The prevalence of Johne's disease in New Zealand: A review of our current understanding

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Executive Summary

This review was commissioned by the Johnes Disease Research Consortium (JDRC) in May of 2011 and produced by the authors during June and July of 2011. The objective of this review was to define, to the best of current knowledge and ability and within the time frame allotted, the prevalence of Johne's disease (JD) in the major farmed species in New Zealand at the present time, against which control efforts can be measured. The terms of reference of the review include defining the disease; assessing the standard of diagnostics available; and defining prevalence, with specific regard to clinical animals, shedding of organisms, and *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in product.

The review commenced with a comprehensive literature search of the subject from a variety of sources. These included peer reviewed papers; non-peer reviewed scientific articles; reports and books; and also included material not in the public domain that was made available to the authors. Over 350 scientific articles were sourced and reviewed during this process. A second and key part of the review process consisted of interviews and discussions with key figures who have contributed significant understanding to JD in New Zealand.

This review is not intended as a comprehensive perspective on the current state of JD understanding in New Zealand. Rather, it has been conducted within the confines of a timeline to feed into the JDRC strategic review as a formative part of their benchmarking process.

This review begins with an overview of JD in New Zealand, including a discussion about the implications of JD for the New Zealand livestock industry and a brief review of the historical data on prevalence available (Chapter 1). Because of the insidious nature of the disease, clinical determination is challenging, and therefore most reports will underestimate the prevalence of JD. Moreover, JD was a notifiable disease for a period, and this inevitably lead

to a stigmatisation of being seen as being a ‘JD positive’ farm. To some extent, this perspective is still true amongst many dairy farmers today. Furthermore, a distinction needs to be made between prevalence, which is the burden of disease in a population at any given time, and incidence, which is the rate of new cases over a period of time. For an insidious clinical disease such as JD, this distinction is important when discussing certain issues.

Infection with MAP presents with a whole gamut of potential classifications. There is no consistent terminology applied to MAP infection. There is a reasonably clear understanding within the literature on what constitutes clinical JD, but nevertheless, many studies refer to JD infection when they mean MAP infection; or use the terms interchangeably. Subclinical infection, latent infection, and affection or affected are all terms which are encountered, without often a clear definition of what they may refer to. The reviewers have attempted to clarify their approach to classification by proposing, for the purposes of this review, essentially two separate and distinct states (Chapter 2). The first is MAP detected; and the second is non-MAP detected.

Under the first category is included a subgroup of animals that are clinically affected- these animals have JD. And a second subgroup of animals is outlined in which MAP can be detected but where no obvious clinical signs are seen. These are subclinically infected. The second group of animals, those of non-MAP detection, allow for the possibility that, given the poor sensitivity of tests, MAP is present but not detectable. By definition, it is not possible to say how large a group this is. It is critical to clarity of understanding that the definition of MAP status is clear in the literature. An attempt is made to define these classifications as far as possible.

There are a number of diagnostic tests available to determine MAP infection, and all have limitations (Chapter 3). In particular, sensitivity and specificity pose some significant problems when interpreting their output. Others, such as faecal culture, have high sensitivity and specificity but are extremely time consuming. Serological tests are quick and simple, but

are more effective later in the disease than earlier. This is because in the early stages of infection cell-mediated immune responses dominate the host response. However, the relative stage of infection of an individual is obviously unknown prior to testing.

Other tests have more specialised, but important roles. Bulk milk ELISA allows rapid and cost-effective screening of dairy herds although it lacks sensitivity. Polymerase chain reaction (PCR) techniques can be potentially very sensitive, but may lack specificity. A recent study utilised latent class modelling to adjust for the imperfect sensitivities of two tests in developing prevalence models.

The published prevalence of JD and of MAP is reviewed (Chapter 4). Whilst there are a reasonable amount of data available, very little of it could be assessed in a genuine meta-analytical process, simply because the methodologies, sampling frames, tests and testing regimes, and even the definition of outcomes are frequently quite different to each other or often simply poorly defined. It would be of advantage for the JDRC to develop some guidelines on the parameters, definitions and outcomes used in studies in all species so that direct comparisons between studies can be made more easily in the future. This is particularly the case with regard to case definition. For this review, in the absence of a formal meta-analytical approach, an attempt has been made to review all available material and at least report it. The critical output is summarised in the graphs and tables in the appendices at the end of the review.

Among the data for all species, a prevalence difference between the South Island and the North Island was often found. This may be attributable to shifting populations within and between the two islands; or it may be due to management or environmental differences. However, the data suggest, for example, that in both dairy and deer, MAP is more prevalent in the SI than in the NI; whereas it is more common in sheep in the NI. In a similar fashion, Jersey cows appear more prone to clinical JD than other breeds, so it can be inferred that there is a strong genotype and possibly phenotype role at work.

The harder people look for MAP the more is found (Chapter 5). It may be that, under some systems, MAP in carcasses- and hence in animals- is ubiquitous. Certainly overseas data suggest that high levels of prevalence are seen under some of their more intensive systems. However, as MAP becomes more prevalent, or as detection rates improve, it is necessary to question the role of MAP in the disease. For if every animal is hypothetically infected with MAP then detection of (and hence prevalence of) MAP is of little consequence. What becomes increasingly important is the role of co-factors which trigger either increased shedding, or clinical JD, or both.

The most comprehensive and robust data available on JD or MAP prevalence come from analysis of lymph nodes at slaughter in deer and from a comprehensive study performed by Massey University on all species (although dairy cows were not sampled, only the farmers were surveyed). Other data are available, but they are in a more piecemeal fashion and less reliable. However, certain trends shine through, so that there is some degree of comfort that around 60% or more of deer carcasses in slaughterhouses have been found to have MAP infection. Interestingly, although the data are conflicting, the figure of ~60% MAP prevalence is also found in both sheep and dairy cattle work in other studies.

The prevalence of clinical JD is particularly challenging to establish. The insidious nature of the disease does not lend itself to easy identification; nor does MAP lend itself to simple post mortem verification. Researchers are frequently left to rely on farmer survey data as a very unreliable tool to determine JD prevalence, and indeed incidence. The Massey Epidemiological Survey tried to minimise the bias inherent in these surveys by including a whole section on leptospirosis, in an effort to also pull data from people who weren't so pre-occupied with JD. Thus, their findings are probably among the most robust with regard to prevalence.

MAP in product is a critical issue (Chapter 5), because it cuts to the very heart of the JD issue within New Zealand. Being so dependent on primary produce, New Zealand is

particularly sensitive to consumer perception around the methods of food production and the safety of that product. There has been a reasonably informed debate on the role of JD in Crohn's disease (CD) in humans for a number of years. Certainly, there is an association, but there is no direct causal relationship yet established. The dairy industry is particularly vulnerable because, not only is it New Zealand's most important industry and exporter, but for some reason the popular debate amongst the consumer is more concerned with the risk of MAP in milk rather than meat.

MAP can find its way into both products- neither is overly desirable from the consumer perspective, notwithstanding that for all that is known they may already live in a sea of MAP. However, rigorous quality control around processing of both meat and milk can significantly reduce the risk in both products. The risk of MAP being found in pasteurised commercial milk has been demonstrated to be highly unlikely if appropriate procedures are followed.

Ultimately, the public seek assurance that their food is safe; or at least as safe as possible. More importantly, they expect all steps to be taken to identify and minimise risk. The knowledge that MAP can make its way into food products poses great danger to NZ Inc. The positive effects of many (all?) JD control and management plans are frequently called into question. However, it may be better to be doing something, however imperfect, than nothing. Furthermore, at the very least an appropriate control programme should aim to reduce the prevalence of MAP in the farming environment, pre and post slaughter/production. It is outside the scope of this review, but a form of control and management, or risk management plan, is crucial to develop for the industry. It may appear unworkable, but a similar plan imposed upon on the industry from outside would be quite a significant threat to current production processes.

Partly because of the extensive nature of New Zealand's livestock farming, partly because of size and history, and perhaps also for other more esoteric reasons, the livestock industries do not record individual disease as comprehensively as may be expected. Consequently,

determining both clinical JD prevalence and incidence from records and data mining is challenging (Chapter 6). Use has been made of cull cow records, but the majority of data comes from surveys. Surveys themselves present significant and well understood challenges and risks of bias. Furthermore, in the absence of quality data, farmers in New Zealand tend to have a dichotomous approach to JD- either they never see it, and any clinical cases are put down to something else; or everything sick and dying they see is attributable to JD.

Patterns emerge in the literature, however. So it appears that clinical JD is present at a fairly low level in all species, but in all species there is a significant tail of the population where within-herd prevalence (and incidence) is particularly high. In deer in particular, this may reach 20%. For sheep and dairy farms, there are likely a smaller proportion of farms with a lower- but still significant- within-farm prevalence. In these 'tails' of each industry there are likely to be significant economic loss; and also the greatest risk of transmission of MAP, both into new herds and possibly species, and across into the food chain. The most robust data we have on the prevalence of clinical JD- positive herds suggest that around 20% of both dairy and sheep farms may experience clinical JD. The figure for deer farms is higher, possibly up to 34%. It is possible that all figures are underestimating the true prevalence of farms where MAP infection causing clinical disease exists.

With the prevalence of MAP, the situation is quite different. High levels of within-herd MAP prevalence have been found in deer, serologically, through faecal cultures and in slaughter surveys. The data from deer consistently suggest that within-herd MAP detection is more likely than non-detection, with a figure of around 60%. Similarly, at a herd level, the prevalence of MAP infection appears more common than the absence. For dairy farms, there are conflicting data, from low levels of herds detected with MAP by bulk milk ELISA (around 3-5%), and subsequent estimates of within-herd prevalence of around 2%; to reports suggesting up to 65% of affected herds may be detected with MAP. However, within-herd prevalence data are very scant for dairy cattle, as they are for all species except deer.

In sheep, flock prevalence of MAP is not dissimilar to deer, being around 68%. However, recent work suggests that the prevalence of MAP-positive flocks is higher in the North Island than in the South Island, in contrast to deer and dairy, both of which have a higher prevalence of MAP-infected herds in the South Island.

Given the sensitivity of diagnostic tests, it should be noted that MAP infection is essentially a proxy for MAP detection. As diagnostic techniques improve and analytical techniques are refined, the proportion of herds or flocks designated MAP positive is likely to only increase.

Looking at an industry level, a large amount of data are available within the deer industry, which is disproportionate in scale for the size of the industry. The deer industry has put a lot of focus on JD and MAP over its recent history. In contrast, the dairy industry, which contributes over a third of the GDP of all primary sector industries (which includes all meat and milk industries, horticulture, fishing, forestry and mining combined), is less well represented. In a similar vein, it can be argued that the dairy industry has the most to lose from any loss of consumer confidence associated with MAP risk, unless it is possible to demonstrate a clearer understanding of MAP/JD within the industry.

There are clear gaps in the knowledge of MAP and of JD across all industries. Some of these relate to the economic impact of MAP/JD presence; some to prevalence and incidence and their detection; and some to co-factors which trigger increased risk. Most importantly, the most economically important sector of our livestock industries has been largely overlooked in recent years. The reviewers propose (Chapter 7) that greater focus is paid to the dairy industry with regard to understanding the current situation of MAP/JD prevalence. Furthermore, that industry is best placed to undertake comprehensive economic analyses and both longitudinal and intervention studies which would enhance the understanding of MAP/JD overall, and which critically could and should lead to the development of a comprehensive approach to the management and control of JD/MAP.

The goal of any control and management programme should ultimately be to reduce the risk of MAP in the food chain and enhance consumer confidence in the product. To be successful, any programme needs to succinctly identify its outcomes prior to its development, and be able to measure progress as it develops. The measurement of consumer confidence is challenging, and proxies may be necessary. The current understanding of the prevalence of both MAP and JD across all species is not yet comprehensive enough in all areas to adequately develop an appropriate food safety and assurance programme. However, given the vital role of the livestock industry to New Zealand, and the significant risk attached to any disruption of consumer demand, the importance of getting into a position where it is possible to successfully implement an appropriate programme cannot be understated.

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Acronyms

CD- Crohn's Disease

CFT- complement fixation test

CFU- colony-forming units

CMI- cell mediated immunity

ELISA- Enzyme linked immunosorbant assay

AGID- Agar-gel immunodiffusion

HTST- high temperature short time

IFN-γ –interferon gamma

IL2- Interleukin 2

JD- Johne's Disease

JDRC- Johne's Disease Research Consortium

JML- Johnes Management Limited

LCA- latent class analysis

LIC- Livestock Improvement Corporation

MAC- Mycobacterium avium complex

MAF- Ministry of Agriculture and Forestry

MAP- Mycobacterium Avium Paratuberculosis

OIE- Office International des Epizooties, now known as the World Organisation for Animal Health

PCR- Polymerase Chain Reaction

PI –production index

PPD- purified protein derivative

DNA- deoxyribonucleic acid

SCC- somatic cell count

SSR- short sequence repeats

TH1- T helper cell 1

TH2- T helper cell 2

VNTR- variable number tandem repeats

ZN- Ziehl-Nielssen

Chapter 1. Introduction

The Aetiology and Pathogenesis of Johne's Disease

Johne's disease (JD) is a chronic, progressive, infectious, bacterial enteritis that primarily affects ruminant animals. The disease causes wasting and often profuse watery diarrhoea, culminating in the death of the animal.

The causative agent of JD is the acid-fast, slow-growing bacterium, *Mycobacterium avium* subspecies *paratuberculosis.* It is often designated, together with *M. intracellulare* and two other subspecies of *M. avium* (*M. avium avium* and *M. avium silvaticum*) as part of the *M. avium* complex, or MAC (Thorel et al., 2001). However, the classification of this genus is controversial and some would advocate that the causative agent of JD should be considered a separate species, *Mycobacterium paratuberculosis* (Chiodini, 2005; Clarke, 1997). In this review, the official taxonomy will be adhered to, and the organism referred to as *M. avium paratuberculosis*, or MAP.

Mycobacteria have lipid-rich, impermeable cell walls and can survive for long periods in soil, faeces and water. Young animals are the most susceptible to infection. In calves, the greatest risk of infection with MAP occurs before 30 days of age, although clinical signs do not usually develop until cattle are at least two years old (Chiodini et al., 1984). Sporadic cases due to infection of older animals do occur, however (Clarke, 1997). The most common route of infection is ingestion of the organism (Stabel et al., 2009). This usually occurs when young animals suckle teats contaminated with faecal material or graze contaminated pasture, but direct secretion of mycobacteria into milk or colostrum also occurs. Intrauterine infection has

been documented (Hasonova et al., 2009), particularly in deer (Mackintosh and van Kooten, 2005), but it is not known whether animals infected in utero inevitably develop clinical disease (Thompson et al., 2007).

After ingestion, MAP organisms are engulfed by epithelial cells overlying the intestinal Peyer's patches, especially in the ileum (Begg et al., 2005; Sweeney et al., 2006a). The mycobacteria are then transported to macrophages in adjacent tissue layers. The complex cell walls of mycobacteria may help them to survive the killing mechanisms of the macrophages, although a number of other mechanisms are also involved (see review by Clarke, 1997). Mycobacteria that succeed in establishing in the intestinal macrophages stimulate a host immune response. Most host animals are capable of eventually clearing the infection, but in some sub-clinically infected animals, small numbers of bacteria are able to survive for prolonged periods. These animals may eventually go on to develop clinical disease (Gilmour et al., 1977). Others may remain as asymptomatic carriers and continue to shed bacteria without developing disease (Chiodini et al., 1984). The triggers required for this to occur are largely unknown, but stress often plays a role. Whether or not disease expression occurs is probably dependent on the strength and persistence of the host cell-mediated immune (CMI) response (Chiodini et al., 1984; Gilmour et al., 1977). Because mycobacteria reside within cells, they do not stimulate a humoral immune response until late in the course of infection, when the death and rupture of infected cells and consequent release of mycobacterial antigen stimulates the formation of antibody. By this stage, the CMI is declining (Begg et al., 2005; Clarke, 1997).

The clinical signs of JD are due to the host immune reaction to the mycobacteria (Chiodini et al., 1984). The influx of inflammatory cells into affected tissues impairs circulation and the release of inflammatory mediators may also cause damage. The damaged intestine fails to absorb dietary protein and leaks fluid, resulting in a protein-losing enteropathy and

progressive emaciation. Diarrhoea usually results, although it may be intermittent and is not common in sheep, as it is in deer or cattle. The clinical course of the disease is usually 1-6 months in cattle and sheep. In deer, there is evidence that the younger the animal at clinical onset, the faster the progression of the disease (Mackintosh et al., 2004).

There is no effective treatment for JD (Chiodini et al., 1984). There is a vaccine available in New Zealand for sheep (Gudair^{TM}), but it does not prevent the disease, only reduces clinical signs (Britton, 2001). It also interferes with routine skin tests for tuberculosis (Mackintosh et al., 2005), so is not licensed for use in cattle or deer.

Throughout this review, unless otherwise stated, JD refers to the clinical manifestation of Johne's disease following MAP infection.

Implications of Johne's Disease for New Zealand Livestock

Clinical JD could cause losses to producers due to reduced meat and milk production, reduced life expectancy of affected animals, reduced price of cull animals and through increased animal health costs associated with diagnosis or attempted treatment for other causes of wasting. Subclinical disease may also result in losses, although these are more difficult to measure.

In a study of six Taranaki dairy herds known to have clinical cases of JD, production data were correlated with the infection status of all cows within each herd (Milestone and de Lisle, 1986). Four of the affected properties had total milk fat production per cow that was actually greater than the average for the region. A production index (PI) was also calculated for each cow which took into account other factors such as age and calving date as well as milk production. In all cases, cows shedding MAP had a lower PI than cows that were not

shedding MAP, but this was only significant on two farms. The economic losses associated with the lost production were calculated and varied widely. On the least affected properties, the financial costs of JD were minimal, but on the worst affected properties the costs were substantial.

In a more recent study of nine Wisconsin dairy herds over a six year period, JD had no noticeable effect on milk production (Collins et al., 2010). In contrast, a JD-infected Irish dairy herd experienced significant reductions in milk yield and prices of culled animals, combined with an increase in the number of animals culled (Richardson and More, 2009). These losses were due to clinically affected animals only; sub-clinically infected animals (identified by serological tests) had similar milk production to uninfected animals in the herd. (However, the possibility that some animals which tested negative were actually infected and therefore skewing the production data should not be overlooked). Culling for infertility dropped from 4-14% before a JD control scheme was implemented, to 3-4% after the scheme, and the authors base this fall largely on the control of JD.

Because MAP is secreted into milk (Sweeney et al., 1992), it might be expected to increase somatic cell counts (SCC). This is important, because in New Zealand, financial penalties are imposed on milk producers when SCC in bulk tank milk increase above a predetermined level. Increased SCC have been observed in at least one study (Baptista et al., 2007) but not in others (Collins et al., 2010; Milestone and de Lisle, 1986). Whether or not JD results in sufficiently increased SCC to have economic effects is therefore unknown.

In the 1980s, JD was reported as the biggest cause of ill-thrift and diarrhoea in adult sheep in New Zealand (Gumbrell, 1986) and it was estimated that 0.8% of North Island flocks and 10% of South Island flocks were affected. More recently, in order to quantify the production loss associated with infection, a naturally infected experimental sheep flock was monitored

over eight years (Morris et al., 2006). A post-mortem examination was performed on all ewes over two years of age that died during the study to establish the rates of MAP infection, and production measures were recorded. Live weight, fleece weight, number of lambs born and overall lifetime production were all significantly reduced in ewes with JD. The overall loss of productivity was estimated to be 46% compared with healthy ewes. A production loss of this magnitude would be expected to have significant financial costs to farmers. However, another study examining the effects of subclinical infection in vaccinated and unvaccinated sheep failed to show any production loss in sub-clinically infected ewes (Thompson et al., 2002).

Whereas clinical cases of JD tend to be sporadic in sheep and cattle, affecting only a small percentage of the adult animals each year, it is not uncommon to have large outbreaks of disease in mobs of yearling deer, affecting up to 10% or more of the mob (Mackintosh and Wilson, 2003). Such outbreaks can have serious financial consequences for farmers. Sporadic deaths in adult deer also occur.

Subclinical effects of MAP infection have been better documented in deer than in other species. Abattoir surveillance data show that sub-clinically infected deer have carcass weights up to 30% lower than uninfected deer (Hunnam et al., 2009). Reduced fertility in hinds (Thompson et al., 2007) and reduced velvet production in stags (Wilson et al., 2009) can result from subclinical infections. There are also additional testing costs associated with the necessity to distinguish MAP infection from tuberculosis in deer that react to the tuberculosis skin test, or that present with tuberculosis-like lesions at slaughter (Mackintosh et al., 2004).

The most comprehensive analysis of the economic effects of JD on the New Zealand livestock industries was carried out by Brett in 1998. She estimated that the overall cost of clinical disease to the sheep, cattle and deer industries at that time was about $29.2 million (Brett, 1998) (approximately $40.6m in today's prices) and concluded that the cost of JD was

not large in comparison with other diseases. However, she conceded that the lack of information on subclinical effects of the disease and on disease prevalence impeded accurate analysis. Despite this apparent low cost of the disease, the models presented indicated that control could be economically feasible but needed to be tailored for specific situations.

Historical Data on the Prevalence of Johne's Disease in New Zealand

The association of JD with acid-fast bacteria in animals with enteritis was first described by Johne and Frothingham in Germany in 1895, but there are many earlier reports of chronic diarrhoea and wasting in European cattle that are consistent with a diagnosis of JD. Johne and Frothingham thought that the disease was an atypical form of tuberculosis and it was Bang in 1906 who first recognised it as a separate entity and proposed the name Johne's disease (Twort and Ingram, 1913).

The history of JD in New Zealand has been reviewed by De Lisle (2002). It was diagnosed first in 1912 in an imported cow, and then again in 1928 in Taranaki. Further recognition of the disease in dairy herds throughout Taranaki and Waikato lead to the scheduling of JD as notifiable under the Stock Act in 1931. By the late 1950s there were a handful of cases reported in South Island dairy herds (Chandler, 1957) and the disease has since continued to spread in both islands.

The first case of JD in sheep was reported in 1952 in South Canterbury, but there had been sporadic cases of wasting and death in adult ewes on the property for at least the previous ten years (Williamson and Salibury, 1952). At the time, JD was still uncommon in cattle in the South Island and the source of the infection was never traced. By 1956, there were cases on at least 14 farms in South Canterbury and two near Ashburton, with between 0.5-4% of the ewe

flock affected on each farm (Armstrong, 1956). On every farm the disease had been present for a number of years before diagnosis; possibly more than 30 years in two cases. Johne's disease was first diagnosed in Southland in 1966 (Davidson, 1970) but not in North Island sheep until 1972 (Gumbrell, 1986). Although not confirmed, the initial North Island case was thought to have come from South Island sheep, rather than North Island cattle. In 1979, Manktelowe predicted marked increases in the disease over the next two decades; by 1986 the number of infected farms had far exceeded expectations (see Gumbrell, 1986).

The first case of JD diagnosed in deer was in a Rusa deer in Northland in 1979. The animal died without showing signs of wasting or diarrhoea, but enlargement of mesenteric lymph nodes and histological lesions in the ileum lead to a diagnosis of JD. The first clinical cases of JD were diagnosed on a number of farms throughout the North Island in 1985 (Gumbrell, 1987).

In considering the early history of JD in New Zealand, two things are apparent. First, the patterns of disease spread in cattle and sheep are different, suggesting that the disease does not spread readily from one species to the other. More recent work confirms this (e.g. Collins and de Lisle, 1990; Collins et al., 2011; Moloney et al., 2003), and will be discussed later in this review. Second, it has been common, at least historically, for the disease to be present in an area for quite some time before it is diagnosed. As well as contributing to the spread of the disease, this lack of recognition of infection has made it difficult to obtain accurate estimates of the prevalence of JD throughout New Zealand. However, farmers can be reluctant to have JD diagnosed on their property, even when it is suspected, due to the risk (real or perceived) that it will affect their ability to sell stock (Chandler, 1957; de Lisle, 2002). Johne's disease was removed from the list of notifiable organisms in 2000 (Poland, 2001), which may have led to better reporting of disease (Voges, 2008), but there are still significant gaps in the

understanding of JD prevalence in New Zealand, the specifics and implications of which will be discussed later in this review.

Chapter 2. Definitions

Studies examining the prevalence of JD in New Zealand do not all measure prevalence the same way. In order to understand the scope and limitations of each study, it is necessary to have an appreciation of what was actually measured.

The definition of what constitutes an infected animal may vary from study to study. Formal definitions are hard to find. In a 1990 survey carried out by MAF Quality Management in New Zealand, an infected property was considered to be one on which animals had had positive faecal culture results, had been found to have acid fast organisms in their faeces or on post-mortem examination, or had histological evidence of a granulomatous ileitis/lymphadenitis with acid-fast organisms present (Nuttall, 1991). Similarly, the Australian National Johne's Disease Program Standard Definitions and Rules defines an infected animal as one which is confirmed as infected by histopathological or bacteriological examination (Anonymous, 2010a). For practical purposes, these definitions are necessarily based on responses to diagnostic tests, but because of the limitations of the available tests, such definitions do not reflect true disease states.

The reviewers have been unable to find a standard, agreed definition of the various manifestations of MAP infection. For the purposes of this review it is proposed to use the following categories of animals:

1. MAP detected. MAP is detectable in the animal by one or many of the various diagnostic techniques discussed later in this review.

Within this category are animals that are:

a. *Clinically affected.* They have clinical signs of disease.
b. *Subclinically infected.* They have no clinical signs, but may have other gross post mortem changes (e.g., enlarged lymph nodes) or have a positive diagnostic test, or have a measurable productive loss that is associated with MAP infection.

2. Non-MAP detected. MAP is not detectable using existing tests, or has not been detected using one or many of existing tests.

Within this category are animals that:

a. Have not been tested but may or may not have MAP
b. Have been tested, have MAP, but this has not been detected by diagnostic tests
c. Have been tested and do not have MAP

Note that, in the literature and in research generally, other categories of MAP-associated stock are mentioned such as 'latently infected', 'pre-clinical', 'affected', etc. However, there is no general agreement on the meaning and implications of these terms, and for the purposes of this review the categories stated are used. The implications of this classification will be discussed later in the review, but below is a summary of the key states identified above.

1a- Clinical Johne's disease

Johne's disease is the clinical entity that represents the 'final stage' of MAP infection. Affected animals present with clinical signs such as diarrhoea and wasting, terminating in death (if the animal is not slaughtered for humane reasons). Twort and Ingram, in the early

days of JD research, defined the disease as “A chronic specific enteritis, affecting cattle, more rarely sheep and deer, and probably goats and allied animals, caused by the multiplication, in the intestinal mucous membrane and mesenteric glands, of a specific micro-organism known as "Johne's bacillus," which produces a diffuse thickening of the bowel and an interference with food absorption, leading to diarrhoea and wasting” (Twort and Ingram, 1913). This definition could still be applied today.

Animals with clinical JD can often be identified with a reasonable degree of certainty by history and clinical signs alone. They usually shed large numbers of bacteria in their faeces, which enables them to be readily identified by faecal culture, but this also means they pose a major threat in terms of disease transmission. In the clinical stages of the disease, the host immune response to the MAP organism is dominated by a TH2 immune response. As such, the animals usually produce antibodies and test positive to commonly used serological tests (O’Brien et al., 2003). At the same time, the TH1 response that dominates in earlier stages of infection generally wanes, so that tests for cell-mediated immunity tend to return negative results.

The prevalence of clinical JD is therefore significantly lower than the prevalence of MAP infection in a population. Note also that the distinction between incidence and prevalence means that, with regard to JD, the incidence of clinical JD may fall before the prevalence falls, because new cases may not arise, but the prevalence of existing clinical cases may continue to be high. Incidence conveys information about the risk of contracting the disease at any given time, whilst prevalence indicates how widespread disease is within a population.

1b- Subclinical disease or MAP infection

MAP infection is the presence of MAP bacteria in animal tissues, with or without the subsequent development of clinical disease. In the early stages of infection animals do not show any clinical signs of disease. Some (perhaps most) animals go on to clear the infection without ever developing JD. Others remain sub-clinically infected for a period of months or years, and some of these eventually go on to develop clinical JD (Chiodini et al., 1984; Clarke, 1997; Gilmour et al., 1977). Estimates of the prevalence of MAP infection should thus include animals in all possible stages of infection.

Sub-clinically infected animals are difficult to identify using currently available tests, and so the prevalence of subclinical infection is hard to determine. In the early stages of infection, animals may be difficult to detect by faecal culture because they shed few bacteria in their faeces or do so only intermittently. They do not usually produce antibodies that can be detected by serological tests, but they may react positively to tests for cell-mediated immunity. When attempting to determine the prevalence of MAP infection, and the incidence rate of new infection, sub-clinically infected animals present the greatest challenge.

2. Non-MAP detected

These animals may genuinely not have MAP; they may not have been tested; or they have been tested but because of the poor sensitivity of many of the tests MAP has not been detected. It is worth noting that as the diagnostic tests improve and the understanding of MAP diagnostics increases, an increasing proportion of animals are being diagnosed with MAP

infection. Given the limitations of our diagnostics it is not inconceivable that the presence of MAP in farmed animals is the normal state.

Chapter 3. Diagnostic Testing for *Mycobacterium paratuberculosis*

Difficulties in determining sensitivity and specificity of tests

The results of any diagnostic test must be interpreted in the light of the known sensitivity and specificity of the test. However, the determination of sensitivity and specificity is fraught with difficulty.

The sensitivity of a test is the accuracy with which the test correctly identifies a truly infected animal. In order to determine this, the test must be performed on a large number of known infected animals. The problem is to establish a population with known infection status in the absence of a "gold standard" test that will identify 100% of infected animals. In the case of MAP, infected animals are difficult to identify early in the course of the disease, so a known-infected population is likely to contain a larger proportion of clinically affected animals than sub-clinically infected animals. Sensitivity estimates determined under these conditions may not be representative of the sensitivity of the test in the field (Worthington, 2004).

The specificity of a test is the accuracy with which the test will correctly identify non-infected animals, and is established by assessing the results of the test in a known non-infected population. Mycobacteria are ubiquitous in the environment, so non-infected animals may be sensitised and react to tests with low specificity, but the frequency with which this happens is difficult to determine (Worthington, 2004).

There is currently no gold standard test available for MAP. Tests for MAP are most often validated by comparison with post-mortem examination or faecal culture (Norton et al.,

2010). However, the slaughter and post-mortem examination of the large number of animals required to validate a diagnostic test is expensive and cumbersome, and faecal culture is not 100% reliable. Serial sampling of animals over a prolonged period is another option, but is again expensive and laborious. It is not surprising then, that the reported sensitivity and specificity of diagnostic tests varies widely.

Direct Microscopy

One of the simplest tests for MAP is Ziehl-Neelsen (ZN) staining of smears of faeces, mucosal surfaces or the cut surfaces of lymph nodes for microscopic examination (OIE, 2008). The acid-fast organisms stain bright red. Fluorochrome stain (auramine-O or auramine-rhodomine) can also be used. However, the test is not specific for MAP, as other mycobacteria and some non-mycobacterial species such as *Nocardia* species are also acid-fast. Experience is needed to be able to distinguish MAP from other acid-fast organisms (OIE, 2008; Payeur, 2005) and other tests may be required for confirmation. The organism may not be present in the faeces of infected animals in the early stages of the disease, and is easily missed if levels of faecal shedding are very low. Smears are therefore not sensitive or specific enough for definitive diagnosis.

Histopathology

Histology allows direct visualisation of pathology and organisms in thin slices of tissues and is usually considered the gold standard test if post-mortem samples are available. If gross lesions are visible, this test is very sensitive, but in the absence of obvious lesions a large number of sections may need to be examined in order to detect MAP, especially in animals with low levels of infection.

The tissues of most value for diagnosis of MAP infection are the terminal ileum, the ileocaecal valve and the associated lymph nodes (Chiodini et al., 1984). In deer, these lymph nodes may be more useful than the intestinal tissues for demonstrating infection in mild cases of disease (Clark et al., 2010), although the lesions in deer may be hard to distinguish from those of tuberculosis (de Lisle et al., 2003).

The characteristic lesions of JD are accumulations of epithelioid cells and multinucleate giant cells in the mucosa, submucosa, Peyer's patches and the cortices of regional lymph nodes (Clarke, 1997). Clumps of acid-fast organisms (0.5-1.5µm diameter) may also be present in the cells (Payeur, 2005).

Clark *et al.* (2010) developed a histopathological grading system to correlate the severity of clinical disease in red deer with the histological lesions seen at post-mortem. Paucibacilliary (containing few bacteria) lesions were seen mainly in animals with mild clinical disease, and multibacilliary (containing many bacteria) forms correlated with more severe disease. Langhans giant cells were more common in paucibacilliary forms of the disease.

Culture techniques

Culture of MAP from the tissues or faeces of an infected animal is often used as a gold standard diagnostic test. Faecal culture can detect infected animals up to 6 months before development of clinical signs (OIE, 2008), and identifies animals within a flock or herd most likely to be contributing to pasture contamination. However, MAP is a fastidious organism and difficult to grow. It needs specialised media, and has an obligate requirement for mycobactin, an iron-binding chemical necessary for transport of iron into the cell. Inability to synthesise mycobactin is a unique characteristic of MAP.

MAP grows very slowly in culture and is easily overwhelmed by faster growing organisms, so the initial step in the culture process involves decontamination to remove these where possible. Inevitably, some MAP organisms will be lost in the process, reducing the sensitivity of the culture technique (Gao et al., 2009).

The sensitivity of faecal culture is also limited by the fact that many infected animals in herds with lower levels of infection may not be actively shedding bacteria in their faeces at the time of testing. Whitlock *et al.*, (2000), by repeatedly testing animals in ten herds at six monthly intervals estimated that 70% or more of the infected animals were not shedding bacteria at a given time. Only 38% of animals later found to be infected were detected at the time of first sampling. The sensitivity of faecal culture in deer varies between farms, partly as a result of different levels of faecal shedding, but also due to differences in prevalence and the proportions of animals in clinical or subclinical stages of the disease (Wilson et al., 2009).

For detection of MAP in the tissues of infected animals, the sensitivity of culture can be limited by the selection of too few sites for sampling, especially in the early stages of infection (Sweeney et al., 2006a). The ileocaecal lymph node is usually recommended as the primary tissue to collect for culture, but MAP is not consistently isolated from this site. The sensitivity of culture can be improved by including additional jejunal and ileal samples, particularly during the early stages of infection.

Faecal or tissue culture is generally considered to have 100% specificity for MAP. However there may be instances when animals ingest the bacteria, which pass through the gastrointestinal tract in approximately 48 hours and are shed in faeces without establishing an infection in the host (Sweeney et al., 2006a). Although probably rare, it is possible that false positive faecal cultures could occur due to such passive shedding of the organism.

One of the major drawbacks of faecal culture is the time required to perform the test. The Gribbles Veterinary Laboratories in New Zealand report that on average cattle strains of MAP take 9-16 weeks to grow and sheep strains 13-20 weeks, and these times can be extended even further if tests need to be repeated for any reason (Gribbles Veterinary, 2006).

Traditional culture techniques use solid media, both with and without the inclusion of mycobactin, to demonstrate mycobactin-dependency of the organism (OIE, 2008). Radiometric liquid culture techniques incorporate radiolabelled palmitate, which is metabolised by mycobacteria to labelled $^{14}CO_2$, thus indicating growth of the organism. Other non-radiometric liquid culture systems have now been developed and are used in some labs (OIE, 2008; Payeur, 2005). Herrold's egg yolk medium (HEYM) is commonly used but Middlebrook 7H10 may be required for ovine strains.

Collins (2005) discussed some of the issues associated with culture techniques, including non-standardisation of techniques between laboratories and the labour intensity required. Contamination of samples by non-mycobacterial organisms despite decontamination steps can result in the loss of more than 10% of samples. Contamination rates can be lowered by using smaller amounts of faeces in the culture, but this is to the detriment of the test sensitivity. Stabel et al (1997) similarly found that methods effective at reducing contamination have lower sensitivity. The limits of detection of faecal culture have been reported to be in the region of 10-50 colony-forming units (CFU)/g faeces (Schroen et al., 2003a). For comparison, the animals from which the faeces were obtained (sub-clinically infected deer) were excreting 10^1 to 10^6 CFU/g faeces.

An Australian study comparing the results of various diagnostic tests for use in deer found that the culture of tissue samples collected at post-mortem examination was the most sensitive method (92%) for diagnosing MAP infection in this species (Schroen et al., 2003a).

Faecal culture detected 49% of the infected animals and histopathology 66%. It was also noted that the results of these three tests did not correlate well with one another.

A number of workers have investigated the use of pooled faecal cultures as herd screening tests that would reduce both the cost and the labour involved in testing large numbers of animals. In sheep flocks, Whittington et al (2000a) found that pooled faecal cultures containing one faecal pellet each from 50 animals detected all infected flocks when the infected animals within the flock were shedding high numbers of bacteria in their faeces. When the infected animals were only shedding low numbers of bacteria, then only half the infected flocks were detected by pooled faecal culture. This would limit the usefulness of pooled faecal culture in flocks with high levels of paucibacilliary disease. However, the test was 30% cheaper to run than the enzyme-linked immunosorbant assay (ELISA) test, and labour costs for sample collection were also reduced, making it very economical. Six pools per flock had to be tested to be 95% confident of detecting flock infection if within-flock prevalence was greater than 2%. Pooled faecal culture was found to be more sensitive than agar-gel immunodiffusion (AGID), the recommended serological test for MAP in sheep.

In deer, pooled faecal culture with a pool size of up to 50 animals identified 100% of infected pools when one positive animal shedding large numbers of bacteria was included in the infected pool. However, the sensitivity of pooled faecal culture decreased to 25% for pool sizes of 10 to 50 when animals shedding medium numbers of bacteria were included. When positive animals shedding only low numbers of bacteria were included, faecal culture failed to detect positive pools including more than 10 animals, and only detected 25% of positive pools when the pool size was 10 (Mackintosh and van Kooten, 2005). In another study in deer, using a pool size of 10 and six pools per farm, 70% of infected herds were detected. This was increased to 82% if specific animals in the herd exhibiting symptoms of scouring and weight loss were targeted for the collection of samples (Glossop et al., 2007b). The

detection limit of pooled faecal culture in deer has been estimated as approximately 100 CFU/g faeces for a pool size of 10-20 samples (Schroen et al., 2003a).

MAP can be isolated from the milk of affected dairy cows (Sweeney et al., 1992), so milk culture offers a potential alternative to faecal culture as a diagnostic test in dairy herds. Stabel et al (2004) found that while there were no apparent increases in faecal shedding of MAP in 12 recently calved, sub-clinically infected cows, levels of MAP in colostrum at this time did increase. The levels of antibody to MAP and gamma interferon (IFN-γ) activity decreased after parturition, suggesting that serological tests would have reduced sensitivity. Milk culture may therefore be an effective diagnostic technique for detection of MAP. Gao *et al.*, (2009) found that milk and faecal cultures were equally sensitive at detecting infected animals within a herd, but because there was no correlation between milk test results and faecal test results, suggested that both should be used concurrently. They estimated that milk culture alone would fail to detect 29% of infected animals, while faecal culture alone would miss 41%. Pillai and Jayarao (2002) found that milk cultures were far less sensitive than PCR on milk samples (4% compared with 33%).

Mycobacteria can survive well in soil because they have a low metabolic rate and have cell walls with a high lipid content. Survival is enhanced in wet, shaded conditions (Schroen et al., 2003b). Cultures of environmental samples might therefore present further opportunities for the detection of MAP. In a Dutch study, 246 herds with at least one ELISA positive cow present were identified. Samples were collected from alleyways and from the slurry pit, and individual faecal cultures were performed on all cows that had had a positive ELISA test (Weber et al., 2009). Faecal cultures identified 88% of the herds as having at least one infected cow. Culture of the slurry samples detected 92% of the faecal culture positive herds, and alleyway samples detected 88% of faecal culture positive herds. Testing both alleyway and slurry samples detected 95% of faecal culture positive herds. In a similar study, Berghaus

et al., (2006) collected three composite samples from the exit to the milking shed, the sick cow pen and the wastewater lagoon on 23 California dairy farms. The sensitivity of testing three environmental samples to detect an infected herd was equivalent to performing ELISA tests on 60 cows or pooled faecal culture of 60 cows for detection of infected herds, but labour and testing costs were much lower. Environmental sampling might thus be useful as a herd screening test in some situations.

Cell-Mediated Immunity

(a) Intradermal testing

Intradermal testing is a measure of the delayed-type hypersensitivity reaction that occurs when antigen is injected into the skin of a previously sensitised animal. Either avian purified protein derivative (PPD) tuberculin or johnin antigens have been used. Changes in skin thickness greater than 2mm at 72 hours after injection indicate that a delayed hypersensitivity reaction has occurred (OIE, 2008). The sensitivity of the test has been estimated at 54% and the specificity at 79% (Chiodini et al., 1984), so this test is not often used for the diagnosis of MAP infection.

(b) Lymphocyte stimulation

The lymphocyte stimulation test also measures delayed hypersensitivity in response to johnin, but the antigen is injected intravenously. After six hours, the test is considered positive if the animal's body temperature has risen by more than 1.5 degrees C, or if there is an increase in the neutrophil:lymphocyte ratio of greater than 2:1. Measuring the temperature is less laborious than measuring the neutrophil:lymphocyte ratio, but is also less accurate. Occasionally anaphylactic reactions can occur. With a sensitivity and specificity of around

50% (Chiodini et al., 1984; Worthington, 2004), lymphocyte stimulation is not accurate enough to be routinely used.

(c) Measurement of Cytokines

Tests for cell-mediated immunity should in theory be better than tests for antibody for identifying sub-clinically infected animals, since cell-mediated immune responses dominate during the early stages of MAP infection. Interferon gamma (IFN-γ) and interleukin 2 mediate the TH1 responses seen early in the course of infection and could be measured to indicate infection(O'Brien et al., 2003). However, cytokines such as these are difficult to measure because they are only produced in small quantities and are often attached to the surfaces of the cells that produce them. Detection therefore requires the use of specific monoclonal antibodies that are difficult to produce. Alternatively, real-time polymerase-chain reaction (PCR) can be used to detect the mRNA that is involved in the production of cytokines, with the number of amplification cycles required before the product is detected being proportional to the amount of cytokine produced.

A commercially available test for bovine tuberculosis has been used for the diagnosis of MAP infection (Kalis et al., 2003). Sensitised lymphocytes incubated with avian PPD, bovine PPD or johnin antigen release IFN-γ that can subsequently be detected by an ELISA using monoclonal antibodies to IFN-γ. However, due to lack of information on the use of this test for MAP, interpretation of the results is difficult (OIE, 2008). Kalis *et al*., (2003) developed a new algorithm for test interpretation, which improved the specificity of the test from 66-67% when interpreted according to the manufacturer's instructions, to 93.6%. The low specificity is typical of tests for IFN-γ, since mycobacteria other than MAP will stimulate its release. Unless MAP-specific antigens can be identified that are released in sufficient quantities for

laboratory detection, the use of tests for cell-mediated immunity is likely to remain limited (Collins, 2005).

The other difficulty that arises from measuring cell-mediated immunity is that it is not known whether animals mounting a TH1 immune response will actually go on to develop clinical disease, whether they will remain carriers, or whether the immune response will successfully clear the infection (Collins, 2005). Determination of sensitivity and specificity is therefore problematic. Because the TH1 response to MAP tends to wane over time, tests for cell-mediated immunity are less useful in animals in later stages of the disease.

Complement Fixation and Agar Gel Immunodiffusion

The complement fixation test (CFT) was the first serological test available for the diagnosis of JD (Sockett et al., 1992). It performs well in cases of clinical disease, but is generally considered to have a lower sensitivity than the ELISA test for detection of subclinical infection (OIE, 2008). The sensitivity and specificity of four serological tests for MAP were assessed by Sockett *et al*., (1992), using serum samples from cattle from certified-free dairy herds and from cattle that had previously been identified as faecal culture positive for MAP but were not exhibiting clinical signs of JD. The complement fixation test had a sensitivity of 38.4% and a specificity of 99% in this study. When the infected animals were divided into those that were shedding MAP in their faeces at the time of blood sample collection and those that were not, the sensitivity of the CFT was 55% and 15% respectively.

The agar gel immunodiffusion (AGID) test has been widely used as a test for MAP, particularly in sheep flocks. The sensitivity of the AGID varies between flocks depending on body condition score (and other unidentified factors) (Sergeant et al., 2003). The sensitivity was also shown to vary from around 10% to around 60% depending on the prevalence of

clinical and subclinical disease in the flock (Sergeant and Marshall, 2000). The specificity of the AGID in this study was 99.9%.

In cattle, the sensitivity of the AGID was 41% in animals shedding MAP in their faeces but only 4% in non-shedding infected animals. The average sensitivity was estimated to be 26.6% and the specificity was 100% (Sockett et al., 1992). The sensitivity of the AGID in this study was considerably lower than that of the other serological tests evaluated.

The AGID was considered to be the best test for JD in deer before the validation of ELISA tests for use in this species (Mackintosh, 1999), but the ELISA is now more commonly used.

Enzyme-Linked Immunosorbant Assay

The enzyme-linked immunosorbant assay (ELISA) is probably the most widely used serological test for JD. Earlier versions of the ELISA suffered from poor specificity, but this can be overcome by pre-absorbing cross-reacting antibody with *M. phlei*, an environmental bacterium (Yokomizo et al., 1985). ELISA tests have been validated for use with milk as well as serum (Collins et al., 2005; Salgado et al., 2005).

Sensitivity estimates for ELISA tests have ranged between 25 and 58.8% (Collins et al., 2005; Sockett et al., 1992; Whitlock et al., 2000). However, as with other serological tests, the sensitivity increases in the later stages of disease. In one of the above studies, the sensitivity was as low as 15% in animals with low levels of faecal shedding but 87% in clinically affected animals (Whitlock et al., 2000). The specificity estimates in the same studies ranged from 84.9% to 100%, with most estimates being around the higher end of this range. There also tend to be differences in sensitivity and specificity between laboratories performing the same test (Collins et al., 2005).

ELISA tests can be performed on milk as well as serum. The sensitivity of the ELISA test on individual milk samples is similar to that of serum ELISA testing, but the test is less expensive as samples can be collected during normal milking and herd testing (Collins, 2005).

Repeated testing of dairy cattle has been carried out to estimate the age at which infected animals are first likely to be detected as being infected with MAP. Faecal and serum samples were collected yearly or twice yearly from 12 herds, over a nine year period. Only 46% of the faecal culture positive animals had one or more positive ELISA tests. Of the remainder, 50% became positive on faecal culture at a testing date earlier than the date of the first positive ELISA test, 38% became positive on both tests on the same date and only 12% returned a positive ELISA result before the first positive faecal culture (Sweeney et al., 2006b). Approximately 8% of the faecal culture negative cows were positive on at least one ELISA test. Although neither test was perfect, faecal culture tended to identify infected animals earlier than the serum ELISA. These results are not consistent with a similar study compared the use of a milk ELISA test with faecal culture for the early detection of MAP infection (Nielsen, 2008). The milk ELISA identified infected cows earlier than the faecal culture, suggesting that cows secreted antibody into milk before they began to shed mycobacteria in their faeces. There is evidence that the sensitivity of detection of both milk and serum ELISA tests increases with the level of faecal shedding, however (2006; Nielsen, 2008).

In New Zealand, Griffin et al (2005) developed an ELISA for use in deer. Two antigens were chosen that react to IgG1 antibody, which is considered specific for seroreactivity to mycobacterial disease in deer. The use of denatured purified protein derivative as the test antigen resulted in a test sensitivity of 84%. Un-denatured protoplasmic antigen resulted in a

sensitivity of 88%. When the two antigens were used serially, the test sensitivity increased to 91%, with a specificity of 99.5%. These results indicated that the ELISA test might be more useful in deer than faecal culture, for which the sensitivity was estimated to be 67.5%. The ELISA was less sensitive in sub-clinically affected animals than in those with clinical disease. The high sensitivity of the ELISA in deer compared to other species was suggested to be a result of the propensity for deer to develop clinical JD at a younger age than other species, with a concomitant earlier development of antibody (Griffin et al 2005).

Polymerase Chain Reaction

Polymerase chain reaction (PCR) techniques are based on the use of genetic probes to detect mycobacterial DNA in faecal, blood, tissue or milk samples. The technique incorporates an amplification step, allowing the target DNA in the sample to be tested to be multiplied up and therefore more easily identified. The specificity of PCR depends on the selection of the probes used for identification of the DNA.

A unique DNA sequence, known as an insertion sequence and designated IS*900*, was identified in the MAP genome (Green et al., 1989), and has subsequently been used successfully as the basis of PCR tests for MAP (Miller et al., 1999; Pillai and Jayarao, 2002; Vary et al., 1990). More recently, IS*900* has been identified in mycobacteria other than MAP (Bölske et al., 2003; Englund et al., 2002; Taddei et al., 2005). Although this is a relatively rare occurrence, it could occasionally result in false positive results on PCR tests based on IS*900*. Consequently, other DNA sequences of DNA have been investigated for potential use in PCR tests for MAP (Stabel and Bannantine, 2005; Strommenger et al., 2001; Vansnick et al., 2004).

Because PCR techniques amplify the DNA present in a sample, they have the potential to be very sensitive tests. However, biological samples tend to contain large amounts of non-target DNA as well as the DNA of interest, and the dilution factor may be such that in the small aliquots used for PCR tests, the target DNA may not be present (D. Collins and G. de Lisle, personal communication). This can limit the sensitivity of PCR tests. Van der Giessen et al., (1992) evaluated three PCR tests and found the sensitivity to be poor, ranging from 3 to 23%. More recently, the sensitivity of two PCR tests used for milk was estimated to be 41.3% and 77.8%, compared with a sensitivity for faecal culture of 71.4% in the same study (Gao et al., 2009). In a review of diagnostic techniques for MAP, the sensitivity of PCR was similar to or less than that of faecal culture in most of the studies examined (Worthington, 2004).

Pilai et al (2007) developed an IS*900*PCR assay for the detection of MAP in raw bulk milk. The limits of detection were 10-100 CFU/mL of milk, which was similar to the detection limits observed for milk culture. The PCR test was 100% sensitive when performed on milk samples containing 100 CFU/mL, but the sensitivity decreased to 50% when used on samples containing only 10 CFU/mL. When pooled quarter milk samples from 211 cows in five infected herds were tested by PCR and milk culture, 33% of the cows were positive on the IS*900* PCR test, but only 4% were positive by milk culture. Four bulk tank aliquots were then taken from each of these five herds. Fifty per cent were positive by PCR, compared with 5% by culture, indicating that PCR is a relatively sensitive test for bulk milk screening.

In addition to diagnosis of MAP infection, PCR techniques can be used to distinguish different subtypes of MAP (Bauerfeind et al., 1996; Collins et al., 2002; de Lisle et al., 2006), which may facilitate studies of the epidemiology of infection.

Latent Class Analysis

Latent Class analysis (LCA) is not a diagnostic technique but it is a useful analytical process used in parallel with 2 or more diagnostic techniques, to refine predictions and develop improved modelling processes. LCA was first developed in the 1950's, and has been used most extensively in the social sciences. It may also be referred to as finite mixture modelling. More recently, its use has spread into medical scientific literature, and indeed the OIE have now included LCA in their standards for evaluation diagnostic tests (OIE, 2010).

Originally developed to analyse clustering based on dichotomous observed variables (Lazarsfeld, 1950), its use has been modified and extended into obtaining maximum likelihood estimates (Goodman, 1974), log-linear models containing missing cell counts (Haberman, 1979), and more recently models containing continuous covariates, ordinal variables and repeated measures have been developed (Hagenaars, 1990; Vermunt, 1997).

In essence, LCA seeks to use at least 2 imperfect tests with an assumption of imperfection, and develop a model from these. Typically, there needs to be an assumption of conditional independence between the 2 tests. Toft attempted to circumvent this problem by using a third test which was conditionally independent of the first two (Toft et al., 2003a). In another study, it was suggested that case definition was more important than the assumption of conditional independence, and that having the 2 tests measuring the same thing was more critical (Toft et al., 2003b)

LCA was used to analyse the data from the Massey Epidemiology project to determine prevalence from the two imperfect tests used (faecal BACTEC and serum Paralisa). Separate models were constructed for sheep, deer and beef cattle. LCA has become a useful tool in

similar situations where two or more tests are imperfect and the population prevalence is unknown. For example, it has been used in a recent paper where mastitis in goats caused by *Staph*. *aureus* was determined by somatic cell count (SCC) and also by bacteriological culture (Koop et al., 2011); and in a paper utilising 3 imperfect tests in the diagnosis of ketosis in dairy cows (Krogh et al., 2011).

Chapter 4. The Published Prevalence of Johne's Disease in New Zealand Farmed Livestock

Dairy cattle

(a) Background

The first case of JD reported in a New Zealand dairy herd was in 1912 in an imported Jersey cow (Stephens and Gill, 1937 - cited in de Lisle, 2002). The disease was next reported in 1928 in Taranaki and was subsequently diagnosed in herds throughout Taranaki and Waikato (de Lisle, 2002).

Once JD became notifiable, animals diagnosed with the disease had to be slaughtered, generating records which allowed some estimate of disease prevalence to be made. Assessment of the true prevalence (actually incidence) of infection was hampered by farmers' reluctance to report the disease and the fact that few suspected cases were submitted for laboratory testing. Chandler (1957) noted a 500% increase in condemnations due to JD recorded by the Department of Agriculture over the eight years between 1946 and 1954, mainly in Taranaki. This was partly due to improvements in diagnostics, but also to increasing prevalence of the disease, and possibly an increased awareness of the disease. Over the same period, stained tissue smears examined at Wallaceville Animal Research Station indicated that the disease was also present in Waikato, North Auckland, Horowhenua, Hawke's Bay and Masterton, plus in a very small number of cases from Canterbury and Southland. The spread of JD between regions followed the pattern of cattle movements.

By 1986, JD was recognised as a major problem in some Taranaki herds. In the previous five years, the disease had been definitively diagnosed on 16% of farms in the region and was suspected in another 31% of herds (Milestone and de Lisle, 1986). A study of six properties on which the disease was known to be present yielded estimates of within-herd prevalence (based on faecal culture results of all cows in the herd) of approximately 5-16%. Many cull cows from these properties that had negative faecal culture results were subsequently shown to be infected by tissue culture and histology at post-mortem examination. When these sub-clinically infected animals were taken into account, the prevalence of infection on these six farms was estimated to range from 30-65%.

In 1990, the MAF surveillance data for JD in the Waikato, Bay of Plenty and Rotorua over the previous five years were analysed (Ryan, 1991). The percentage of infected dairy herds in each district within these regions ranged from 4.7 to 13.6%. During this period, 85% of new livestock JD notifications were cases in dairy cattle. These prevalence levels were apparently similar to those recorded from other parts of New Zealand, although no figures were given for other regions.

Since JD in dairy cattle ceased to be notifiable in 2000, there has been less reluctance among farmers to report cases of the disease (Voges, 2008). The MAF laboratory submission data (see Table 2) show a steady increase in diagnosed cases of JD in cattle since 1990 but this does not necessarily indicate that either MAP infection in dairy herds is also increasing, or that clinical JD is increasing (although it seems likely). More comprehensive data are required to assess the current prevalence of disease.

(b) Survey data

In 1999, a questionnaire was sent to dairy farmers in the Waikato, Taranaki, Wairarapa, and Wellington/Manawatu/Wanganui regions, asking about specific management practices and the incidence of JD in herds (Heuer et al., 2003; Norton et al., 2009). Approximately half of the 427 respondents had had no known cases of JD on their farms and were designated as controls. The others were designated Case 1 or Case 2 based on the number of clinical cases of JD per cow year over the previous five years. The effect of different risk factors on the occurrence of JD was examined by multinomial logistic regression modelling.

The proportion of Jersey cows in the herd was one of the biggest risk factors for the presence of clinical JD on a farm, possibly due to a greater susceptibility of this breed for the disease. The purchase of bulls, and to a lesser extent, heifers, was also a major risk factor. In the case of bulls, the increased risk was proportional to the number of different properties from which the bulls were acquired. It is not surprising that the movement of cattle between properties would spread disease, but as the authors pointed out, it is not obvious how infected bulls would transmit infection to calves, the most susceptible population (Norton et al., 2009).

Other factors that were reported in this study to have been associated with increased risk of disease were contact between calves and cows in hospital paddocks, larger herd sizes and greater stocking densities, purchase of replacement heifers and greater numbers of inductions (more than 5% of the herd per year). The latter may be a reflection of poor management practices in general, rather than a causal association (Norton et al., 2009).

On average, calves were introduced to the adult stock at 15-16 months of age, but delaying this by eight months appeared to reduce the risk of clinical JD on the farm (Norton et al., 2009). This is likely to be due to the decreased susceptibility to infection of older animals.

Grazing animals off the farm was practiced by 80% of farmers, but was not associated with a significantly increased risk of JD. However, there was little co-grazing of cows with other stock (except occasionally with sheep). Although 95% of farms reported the presence of rabbits on the farm, this was not associated with an increased risk of JD (Norton et al., 2009).

The main limitations of survey data as means of assessing the prevalence of disease are that they rely heavily on both the ability of farmers to diagnose the disease, and their willingness to report it. The main clinical signs of JD, weight loss and diarrhoea, are very non-specific and can easily be mistaken for signs of other common conditions such as gastrointestinal parasitism. This could result in both false positive and false negative diagnoses. The frequency and care with which farmers observe their stock for clinical signs will also affect the accuracy of diagnosis. In the absence of any form of confirmation of the presence of the disease, survey data will not give a reliable estimate of disease prevalence. In the survey described here, farms known to have JD cases were deliberately targeted for inclusion in the study, elevating the apparent prevalence of disease, which was 0.23 cases of clinical JD per cow year over the five year period (Heuer et al., 2003). While no meaningful estimates of true disease prevalence can be obtained from these data, the reported low levels of JD on farms chosen for high disease prevalence does suggest that the overall rates of disease are lower than might be inferred from the historical data.

(c) LIC Data

The Livestock Improvement Corporation (LIC) national database contains a wealth of information that can be mined for data relating to dairy cows. Voges (2008) analysed the data pertaining to cows that had been listed in the LIC culling records as having been culled for JD. He examined records from the 1998/1999 season through to the 2006/2007 season,

looking at the effects of age, breed, season and geographical region on the prevalence of JD in dairy herds, within the acknowledged limits of the data.

It is not compulsory for farmers to record JD as the reason for culling, so it is likely that many (possibly most) cows culled due to JD were not reported. In addition, only clinical cases of JD would be recognised and culled, so the data reflected the prevalence (or incidence) of clinical JD, but obviously not the true prevalence of MAP infection. On the other hand, the records were likely to include cows for which a definitive diagnosis was not made and which may not have been true cases of JD. Wherever possible, records that were apparently inaccurate were removed before analysis.

The data analysis showed that there were very few culls for JD in cows less than three years old, but the risk of being culled for JD increased with age to a peak at about eight years old (Voges, 2008). This pattern would be expected to occur in cows affected by JD and supports the integrity of the data. Data for each season rather than each calendar year were used, to eliminate the confounding effects of herd movements, which were sometimes too difficult to trace.

On average, 8.35% of New Zealand dairy herds recorded JD culls over the nine seasons of the LIC analysis. The number of herds recording JD culls didn't change over the study period, but the number of herds declined, so herd prevalence increased slightly from 7.7% to 9.5% over this time. Only 7.4 % of North Island herds were affected, in comparison with 13% of South Island herds (Voges, 2008). The regional culling rates and prevalence are shown in Appendix 1.

The average size of the herds recording JD culls was larger than the national average by about 50%. This is in agreement with the findings of Norton *et al*., (2009). It would be interesting to compare regional herd sizes over the period of the LIC data analysis and

identify any correlation between herd size and regional prevalence. With the continuing expansion of South Island dairying and growing herd sizes, further analysis of more recent data could be revealing.

Jersey cows were disproportionately represented in the LIC JD culling records. Although they made up only 15% of the national herd, they accounted for 38% of the cows culled for JD, giving them a relative risk of being culled of 4.25 compared with Holstein-Friesian cows (which made up 52% of the national herd but accounted for only 31% of JD culls). As might be expected, cross-bred cows had an intermediate relative risk (1.79) of being culled for Johne's. Jersey cows were more likely to be culled alive than to die from JD than other breeds, probably reflecting breed differences in the development of clinical disease.

Forty per cent more cows were culled for JD between August and October each year, presumably because the stress of calving triggered the onset of clinical disease in sub-clinically infected animals (Voges, 2008).

Within herds that culled for JD, the disease prevalence was about 0.45% across the study (Voges, 2008). Thus 0.055% of the national dairy herd were culled for JD each year, and this percentage remained static over the nine seasons of the study. These data are not an estimate of the true prevalence of JD in New Zealand, but rather an indication of the general trends occurring in the dairy cattle population. From this study, it appears that the prevalence of JD in dairy herds has been stable for a number of years.

The number of New Zealand dairy herds in 2009/2010 (11,691) was almost the same as it was in 2006/2007 (11,883). However, the total number of dairy cows in New Zealand had increased by almost half a million (Anonymous, 2010b). If herd size is a risk factor for the occurrence of JD (Norton et al., 2009), it remains to be seen what effect this is likely to have on JD prevalence.

(d) LIC study for the JDRC

In December 2008, the LIC began collecting blood samples from dairy cattle with the aim of establishing an archive of DNA samples for use in future genomic studies relating to MAP infection (Anonymous, 2011c). As part of this study, a bulk milk ELISA for screening herds was evaluated. The ELISA was to be used to identify cows infected with MAP for blood sampling (Voges et al., 2009). The Pourquier indirect ELISA had 50-75% sensitivity and 100% specificity when used on pools of ten herd test milk samples and was chosen for use as the potential screening test.

As a pilot study, bulk milk from 154 herds known to have culled cows for JD, 64 high risk herds and a further 216 herds from low-prevalence areas of New Zealand was sampled using the ELISA. The herds were chosen based on information from the LIC database (see Voges, 2008). Confirmatory testing was carried out on pooled milk samples (ten cows per pool) on samples from 64 of the herds representing a range of MAP infection levels (based on the vat test results). Individual confirmation testing was then performed on any pools that were positive for MAP.

The vat bulk milk sample results correlated well with the pooled and individual results (Voges et al., 2009). Blood samples from reactor cows correlated reasonably well with the herd test results, and more than 80% of the cows that were positive on both the blood and milk tests were also positive on faecal culture (Voges, personal communication). These data are still being analysed. The vat test also reduced the amount of testing required by 75% and was thus accepted as a potential screening test for MAP infected herds.

About 2% of the 18,922 cows screened in the pilot study were positive for MAP (Anonymous, 2011c). This is higher than the prevalence for individual cows cited in the

earlier LIC study (Voges, 2008), as might be expected for a number of reasons. Although herds with a range of vat test results were chosen for inclusion in the pilot screening study, the selection was biased in favour of herds with high reactor levels (Voges, personal communication). This would result in an elevated estimate of prevalence. ELISA testing would also be expected to identify some animals that are not yet showing clinical signs of JD (despite the lower sensitivity of ELISA tests in these animals), whereas the earlier study would be expected to reflect only clinical disease prevalence.

Breed prevalence followed the same trends as in the earlier study, with 3.1% of Jerseys, 2.7% of crossbreds and 0.9% of Holstein-Friesian cows testing positive for MAP. The age distribution was also as expected, with prevalence of less than 1% in young animals and a peak prevalence of 3.55% in eight-year-old cows (Anonymous, 2011c).

The pilot study was followed by large-scale screening of 5000 herds over two years using the ELISA on vat milk samples (Anonymous, 2011c). Based on the level of the vat test result, the reactor herds were categorised as positive (3% of herds), suspect (2% of herds) or check (0.5% of herds). Approximately 400 herds were selected on this basis for further testing of pooled milk samples during herd testing, followed by individual testing of cows in reactor pools. Positive individuals were confirmed by serological tests. This testing is on-going at the time of writing, so the results are not available.

Once analysed, it may be possible to make some estimate of the prevalence of MAP infection in New Zealand dairy cattle from these data. However, it must be borne in mind that the screening data were collected to target MAP-infected herds for the collection of DNA from infected animals, so it may be difficult to estimate the true prevalence of MAP infection from these results. Further screening primarily for the purpose of estimating prevalence might be an avenue for further research, if feasible. Farmer reception of the process was good, and

many were prepared to pay for testing by this method (within reason) to reduce the impact of JD in their herds (Anonymous, 2011c).

Deer

(a) Background

Although JD was first diagnosed in deer in 1979 (Gumbrell, 1987), MAP was not isolated from deer until 1985. Over the next three years, three more cases of MAP infection were identified, and eighteen in the following three years (de Lisle et al., 1993). This number had grown to 619 by 2000, representing approximately 6% of herds (de Lisle et al., 2003), and about 12% of herds were infected by 2008 (Verdugo et al., 2008b). Only a few of the affected animals had clinical JD; some were identified during Tb skin testing but most diagnoses originated from post-mortem examination of lymph nodes at slaughter premises.

Clinical JD in deer can present as sporadic losses of adult animals, as seen in sheep and cattle, but large outbreaks in young animals also occur. Animals as young as eight months old may be affected, showing signs of diarrhoea, ill-thrift and rough hair coats (Mackintosh and de Lisle, 1997). Mackintosh *et al*., (2008) orally dosed weaner, yearling and adult female deer with a bovine strain of MAP to determine the susceptibility of each age group to infection. One third of the weaners developed JD but none of the yearlings or adults displayed clinical signs. There were also distinct decreases with age in the number of animals that were positive on faecal culture or Paralisa, and in the number that had visible lesions at slaughter after 50 weeks. These results indicated a significant reduction in susceptibility to infection with increasing age.

The risk of intrauterine transmission of MAP may also be higher in deer than in other species. MAP was cultured from nine out of ten foetuses from infected hinds in one study (Mackintosh and van Kooten, 2005), and from fourteen out of eighteen foetuses in another (Thompson et al., 2007).

(b) Abattoir surveillance data

Due to the large numbers of animals that pass through abattoirs every year, abattoir surveillance schemes can be an invaluable source of data for monitoring disease prevalence. Deer slaughter premises in New Zealand often perform mycobacterial cultures on deer tissues displaying lesions as part of the tuberculosis control scheme. Since 1990, because the lesions of tuberculosis and MAP infection are both grossly and histologically similar in deer, tissues cultured for *M. bovis* have been cultured for MAP as well (de Lisle et al., 2003). Monitoring of MAP in deer has been facilitated by the creation of a national database for abattoir surveillance administered by Johne's Management Ltd (JML) (Hunnam et al., 2009; Lynch, 2007). Carcase information is sent to the database for all deer slaughtered in New Zealand (Hunnam et al., 2009). Prevalence data for MAP are more abundant for deer than for other species, largely due to abattoir monitoring.

Only healthy deer are supposed to be transported for slaughter, so the majority of animals with lesions of MAP detected at deer slaughter premises are sub-clinically infected (Hunnam et al., 2009; Verdugo et al., 2008b). However, the early lesions of MAP are not evident at post-mortem inspection, so it is possible that many affected deer with mild clinical signs are

sent for slaughter inadvertently; that is, the farmers do not recognise the disease when it is present (Mackintosh and de Lisle, 1997).

Abattoir monitoring for MAP is probably more effective in deer than in sheep or cattle because the typical gross pathological lesions are different. In deer, MAP infection tends to manifest as a regional lymphadenopathy, rather than the granulomatous enteritis common in other species (Glossop et al., 2007a). Lymphadenopathy is very non-specific for MAP infection but the detection of enlarged lymph nodes flags the possibility of infection, which can then be confirmed by culture. While the majority of lesions are found in the mesenteric lymph nodes, extra-intestinal lesions may also occur in deer, often in the absence of intestinal lesions (Verdugo et al., 2008b).

The success of abattoir surveillance for MAP depends on the ability of meat inspectors to accurately detect enlarged lymph nodes. A study was carried out in which meat inspectors from deer slaughter premises throughout the country were presented with a series of life size photographs of normal and enlarged mesenteric lymph nodes. On average, the meat inspectors correctly identified 68% of enlarged lymph nodes and 65% of normal lymph nodes (Glossop et al., 2007a). However, individual meat inspectors who correctly identified more enlarged lymph nodes also tended to incorrectly identify more of the normal lymph nodes as enlarged; that is, there was an inverse relationship between sensitivity and specificity. Discrete lesions were identified more accurately than the diffuse enlargement or focal discolouration that is more typical of MAP infection. There were regional differences in the ability of meat inspectors to correctly identify enlarged lymph nodes and better results were obtained by meat inspectors who had spent time inspecting deer carcases (as opposed to other species) in the previous four weeks.

Meat inspectors usually assess lymph nodes by manual palpation and incision to allow inspection of the interior of the node, as well as by visual inspection. A second study was thus carried out to determine the sensitivity and specificity of detection of enlarged lymph nodes under these circumstances (Glossop et al., 2008b). Only four meat inspectors were included in the study. Only 25.4% of enlarged lymph nodes were correctly identified, but 98.4% of normal lymph nodes were correctly identified. However, many of the normal lymph nodes incorrectly classified as enlarged were subsequently found to contain pathological lesions, so the true specificity for detection of abnormal lymph nodes was 99.9%. It is likely that the texture of the lymph nodes influenced the decision-making process. Taken as a whole, the assessment of enlarged lymph nodes at meat inspection is likely to considerably underestimate the prevalence of MAP infection in deer herds.

Between 2007 and 2008, information from over 1,000,000 carcases was added to the JML database (Hunnam et al., 2009). Not all meat inspectors consistently record observed enlarged lymph nodes, however. An analysis of these data indicated that enlarged lymph nodes were detected more frequently in the South Island than the North Island. This was consistent with the regional differences found in the sensitivity of detection of enlarged nodes by meat inspectors (Glossop et al., 2007a), but might also have indicated a higher prevalence of infection, particularly in Otago and the West Coast of the South Island (Hunnam et al., 2009).

In weaners and yearlings, the highest prevalence of enlarged lymph nodes recorded was in summer, and the lowest in winter, but there was no seasonal effect seen in older animals

(Hunnam et al., 2009). This pattern in young deer does not reflect the seasonal incidence of JD in deer and the significance of seasonal changes in lymph node enlargement is unknown.

The analysis of the JML database also demonstrated a decrease in carcase weights in animals with suspected MAP infection (Hunnam et al., 2009). In young deer with enlarged visceral lymph nodes, there was a 5-6% decrease in carcase weight (around 3kg) compared with animals with normal lymph nodes. In adults, the difference was 13-30% (7-27kg) in adults.

Carcase weights may be more subtly affected in deer with subclinical MAP infection but no enlargement of visceral lymph nodes (Stringer et al., 2009). At each of four deer slaughter premises, normal-looking jejunal lymph nodes were collected from fifteen lines of deer. Four carcases were sampled from each line. The lymph nodes were cultured for MAP, and the carcase weights recorded. The results were weighted for the number of deer killed at each premises and the national distribution of deer herds.

In the North Island, 29% of carcases and 44% of herds were culture positive for MAP. In the South Island, 51% of carcases and 67% of herds were positive (Stringer et al., 2009). The difference in prevalence between the islands was statistically significant for carcases, but not for herds. Nationally, the carcase prevalence of MAP was 45% and the herd prevalence was 59%. Although lymph nodes were not cultured from animals that had visible lesions, the lines from which these animals came were classified as suspect. Cultures of normal lymph nodes from other animals within these suspect lines were more likely to be positive for MAP than cultures from non-suspect lines. Notably, the average carcase weight of affected and non-affected individuals was similar, but the average weight of carcases from lines with at least

one animal positive for MAP was lower than the average weight of lines from which MAP was not isolated.

Abattoir surveillance data have also been used to examine the relationship between suspected MAP infection and the presence of sheep and/or cattle on properties farming deer. Data on the occurrence of enlarged lymph nodes at slaughter were collected from 13 abattoirs via the JML database. Almost 350,000 carcases from 1954 farms were examined. The slaughter data were matched with records from the Assure Quality Agribase pertaining to the population of other livestock species on the farms from which the deer originated, using multivariate logistic regression (Verdugo et al., 2008b). Fourteen per cent of the farms studied had deer with enlarged lymph nodes at slaughter. It was assumed that these were mainly due to MAP infection. Deer were more likely to have enlarged lymph nodes if they originated from properties where cattle, or cattle and sheep, were also present, than if they came from properties running only deer. However, deer from properties on which sheep were also present were less likely to have enlarged lymph nodes at slaughter. One possible explanation suggested was that strains of MAP infecting sheep have lower pathogenicity for deer. Exposure to less pathogenic strains might also stimulate an immune response and have a protective effect against more virulent strains. However, it was acknowledged that these explanations were highly speculative.

It is evident that the abattoir surveillance data can provide a wealth of information relating to disease prevalence in deer. However, in the absence of confirmation of MAP infection by culture or PCR, much of this information must be treated with caution. In New Zealand, abattoir monitoring for MAP is currently only carried out on deer carcases, but in Australia it

has also been used for the detection of sheep flocks with moderate to severe infection prevalence (Anonymous, 2005).

(c) Epidemiological survey data

Most of the research into the prevalence of JD in New Zealand deer relates to red deer or wapiti (elk). Red deer constitute 85% of the deer in New Zealand and the rest are mainly wapiti. However, fallow deer make up about 3% of the New Zealand deer population. In a postal survey of 52 fallow deer farms, none of the 20 respondents reported seeing clinical JD in their stock. However, one deer was tentatively diagnosed by the authors as having JD because it had intractable diarrhoea and weight loss (Hell et al., 2008). In the same survey, 40% of 342 farmers who owned red deer, elk or red deer-wapiti crosses reported clinical JD on their farms. The farmers were also questioned about species other than deer that grazed on the deer pasture. Fallow deer farms were less likely to stock other ruminant species than red deer/elk/wapiti farms (42% compared with 74% respectively). Beef cattle, adult sheep and goats were the species most commonly grazed on fallow deer pastures; other species of deer were also present on four of the fallow deer farms. However, it was difficult to draw any conclusions about the risk of grazing with other species for the development of JD in deer, since no clinical JD was reported in non-deer species on any of the fallow deer farms. Forty per cent of the red deer/elk/wapiti farmers reported clinical JD in their deer, but no data on the reported incidence of clinical JD in other stock on these farms were presented.

A case-control study was performed in 2005, looking at data obtained from 174 deer farms of known JD status (based on tissue culture or pooled faecal culture results). Eighty-one of these

herds were also followed in a three-year longitudinal study (Glossop et al., 2008a). Herds were classified as either clinical or non-clinical based on the farmer's assessment of the presence of clinical disease on the farm. Although the primary aim of the study was to investigate risk factors for JD, the prevalence of the disease was also assessed. The selection of deer herds in this study was not completely random, as it depended on voluntary participation by the farmers involved.

Overall, 74% of the herds in the study had clinical cases of JD, and the average within-herd prevalence was 0.31% in 2005, increasing to 0.54% by 2007 (Glossop et al., 2008a). However, there were considerable differences between herds, with some having very low levels of disease, and others having up to 12% of the herd affected, and up to 20% in some classes of stock. Weaners, yearling hinds and adult hinds had the highest rates of disease. Eighty-three per cent of South Island herds were classed as clinical in comparison with 53% of North Island herds. A seasonal effect was also seen, with more clinical cases occurring in winter and spring.

The risk factors for weaners developing clinical JD were assessed in a smaller study within the case-control study described above (Glossop et al., 2007b). As seen in other studies (Heuer and Wilson, 2011; Verdugo et al., 2008b), grazing with sheep reduced the risk of weaners developing clinical disease (as determined by the farmer), possibly because the sheep strains of MAP are less pathogenic for deer. Herds in which the average age of the breeding herds was less than five years, and herds which bought in yearlings had a lower risk for clinical JD in weaners, but the reasons for this were unknown. Weaners were more likely to develop clinical JD if they grazed with yearling beef cattle; grazing with beef cattle has been associated with an increased risk of infection in other studies (Heuer and Wilson, 2011;

Verdugo et al., 2008b) but it was not clear why yearlings were particularly incriminated. Irrigation of the deer pasture was also a risk factor for the development of the disease, possibly by improving the longevity of MAP in the environment.

A further study was carried out by Massey University to examine the epidemiology of JD in deer and other species (Heuer and Wilson, 2011). This study will be discussed in a separate section of this review.

Sheep

JD in sheep is probably much more prevalent than reported, due to the sporadic nature of the disease and the relatively low value of sheep. Unless there are significant losses due to ill-thrift and death, farmers will be unlikely to seek a definitive diagnosis (de Lisle, 2002). The disease is often masked by the presence of other causes of ill-thrift in the flock, such as poor nutrition or gastrointestinal parasitism and the disease is often overlooked for long periods before losses become severe enough to stimulate investigation (Williamson and Salibury, 1952). The lack of reliable tests to diagnose subclinical disease compounds the problem.

The predominant clinical sign of JD in sheep is ill-thrift; diarrhoea is less common in sheep than in cattle and deer. Typically, affected ewes begin to lose weight in the (West et al., 2002) winter and die around the time of lambing. The stress of pregnancy and parturition is likely to play a role in triggering the onset of clinical disease. The peak of faecal mycobacterial shedding thus occurs when lambs are at their most susceptible to infection.

Although the initial discovery of JD in New Zealand sheep did not occur until 1952, it is clear that the disease had already been present in South Island flocks for many years. By 1986, about 2% of New Zealand flocks had confirmed and 5% suspected Johne's infections

(Gumbrell, 1986). South Island flocks were predominantly affected, but the rate of diagnosis of new infections was increasing in both islands.

In the light of concerns expressed in the 1980s at the dearth of information on the epidemiology and prevalence of ovine JD in New Zealand (Bruere, 1986), it is surprising that there has subsequently been little research carried out. The most relevant recent information is presented in the sections of this review relating to MAF data and the Massey University epidemiology study.

Beef Cattle

The information available on the prevalence of JD in beef cattle in New Zealand is scant. Some data has been collected by MAF, but data for dairy and beef cattle tends to be grouped together under the general heading of cattle, so is not very useful for determining differences between the two. The most detailed information available comes from the Massey University epidemiology study. The herd sampling that was carried out indicated that approximately 31% of New Zealand beef herds had evidence of MAP infection. The regional distribution of infected herds appeared to be more uniform for beef cattle than for other farmed species, with 33% of North Island herds and 27% of South Island herds affected (Heuer and Wilson, 2011). Based on farmer assessment of the incidence of JD in their herds, the highest incidence was in Wairarapa and the lowest in Southland, but levels across the rest of the country were remarkably consistent (Verdugo and Heuer, 2010). The methodology of the study and the results will be described in more detail in the relevant section of this review.

MAF Data

(a) Analysis of the MAF Register of Infected Properties

Until JD ceased to be a notifiable disease in 2000, regional MAF offices were required to maintain registers of livestock properties on which JD had been diagnosed. These lists mainly included properties on which a definitive diagnosis of JD had been made as a result of faecal culture, histology or faecal smears combined with serology. In 1990 and 1993, MAF offices throughout New Zealand were asked to supply lists of JD infected properties current at the end of May (Nuttall, 1991; Staples, 1994). Properties on which the diagnosis of JD was based on serological tests only were not included in these survey (Staples, 1994). A summary of the survey results is presented in Table 1. It should be noted that these data are cumulative, so the 1993 figures include affected flocks and herds that were recorded in 1991.

Table 1. The cumulative occurrence of JD in New Zealand farmed livestock at the end of May in 1991 and 1993 as recorded by MAF regional and district offices. For cattle and sheep, the percentage of herds or flocks with recorded cases of JD is shown, with the total number of herds in brackets. For deer and goats, the total number of affected herds or flocks is shown. (From Nuttall, 1991 and Staples, 1993).

	Cattle		**Sheep**		**Deer**		**Goats**	
	1991	**1993**	**1991**	**1993**	**1991**	**1993**	**1991**	**1993**
North Island	8.2%	6.3%	1.9%	3.5%	3	14	12	18
	(12,946)	(27,594)	(10,376)	(11,246)				
South Island	13.0%	5.8%	4.9%	6.8%	5	15	4	8
	(1,262)	(5,749)	(11,229)	(12,286)				
New Zealand	8.6%	6.2%	3.5%	5.2%	8	29	16	26
	(14,208)	(33,343)	(21,675)	(23,532)				

Despite the fact that JD was notifiable at the time of these surveys, underreporting was common (Staples, 1994), due to the detrimental effects a diagnosis of JD would have on the ability of farmers, particularly stud breeders, to sell livestock. Subclinical cases were also likely to go unrecognised. The data recorded by MAF offices are therefore likely to significantly underestimate the real prevalence of JD, but can indicate general changes in prevalence.

From the data in Table 1, the prevalence of JD in cattle appeared to decrease slightly between 1991 and 1993, but this was probably due to the large increases in the number of herds over the same period. The actual number of infected herds rose by 820 (Staples, 1994). The increase in the number of sheep flocks over the same period was much smaller and the percentage of notified flocks increased, suggesting a steady increase in ovine JD. Disproportionate numbers of the affected properties were in the South Island, for both cattle and sheep. The West Coast of the South Island was the worst affected area for bovine JD, although Waikato and Taranaki also had large numbers of affected herds (Nuttall, 1991; Staples, 1994). The data provided did not allow differentiation between beef and dairy herds, but less than 5% of beef herds were considered to be infected in 1991 and less than 2% in 1993. Little detail was available for deer and goats because of the low number of cases recorded, but the prevalence of JD recorded in both species increased between the two surveys.

(b) Laboratory Surveillance Data

From 2000, with the introduction of the National Pest Management Strategy for MAP, JD ceased to be notifiable (Poland, 2001) and the MAF register of infected properties lapsed. However, JD remained on the list of endemic animal diseases that are of surveillance interest.

As such, laboratory diagnoses of JD are recorded by MAF and published annually in *Surveillance*. A summary of JD cases recorded by MAF from 1999 to 2009 is presented in Table 2.

Table 2. Cases of JD diagnosed at veterinary laboratories in New Zealand from 1999 to 2009. Data from MAFBNZ annual reports of cases of surveillance interest, published in *Surveillance*. Note that in 2006, MAF changed the definition of a surveillance case to require evidence of flock or herd involvement rather than individual animal cases.

Species	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Cattle	57	79	42	120	306	181	430	307	63	341	664
Sheep	53	58	53	42	46	37	37	33	26	15	22
Deer	7	8	11	34	35	14	12	8	7	8	7
Goats	2	4	1	9	3	4	5	10	1	-	-
Lamoids	0	0	0	0	1	0	1	0	0	-	-

The limitations of laboratory surveillance data are similar to those of the MAF notification data presented by Nuttall and Staples (Nuttall, 1991; Staples, 1994). Although general trends are evident, laboratory surveillance data will underestimate the prevalence of MAP infection. The majority of samples submitted to veterinary diagnostic laboratories would be from animals that were showing clinical signs of, or had died of, JD, so there is no indication of the rate of subclinical infections. In addition, it is not known what proportion of suspected cases actually result in laboratory submissions, as many clinically affected animals are slaughtered without a diagnostic workup. The figures given are for New Zealand as a whole, so regional differences cannot be analysed. The data may also include multiple diagnoses from a single infected property within each year, so the actual number of herds or flocks may be lower than the number of cases.

The data presented in Table 2 suggest that the incidence of JD in cattle increased overall between 1999 and 2009, although there are some major fluctuations. Over the same period, cattle numbers (both beef and dairy) in New Zealand increased by approximately a million (Anonymous, 2010b, 2011a, b), so an increase in laboratory submissions is perhaps not surprising.

Laboratory diagnoses of JD in sheep seem to have been steadily decreasing. This follows the general trend in sheep numbers, which decreased by about 13 million between 1999 and 2009 (Anonymous, 2011a, d). However, the decline in the number of diagnoses does not appear to have kept pace with the decrease in the sheep population, and might suggest that the prevalence of ovine JD is actually stable or increasing. More information on the correlation between the rates of laboratory submissions and disease prevalence would be required to draw any conclusions. The reduced laboratory diagnoses of JD in deer since 2006 do not reflect reports by other authors (e.g. Glossop et al., 2008a; Hunnam et al., 2009; Wilson et al., 2009) that the prevalence of JD is high and possibly increasing

Diagnoses of JD in goats, camels and llamas are sporadic, as would be expected due to the relatively small numbers of these species in New Zealand.

Massey Epidemiology Study

The most comprehensive data on the prevalence of JD and MAP infection in New Zealand livestock come from the epidemiology study carried out by Massey University researchers as part of the JDRC Epidemiology Objective. As well as the prevalence of infection, this study examined the effect of species interactions on occurrence of clinical JD in all the major farmed species (Heuer and Wilson, 2011). The Massey study consisted of an initial postal

survey, followed by collection of blood and faecal samples for testing, and finally the development of a computer model for the assessment of disease control options.

(a) Methodology

The postal survey was sent to almost 8000 farmers throughout New Zealand in 2008, with a response rate of 24.3% (1940 surveys). The respondents were asked about clinical cases of JD on their farms over the previous three years, production measures that might be affected by JD, and the grazing management of livestock species on the farm. Questions about leptospirosis were also included in the survey, to minimise response bias by ensuring that the responses received were not restricted to farmers with an interest in JD. The data analysis included an analysis of the effects of species interactions in the incidence of clinical JD (Heuer and Wilson, 2011). Although the response rate was low, the inclusion of leptospirosis in the survey made this survey far less susceptible to bias than all other JD surveys to date.

The information from the postal survey responses was used to stratify the farms on the basis of which species or combination of species was present. A subsample of 238 farms was then selected across the seven strata for serum and faecal sampling in 2009/10. Farms with and without clinical JD were included (Heuer et al., 2011a). Samples were collected from 20 animals from each species on these farms. The faecal samples were pooled and cultured by BACTEC culture and the serum samples were frozen for strain-typing, but tested by ELISA if the faecal cultures were negative. A further survey of production measures was carried out when the samples were collected.

Similar testing was carried out on 110 Landcorp farms, in addition to the farms identified as a result of the postal survey. The information gathered from these farms was similar, but included data on the movement of animals between farms.

In order to overcome the inherent inaccuracies involved in the available diagnostic tests, the results of the serum and faecal testing were subjected to Bayesian latent class modelling. This first required the development of a model to assess the sensitivity and specificity of the tests used. Serum and faecal samples from clinically normal yearling deer were used to develop this model. Paired samples were collected from 20 animals per farm on 20 South Island deer farms and 17 North Island deer farms. Both JD positive and JD negative properties were chosen for sample collection. The faecal samples were subjected to BACTEC culture and the serum samples were analysed by the Paralisa test. These results were then used to develop the initial latent class model.

Separate latent class prevalence models were then used to analyse the original data for sheep, deer and beef cattle. The original data do not account for inaccuracies in test results due to imperfect sensitivity and specificity of the diagnostic tests. In this study, the faecal culture procedure was shown to have an estimated sensitivity of 77% and a specificity of 99%. The Paralisa test in this study was reported to have a sensitivity of 19% and a specificity of 94% (Heuer and Wilson, 2011). This sensitivity is significantly lower than that reported previously (Griffin et al., 2005). Bayesian latent class analysis allows adjustment of the results to reduce the effects of these inaccuracies. There may also have been some bias introduced into the results by the stratified selection of properties for sampling according to species present. The results were therefore weighted after analysis to reflect the species distribution in the original survey data (Heuer et al., 2011a).

(b) Prevalence Data

The prevalence data obtained from the epidemiology study are shown in Table 3. The results obtained from the postal survey indicated the incidence of clinical cases of JD over the previous three years, as farmers were unlikely to have identified and reported subclinical cases. Many of the clinical cases reported in the farmer survey were not confirmed by veterinary opinion or laboratory testing. The observational skills of the farmers and their ability to recognise JD would therefore influence the results. The possibility was raised that farmers who had had cases of JD in their stock might be more likely to respond to the survey, thus skewing the results. However, only 20% reported clinical cases, despite 97% claiming knowledge of JD (Verdugo, 2009).

The farm sampling data shown in Table 3 are the adjusted values after latent class analysis, and indicate the prevalence of subclinical and clinical MAP infection at the time of sampling. The Landcorp data are incomplete as they are still being analysed, but the available data are shown for comparison. However, the epidemiology of infection on Landcorp farms may not reflect the epidemiology on other farms in New Zealand due to differences in management practices.

Table 3. The percentage of herds and flocks with JD (survey data) and MAP infection (sampling data) in New Zealand as determined by a farmer postal survey, by faecal and serum testing on 238 farms and by faecal and serum sampling on 107 Landcorp farms (Heuer and Wilson, 2011; Heuer et al., 2011b). Sampling results are presented after latent class analysis.

	Farmer Survey Data			Farm Sampling Data			Landcorp Sampling Data		
	NI	SI	NZ	NI	SI	NZ	NI	SI	NZ
Sheep	19.4	16.9	**18.2**	74	60	**68**	32	29	-
Beef	4.5	3.7	**4.2**	33	27	**31**	28	8	-
Deer	25.3	38.1	**33.8**	42	73	**60**	60	66	-
Dairy	18.5	26.3	**21.5**	-	-	**-**	22	48	-

Nearly 70% of New Zealand sheep flocks showed evidence of MAP infection (Table 3) with overall prevalence slightly higher in the North Island than the South Island. The highest incidence of clinical disease in sheep was in Hawkes Bay, Wairarapa and Marlborough (Verdugo and Heuer, 2010). In deer herds, the prevalence of MAP infection was 60%, with more South Island herds infected. Of note, the highest clinical incidence in deer was in Southland, Canterbury and Manawatu/Wanganui, some of the lowest prevalence areas for sheep. The infection prevalence in beef herds was about half those of deer and sheep and was similar in both islands, although Wairarapa farms had the highest rates of clinical disease.

The farm survey and Landcorp data (Table 3) suggest that infection rates in dairy cattle might be similar to those in sheep and deer, and highest in the South Island, but the farm sampling did not include dairy cattle. The geographical distribution of clinical disease in dairy cattle followed a similar pattern to that in deer (Verdugo and Heuer, 2010).

The high prevalence of MAP infection in sheep flocks and beef herds in comparison to the numbers of flocks and herds reported to have clinical cases could indicate that sheep and beef farmers are less likely to notice it in their animals (Heuer et al., 2011a). The less intense nature of sheep and beef farming probably contributes to this. Beef animals also have a lower overall prevalence. Nearly half of the beef herds sampled were finishing units, with no breeding stock. Verdugo *et al*., (2010) proposed that lack of contact between older animals that might be shedding MAP in their faeces and young, susceptible animals possibly accounted for lower infection rates on beef finishing units. However, beef calves are left with their dams for several months, whereas dairy calves, which have apparently higher rates of clinical JD, are removed at birth. This suggested that transmission from cows to their calves is not the most important route of infection, and possibly transmission via pasture plays a

major role under New Zealand conditions (Heuer and Wilson, 2011). The role of intra-uterine infection also needs to be established.

One major drawback of the Massey epidemiology study was that it did not include blood and faecal sampling from dairy cattle. The survey data alone are not sufficient to estimate the prevalence of infection due to the limitations of survey data already discussed. A second limitation of the study was that no estimates of within herd prevalence of MAP infection or JD were made. It seems likely that a relatively small number of farms have significant problems with JD, but this cannot be ascertained from the available data.

(c) The Effects of Grazing Interactions on the Prevalence of Johne's Disease

New Zealand farms often run two or more species of livestock on the same pasture, either concurrently or consecutively. Because MAP may survive in soil for prolonged periods (Chiodini et al., 1984; Schroen et al., 2003b), shared pasture could pose a risk for inter-species transmission of MAP.

In the Massey postal survey, respondents were asked about the frequency with which different species on each farm grazed the same pasture, either at the same time or sequentially (Heuer and Wilson, 2011; Verdugo and Heuer, 2010; Verdugo et al., 2008a). The effects of species associations on the risk of clinical JD and MAP infection in each species were assessed.

Sheep grazed with deer had lower incidence of clinical JD but no change in MAP infection rates. The same was true of deer grazed with sheep, indicating that co-grazing of deer and sheep could be mutually beneficial (Heuer et al., 2011a).

Sheep grazed with beef cattle were more likely to have MAP infection, but no change in clinical disease incidence, whereas beef cattle grazed with sheep were more likely to have both MAP infection and clinical disease. Beef grazed with deer had lower prevalence of MAP infection and lower incidence of clinical disease, but deer grazed with beef had higher rates of both (Heuer et al., 2011a).

Sheep that grazed with both beef and deer had higher rates of clinical disease without an increase in prevalence of MAP infection. This might be explained by a tendency for farmers grazing all three species to be more aware of JD in their flocks. However, the lower disease incidence reported in sheep grazed with deer does not really support this (Heuer and Wilson, 2011). These results are very similar to those of Verdugo *et al*., (2008b) correlating the risk of detection of enlarged lymph nodes in deer at slaughter with the presence of sheep and cattle on farms, and support those previous findings.

Overall, the findings suggest that allowing sheep and deer to graze together but reducing contact between either of these species and beef cattle, might help to reduce JD incidence on farms.

The Landcorp data on animal movements are not yet available, but may provide additional insight into the risks for MAP transmission between farms, as well as within farms.

(d) Modelling

The prevalence data accumulated during the epidemiology study and the information on the effects of species co-grazing were used to generate a single farm, multi-species simulation model (Heuer and Wilson, 2011). The effects of changes in environmental, host and pathogen-related factors on the prevalence of MAP can be assessed. In particular, factors

such as grazing management and other human interventions have been incorporated in the model. The model was developed in collaboration with Cornell University and is still being refined. It is envisaged that the final model will incorporate economic and production factors as well, to give a cost-benefit analysis of possible interventions.

Strain Typing

Isolates of MAP obtained from sheep have historically been more difficult to culture than isolates obtained from cattle, due to differences in the strains of MAP that infect sheep and cattle (Whittington et al., 2000b). Differences between sheep and dairy cattle in the geographical distribution of JD early in the history of the disease in New Zealand also intimated the existence of separate strains (Collins and de Lisle, 1990). Individual strains may have different pathogenicity or a predilection for different host species, which is probably why the transmission of JD between sheep and cattle appears to be rare (Moloney et al., 2003). Strain typing will enable better geographical or temporal tracking of the transmission of isolates and also a better understanding of the risks of transmission between species.

Ris *et al*., (1987) conducted a simple experiment to see whether transmission from cattle to sheep occurred under New Zealand conditions by allowing six yearling Romney ewes to graze behind cattle with clinical JD. After two years, the ewes showed no signs of JD and were slaughtered. At post-mortem examination there was no evidence of MAP infection in the intestinal tissues, but MAP was cultured from the ileum and mesenteric lymph nodes of four of the ewes.

The development of tests to characterise genomic DNA enabled MAP strain types to be distinguished genetically (Collins et al., 2002; Collins et al., 1990). When DNA restriction

endonuclease techniques were used to type New Zealand and overseas isolates of MAP, all of the New Zealand cattle isolates fell into one strain type (C) and all of the sheep isolates into a second (S). The overseas isolates belonged to the same two groups, although Canadian sheep isolates were of the cattle type rather than the sheep type. These findings tended to support the idea that the different types do not transmit readily between cattle and sheep (Collins and de Lisle, 1990). However, isolates from New Zealand deer and goats fell into both categories.

Further strain-typing was carried out on 20 archived isolates from deer, collected between 1985 and 1991. Three isolates were of the sheep type and the remainder were the cattle type (de Lisle et al., 1993). More recently, 72 MAP isolates from naturally infected, clinically diseased deer were all typed as bovine strains (O'Brien et al., 2006). Many of these strains were cultured from the lymph nodes of deer with suspected tuberculosis and may not truly represent the relative prevalence of different strains of MAP in deer (Wilson et al., 2009). However, experimental challenge studies also indicated that ovine strains were less pathogenic for deer than bovine strains (Mackintosh et al., 2007; O'Brien et al., 2006).

MAP isolates can be typed by examining differences in repetitive DNA sequences within the MAP genome known as variable number tandem repeats (VNTR), or short sequence repeats (SSR). For the JDRC study, eight VNTR and two SSR were used for preliminary subtyping of MAP isolates obtained from LIC and the Massey epidemiology study (Collins et al., 2011). Of the original DNA sequences chosen, 5 VTNR and the two SSR gave the best results and were applied to 65 Type C isolates, and later to 58 Type S isolates. Good discrimination of Type C isolates was obtained, but there was less variation in the Type S isolates.

The typing process was then applied to 200 dairy cattle isolates from LIC and 154 isolates from the Massey epidemiology study collected from properties farming two or more species

(beef cattle, sheep and deer). Most of the Massey isolates were from pooled samples. All the isolates were first divided into Type C or Type S isolates by PCR (Collins et al., 2011). The strain-typing study is on-going, so only half of the results for the LIC samples were available.

One sequence (VNTR3) was present as a single copy in all Type S subtypes, and as two copies in all Type C subtypes. Of the LIC isolates, 23% of the samples had more than one subtype present, indicating plural infections in a single animal. Of the Massey samples, 8% had multiple subtypes. Although most of these were pooled samples, it still indicated that multiple strains of MAP were circulating in some herds (Collins et al., 2011).

Unexpectedly, 13% of the dairy cattle isolates and more than half of the beef cattle isolates were of the S type. When 121 New Zealand isolates archived over the previous 25 years were subsequently subtyped using the same procedure, all the sheep isolates were found to be type S and all the cattle and deer isolates were found to be type C (Collins et al., 2011). The results for the archived samples support the results of previous research indicating that there was little MAP transmission between cattle and sheep, but the more recent research suggests that this may no longer be the case. Some subtypes found in the archived material, including one common one, were not isolated in the Massey or LIC studies. It is likely that the subtypes common in New Zealand have changed over time, possibly because more pathogenic strains were better able to survive and less pathogenic strains died out. Future longitudinal studies employing strain typing techniques could provide information about such trends.

Although six type S subtypes were identified, nearly all of the type S isolates from the Massey, LIC and archived samples proved to be the same subtype (Collins et al., 2011). It is possible that this is the only common S subtype in New Zealand, but the identification of DNA sequences enabling further discrimination of this subtype would be helpful.

Five sheep had type C subtypes and most of the deer isolates were type C. The type C strains appeared to be more variable than type S strains. The most common C subtype from the Massey samples (56 isolates) was very common in deer but was only rarely isolated from dairy cattle. It is not known whether this is a New Zealand cattle subtype that has greater pathogenicity for deer, or whether it was brought in to New Zealand in imported deer and is not yet widespread in the cattle population (Collins et al., 2011). However, it was isolated from 45 farms where clinical JD occurred, but was not found on any farms without clinical disease (Heuer and Wilson, 2011). There were also 56 Massey isolates of an S subtype, but this was found on farms both with and without clinical disease (roughly 50% of each), suggesting that there are indeed differences in pathogenicity between these two subtypes. In all, 97% of the type C isolates were from farms with clinical JD compared with 46% of the type S isolates. The type C strain that was most commonly isolated from dairy cattle in the LIC samples (74%) accounted for only 12% of the type C isolates from the sheep, beef and deer samples (Collins et al., 2011). Lack of contact between dairy cattle and the other species studied may result in differences in the predominant subtypes.

The JDRC strain typing study has provided useful information about the subtypes present in different livestock species. If this information can be correlated with the farm prevalence data as planned, it may help to determine whether there are differences in virulence between MAP subtypes and identify patterns of infection. The study has also provided some insight into the relative pathogenicity of different MAP subtypes for different host species, which will advance the understanding of the epidemiology of infection, particularly on properties where different farmed species frequently interact.

Chapter 5. MAP in Animal Product

The main concern raised by the presence of MAP in animal products is the possibility that human Crohn's disease, which bears many similarities to JD, might be caused by MAP. There is much debate over the link between Crohn's disease and MAP; the evidence for a causal association is conflicting (Anonymous, 2000; Jenson and Kennedy, 2009; Ryan and Campbell, 2006).

There is little doubt, however, that MAP can be found in animal products. The risk of MAP being present in livestock products such as meat and dairy products in New Zealand has been reviewed (Ryan and Campbell, 2006). It is reported that MAP can be isolated from milk (Sweeney et al., 1992), raw cheese (Stephan et al., 2007) and meat (Gwozdz et al., 1997; cited in Ryan and Campbell, 2006). However, different processes have been demonstrated to produce different levels of destruction of MAP. In laboratory simulated pasteurisation studies, several bovine and human MAP strains survived heat treatment, especially when the samples were cooled quickly. Both low temperature (63°C for 30 mins) and high temperature (72°C for 12s) treatments resulted in survival of MAP organisms. Human isolates had much higher survival rates than animal isolates (Chiodini and Hermon-Taylor, 1993). Gao et al., (2002) tested spiked milk samples for the presence of viable MAP after regular batch pasteurisation (63°C for 30 minutes) or high temperature, short time (HTST) pasteurisation (72°C for 15s) and found that viable MAP was recovered from two samples subjected to HTST, but not from any samples subjected to batch pasteurisation.

Of particular relevance to New Zealand, when studied under conditions resembling those found in commercial processing plants instead of laboratory conditions, pasteurisation was likely to successfully destroy MAP in dairy products (Pearce et al., 2001). Unfortunately, this result is not reflected in studies of other processes used around the world.

In another study, MAP was detected by PCR in commercially pasteurised milk, and some viable organisms were demonstrated by culture (Millar et al., 1996). Grant et al., (2002a; 2002b) also found that MAP in raw milk survived HTST pasteurisation, particularly when larger numbers of organisms were detected in the raw milk. Viable MAP was recovered from laboratory HTST pasteurised milk when concentrations of MAP exceeded 10 organisms per mL (Sung and Collins, 1998).

Viable MAP was isolated from 1.6% of commercially pasteurised milk bought from retail outlets in the Czech Republic (4 out of 244 samples) (Ayele et al., 2005), and from 2.8% of retail milk samples tested from California, Wisconsin and Minnesota (Ellingson et al., 2005). Although contamination of milk samples after pasteurisation could account for positive cultures, this does not appear to have been the case in these studies.

Of particular concern is the detection of MAP in powdered milk products designed for consumption by infants (Hruska et al., 2011). Of 51 samples of commercial products examined, 35% were found by PCR to contain MAP in concentrations ranging from 48 to 32,500 cells per gram of powder. The mycobacteria were not cultured to determine the viability of the organisms; however, the release of immunomodulatory mediators such as heat shock protein and muramyldipeptides from dead mycobacteria might still pose a risk for neonates with undeveloped immune systems, even if mycobacterial infection does not develop.

Although MAP establishes infection via the intestinal lymphatic tissue and is primarily found in the intestines and mesenteric lymph nodes of infected animals, bacteraemia in the later stages of infection results in the presence of MAP throughout the body (Clarke, 1997). Infection of muscle tissue could therefore result in viable MAP occurring in meat. In a Danish study, MAP was detected in the cheek muscles of 4% of carcases by PCR, and viable organisms were cultured from 0.4% of these animals (Okura et al., 2011), suggesting that MAP infection can result in low level carcase contamination.

In the United States, the hides and carcases of cattle from cull cow slaughter facilities and also from fed cattle slaughter facilities were swabbed for MAP PCR, and ileocaecal lymph nodes were also collected. At the cull cow slaughtering facilities, 34% of the cattle lymph node samples were positive for MAP on PCR, but almost 80% of the hides were positive, suggesting that there was a significant amount of hide cross-contamination occurring. After processing, only 11% of the carcases were positive on PCR after processing. Although 51% of the carcases were positive for culture before processing, only 1% were positive afterwards, suggesting that the processing operation removed most MAP from carcases. The difference between the 11% PCR positive and the 1% culture positive is likely that PCR was detecting non-viable MAP as well as viable organisms.

The procedures used for decontamination of the carcases were not described. MAP infection rates were lower for cattle at fed cattle slaughtering plants, 0.4% of lymph nodes, 1.2% of hides and 1.2% of carcases were positive on PCR, but no MAP was cultured either before or after processing (Wells et al., 2009). It seems likely that MAP is more readily isolated from the carcases of older cattle, which are more likely to be in advanced stages of infection and shedding larger numbers of mycobacteria.

Whilst the studies carried out to mimic the New Zealand approach to milk processing demonstrated that detection of MAP in pasteurised milk was highly unlikely, the remaining global data are conflicting with respect to the risk to dairy products. More needs to be done to reassure consumers that the safety of dairy products from New Zealand is superior to those from elsewhere.

Chapter 6. Discussion

1. *The prevalence of clinical JD*

It should come as no surprise that the reviewers have a very limited assessment of clinical JD in New Zealand. Farmed livestock disease recording in general in New Zealand is among the poorest of developed countries. Indeed, even for easily recognisable, treatable clinical disease – such as mastitis or lameness amongst dairy cows- it is difficult to reliably identify the current disease prevalence, or indeed reliably estimate disease incidence rates.

New Zealand has no culture of disease recording among farmed livestock, when compared to most European countries. There is no centrally maintained disease database, and virtually no requirement to record disease. Added to that, the size and scale of most New Zealand farming enterprises mean that individual animals are frequently not monitored, and hence New Zealand farmers are consequently poorly trained in disease recognition and treatment at an individual level.

Because disease recording in all stock is not mandatory (with the exception of dairy cows, although most farmers would still only record disease where treatment is given) assessments of prevalence and incidence largely depend on farmer recall, most often by survey. With an insidious, mostly sporadic disease like clinical JD, it is not surprising that recognition and identification by farmers is poor, and recording at the time of incident is virtually non-existent. Furthermore, accurate identification of cases is challenging for most farmers. Clinical JD can easily be mistaken for the many other chronic, sporadic wasting diseases of livestock, especially where scant attention is paid to the initial stages of most disease states.

And conversely, in the absence of knowledge of differential diagnoses, many other diseases are often identified mistakenly as clinical JD.

Allied to this quagmire of disease identification and recording are the many, well-recognised problems in using survey data of any disease for analysis. Farmer recall is poor- especially without records- and the risk of bias is high. Farmers may not want to remember they have clinical JD; or conversely, farmers may incorrectly attribute all ills to JD. Response rate to surveys is traditionally poor, and leads to valid concerns that those who respond do not represent the true population.

However, well-constructed surveys are possible. Collecting information on other diseases to minimise bias is a clever means of reducing bias in surveys, and was probably best demonstrated within the JD community with the Massey Epidemiological Survey, where information on leptospirosis was collected simultaneously with information on JD. Still, the response rate here was only 24% (Heuer and Wilson 2011).

Nevertheless, despite the vagaries of collecting this sort of information, certain patterns are constant in the data:

- Clinical JD is not highly prevalent in most species (Figures 2-6; Appendix 1)
    - For dairy cattle, a *herd* prevalence of 4-21%
        - The within-herd prevalence is unknown but possibly 0.055%
    - For deer, a *herd* prevalence of 34-74%
        - The within-herd prevalence is varied but 0-20%
    - For sheep, a *flock* prevalence of 2-18%
        - The within-flock prevalence is unknown
- Many farms have virtually no clinical JD, or have it at such a low level it doesn't appear on their radar.

- A small group of all types of farms – certainly deer and dairy; probably also sheep and beef- have significant clinical JD associated with significant losses. This severe prevalence appears most common amongst farmed deer.
- Thus, a 'mean prevalence' of clinical JD is a concept that is of little use in describing the disease.

2. *The prevalence of subclinical MAP and the performance of diagnostic testing*

Because of the nature of subclinical infection of MAP, and the requirement for diagnostic tests, far more robust data are available on the prevalence of MAP in farmed species than on the prevalence of clinical JD. This is because formalised diagnostic testing allows for appropriate sampling strategies and objective measures of disease. However, these data are limited by the sensitivity and specificity of tests, as has been previously discussed. Indeed, as our understanding of both MAP and the tests for MAP improves, MAP is being found in more animals than ever before. In the US, researchers are finding MAP prevalence rates amongst slaughter of over 90% in intensive carcass testing (Heuer, pers comm), suggesting that if a researcher looks hard enough, it can be found. It may be that the presence of MAP in intensively farmed livestock is the normal state; and of course it will always be impossible to prove that this is not the case.

Despite the inconsistency in diagnostic testing and testing procedures, a certain consensus shows through in terms of the prevalence of MAP in farmed livestock:

- MAP infection is common. Infection with MAP is more common than not (Figures 2-6; Appendix 1).
    - For dairy cattle, estimates of MAP infection range from 2-65%

- For deer, from 6-67%
- For sheep, 68% is reported

In these data, the most reliable and consistent reports appear to suggest that around 65% of animals from *all* species have been reliably found to be infected with MAP.

Thus, MAP is present in large proportions of the population of farmed livestock in New Zealand, and it is also probably common in most environments where livestock are farmed. Given this, and the inevitable debate that always arises about diagnostic sensitivity and specificity, the question arises, *does the actual prevalence of MAP matter*? The fact that MAP is far more common than disease suggests that identification of a co-factor (or co-factors) which lead to the development of clinical JD in the presence of the necessary factor, MAP, is of more importance. It is said that stress- starvation, inclement weather, concurrent disease, stocking density- is a co-factor. But there is obviously a critical factor beside stress that leads individuals to develop clinical JD when others around do not.

Genotype may well be critically important in the development or non-development of clinical JD in the presence of MAP, and there is currently a lot of work in this field. However, different genotypes may still require co-factors to trigger clinical disease.

Furthermore, the triggering of an animal carrying MAP into an animal presenting with clinical JD is not necessarily the main issue regarding MAP in farmed livestock. The concern over the association with MAP and CD means that consumers do not want MAP in product, and furthermore they want to know that steps are being taken to minimise this risk. Given the understanding that MAP is present in the majority (if not all) of farmed livestock, albeit at a very low level, the goal should be to minimise the amount of MAP present in livestock systems. What is known is that animals infected with clinical JD shed large amounts of MAP,

and hence become critically important in both the epidemiology of MAP exposure, and thus the risk of MAP in the end product.

3. MAP in animal products

The presence of MAP in animal products needs to be minimised, and consumers need reassurance that all steps are being taken to achieve this. The link between MAP and CD still remains only a link, with no evidence of causality. However, the debate on food safety is no longer about causality, but about linkages.

Consumers no longer just 'believe' the science behind food safety arguments- and it may be possible to lay this particular mindshift onto the BSE crisis in the UK and latterly Europe. In that debate, the inability of science to ever prove a negative (ie, that eating meat infected with BSE did *not* lead to human disease), coupled with some poor management of the emerging disease, lead, with hindsight, to a critical shift in consumer attitudes towards animal food products and their safety. Thus, it is no longer acceptable to hope that a product is safe; the consumer will seek very strong reassurance that the livestock industry are doing absolutely all they can to make products safe. Causality has given way to association in the mind of the consumer.

It is known that it is possible under some circumstances for MAP to make its way into both meat and milk. In milk, the results of pasteurisation are conflicting as has been described (Ayele et al., 2005; Chiodini and Hermon-Taylor, 1993; Ellingson et al., 2005; Gao et al., 2002; Pearce et al., 2001). In meat, that minimisation of risk is more readily done post purchase by appropriate food preparation, however, there is still a risk of MAP post-processing (Wells et al., 2009). Conversely, however, in the public and pseudo-scientific

forum of debate around the risk of MAP to humans, milk appears to be the product of most concern to consumers.

In this context, the absence of understanding of the current JD/MAP situation within the dairy industry poses a significant risk to that industry. New Zealand's dairy industry is world leading and is recognised as such globally, with Fonterra accounting for 37% of all internationally traded whole milk powder sales and 42% of all butter sales (Anonymous, 2010c). It is of immense economic value to New Zealand, generating NZ$16billion in revenue in 2010 (Anonymous, 2011e), and the strong performance of Fonterra in recent years has probably done more to protect New Zealand from the worst excesses of the global recession than any direct government intervention. The dairy industry is estimated at accounting directly for 2.8% of New Zealand's GDP; and around 26% of New Zealand's total exports (NZIER, 2010).

4. Other issues

Overseas studies demonstrate varying economic impacts of MAP infection and of JD, and lead to disparate perspectives of the economic importance of the disease to New Zealand. Furthermore, these perspectives inform views around control and management programmes. The economic effects of MAP infection are clearly very variable, but may be severe on the worst affected farms. The only New Zealand study where the economic impact was examined concluded with the view that there was insufficient information, particularly around prevalence, to make an informed assessment (Brett, 1998). There is no doubt that within-herd prevalence varies considerably. For farms with a high prevalence, the cost of both clinical JD and MAP infection is likely to be high, and it is likely to be cost-effective to

implement better control of JD. The direct economic benefits of control measures may be more doubtful on farms with a low prevalence of disease.

In addition to the costs of disease, the welfare aspects of JD should not be ignored. Ruminants are stoic animals and it is easy to overlook the physical distress that such a debilitating disease may cause. Apart from the ethical responsibility to ensure the wellbeing of farmed livestock, consumer awareness of animal welfare issues might provide additional motivation for improvements in JD control. The authors were unable to find any information in the literature analysing the welfare implications of JD, which, in itself, may tell a story.

Chapter 7. Conclusions, Recommendations and Summary

Conclusions

It would be disingenuous for the authors of this review to pretend that there isn't some degree of dissent amongst the various significant players within the JD community on the next critical steps forward for the JDRC, and for the livestock industries as a whole concerning JD. However, there are more areas of agreement then otherwise, and more areas of collaboration than contention- indeed, the development of and progress made by the JDRC itself since its inception is indicative that pan-industry collaboration is eminently possible.

For the purposes of this summary section of the review, it is worthwhile initially to identify areas over which the authors have found little or no disagreement amongst both the literature and experts in the field:

- MAP prevalence in deer certainly, and in sheep probably, is more common than otherwise (non-detection)
- *Mean* herd/flock prevalence of clinical JD is not a particularly helpful concept
- A small number of deer farms certainly, and dairy and sheep farms probably, are severely affected with a high prevalence and incidence of clinical JD and of MAP infection and for these farms there is likely a high economic impact
- Thus, an understanding of within-herd prevalence, and most importantly, the prevalence of herds/flocks with high within-herd prevalences and incidences, is important

- There is most merit in targeting these ‘high-prevalence/incidence’ farms for control and management options
- The dairy industry is poorly represented in all recent prevalence/incidence work.
- Maintenance of marketability of product is of critical importance for all species, but especially for the dairy industry
- The economic aspects of either JD or MAP infection in any species are not understood
- The role of co-factors in triggering animals from MAP infection to clinical JD in any species is not understood
- The recent dynamics of the dairy industry (significant increase into the lower South Island) is an area where there is very little understanding of the JD situation, particularly given that there is significant grazing off of youngstock on predominantly deer and sheep blocks.

There are other areas that are more contentious. The definition of prevalence itself is one where debate is broad. The relative merit of control and management programmes, and the role of shedding and of co-factors in developing clinical disease are also areas of divergent perspectives. Looming unspoken behind everything that concerns JD and MAP in the livestock industries is the issue of consumer perception and product assurance.

Much excellent research work has been performed over many years in deer. We have a reasonable understanding of the levels of MAP in carcasses from slaughterhouse surveys and other reports. The Massey Epidemiological Study has produced a comprehensive analysis of the prevalence of both JD and MAP in the deer, sheep and beef industries. It minimised the risk of bias attributed to surveys by careful study design; and significantly improved our

predictive capabilities by the use of latent class analysis modelling techniques using existing imperfect tests.

For whatever reason, the dairy industry has received scant attention, which, given its size and importance to New Zealand as a whole, seems disproportionate. The dairy industry would appear to have the most to lose by the absence of comprehensive data; and yet potentially the most to gain by the demonstration of a clear understanding of JD/MAP within the industry, and the development of some form of control and management plan to reassure consumers with regard to food safety. For the dairy industry, food safety is not just about milk, for approximately 1 million dairy-bred animals enter the food chain annually in New Zealand. Moreover, damage to the perception of the dairy industry would, by implication, lead to damage to other livestock industries also.

Whilst control and management programmes have had very mixed success overseas, there can be no doubt that consumers will at some stage demand reassurance that the livestock industries are doing all they can to minimise any risk of MAP entering the food chain. Currently this is not the case.

Recommendations

The dairy industry could lead the way in this by developing a comprehensive understanding of the prevalence and incidence of JD and MAP within the national herd, with particular attention to the prevalence of herds with higher within-herd prevalence of both MAP and JD. This could also embrace longitudinal studies to determine co-factors for disease development; an economic analysis of both clinical and subclinical infection; and ultimately

develop a model for an appropriate control and management programme that is tailored to New Zealand's unique industry and role as exporter of 35% of the world's milk products.

The authors believe that a pan-industry approach to food safety around JD/MAP would be ideal, but would be incredibly challenging. However, by looking closely at the role of MAP/JD within the dairy industry it would be possible to illuminate the major black holes currently existing in our understanding of JD; whilst at the same time developing a template for control, management and food safety assurance that could then be more readily applied to the other livestock industries. The dairy industry is best placed to achieve this because it has better access to individual animal data and recording and has more capability than the other industries.

The authors believe that, as detection rates of MAP improve, thus increasing the apparent prevalence, the onus on primary producers to be seen to be minimising the risk to consumers from MAP in their product will significantly increase. Currently the absence of a coordinated strategy to reassure consumers that JD/MAP within New Zealand's food production industry is both well understood and is being well managed poses one of the greatest risks to New Zealand livestock farming.

Summary

The prevalence of JD in particular, and of MAP in some instances, is still poorly defined. Studies examining clinical JD mostly measured (annual) incidence, and mostly at a farm level. In the opinion of the authors, the following represents the best current understanding of the existing situation.

i. <u>Deer</u>

- MAP prevalence at a herd level consistently appears at around 60% of herds (Heuer & Wilson, 2011; Heuer et al., 2011a; Stringer et al., 2009).
- However, survey data suggests farmers report clinical cases of JD in up to 74% of farms (Glossop at al., 2008a).
- These two figures do not line up well. It is unlikely that clinical JD occurs in a higher proportion of farms than have MAP infection. Therefore, clinical JD is likely over-reported (the figure is likely nearer 30% (Heuer & Wilson, 2011; Heuer et al., 2011a)) or, MAP infection is under-detected.

ii. Sheep

- MAP prevalence at a flock level is around 68% of flocks (Heuer & Wilson, 2011; Heuer et al., 2011a).
- 20% of farmers reported clinical JD over a 3 year period (Heuer & Wilson, 2011; Heuer et al., 2011a), and 7-8% of sheep farmers report clinical JD per year (Gumbrell, 1986; Nuttall, 1991; Staples, 1994).
- The clinical JD figures seem in accordance; and further, the MAP prevalence estimates are strikingly similar to deer.

iii. Beef cattle

- MAP prevalence at a herd level is around 31% of herds (Heuer & Wilson, 2011; Heuer et al., 2011a).

- Farmer assessment of clinical JD is 4%, although this appears to be a 3-year incidence rate. Clinical cases are likely under-reported.

- If MAP prevalence and JD incidence are genuinely of a lower level in beef herds than in sheep and deer herds, this difference needs to be explained and understood.

iv. Dairy cattle

- Reported MAP prevalence at a herd level varies considerably between studies. The bulk milk Elisa work suggests a prevalence of around 5% of herds (Voges et al., 2009), which is markedly lower than the estimates from the Massey work, of 22-48% (Heuer & Wilson, 2011; Heuer et al., 2011a) herd prevalence.

- A number of studies give a herd prevalence (annual incidence) of JD of around 7-14% (Nuttall, 1991; Ryan, 1991; Staples, 1994; Voges, 2008), and the Massey study reports 21% over 3 years (Heuer & Wilson, 2011; Heuer et al., 2011a). The accord in these figures suggests an annual incidence of around 8-10% of herds identifying clinical JD.

- The figure of around 10% of farms reporting JD per annum does not accord with the reported MAP prevalence from bulk milk Elisa studies. Furthermore, the bulk milk data is strikingly lower than the Massey study, and that reported in other species. It is difficult to place the bulk milk data in the context of all other knowledge of MAP/JD in both dairy cattle and other species.

- Dairy cattle currently represent the biggest gap in any consensus on MAP/JD.

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Appendices

Appendix 1- Figures

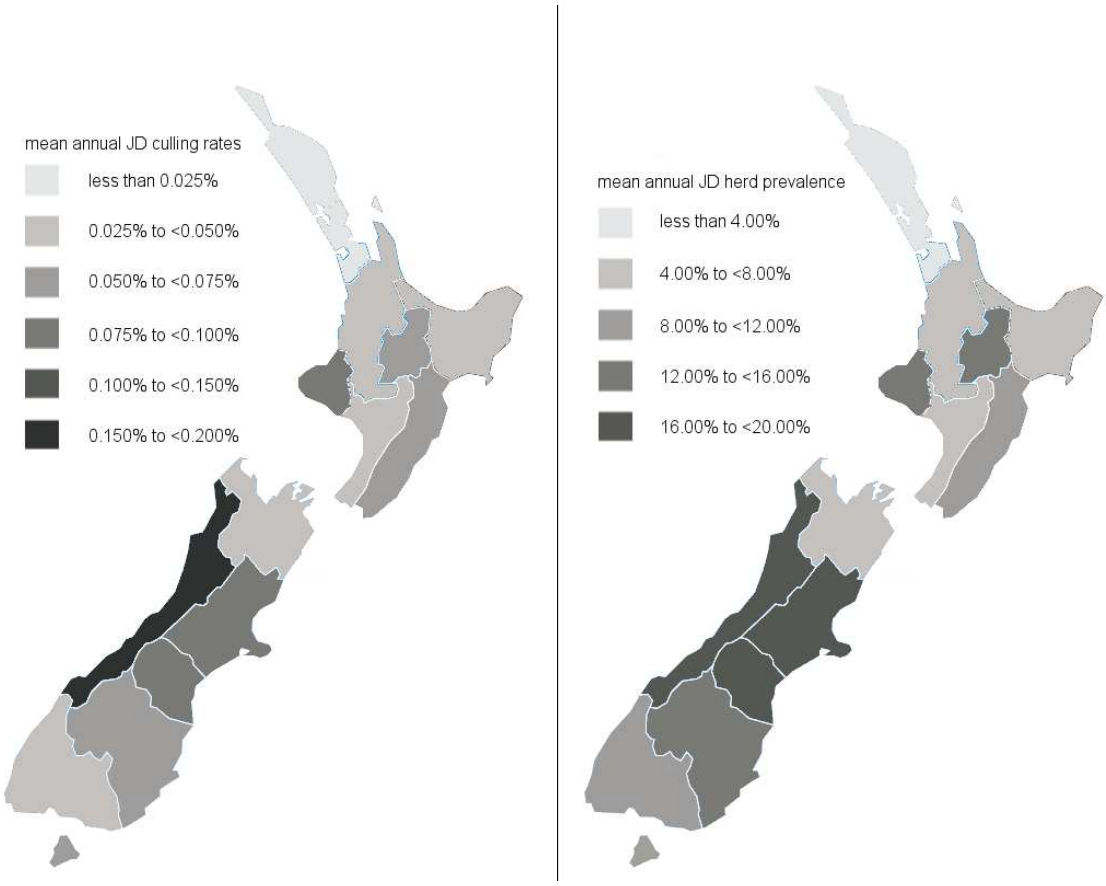

Figure 1. The mean annual JD culling rates (annual incidence) and herd prevalence by region between 1998/99 and 2006/7 in New Zealand. Reproduced from Voges (2008).

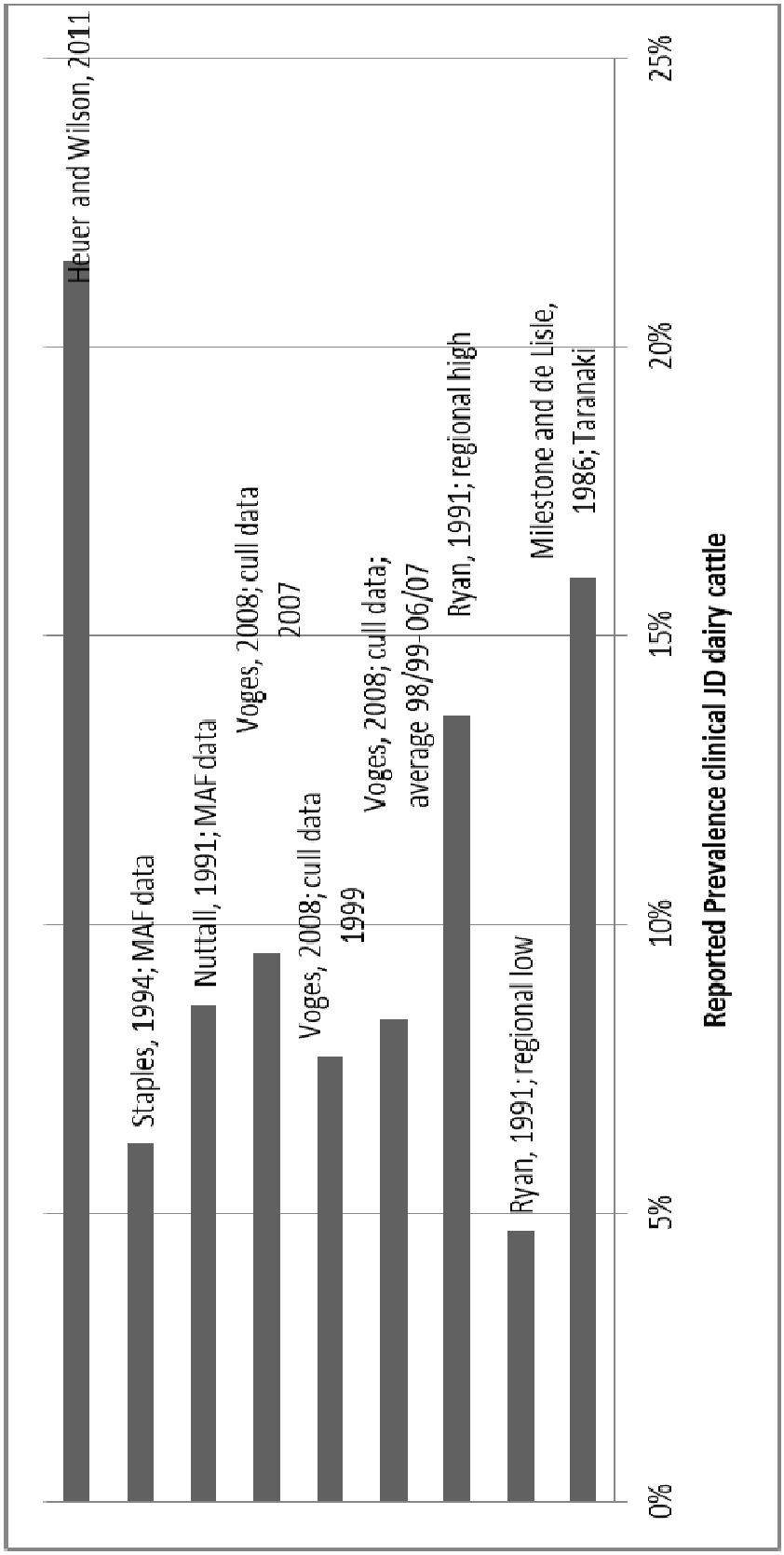

Figure 2. The estimated herd prevalence of JD infection found in dairy cattle in New Zealand

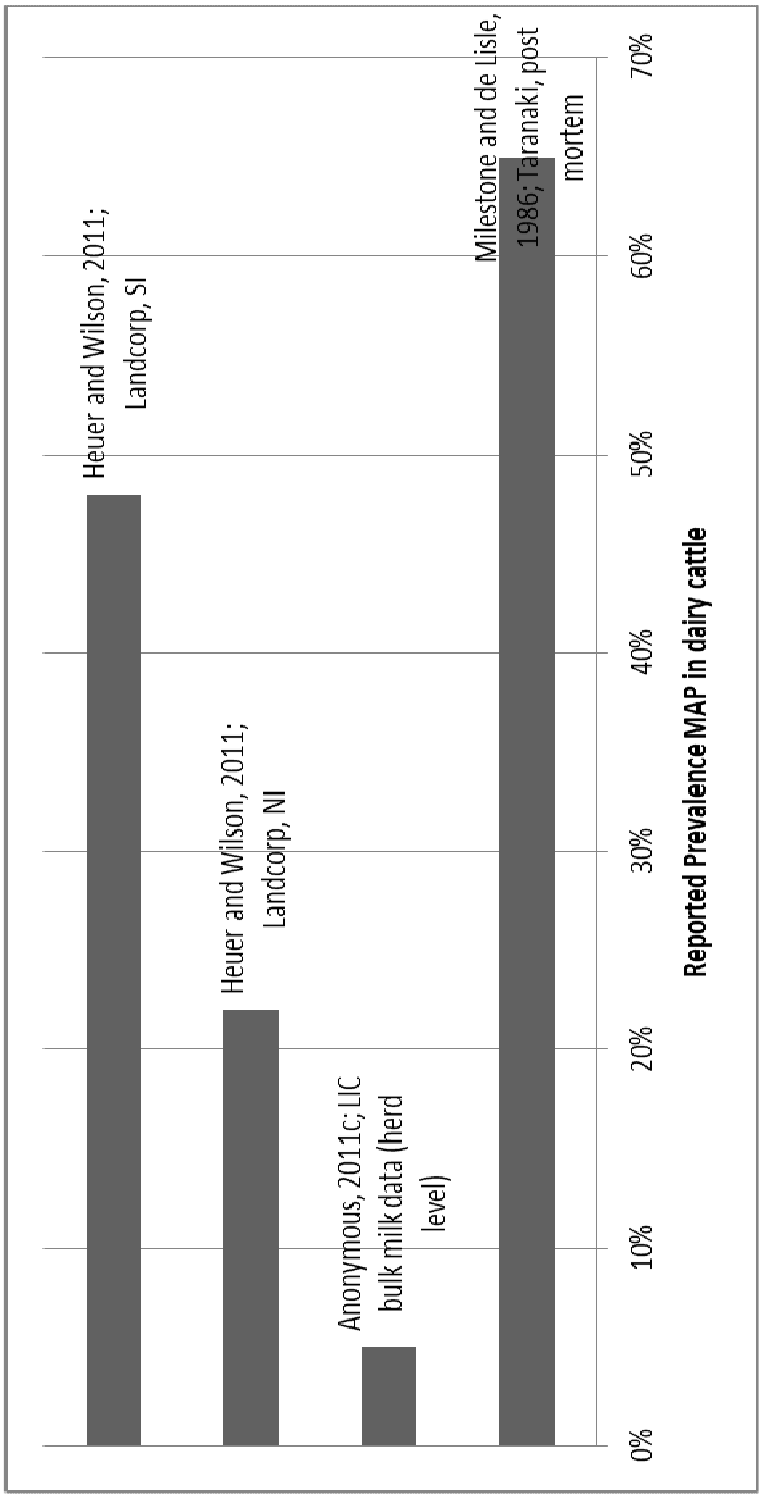

Figure 3. The estimated herd prevalence of MAP infection found in dairy cattle in New Zealand

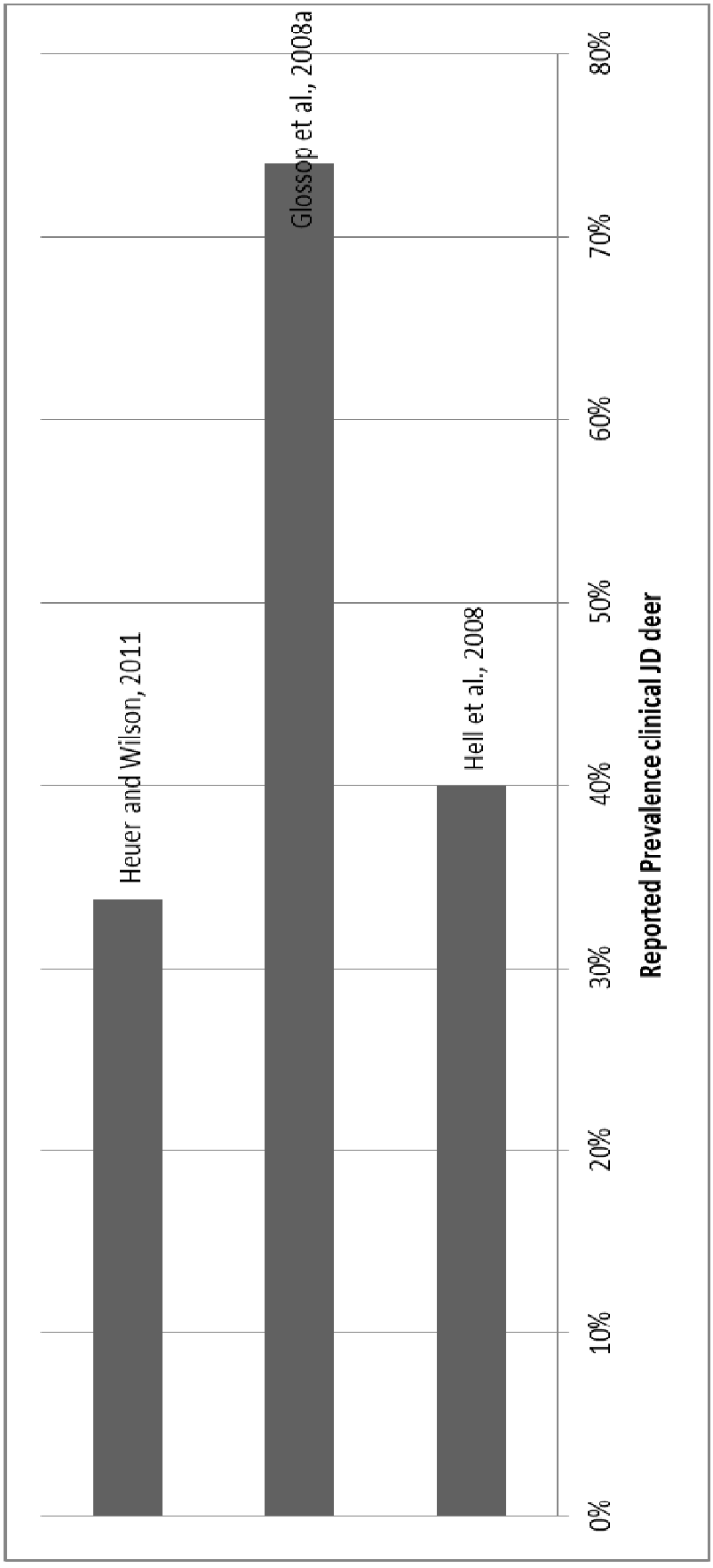

Figure 4. The estimated herd prevalence of clinical JD found in deer in New Zealand

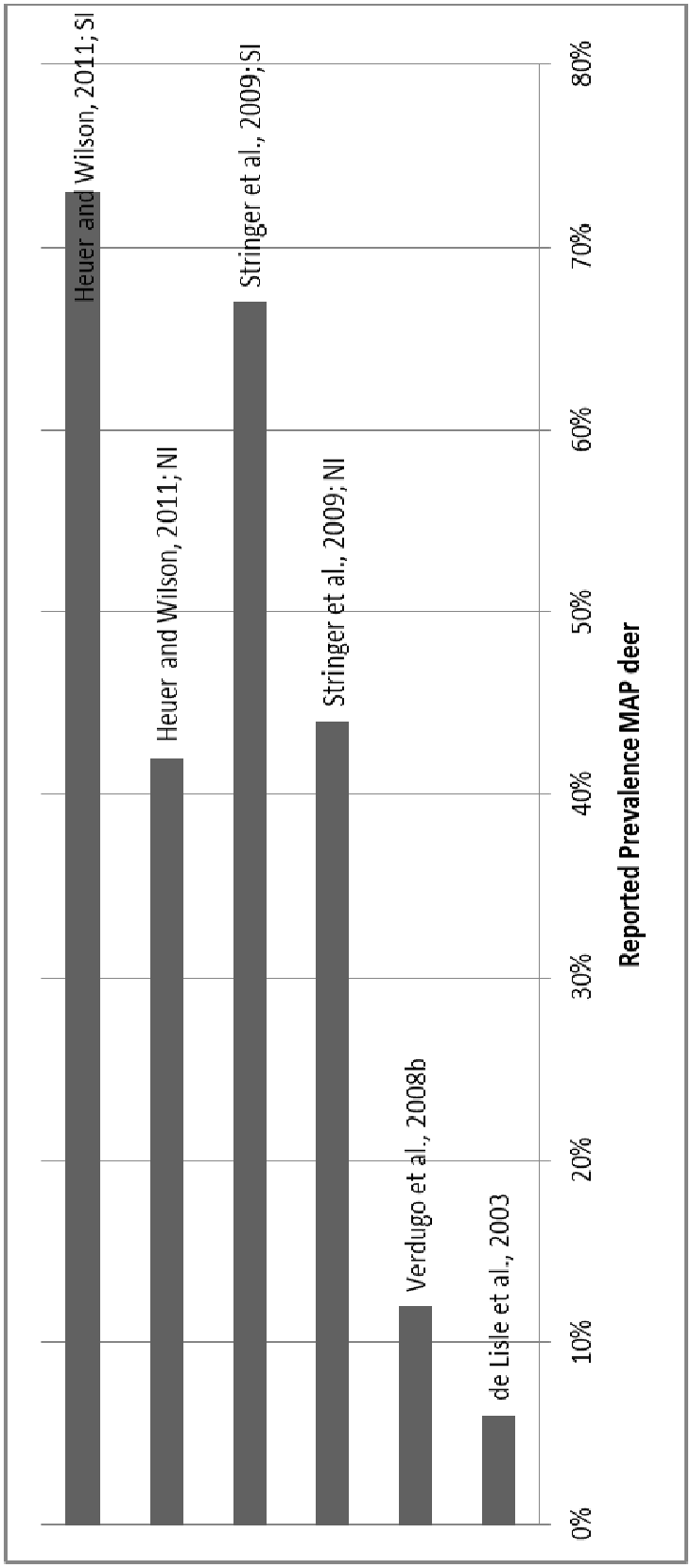

Figure 5. The estimated herd prevalence of MAP infection found in deer in New Zealand

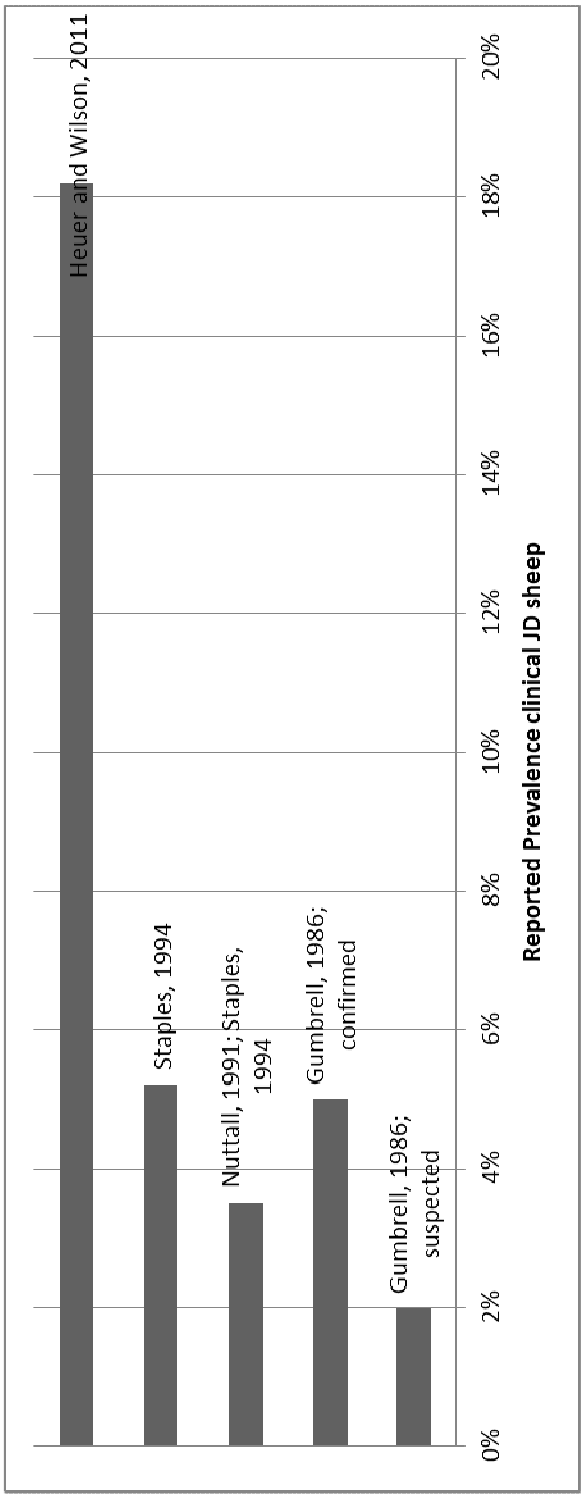

Figure 6. The estimated flock prevalence of clinical JD found in sheep in New Zealand

Table 4: Diagnostic tests used in JD/MAP and their reported sensitivity/specificity

Test	Level of Faecal Shedding	Cattle		Ref	Sheep		Ref	Deer		Ref	Detection limit	Comments
		Sensitivity	Specificity		Sensitivity	Specificity		Sensitivity	Specificity			
Culture												Animals must be shedding mycobacteria. Sensitivity increases with repeated testing. Very slow.
Individual faecal culture		4-59%	100%	3				49%-77%	99.9-100%	12,6	10-50cfu/g	
Pooled faecal culture												Useful for screening
	High				100% (pool size 50, 6 pools/flock)	100%	17	100%	100%	8	100cfu/g (pool size 10-20)	
	Low				50% (pool size 50, 6 pools/flock)	100%	17	0% (pool size >10), 25% (pool size 10)	100%			
								70-82% (pool size 10, 6 pools/herd)	100%	4		
Individual milk culture		71%	100%	3								
Pooled milk culture			100%									Useful for screening flocks/herds
Tissue culture								92%	100%	12		
Histology								66%	100%	12		May need to examine a large number of sections to find mycobacteria/pathology
Tests for Cell-Mediated Immunity												Only likely to detect early (pre-clinical) stages of
Skin test		54%	79%	1								disease
Lymphocyte stimulation test		50%	50%	1,18								
γ-interferon		Not reported	66-94%	7								
Serological Tests												Only likely to detect later stages of infection after animal has seroconverted.
CFT		38%	99%	13								
	High	55%	99%	13								
	Low	15%	99%	13								
AGID		27%	100%	13	10-60%	100%	11					
	High	41%	100%	13								
	Low	4%	100%	13								
ELISA		25-58.8%	84.9-100%	2,13,16				91%	100%	5		
	High	87%		16								
	Low	15%		16				19%	94%	6		
PCR		3-33%	100%	9,14								
Milk PCR		41.3-77.8%	100%	3							10-100 cfu/ml milk	
Bulk milk PCR		50%	100%	10								Useful for screening flocks/herds
Environmental Sampling		88-95%	100%	15								Useful for screening herds

References Table 4

1 Chiodini 1984
2 Collins et al, 2005
3 Gao et al 2009
4 Glossop et al 2007b
5 Griffin et al 2005
6 Heuer and Wilson 2011
7 Kalis et al, 2003
8 Mackintosh and Van Kooten, 2005
9 Pillai and Jayarao 2002
10 Pillai et al 2007
11 Sergeant and Marshall 2000
12 Shroen et al 2003a
13 Sockett et al 1992
14 Van der Giessen et al, 1992
15 Weber et al 2009
16 Whitlock 2000
17 Whittington 2000a
18 Worthington 2004

Table 5: Summary of best estimates of JD prevalence/incidence and MAP prevalence by species

Species	Year	Study type	Method of diagnosis	Herd Incidence/Prevalence			Within-Herd Incidence/Prevalence		
				NI	SI	NZ	NI	SI	NZ
Clinical Johne's Disease									
Dairy cattle	1998-2007	LIC culling data[1]	Farmer opinion	7.4%	13%	8.35%			0.45%
Dairy cattle	2009	Massey JDRC survey[2]	Farmer opinion	18.5%	26.3%	21.5%			
Red Deer/Wapiti	2008	Fallow deer survey[3]	Farmer opinion			40%			
Fallow Deer	2008	Fallow deer survey[3]	Farmer opinion			0%			
Deer	2008	Farmer survey[4]	Farmer opinion	56%	83%	74%			0.31%-0.54%
Deer	2009	Massey JDRC survey[2]	Farmer opinion	25.3%	38.1%	33.8%			
Sheep	2009	Massey JDRC survey[2]	Farmer opinion	19.4%	16.9%	18.2%			
Beef cattle	2009	Massey JDRC survey[2]	Farmer opinion	4.5%	3.7%	4.2%			
Subclinical MAP Infection									
Dairy cattle		LIC JDRC study[5]	Bulk milk screening			3%-5.5%			2%
Deer	2009	Abattoir surveillance[6]	Tissue culture	44%	67%	59%	29%*	51%*	45%*
Deer	2009-2011	Massey JDRC sampling[2]	ELISA/faecal culture	42%	73%	60%			
Sheep	2009-2011	Massey JDRC sampling[2]	ELISA/faecal culture	74%	60%	68%			
Beef cattle	2009-2011	Massey JDRC sampling[2]	ELISA/faecal culture	33%	27%	31%			

*Individual carcass prevalence rather than within herd prevalence

References

1 Voges 2008
2 Heuer & Wilson 2011; Heuer et al 2011a
3 Hell et al, 2008
4 Glossop et al 2008a
5 Voges et al 2009
6 Stringer et al 2009