_8}{dt} = (\sigma_{sP} * (\eta_2 * (sPp_2 + sPnp_2) + \eta_3 * (sPp_3 + sPnp_3)) + \sigma_{sM} * \eta_3 * sM_3) * (1 - I_4) - \psi * E_8$$

D.3 Beef

D.3.1 Population and epidemic dynamic:

$$\frac{d(bS_1)}{dt} = (\omega_b - \zeta_b) * bN_5 - (\mu_{b1} + \rho_{b1}) * bS_1 - \lambda_{b1} * bS_1$$

$$\frac{d(bS_2)}{dt} = \rho_{b1} * bS_1 - (\mu_{b2} + \rho_{b2}) * bS_2 - \lambda_{b2} * bS_2$$

$$\frac{d(bR_3)}{dt} = \rho_{b2} * bS_2 - (\mu_{b3} + \rho_{b3}) * bR_3$$

$$\frac{d(bR_4)}{dt} = \rho_{b3} * bR_3 - (\mu_{b4} + \tau_b + \theta_b) * bR_4$$

$$\frac{d(bTr_1)}{dt} = \chi_{b1} * \zeta_b * bN_5 + \chi_{b1} * \lambda_{b1} * bS_1 - (\mu_{b1} + \rho_{b1}) * bTr_1 - \delta_{b1} * bTr_1$$

$$\frac{d(bL_1)}{dt} = \delta_{b1} * bTr_1 - (\mu_{b1} + \rho_{b1}) * bL_1$$

$$\frac{d(bLs_1)}{dt} = (1 - \chi_{b1}) * \zeta_b * bN_5 + (1 - \chi_{b1}) * \lambda_{b1} * bS_1 - (\mu_{b1} + \rho_{b1}) * bLs_1$$

$$\frac{d(bTr_2)}{dt} = \chi_{b2} * \lambda_{b2} * bS_2 + \rho_{b1} * bTr_1 - (\mu_{b2} + \rho_{b2}) * bTr_2 - \delta_{b2} * bTr_2$$

$$\frac{d(bL_2)}{dt} = \delta_{b2} * bTr_2 + \rho_{b1} * bL_1 - (\mu_{b2} + \rho_{b2}) * bL_2$$

$$\frac{d(bLs_2)}{dt} = (1 - \chi_{b2}) * \lambda_{b2} * bS_2 + \rho_{b1} * bLs_1 - (\mu_{b2} + \rho_{b2}) * bLs_2$$

$$\frac{d(bL_3)}{dt} = \rho_{b2} * (bTr_2 + bL_2) - (\mu_{b3} + \rho_{b3}) * bL_3$$

$$\frac{d(bLs_3)}{dt} = \rho_{b2} * bLs_2 - (\mu_{b3} + \rho_{b3}) * bLs_3$$

$$\frac{d(bL_4)}{dt} = \rho_{b3} * bL_3 - (\mu_{b4} + \tau_b + \nu_{b1}) * bL_4 - \delta_{b3} * bL_4$$

$$\frac{d(bLs_4)}{dt} = \rho_{b3} * bLs_3 - (\mu_{b4} + \tau_b + \nu_{b1}) * bLs_4 - \delta_{b4} * bLs_4$$

$$\frac{d(bYls_4)}{dt} = \delta_{b3} * bL_4 + \delta_{b4} * bLs_4 - \delta_{b5} * bYls_4 - (\mu_{b4} + \tau_b + \nu_{b2}) * bYls_4$$

$$\frac{d(bYhs_4)}{dt} = \delta_{b5} * bYls_4 - (\mu_{b4} + \tau_b + \nu_{b3} + \mu_{bC} + \alpha_b) * bYhs_4$$

$$\omega_b = (\mu_{b1} * (bS_1 + bTr_1 + bL_1 + bLs_1) + \mu_{b2} * (bS_2 + bTr_2 + bL_2 + bLs_2) + \mu_{b3} * (bR_3 + bL_3 + bLs_3) + \mu_{b4} * (bR_4 + bL_4 + bLs_4 + bYls_4 + bYhs_4) + \tau_b * (bR_4 + bL_4 + bLs_4 + bYls_4 + bYhs_4) + \mu_{bC} * bYhs_4 + \nu_{b1} * (bL_4 + bLs_4) + \nu_{b2} * bYls_4 + \nu_{b3} * bYhs_4 + \theta_b * bR_4 + \alpha_b * bYhs_4) / bN_5$$

$$\zeta_b = \omega_b * (\zeta_{b1} * bYls_4 + \zeta_{b2} * bYhs_4) / bN4$$

Beef population size per age category and total herd size

$$bN1 = bS_1 + bTr_1 + bL_1 + bLs_1$$

$$bN2 = bS_2 + bTr_2 + bL_2 + bLs_2$$

$$bN3 = bR_3 + bL_3 + bLs_3$$

$$bN4 = bR_4 + bL_4 + bLs_4 + bYls_4 + bYhs_4$$

$$bN5 = bN1 + bN2 + bN3 + bN4$$

D.3.2 Force of infection equations:

$$\lambda_{b1} = 1 - \exp(-\beta_b * DA_{b1})$$

$$\lambda_{b2} = 1 - \exp(-\beta_b * Sus_b * DA_{b2})$$

$$DA_{b1} = bBL + (sBL * I_3)$$

$$DA_{b2} = E_1 * I_4 + E_5 * (1 - I_4)$$

where;

$$bBL = E_2 * I_4 + E_6 * (1 - I_4)$$

$$sBL = E_3 * I_4 + E_7 * (1 - I_4)$$

D.4 Sheep

D.4.1 Population and epidemic dynamic:

$$\frac{d(sS_1)}{dt} = \omega_s * sN_5 - (\mu_{s1} + \rho_{s1}) * sS_1 - \lambda_{s1} * sS_1$$

$$\frac{d(sS_2)}{dt} = \rho_{s1} * sS_1 - (\mu_{s2} + \rho_{s2}) * sS_2 - \lambda_{s2} * sS_2$$

$$\frac{d(sS_3)}{dt} = \rho_{s2} * sS_2 - (\mu_{s3} + \rho_{s3}) * sS_3 - \lambda_{s3} * sS_3$$

$$\frac{d(sS_4)}{dt} = \rho_{s3} * sS_3 - (\mu_{s4} + \tau_s + \theta_s) * sS_4 - \lambda_{s4} * sS_4$$

$$\frac{d(sPp_1)}{dt} = \chi_{s1} * \lambda_{s1} * sS_1 - \delta_{s1} * sPp_1 - (\mu_{s1} + \rho_{s1}) * sPp_1$$

$$\frac{d(sPp_2)}{dt} = \chi_{s2} * \lambda_{s2} * sS_2 + \rho_{s1} * sPp_1 - \delta_{s2} * sPp_2 - (\mu_{s2} + \rho_{s2}) * sPp_2$$

$$\frac{d(sPp_3)}{dt} = \chi_{s3} * \lambda_{s3} * sS_3 + \rho_{s2} * sPp_2 - \delta_{s3} * sPp_3 - (\mu_{s3} + \rho_{s3}) * sPp_3$$

$$\frac{d(sM_3)}{dt} = \delta_{s3} * sPp_3 - (\mu_{s3} + \mu_{sC3} + \rho_{s3} + \alpha_s) * sM_3$$

$$\frac{d(sPp_4)}{dt} = \chi_{s4} * \lambda_{s4} * sS_4 + \rho_{s3} * sPp_3 - \delta_{s4} * sPp_4 - (\mu_{s4} + \tau_s + \nu_{s2}) * sPp_4$$

$$\frac{d(sM_4)}{dt} = \delta_{s4} * sPp_4 + \rho_{s3} * sM_3 - (\mu_{s4} + \mu_{sC4} + \tau_s + \nu_{s3} + \alpha_s) * sM_4$$

$$\frac{d(sPnp_1)}{dt} = (1 - \chi_{s1}) * \lambda_{s1} * sS_1 - \gamma_s * sPnp_1 - (\mu_{s1} + \rho_{s1}) * sPnp_1$$

$$\frac{d(sR_1)}{dt} = \gamma_s * sPnp_1 - (\mu_{s1} + \rho_{s1}) * sR_1$$

$$\frac{d(sPnp_2)}{dt} = (1 - \chi_{s2}) * \lambda_{s2} * sS_2 + \rho_{s1} * sPnp_1 - \gamma_s * sPnp_2 - (\mu_{s2} + \rho_{s2}) * sPnp_2$$

$$\frac{d(sR_2)}{dt} = \gamma_s * sPnp_2 + \rho_{s1} * sR_1 - (\mu_{s2} + \rho_{s2}) * sR_2$$

$$\frac{d(sPnp_3)}{dt} = (1 - \chi_{s3}) * \lambda_{s3} * sS_3 + \rho_{s2} * sPnp_2 - \gamma_s * sPnp_3 - (\mu_{s3} + \rho_{s3}) * sPnp_3$$

$$\frac{d(sR_3)}{dt} = \gamma_s * sPnp_3 + \rho_{s2} * sR_2 - (\mu_{s3} + \rho_{s3}) * sR_3$$

$$\frac{d(sPnp_4)}{dt} = (1 - \chi_{s4}) * \lambda_{s4} * sS_4 + \rho_{s3} * sPnp_3 - \gamma_s * sPnp_4 - (\mu_{s4} + \tau_s + \nu_{s1}) * sPnp_4$$

$$\frac{d(sR_4)}{dt} = \gamma_s * sPnp_4 + \rho_{s3} * sR_3 - (\mu_{s4} + \tau_s + \theta_s) * sR_4$$

$$\begin{aligned} \omega_s = & (\mu_{s1} * (sS_1 + sPp_1 + sPnp_1 + sR_1) + \mu_{s2} * (sS_2 + sPp_2 + sPnp_2 + sR_2) + \\ & \mu_{s3} * (sS_3 + sPp_3 + sM_3 + sPnp_3 + sR_3) + \mu_{s4} * (sS_4 + sPp_4 + sM_4 + sPnp_4 + sR_4) + \\ & \tau_s * (sS_4 + sPp_4 + sM_4 + sPnp_4 + sR_4) + \mu_{sC3} * sM_3 + \mu_{sC4} * sM_4 + \nu_{s1} * sPnp_4 + \nu_{s2} * sPp_4 + \\ & \nu_{s3} * sM_4 + \theta_s * (sS_4 + sR_4) + \alpha_s * (sM_3 + sM_4)) / sN_5 \end{aligned}$$

Sheep population size per age category and total flock size

$$sN1 = sS_1 + sPp_1 + sPnp_1 + sR_1$$

$$sN2 = sS_2 + sPp_2 + sPnp_2 + sR_2$$

$$sN3 = sS_3 + sPp_3 + sM_3 + sPnp_3 + sR_3$$

$$sN4 = sS_4 + sPp_4 + sM_4 + sPnp_4 + sR_4$$

$$sN5 = sN1 + sN2 + sN3 + sN4$$

D.4.2 Force of infection equations:

$$\lambda_{s1} = (1 - \exp(-\beta_{s1} * sBL)) + (1 - \exp(-\beta_{s2} * bBL * I_3))$$

$$\lambda_{s2-3} = 1 - \exp(-\beta_{s2} * DA_{s2-3})$$

$$\lambda_{s4} = 1 - \exp(-\beta_{s2} * DA_{s4})$$

$$DA_{s2-3} = E_4 * I_4 + E_8 * (1 - I_4)$$

$$DA_{s4} = sBL + (bBL * I_3)$$

D.5 Test and cull program

$$TC_s = \text{if} \cdot \text{time} = 1 \cdot \text{then} \cdot 1 \cdot \text{else} \cdot 0$$

$$TC_b = \text{if} \cdot \text{time} = 1 \cdot \text{then} \cdot 1 \cdot \text{else} \cdot 0$$

$$\nu_{s1} = TC_s * Se_{ELISA-s1}$$

$$v_{s2} = TC_s * Se_{ELISA-s2}$$

$$v_{s3} = TC_s * Se_{ELISA-s3}$$

$$\theta_s = TC_s * (1 - Sp_{ELISA-s})$$

$$v_{b1} = TC_b * Se_{ELISA-b1}$$

$$v_{b2} = TC_b * Se_{ELISA-b2}$$

$$v_{b3} = TC_b * Se_{ELISA-b3}$$

$$\theta_b = TC_b * (1 - Sp_{ELISA-b})$$