The Epidemiology of Johne's Disease in New Zealand Dairy Herds

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Abstract

Johne's disease (JD), caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is a chronic, debilitating enteritis of cattle, other domestic livestock and some wildlife species. JD was first identified in the late 1800s and today it is a worldwide problem in dairy cattle. Heavily infected cows have reduced milk production, a higher risk of removal from the herd and low slaughter value. Several countries have implemented national level control strategies.

In New Zealand, JD was first reported in 1912 and today the prevalence of infected dairy herds is thought to be high. To improve our understanding of the epidemiology of JD and to evaluate the feasibility of a national control strategy, four studies were conducted.

The first study was a questionnaire based case-control study to identify associations between management practices and the occurrence of clinical JD on farms from four regions of New Zealand. The second study was on the effect of sub-clinical JD on milk production and the risk of removal from the herd in four dairy herds over four milking seasons. The effect of misclassification of disease status on productivity estimates was also studied. In the third study diagnostic test result data from the productivity study was combined with a novel Bayesian regression model to estimate performance of the ELISA and faecal culture tests as a function of covariates and utilising repeated tests on individual cows. Finally, results from these three studies were used to adapt an existing JD simulation model, 'JohneSSim', to represent the epidemiological behaviour of JD in New Zealand dairy herds. Control strategies for the disease were simulated and evaluated based on their cost effectiveness.

Of the 427 farmers responding to the questionnaire, 47% had suspected clinical cases of JD in their herd in the preceding 5 years. Only 13% of suspected infected herds had an average incidence of greater than 0.5 cases per 100 cow years at risk. The disease was not considered a serious problem by 20% of herd managers who reported the presence of disease in the preceding 5 years. The presence of Jersey cows in the herd and the purchase of bulls had strong positive associations with the presence of clinical JD. Grazing calves in the hospital paddock, larger herds, the purchase of heifers, and the use of induction were also positively associated with JD.

In the productivity study the herd-level prevalence of JD by ELISA and/or faecal culture ranged from 4.5% (95% CI 2.6–6.9) to 14.2% (95% CI 9.2–20.6). Daily milksolids production by JD positive cows was 0.8% (95% CI -6.1%–4.5%) less than that of JD negative cows. However in herd D, JD positive cows produced 15.5%, (95% CI 6.75%–24.2%) milksolids less than JD negative herd mates daily. This equates to a loss of 53kg of milksolids/305 day lactation, or NZD 265/lactation, given a price of NZD 5/kg of milksolids. In herd D only, the annual hazard ratio of removal for JD positive cows was significantly increased. It was 4.7 times and 1.4 times higher in cows older than 5 years and younger than 5 years. The results were insensitive to misclassification.

Analysis of the diagnostic test data demonstrated the strengths of our Bayesian regression model. While overall estimates of sensitivity and specificity by this method were comparable to estimates by existing methods, it showed a broad trend of increasing sensitivity in higher parity groups and higher sensitivity in early, relative to late, lactation. It also showed that estimates of prevalence may in fact decline with repeated, relative to single, testing. Our novel approach demonstrated trends that could not be shown by existing methods, but could be improved by application to a larger data set.

Simulation showed that control strategies for JD based on either test-and-cull, vaccination, breeding for genetic resistance, or removal of offspring from clinically affected cows, were not cost effective for the average infected herd. Improvement of the hygiene associated with calf management provided the greatest reduction in the within-herd prevalence of JD.

While JD is present in a high proportion of New Zealand dairy herds, the incidence of clinical cases is usually low, and most farmers consider it to be of little importance. However, JD causes significant losses in productivity in some herds. The disease would probably be best controlled on a herd-by-herd basis, given the limited success of national-scale control programs for JD in other countries. The education of dairy farmers regarding risky management practices, and the offer of a risk assessment to farmers wishing to control the disease, would provide a combination of wide reaching and targeted approaches, of low cost, for JD control.

It seems likely that JD will persist in some capacity in the years ahead, but will remain of minor concern next to major animal health issues, such as infertility and mastitis. Clarification of the effect of genetic strain on the virulence of MAP may help explain differences in the effect of the disease between herds. This knowledge could then be used to further improve the efficiency of JD control.

For Jess.

Acknowledgments

Nomenclature

AGID	Agar gel immuno diffusion
AIDS	Acquired immunodeficiency syndrome
CFU	Colony forming units
CFT	Complement fixation test
CI	Confidence interval
CWD	Cell wall deficient
ELISA	Enzyme linked immunosorbent assay
FC	Faecal culture
ICM	Improved calf management
IEL	Intraepithelial lymphocyte
JD	Johne's disease
LAM	Lipoarabinomannan
LIC	Livestock Improvement Corporation
MAP	*Mycobacterium avium* subspecies *paratuberculosis*
MIRU-VNTR	Mycobacterial interspersed repetitive units variable number tandem repeats
MS	Milksolids
NPV	Net present value
NZD	New Zealand dollars
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
RPO	Retention pay-off
USD	United States dollars

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Chapter 1.

General introduction

Mycobacterial disease is an ancient scourge. It has beset human kind throughout known human history and prehistory (Daniel 2006). In the latter part of the 20th century and in developed countries, mycobacterial disease has become comparatively rare as the understanding of mycobacteria has improved and with the discovery of streptomycin, the first bactericidal agent effective against tuberculosis (Schatz et al 1944). Mycobacteria also infect animals including domestic livestock. In livestock it is not cost effective to treat for the disease and the cycle of infection is difficult to break. Two primary examples are *Mycobacterium avium* subspecies *paratuberculosis* (MAP), the cause of Johne's disease and *Mybocacterium bovis* the cause of bovine tuberculosis (OIE 2001).

The causal agent of Johne's disease was first isolated by Dr Heinrich Albert Johne (Johne and Frothingham 1895) in Germany, and the disease was subsequently given its name by Bang in 1906 (Bang 1906). In the early 1900s Johne's disease was detected in a number of European countries and the United States of America (Twort and Ingram 1912). In the decades that followed it spread throughout the world due to the international trade of cattle. Today it is considered endemic in the major dairy producing nations.

The clinical signs of Johne's disease are progressively worsening diarrhoea and weight loss, culminating in death. They are usually seen in older cattle. These signs stem from reduced absorptive capacity of the gut due to deterioration and corrugation of the small intestine as a result of intestinal lesions infected with MAP. MAP may be shed in large numbers in the faeces of clinically affected cows.

The epidemiology of Johne's disease in dairy cattle is moderately well understood. It is characterised by relative susceptibility of calves to infection, and a latent period of several years prior to the appearance of clinical signs. It is typically widespread amongst herds but with low to moderate prevalence within herds (Chiodini et al 1984a). Productivity is reduced due to the disease, as clinically infected cows have reduced milk production, a high removal rate, and low slaughter value. On a national scale, this loss of productivity may represent substantial financial losses to the dairy industry (Ott et al 1999).

National scale, coordinated control strategies for the control of Johne's disease have been undertaken by the United States of America (USDA 2005a) and Australia (Animal Health Australia 2003), both of whom compete with New Zealand for dairy product sales on the world market. Control strategies are implemented to reduce the prevalence and economic losses associated with the disease. They may also be used as a marketing tool to promote the health status of dairy products from countries with a control strategy at the expense of countries without, such as New Zealand.

The aim of this thesis was to further the understanding of Johne's disease in New Zealand's unique year round outdoor, pasture based, dairy production system. Of particular interest were

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epidemiological aspects of the disease and the economic attractiveness of a national scale control strategy for the disease.

Detailed descriptions of studies investigating these topics are presented in the format of scientific manuscripts suitable for peer reviewed publication. The first is of a cross sectional nature, investigating Johne's disease on dairy farms in several regions of New Zealand to identify demographic and management related risk factors for the disease. The second study is of a longitudinal nature, in which the cows in four dairy herds were followed for four milking seasons to evaluate the effect of the disease on milk production and risk of removal from the herd. In the third study the performance of diagnostic tests for Johne's disease when applied to New Zealand dairy herds was estimated by a range of analytical methods. In the fourth study, results from the previous studies were used to adapt an existing simulation model of Johne's disease, 'JohneSSim' (Groenendaal et al 2002b), to represent the disease in New Zealand dairy herds. This model is used to compare a range of potential control strategies for their ability to reduce the prevalence of the disease in a cost effective manner.

The thesis begins with a review of previous research on Johne's disease, addressing the field of epidemiology in particular. It also examines factors which may predispose calves to infection, as little information exists on this topic. The thesis concludes with a discussion of the study findings, identification and discussion of key gaps remaining in our understanding of the disease, and finally, some predictions on the future of Johne's disease in the New Zealand dairy herd.

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Chapter 2.

The epidemiology of Johne's disease (*Mycobacterium avium* subspecies *paratuberculosis*) in dairy cattle

S Norton

Introduction

Johne's disease (JD), or paratuberculosis, caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is a chronic granulomatous enteritis and lymphadenitis found in both domestic and wild animals (Chiodini et al 1984a). It has been spreading slowly through domestic livestock populations for at least a century and has become endemic in most countries (Kennedy et al 2001).

The disease is contagious and untreatable. Recent infections are usually sub-clinical (not detectable by ante-mortem testing methods) and may remain so for some years. Clinical signs of disease are intermittent diarrhoea, loss of weight, emaciation and eventual death. The lesions are usually restricted to the gastrointestinal tract from duodenum to rectum, and are most common in the terminal portion of the ileum and regional lymph nodes (Payne and Rankin 1961a). In some cases (transient infection) presence of the causal organism in the faeces indicates its passage through the alimentary tract, while the host remains uninfected (Sweeney 1992).

Reports consistent with the disease date back as early as 1826, when d'Avrol described a form of enteritis which occurred in some cattle with chronic diarrhoea (Johne and Frothingham 1895). Thickening and corrugation of the intestinal mucosa of cattle dying with this enteritis was noted by Hansen and Nielsen in 1881. The disease inherited its name from Dr Heinrich Albert Johne, a German pathologist who first reported the organism in 1895 from a section of infected intestine (Johne and Frothingham 1895). He demonstrated the presence of acid-fast bacilli and found it indistinguishable from the tubercle bacillus. Bang (1906) re-evaluated the disease and named it pseudo-tuberculosis, or JD.

Between 1902 and 1908 the disease was identified in a number of countries including the United States, Germany, Norway, France, The Netherlands, Denmark, Belgium and Switzerland. Today it is considered endemic in the major dairy producing nations. International trade of cattle and sheep has been linked to the introduction of JD in more than 20 of the nearly sixty countries in which JD has been described (Kennedy and Benedictus 2001).

JD has been reported in all domestic ruminant species and some wildlife species, both ruminant and non-ruminant.

Herein, literature describing several aspects of JD is reviewed. First, the causal agent and its infective process is addressed. Literature describing the development of the calf is then reviewed with respect to infection by MAP. Following that, epidemiological aspects of JD on dairy farms are reviewed, and finally, options available for control of the disease. These topics are addressed from an epidemiological perspective but references for the specific cellular and immunological concepts covered are provided.

The pathogen

2.1.1.Physical attributes

The family Mycobacteriaceae contains several species of bacteria for which a single method of classification has yet to be agreed upon. These species are widely distributed in the environment and are either non-pathogenic, opportunistic pathogens, or obligate pathogens. MAP belongs in the last of these three categories. The classification suggested by Runyon (1959) is based on cultural characteristics, mainly the criteria of extremely slow growth and the requirement for exogenous mycobactin. However the 'wood pigeon' strains of *M. avium*, require exogenous mycobactin on primary isolation but lose this dependency on subsequent passage. An alternative method of classification is based on a large number of biochemical tests, which categorise *M. avium* into three subspecies; *paratuberculosis*, *avium* and *silvaticum* (Thorel et al 1990). A third method involves classification based on specific DNA insertion elements such as IS*900* (Kunze et al 1991). It was initially thought that the insertion sequence IS*900* was unique to *Mycobacterium avium* subspecies *paratuberculosis*. However, IS*900* like elements have since been detected in environmental mycobacteria closely related to *M. scrofulaceum* (Cousins et al 1999), from *M. avium* subspecies *avium* strains isolated from HIV infected patients (Naser et al 1999), and from Mycobacterium sp. 2333 which is closely related to *M. cookii* (Englund et al 2002). Consequently, multiple genomic targets must be evaluated for the specific identification of *M. avium* subspecies *paratuberculosis* by polymerase chain reaction (PCR), a process described by Rajeev et al (2005).

Mycobacterium avium subspecies *paratuberculosis* (MAP) is a small (0.5 × 1.5 micron), gram-positive, slow growing, rod shaped, non-spore forming, non-motile, facultative, acid-fast bacteria. The organism is found in clumps, entangled with each other by a network of intercellular filaments (Merkal et al 1973).

There are two forms of MAP; bacillary (cell-wall-competent) and cell-wall-deficient (CWD). The latter is characterized by a complete or partial loss of cell wall components. The CWD form is larger and rounder, and bound only by a plasma membrane. The chemotype profile of the CWD form differs from that of the cell wall competent (CWC) form in that it lacks bands generally associated with cell wall glycolipids (Hines and Styer 2003). The most likely mechanism for establishment of CWD forms seems to be the lytic activity within the macrophage (Willett and Thacore 1966).

The bacillary form, existing in large numbers, is easily detectable and is identifiable by simple chemical tests for the presence of MAP's lipid rich cell wall. In contrast, the CWD form appears in relatively small numbers and is not easily detected. Acridine orange has been shown to stain

most CWD cells orange, indicating the presence of nucleic acids, but will not stain acid fast MAP, other mycobacteria, or microbes (Hulten et al 2000).

CWD forms of MAP are extremely difficult to isolate from infected tissues, and to maintain and harvest in sufficient numbers for study. However a chemical treatment process to generate a high percentage of CWD organisms in culture with minimal loss of viability has been developed (Naser et al 1993) and modified for use with MAP. With this treatment 90% of the MAP organisms converted to CWD forms, whereas in untreated cultures, the CWD form comprises only about 5% of cultured cells (Hulten et al 2000).

In vitro, the absence of the chemicals required to induce the CWD form of MAP causes these cells to revert to the CWC form. MAP is induced into a CWD form over a period of about 8 days, and it returns to the CWC form in a similar period in the absence of sustained treatment (Hines and Styer 2003). Experiments on guinea-pigs showed that the CWD form of *M. tuberculosis* did not acquire pathogenicity before its reversion to the CWC form (Ratnam and Chandrasekhar 1976).

The comparison of CWD MAP with its CWC form reveals differences in morphology, chemotype profile, cell wall constituents and antigens recognized by sera from JD positive cattle. Western blotting shows that CWD MAP has lost bands that migrate in the same region as lipoarabinomannan (LAM) and some other bands (Hines and Styer 2003). LAM is a key immunogen in the cell wall of mycobacteria (Hunter et al 1986).

It is not yet known what, if any, role CWD forms of MAP play in the etiology of JD. Persistence of CWD forms of MAP within tissues may be responsible for triggering an abnormal and/or continuing immune or autoimmune response leading to development of disease.

The cell wall of MAP has properties which help it to gain entry to, and survive and multiply within, the macrophage. The lipid rich cell wall, which constitutes about 40% of the total cell dry weight, is believed to assist MAP in resisting intracellular degradation, adjuvancy, and anti-tumor activity (McFadden 1992).

Three characteristics of the cell wall in particular facilitate invasion of the host. Also of interest is the importance of iron.

The first characteristic is biologically active components embedded in the cell wall. In particular LAM, but also cord factor, macrophage inhibitory factor, and superoxide dismutase. LAM is a highly immunogenic and potent inhibitor of macrophage activation. It down-regulates macrophage effector function by suppressing macrophage activation and T-cell stimulation (Sibley et al 1988; Chan et al 1991; Barrow 1997). The activities of LAM may explain why an immune response is slow to develop following infection. The other components are involved in

the detoxification of reactive oxygen intermediates (Barrow et al 1995; Daffe and Etienne 1999) which aids survival of MAP within the phagosome.

The second cell wall characteristic is the presence of glycopeptidolipids, which accumulate on the surface of MAP. These provide a capsule surrounding the mycobacteria which may act as a passive barrier against attack when the organism is within the phagosomal compartment (Barrow 1997).

The third characteristic is the presence of fibronectin attachment proteins embedded in the cell wall of MAP and some other mycobacteria. Fibronectin is a ubiquitous glycoprotein found in body fluids and the extracellular matrix of vertebrates. MAP may be attached to and internalised by host epithelial cells, depending on the interaction between fibronectin attachment proteins and fibronectin binding proteins. The binding site for mycobacteria opsonized by fibronectin is the βI integrin, which is present on the luminal faces of microfold epithelial (M) cells at high density, but nowhere else on the intestinal epithelium (Clark et al 1998). The interaction between these integrins and fibronectin bound by MAP may explain why M cells are the portals of entry for MAP. However research leading to this understanding is largely *in vitro* based and may not be representative of the *in vivo* situation. The distribution of receptors on cells in culture may be markedly different from that on cells in intact tissue, particularly for intestinal epithelial cells (Kerneis et al 1997). Nevertheless a recent study *in vivo* showed that M cells were selectively invaded by MAP, and that this effect was enhanced when MAP was opsonized with fibronectin. Attenuation of fibronectin attachment protein expression effectively eliminated the selective invasion of M cells by MAP. However, some invasion of villous epithelial cells by MAP occurred by a mechanism independent of fibronectin (Secott et al 2004). For fibronectin binding to play a role in the pathogenesis of infection with MAP, binding must be stimulated as the organism passes through the host digestive tract.

The ability of MAP to bind fibronectin is enhanced by brief pre-treatment with acid of about the pH found in the abomasum (Secott et al 2001). These authors propose that passage through the ruminant abomasum, which maintains a pH of 3, facilitates binding to fibronectin found in bile secretions in the duodenum (Secott et al 2004). Once bound to MAP, fibronectin could bridge the organism to M cells in the terminal ileum leading to translocation across the epithelial barrier via Peyer's patches.

A high concentration of iron stimulates MAP proliferation. MAP requires iron in the form of ferric mycobactin to sustain the respiratory activity necessary for replication, as shown by *in vitro* cultivation (Francis et al 1953). In cattle, high iron concentrations exist in the intestinal epithelium and mesenteric lymph nodes (Kolb 1963). Under certain experimental circumstances simply providing a concentration of iron in excess of that normally required by other

mycobacteria may maintain replication, such as one percent ferric ammonium citrate (Merkal and Curran 1974).

Ferratin and haemosiderin, which contain iron, accumulate in undifferentiated macrophages of most cows with clinical JD. These macrophages often contain MAP bacilli. High intracellular iron concentration in phagocytic cells was positively associated with the numbers of bacilli and the severity of lesions in clinically and subclinically affected cattle (Lepper and Wilks 1988). High iron concentrations also stimulated MAP infection in mice (Lepper et al 1988). An excess of free iron is thought to rapidly catalyse the development of MAP lipids and could also disrupt the activity of phagocytic host cells.

In summary, a single method of classification of mycobacteria has yet to be agreed upon and both culture based and genetic approaches exist. MAP is a small rod shaped bacillus that usually has a thick lipid based cell wall. However some MAP cells may exhibit partial or total loss of the cell wall. Components of the cell wall enhance the invasive ability of MAP. These components include LAM which inhibits macrophage function and fibronection attachment proteins which facilitate MAP uptake by host M cells. Replication by the organism is enhanced in the presence of high levels of iron.

2.1.2. Entry of MAP into the host

Host invasion by MAP is a three stage process. Initially, there is a focal lesion at the site where bacilli enter the intestinal mucosa. The second stage is involvement of the regional lymph nodes. In the third stage there is dissemination of bacilli by lymph- and blood-streams leading to lesions at distant locations in the body such as the liver, kidney and spleen (Payne and Rankin 1961a). The first two stags are of particular interest and described in more detail.

The most important route of infection is via the small intestine following ingestion according to published reviews (Larsen 1972; Julian 1975; Chiodini et al 1984a). Evidence to support this is largely circumstantial and other routes such as the tonsil (Payne and Rankin 1961a) or the lung (Corner et al 2004) have been suggested.

For infection to occur, MAP bacilli passing through the gastrointestinal tract amongst digesta must adhere to the surface of the intestine. Of all MAP organisms that enter the gastrointestinal tract, the proportion that adhere to the intestine is unknown. Intercellular filaments on MAP (Merkal et al 1973) may aid in achieving adherence to the intestinal epithelium. The likelihood of adherence is not uniform throughout the small intestine. It is more likely to occur at the surface of M cells (described in the following paragraph) in Peyers patches because these cells do not possess mucus and microvilli (Featherston 1997), and because adherence by MAP to M cells but not other intestinal epithelium is enhanced by the binding of fibronectin (Secott et al 2004).

M cells are absorptive epithelial cells found in uniform populations shaped as a dome over Peyers patches. Peyers patches are localized collections of lymphoid tissue specific to the gastrointestinal tract (Sell 1987). M cells have a unique capacity for taking up material from the intestinal lumen and transporting it through the epithelial barrier into this lymphoid tissue. M cells also lack digestive enzymes with which to attack MAP (Featherston 1997). This contrasts with other absorptive epithelial cells that take up, but then destroy small particles (Momotani et al 1988; Kato and Owen 1994; Stabel 2000). The immune response to antigen presented within the Peyers patches differs depending on the location of the Peyers patch. In the ileum, a humoral response but not a mucosal response was described following administration of bovine herpesvirus-1 antigen to intestinal loops in lambs. In contrast, antigen presented to the jejunal peyers patches induced a mucosal immune response (Mutwiri et al 1999).

2.1.3. Establishment of infection

In general, antigen released by M cells onto the basolateral side of the intestinal epithelium is subsequently taken up by macrophages in the intestinal mucosa and gut associated lymphoid tissue. To take up antigen, the macrophage first binds to it, then extends a membrane around it, creating a phagosome within the macrophage. The phagosome undergoes a series of events including fusion with lysosomes causing the antigen to be destroyed. MAP is ingested into a phagosome but contrary to other material (Momotani et al 1988; Stabel 2000), MAP persists and replicates. It may escape from the phagosome into the cytoplasm of the macrophage and continue to replicate causing the macrophage to rupture. The mycobacteria released are then engulfed by other macrophages and mycobacterial replication continues within these (Tessema et al 2001). MAP persists by utilising specific uptake pathways into the macrophage and interfering with macrophage processes that would normally destroy phagosomal contents.

The destructive effort applied by a macrophage to the contents of its phagosome varies depending on how the phagosom was created. Macrophages have a range of receptors in their plasma membrane which recognize antibody patterns on the surface of particles. Mycobacteria have several different surface antigens which are recognized by different receptors on the macrophage. Thus the uptake process and intracellular processing experienced by MAP depends on the type of macrophage receptor. By using an uptake pathway that minimizes the destructive effort of the macrophage, for example one that does not result in phagolysosomal fusion, a respiratory burst, or other cytocidal mechanisms, MAP can greatly increase its chance of surviving internalization by a macrophage (Denis 1991).

The known benefits of several uptake pathways were discussed in the review by Tessema et al (2001). Once inside the macrophage, strategies used by mycobacteria to avoid enzymatic and toxic attack are to escape from the phagosome into the cytoplasm (McDonough et al 1993), to

avoid phagosomal maturation and phagosome-lysosome fusion (Frehel and Rastogi 1987), to modify the lysosomal contents (Barrow et al 1995) and utilise passive protection afforded by the cell wall (Orme 1995).

The cytokinetics of infection, adapted from a guinea pig model, were presented by Chiodini (1996). Infection is followed by a lag phase during which little or no replication occurs, hence few MAP organisms are present. At some point the organisms within the lesions enter an exponential growth phase, replicating to create a heavy bacterial burden, perhaps in the order of 10^3 to 10^4 colony forming units (CFU)/g. Prior to this bacterial accumulation, it is considered unlikely that the host would mount a noticeable immune response. A stationary phase, bacteriostasis, follows the growth period as the host's immune mechanisms react. Following this the bacterial burden either declines as the host controls the infection, or a further period of replication ensues, coined the 'progressive phase' in which there would be dissemination of lesions both locally and to more remote locations within the host.

Host susceptibility

Chiodini (1996) contends that in a dairy herd with endemic JD, most cows are likely to be exposed to MAP, but that in general, only a proportion of the herd is infected. This suggests either clearance of infection by the host, or that attempts by MAP to intiate infection are not always successful. Similar observations have been made with tuberculosis; 95% of exposed individuals are successful in eliminating *M. tuberculosis* after infection (Dannenberg 1989; Ellner and Wallis 1989). Additional evidence to support the elimination of infection in a proportion of cases exists in some experimental studies of JD in cattle (Stuart 1965; Stewart et al 2007).

Infection by MAP initially results in a cell mediated immune response, which later wanes as a humoral response becomes stronger. Alternatively, the infection may persist but be contained by the host's immune system, possibly over the entire lifetime of the host (Hagan and Zeissig 1935). The key factors causing the shift from a cell mediated to a humoral immune response are unknown (Chiodini 1996).

The calf is more susceptible to infection than the adult cow and susceptibility is generally believed to wane during the first year and a half of life. The mechanisms responsible for this have not been clarified and conclusive evidence to support the belief is scant. Five of 12 cattle, ranging in age from 18 months to almost 6 years became infected with MAP after experimental inoculation with the scrapings of infected mucosa. Another was suspected of being infected despite failure to detect bacilli at post mortem (Doyle 1953). Larsen et al (1975) reported an experimental infection study of ten cattle: two calves, four nine month old cattle and four adult

cattle. At post mortem examination, five months after inoculation, granulomas or colonies of MAP were detected in all ten. But the severity of infection declined as the age of the group increased. Similar results were reported by Payne and Rankin (1961). They killed a pair of calves and a pair of cows at each of four time points, 2, 3, 4, and 6 months after oral inoculation with 200mg of MAP. A 'primary complex' lesion was evident in all cattle, but viable MAP was recovered from only one adult and at one site. In contrast MAP was recovered from all six calves killed more than two months after infection. The authors concluded that the earlier development of lesions, evident in adults, may be associated with the elimination of the infection, and consequently, resistance to the disease. (Doyle 1953; Payne and Rankin 1961a, 1961b; Larsen et al 1975).

The first eight weeks of life represent a period of great stress to the calf as it struggles to (Davis and Drackley 1998) survive the birth process, achieve homeostasis and adapt to the extra-uterine environment. The following section investigates changes occurring during this period and the effect they may have on susceptibility to infection with MAP (Doyle 1953; Rankin 1958; Payne and Rankin 1961a).

2.1.4.Immunologic development of the neonatal calf alimentary tract

Intestinal mucosal tissues of newborn animals are practically devoid of immunocytes (Porter et al 1974; Wyatt et al 1996). Following emergence from the sterile environment of the uterus, the intestine of the neonate experiences massive antigenic challenge following rapid microbial colonization (Porter et al 1984). In the absence of research specific to calves, it is worthwhile considering some aspects of the immunologic development in the lamb.

In the lamb, the method of digestion while adapting from parenteral to enteral nutrition is quite different from that during normal nutrition. During this period and until the transfer of maternal antibodies to the small intestine stops, digestion takes place within the cell, particularly vacuolated foetal enterocytes, rather than within the lumen of the small intestine (Blum et al 2002). Vacuolated foetal enterocytes, unlike the adult enterocyte, digest material intracellularly, as well as transporting intact proteins from the lumen across the epithelium to the basolateral surface. Only these cells and M cells overlying the Peyer's patches exhibit this capacity. If a similar pattern of development exists in the calf, vacuolated foetal enterocytcs represent a possible means by which MAP may traverse the epithelial barrier of the neonatal calf which is not present in older animals.

Post natal development of the gut mucosal immune system in the calf, specifically, involves the growth and maturation of intraepithelial lymphocytes (IEL). At birth few ileal mucosal lymphocytes are present in the calf, but by 1.5 weeks of age the intestinal villi are populated with large numbers of lymphocytes and by 3 weeks of age lymphocyte numbers are greater yet

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(Wyatt et al 1996). The immunologic capability of the ileum in the calf continues to increase throughout the first six months of life. Cells commonly associated with cell mediated immune mechanisms, CD4+ and CD8+ T cells comprised a significantly smaller proportion of IEL in suckling calves (1–3 weeks of age) than in weaned calves (3–6 months of age) (Wyatt et al 1999).

A major function of mucosal immunity is to down-regulate responsiveness to common dietary antigens, a feature known as oral tolerance. Evidence suggests that immunization to antigens is favored when presentation is by macrophages whereas oral tolerance is favored by antigens processed by the intestinal epithelium (Bruce and Elson 1990). The association between oral tolerance and infection of the host by MAP has yet to be explored.

2.1.5. Physical development of the calf alimentary tract around birth

During the immediate postnatal period, the gastrointestinal tract undergoes profound growth and morphological changes, reviewed by Xu (1996). In the early weeks of life of the young calf, digestion and metabolism are in a transition state, during which processes typical of the monogastric change to those of the ruminant (Huber 1969). Physical elements of the intestinal tract of the calf continue to develop for approximately 3–4 months following birth(Davis and Drackley 1998). These changes may play a role in the waning susceptibility of the calf, as it ages, to infection by MAP.

Development of the rumen is the most physically obvious change in the gastrointestinal tract of the calf. At birth the rumen is essentially undeveloped and is smaller and lighter than the omaso-abosmasum (Becker et al 1951). This relationship changes dramatically and the rumen in the adult constitutes about 87% of the total stomach volume (Warner and Flatt 19644). Rumen activity in 6 week old calves can be considered equivalent to that of the adult's rumen (Huber 1969), provided solid foods have been available, hay in particular, and despite the ongoing development of these tissues in the calf (Roy 1984). A key function of the rumen is degradation of lipids by anaerobic fermentation. The effect of this on the viability of ingested MAP is likely to be slight as degradation of lipids of a waxy nature, such as the cell wall of MAP, is minor compared to triglycerides and galactolipids (van Soest 1982). Also, the period spent in the anaerobic conditions of the rumen is unlikely to be long enough to impair the viability of MAP, which is aerobic.

Abomasal conditions and behaviour also change considerably as the calf ages. In new born calves the rate of secretion of hydrochloric acid is low , but doubles in the first 4 weeks of life. Following consumption of milk, the pH in the abomasum rises from a resting level of about two to about six for some 3 hours. In contrast, abomasal pH in the adult fluctuates about two only slightly in response to feeding (Roy 1984). This adaptation in the calf is likely linked to

minimising degradation of maternal antibodies which are later absorbed through the small intestine (Xu 1996).

The less acidic conditions in the calf abomasum, relative to that in cows, may enhance MAP survival. The degree to which this would improve its viability is difficult to estimate. In the case of *E. coli*, it is proposed that abnormally low levels of acid facilitate adherence to the mucosal surface of the small intestine and subsequent infection (Smith 1925). With respect to MAP, very low pH may impair viability, but it facilitates uptke of M cells by improving the binding of fibronectin to MAP (Secott et al 2001).

In the calf, the pancreas doubles in size during the first two weeks of life, and flow rates of digestive enzymes increase about six fold between 4 and 100 days of age (McCormick and Stewart 1967). These changes are unlikely to affect MAP traversing the gastrointestinal tract. Only a very small proportion of the digestive enzymes degrade lipids, such as found on the cell wall of MAP, and an increasing pancreatic flow rate results in a faster reduction in the pH of digesta leaving the abomasum.

While there are some changes in motility of the small intestine associated with maturation of the calf, overall the changes, including those associated with weaning, are minor (Ruckebusch and Bueno 1973).

2.1.6. Colostrum

Colostrum from the dam provides passive immunity to the neonate in some species, for example cattle, pigs, and sheep. Relative to mature milk, colostrum contains very high amounts of immunoglobulins such as IgG1, IgG2 and IgM, as well as hormones and growth factors (Macy et al 1953). The neonatal calf is dependent upon colostral immunoglobulins, which last for several weeks, for both local intestinal and humoral immunity (Logan and Penhale 1971; Saif and Smith 1985). For about 24 hours after birth non-specific absorption of colostrum occurs in the ileum (Roy 1984; Weaver and Walker 1989).

Bacteria in the intestine may be absorbed in the same manner as colostral components when colostrum is given after the establishment of intestinal microflora. MAP may be present in the colostrum of cows with JD (Streeter 1995) and could also be absorbed in this way. This process has been demonstrated with *E. coli*, which when administered in colostrum, could be cultured from the recipients mesenteric lymph nodes 24 hours later (Corley et al 1977).

The colostrum of a heavily infected cow is likely to be more infectious than that of mildly affected cows for two reasons. Firstly, the concentration of MAP in the colostrum is likely to be higher. Secondly, the presence of maternal antibodies for MAP enhance uptake of the organism into Peyers patches (Momotani et al 1988).

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MAP infection via colostrum could, conceivably, be a pivotal means of infection, despite the low concentration of MAP found in colostrum relative to faeces. Contamination of colostrum with MAP could be via faecal material containing MAP on the udder being ingested as the calf suckles, or via haematogenous spread within the infected dam. The relative importance of these two routes is unknown.

In summary, substantial immunological and physical changes occur in the gastrointestinal tract of the calf in the months following birth. Some of these increase the likelihood that MAP, if present, will initiate infection. Following birth, vacuolated foetal enterocytes provide a means by which MAP may be taken up which is not present in adults. Also the number of intraepithclial lymphocytes present steadily increases, offering greater protection against infection. Physical changes occur to the rumen, abomasum and pancreas with post natal development. The change most likely to predispose calves to infection with MAP is the low levels of stomach acidity during consumption of milk and colostrum. Also of probable importance is non-specific absorption associated with the uptake of maternal antibodies in colostrum. Uptake by this pathway is enhanced by presence of maternal antibodies for MAP, which are likely to occur in the colostrum of heavily infected cows. Overall, the activity of vacuolated foetal enetrocytes and the consumption of colostrum are the aspects of calf-hood most likely to promote infection with MAP, aspects that are absent in the adult cow.

Transmission of infection

Two general statements can be made regarding transmission of JD. The first; the calf is most susceptible to infection and its susceptibility wanes substantially in the first year and a half of life. The second; that the organism is most likely to be transmitted from cows clinically affected with JD as excretion rates are usually high and often by multiple routes. A range of possible transmission routes have been identified. However determining the proportion of infections occuring by each of these methods has proven an enduring challenge. The relative importance of each route is likely to be a function of the concentration of MAP and the likelihood of contact.

Ingestion of infectious faecal material by the newborn calf is thought to be the most important route of transmission, as faeces dried on the udder or in the calf's immediate environment may be heavily contaminated with MAP. Most heavily shedding cows excrete in the region of 1×10^4 CFU of MAP per gram of faeces. Some animals may shed as much as 1×10^6 CFU per gram without showing clinical signs of the disease (Whitlock et al 2005b).

Naturally infected calves less than three months of age may excrete MAP in their faeces and may shed heavily before 14 months of age (Bolton et al 2005). This suggests that calf to calf transmission via faeces may be a more important route of transmission than has historically

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been thought. A single shedding calf residing amongst a non-infected group for some months may represent a greater source of infection than a single dam which has a short contact period with only one calf. High rates of infection have been demonstrated when susceptible calves are housed with experimentally infected calves (Stuart 1965). Faecal shedding by calves 6 months of age was reported by Weber et al (2005) and 5% to 14% of cattle in this study became culture positive before 2 years of age depending on breed and herd size. In high prevalence herds the proportion of animals becoming culture positive increased sharply during calf-hood, and reached a maximum between 9 and 25 months of age (Weber et al 2005).

Transmission of MAP via contaminated milk is also considered important. The organism can be cultured from the milk of about 5–20% of known infected cows (Doyle 1954). Individual excretion rates from six infected cows had a mean of 42 +/-20 CFU/100ml while samples from the tank to which they were contributing contained 12 +/-12 CFU/100ml (Herman et al 2005). While it seems likely that the concentration of MAP in and individual's milk would increase with the severity of disease, data to support this assumption is lacking.

The possibility of transmission of MAP in-utero, first raised by Dunkin (1935), is generally considered of less importance than infections via faeces or milk. Nonetheless, up to 30% of calves from cows shedding large numbers of MAP, or clinically affected with JD, may become infected prior to birth (Lawrence 1956; Doyle 1958; Seitz et al 1989). Subclinically infected cows may also produce infected calves (Sweeney et al 1992). Furthermore, MAP has been cultured from the uterus of subclinically infected cows (Kopecky et al 1967) and the uterine flush fluids of clinically affected cows (Rohde and Shulaw 1990).

The culture of MAP from the genital organs and semen of bulls (Larsen et al 1981; Ayele et al 2004) is probably an artifact of disseminated disease and is unlikely to play an important role in the epidemiology of JD.

Transmission of MAP to young cattle from the environmental directly, or from infected wildlife is discussed in the sections 2.1.6.2 and 2.1.6.3.

In summary, current research indicates that infection of the calf is primarily via faecal-oral contact and by consumption of infectious milk/colostrum. Calf to calf transmission appears to be more important than previously thought, while infections via MAP in the semen, uterine fluids and wildlife are likely to be few. The herd-level control of JD could be refined for greater effectiveness and efficiency if the relative importance of these transmission routes was more clearly understood.

2.1.7.Experimental infection studies

Experimental infection studies offer a method for studying JD under controlled conditions. They are of benefit when studying the pathogenesis of the disease and when comparing diagnostic

tests or vaccines. Their relevance to JD in the field may be called into question because to reliably establish infection, these studies use doses of MAP that are almost certainly much larger than the infective dose typical in the field. However valuable insight into the disease has been provided by these studies.

The oral route has been the most common form of inoculation, either using a single dose (Payne and Rankin 1961a, 1961b; Larsen et al 1974; Larsen et al 1975) or weekly doses repeated for up to 10 weeks (Gilmour et al 1965; Gilmour and Gardiner 1969; Beard et al 2001b; Hines et al 2007), or weekly doses repeated for four weeks and with two further doses at 19 months of age (Embury and Lepper 1984). In these studies the total infective dose varied from 3×10^6 (Sweeney et al 2006a) to 1×10^{10} (Gilmour et al 1965) while 50 grams wet weight (Embury and Lepper 1984), and 200 milligrams of culture (Payne and Rankin 1961a, 1961b; Larsen et al 1974) were also reported.

Intravenous, subcutaneous (Larsen et al 1977) and intratonsillar (Waters et al 2003) routes of inoculation have been tested. Intravenous inoculation resulted in greater dissemination of disease than was evident by subcutaneous or oral inoculation. The intratonsillar route was recommended as an experimental model for future use but should be investigated further as only three calves were used in this study. Curiously, 320 days after infection by this route, MAP was cultured from the duodenum, ileum and jejunum, and from the lymph nodes associated with the jejunum, ileum and colon (Waters et al 2003). More recent experimental infection research supports the hypothesis that the small intestine, rather than the tonsil, is the primary route of entry by MAP (Sweeney et al 2006a), irrespective of the preferred site of initiation of infection.

The most natural method, to date, of exposing the calf to MAP involved housing study calves with previously infected calves for six months. This resulted in the recovery of MAP from the intestines and/or internal organs of 24/29 calves (Stuart 1965). However comparison between these and vaccinated calves was hampered by the unknown infective dose or number of infective exposures.

The pathogenesis of experimental JD in calves and cows has been studied and compared. This research has demonstrated that, in calves, the response to infection varies much between individuals (Larsen et al 1975; Larsen et al 1977; Sweeney et al 2006b). Infection in cows, relative to calves, does not seem to progress despite lesions being more severe and widespread in the initial months following infection. In the third and fourth month after infection when lesions are common in the calf, they are becoming rare and difficult to find in the adult cow (Payne and Rankin 1961a, 1961b). The rapid and severe inflammatory response to MAP in adults, compared to calves, may be the reason that adults are less likely to develop a chronic infection with MAP.

The immune response to infection in the calf has been a key research area explored by experimental infection studies. Lesions are first evident by histology one month after infection (Payne and Rankin 1961a). A humoral immune response is generally detectable about four to six months after infection using fluorescent antibody techniques, and nine to 17 months after infection using the complement fixation test (Gilmour and Gardiner 1969). Antibody response measured with a lipoarabinomannan (a constituent of the cell wall of MAP) enzyme linked immunosorbent assay (LAM-ELISA) commenced three to four months post infection, with highly elevated levels present four to seven months post infection (Waters et al 2003). Persistently elevated proliferation of antibodies was evident six months post infection by flow cytommetry by Koo et al (2004). MAP was first cultured from the faeces of three calves infected via the tonsil at five, five and a half, and nine months, post infection(Waters et al 2003). These findings illustrate that, in the calf, an immune response is slow to develop despite a high challenge dose.

Recently, flow cytommetry has been demonstrated as a consistent and reliable way to monitor the changes in the immune response occurring during disease progression (Koo et al 2004). This technique may supersede the ELISA test in experimental infection studies, but it is unlikely to replace the ELISA test in large scale studies on the grounds of logistics and cost.

In summary, experimental infection studies have contributed to the understanding of JD despite using a heavier challenge dose than is likely to occur in the field. Key characteristics of the pattern of lesion development, faecal shedding and immune response have been demonstrated. Differences in the pathogenesis of disease between calves and cows has also been shown. Variation in the disease process between individuals and the often small number of subjects used in experimental infection studies suggest that the results should be interpreted as indicative rather than definitive. Further refinement of the experimental infection process should be possible in the future, and consequently, a better understanding of the disease in a natural setting.

Genetic aspects of Johne's disease

2.1.8.Heritability of resistance by dairy cows

The heritability of resistance to JD is a tantalizing possibility amongst the methods available for its control. Heritability values range between zero and one, with values near to one indicating strongly heritable traits. In both murine models (Kunze et al 1991) and analysis of cattle data evidence indicates that genetic factors participate in susceptibility or resistance to infection with MAP. In dairy cows the ability to produce antibodies to MAP had a significant heritability of 0.102 using a mixed model and residual maximum likelihood estimation of covariance

components using average information. This result was not correlated with milk yield indicating that it should be possible to breed towards better milk yield and against JD at the same time (Mortensen et al 2004). An identical heritability value of 0.102 was reported, using a threshold model, for having either a positive ELISA or faecal culture test from 4603 Holstein cows from 238 herds by Gonda et al (2006). In a study of 3020 cows for which the infection status for JD was determined at slaughter, the heritability of susceptibility to MAP was 0.06 using a standard polygenic statistical probit model (Koets et al 2000). Based on the studies above, the likelihood of significantly reducing the prevalence of JD via breeding for resistance, is low.

2.1.9. Genetic variation in MAP

Subtle differences in genetic makeup exist between members of any species, for example MAP. Such genetic differences may correspond to differences in physical attributes, such as pathogenicity. The study of genetic variation in MAP has provided epidemiological insight into Johne's disease (Motiwala et al 2003). The epidemiology of other mycobacterial infections has also been studied on a genetic level, such as *M. avium* in humans and pigs (Bono et al 1995), *M. tuberculosis* (van Embden et al 1992; van Soolingen et al 1994) and *M. bovis* (Haddad et al 2004). The most common method for identifying genetic differences within MAP has been by analysis of restriction fragment length polymorphisms (RFLP). In this method, fragments of DNA are cut from a sample of MAP isolates and examined for similarities, differences, and their ability to be allocated into groups. For example, a certain fragment may be characteristic of JD in sheep, while another may be characteristic of JD in cattle (Collins et al 1990). The fragments are cut from the same genomic location on all MAP samples using a restriction enzyme. The particular sequence of DNA used as a starting point for the fragment, for example in MAP, insertion sequences IS*900* (Green et al 1989), or IS*901* (Kunze et al 1991) or the direct repeat (DR) region (Groenen et al 1993), or the poly(GC) rich (PGRS) sequences (Ross et al 1992), and in *M. tuberculosis*, the IS*6110* insertion sequence (Mazurek et al 1991). As yet, there is no clearly established methodological protocol for analyzing genetic differences between MAP isolates, so caution must be excercised when comparing findings between studies. Two alternative means of identifying genetic variation in MAP have been described, both based on polymerase chain reaction (PCR). These are spoligotyping, applied to *M. tuberculosis* (Groenen et al 1993) and analysis of MAP isolates for variable-number tandem-repeats of genetic elements called mycobacterial interspersed repetitive units (MIRU-VNTR) (Thibault et al 2007).

In one of the early genetic studies of MAP, Collins et al (1990) found that the isolates they studied could be allocated into two groups. All strains from cattle fit into one group, defined as the C group, and all sheep strains fit into a second group, defined as the S group. Since then, a

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number of genetic analyses of MAP have been conducted on isolates from a variety of sources including bovine, ovine, caprine, cervid, subhuman primates and humans (Chiodini 1990; de Lisle et al 1993; Pavlik et al 1995; Cousins et al 2000; Machackova-Kopecna et al 2005). In the largest study, DNA from 1008 strains of MAP from 17 countries and bovine, ovine, caprine, and human sources, were digested using *Pst*I and *Bst*EII resulting in 13 and 20 RFLP types respectively (Pavlik et al 1999). The studies of genetic variation in MAP summarized by Whittington et al (2000) show that, almost without exception and regardless of geographic location, isolates from cattle have been of the C type, as have most isolates from goats and deer (de Lisle et al 2003; O'Brien et al 2006). This generalisation was essentially affirmed by Motiwala et al. (2003), who concluded that a high degree of genetic similarity existed between bovine isolates, irrespective of their geographic origin. However, Motiwala et al. (2003) note that a relatively higher degree of genetic heterogeneity exists in MAP isolates from human or ovine sources.

Additional RFLP types may remain to be discovered, as the most effective enzyme for detecting them, *Bam* HI in the study by Cousins et al (2000), was not used in other studies (Collins et al 1990; Whittington et al 2000; Dvorska et al 2004). In general it has been concluded that there is insufficient variation in MAP using RFLP analysis to offer much insight into the epidemiology of JD by this method (Stevenson and Sharp 1997; Cousins et al 2000). Nevertheless, interesting observations based on genetic studies have still been made.

One important observation made with RFLP analysis was that mycobacteria isolated from humans with Crohn's disease were identical to MAP (McFadden 1987). A possible link between MAP and Crohn's disease was first suggested in 1913 (Dalziel 1913). The majority of studies investigating this possibility and published since 2000 have indicated higher detection rates of MAP in Crohn's patients compared with control subjects. Yet the concensus following review by several expert groups in recent years was that the available information remains insufficient to prove, or disprove, the link (Grant, 2005).

A second was that transmission of JD between domestic animals and wildlife may have occurred in the United States of America (Motiwala et al 2003), the Czech Republic (Pavlik et al 2000), and Scotland (Greig et al 1999). A third important observation was that the expression of certain surface proteins varied significantly between a strain of MAP adapted to laboratory conditions and a recently isolated strain. This points to the possibility that virulence may wane as the number of passages since primary isolation increase (Radosevich et al 2007). Hence, in experimental infection studies, only strains of MAP that have recently been isolated from the host should be used.

Other observations relate to the geographic and between species spread of the disease in Australia. Whittington et al (2000) demonstrated that some subtypes of the C strain could be

frequently isolated in some states of Australia, yet were not detected in other states. Furthermore in Victoria another C subtype was commonly isolated from dairy cattle, but not isolated from beef cattle while in New South Wales it was common in both cattle types.

At a finer level of detail, one, two, or three RFLP types of the C strain were identified within individual farms. Within-animal mixed infection is uncommon; there was but one case from 64 animals in which more than one RFLP type was identified (Whittington et al 2000).

Three studies suggest variation in virulence between strains of MAP. In the first study, two strains of MAP isolated from animals, both containing the genetic insertion sequence IS*901*, proliferated more vigorously in mice than two strains lacking IS*901* isolated from AIDS (acquired immuno deficiency syndrome) patients (Kunze et al 1991). While this is not direct evidence that pathogenicity is linked to the presence of IS*901* it shows that the topic is worthy of further investigation. In the second study, pathological differences between lambs experimentally infected with sheep or cattle strains of MAP were demonstrated. Lambs infected with a bovine strain all showed a common lesion pattern, mostly in the mesenteric lymph nodes, while lambs infected with an ovine strain showed more severe lesions which were commonly located in the intestinal lymphoid tissue (Verna et al 2005). In the third study the ability to bind fibronectin varied significantly between two strains of MAP (Secott et al 2001).

In summary, the heritability of resistance to MAP appears to be about 0.1, which is weak. Studies of the genetic variation between strains of MAP have presented the possibility of an association between JD and human Crohn's disease, and transmission of JD between domestic stock and wildlife. They have also provided insight into the distribution of the disease in beef and dairy cattle in Australia, and deer in New Zealand. There is evidence that variation in virulence exists between strains of MAP and this topic is worthy of further investigation.

Prevalence, distribution and risk factors

2.1.10. Prevalence of JD

JD in cattle has been reported on every continent and is thought to be present in most countries. The most comprehensive summary of the distribution of the disease is presented by Kennedy et al (2001). The reported prevalence is at least partially a reflection of the diligence with which veterinarians and animal owners look for and report the disease. As a result, it is difficult to determine the rate of change in the true prevalence. Nevertheless, published estimates of herd-level prevalence provide a useful description of the situation, bearing in mind that they underestimate the true prevalence to varying degrees.

The National Animal Health Monitoring Service study of United States dairy herds, Dairy NAHMS (1996), estimates that 22% of dairy herds and 8% of beef herds were infected at that

time. The prevalence varies considerably between states (Collins et al 1994; Adaska and Anderson 2003; Keller et al 2004; Tiwari et al 2006). JD is also prevalent in Europe, Canada, Australia, and New Zealand (Chiodini et al 1984a; Tiwari et al 2006). In England between 1985 and 1994, 4.9% of 2855 dairy farmers reported seeing clinical cases of the disease in their herd (Cetinkaya et al 1998). A national herd-level prevalence of 18% was reported in all Belgian cattle (Boelaert 2000) and in the Netherlands 55% of 378 herds tested positive (Muskens et al 2000). In seven provinces in Canada the percentage of infected dairy herds ranged from 37 to 74 (Tiwari et al 2006). A comparatively high herd-level prevalence (97.5%) was reported in Brazil in a small sample of 36 dairy herds where 44.6% of the 1316 cows tested positive (Hines et al 2007).

The average incidence of clinical cases within infected herds is generally low. It was estimated at 1.6 per 100 cow years in 1993 and 2.3 per 100 cow years in 1994 in England (Cetinkaya et al 1998), or a cumulative incidence of 1.9% of the herd in 1991 (Cetinkaya et al 1994). In support of this estimate, the sero-prevalence of JD was only 1.78% of over 580 000 cattle from 542 herds in Victoria, Australia by ELISA (Jubb and Galvin 2004).

In the United States of America, of the 748 respondents to a JD questionnaire who had observed cows with clinical signs of the disease in 2002, the majority (74%) reported that fewer than 5% of their herd showed clinical signs of the disease. Seventeen percent reported 5–9.9%, 8% reported 10–14.9% and the remaining 0.7% reported 15% or more of their herd showing clinical signs of JD (USDA 2005b). It is unfortunate that these results were not presented in a form that could be compared with other studies, for example, cases per 100 cow years.

Little is known of the frequency of clinical cases in less developed countries.

In summary, the herd-level prevalence is difficult to determine with accuracry and precision, and appears to vary widcly between the populations studied. Within the majority of infected herds the annual incidence of clinical cases is less than 5% of the herd size.

2.1.11. MAP in the environment

Organisms of the *Mycobacterium avium* complex are ubiquitous and readily recoverable from natural water and drinking water systems worldwide (Du Moulin et al 1988; Aronson et al 1999; Le Dantec et al 2002). MAP specifically, has been cultured from water treatment plants in Northern Ireland (Whan et al 2005) and the river Taff in South Wales, UK (Judge et al 2005).

A range of mycobacteria can be recovered from the environment on dairy farms (Robbe-Austerman et al 2005). The level of contamination varies substantially between locations. Within farm, the locations most likely to be positive will depend on the type of farming enterprise. Contamination in barn-style production systems characteristic of some parts of the US and Europe is more likely when the feed or utensils and machinery used for feeding and

cleaning are contaminated with the faeces of adult cows (Chiodini 1996). Infection via environmental contamination has not been studied in the New Zealand dairy production system. Of 80 Minnesota dairy farms known to be infected with MAP, 77% had culture positive cow alleyways, 68% had positive manure storage facilities, 21% had positive calving areas and 18% had positive sick cow pens (Raizman et al 2004). In contrast, an earlier study found positive cultures were most likely from pasture and exercise lots while cow barns were seldom positive (Whitlock et al 1992). No similar research has been performed on New Zealand dairy farms which keep stock outdoor year-round and use a pasture based feeding regime.

Research on MAP summarised by Whittington et al (2004) demonstrated its prolonged environmental viability under favourable conditions. Of the field conditions studied, survival was longest in tap water stored in the dark at neutral pH (72 weeks). When considering the general trends, MAP survival ranged from 20–45 weeks in water, from 30–40 weeks in bovine faeces spiked with MAP, up to 38 weeks in slurry, and for about 15 weeks in naturally infected faeces. Survival was shorter when exposed to ammonia (1–4 weeks) or silage with low pH or high ammonia levels (2 weeks).

Experiments performed by Whittington et al (2004) using the ovine strain of MAP indicated that the organism survives longest in the shade, possibly due to protection against solar radiation and/or changes in temperature. Interestingly, MAP was recovered from grass that had germinated through infected faecal material in the shade after 24 weeks. The patterns of recovery and change in viable counts indicate that MAP may enter a type of dormancy. MAP was not recovered between 18 and 24 weeks in any experiment but was recovered both before and after this period. A genetic element involved in dormancy responses in other mycobacteria is present in MAP (Whittington et al 2004).

Research in sheep has shown that nematode parasites may act as mechanical vectors for MAP between the environment and the host. The external surfaces of nematode larvae developing from ova within faeces may become contaminated with MAP if the faeces are heavily contaminated with MAP (Whittington et al 2001; Whitlock et al 2005b). The larvae are mobile and highly adapted to maintaining a helminth infection cycle and in this way may aid transmission of the bacterium. MAP was cultured from the third stage trichostrongylid nematode larvae and the washing water used to prepare larvae. The infectious third stage larvae are negatively geotropic and positively phototropic, which results in their migration in a water film up blades of grass and other matter (Soulsby 1968) increasing the likelihood of consumption by a grazing animal.

Environmental factors known to favor the survival of populations of infectious third stage larvae on pasture are akin to those that favor the environmental survival of MAP, for example

protection from solar radiation, protection from heat and availability of moisture (Soulsby 1968).

The nematode larvae may actively facilitate infection by MAP as they are able to penetrate the gastrointestinal mucosa (Anderson 1992). For example, third stage larvae of *Nematospiroides dubius* raised in faeces containing *Salmonella typhimurium* became contaminated with the bacteria and were able to transmit the infection to mice. Furthermore, one thousand fold lower doses of the bacterium were required to establish infection when they were present within the larvae, suggesting that the larvae facilitated infection (Bottjer et al 1978).

In summary, MAP can probably survive in the dairy farm environment for several months, possibly longer under favourable conditions. It is most likely to be found in areas of the farm with large amounts of faecal material. Where present in faeces on pasture, its ability to infect new hosts may be enhanced through fortuitous adherence to nematode larvae.

2.1.12. The wildlife/domestic animal interface

JD was first identified in wildlife in the 1970s in the United States of America (Riemann et al 1979; Williams et al 1979). The first reports of infected wildlife, axis and fallow deer, were thought to be linked to local domestic cattle or the spraying of their faeces on pasture (Riemann et al 1979). Since then MAP has been cultured from wildlife in Europe (Pavlik et al 2000), Scotland (Greig et al 1997) and New Zealand (Glossop, pers. comm.) The epidemiological significance of infected wildlife remains unclear.

MAP has been cultured from a range of wild ruminants such as red, roe and fallow deer (*Cervus* spp.), muoflon (*Ovis musimon*) (Pavlik et al 2000), elk (*Cervus elephus*) and big horn sheep (*Ovis canadensis*) (Manning et al 2003). Three and a half percent of 718 wild ruminants sampled from throughout most of the Czech republic in 1997–1998 were infected (Pavlik et al 2000).

It has also been cultured from rabbits (*Oryctolagus caniculus*) (Greig et al 1997), foxes (*Vulpes vulpes*), stoats (*Mustela erminea*), weasels (*Mustela nivalis*) and badgers, (*Meles meles*) (Beard et al 2001a) cats (*Felis catus*), the raccoon (*Procyon lotor*), opossum (*Didelphis virginiana*) and armadillo (*Dasypus novemcinctus*), and free-ranging birds (Corn et al 2005). Sixteen of the 39 culture positive birds and small mammals described by Corn et al (2005), had tissue samples that cultured positive for MAP indicating infection, rather than simply passage of the organism through the gastrointestinal tract.

Genetic studies provide evidence to support transmission of MAP between domestic animals and wildlife (Pavlik et al 2000; Motiwala et al 2003). However, the frequency and direction of transmission by this route has yet to be clarified.

There are two aspects of MAP in wildlife of particular interest; The first is the rabbit, the second concerns a species of fly, *Eristalis tenax*.

The possible link between JD in rabbits and in cattle has been the subject of several studies in Scotland. A pilot study cultured MAP from 70% of 33 rabbits sampled from four farms in the Tayside region. JD had been diagnosed in cattle on three of these farms (Greig et al 1997). In a subsequent study rabbit infection levels ranged from 0% (0/6) to 53% (8/15) across five farms while no infected rabbits were detected on a further 14 farms. There was a statistically significant relationship between a past or current problem with JD in cattle and infection in the wild rabbit population. Genetic typing could not discriminate between rabbit and cattle isolates (Greig et al 1999) suggesting that a single strain may have been responsible for infection in both hosts. It was then shown that cattle did not demonstrate behavioural avoidance of rabbit faecal pellets when grazing (Judge et al 2005), and that calves could be infected with MAP isolated from rabbits (Beard et al 2001b). Beard et al (2001b) concluded that there was sufficient evidence for wildlife, ie rabbits, to be considered when formulating control plans for JD. This differs from the conclusion by Corn et al (2005), that given the volume of contaminated manure produced by infected domestic livestock, contamination of the environment by wildlife is probably negligible, but that wildlife may have epidemiological significance if transmitting MAP between nearby properties. A link between MAP infection in wildlife and domestic stock remains to be conclusively demonstrated. It should be noted that in the case of *M. bovis*, wildlife reservoirs of infection greatly complicate the control of bovine tuberculosis in New Zealand and in the United Kingdom (Morris et al 1994).

The second aspect of interest relates to the isolation of MAP from the larvae of the syrphid fly, *Eristalis tenax*, which is found in and around dung storage pits on farms in the Czech Republic at various developmental stages in its lifecycle. On 2/7 farms known to be infected with MAP the mycobacteria was recovered from the intestinal tract and internal organs of 24.4% of 368 *Eristalis tenax* larvae examined, but not from the pupae or adults. Fifty or more CFU were detected in 5.4% of the positive pupae (Fischer et al 2005). This finding raises three interesting possibilities. The first is that predation on these larvae may result in infection. The second is that MAP could be disseminated in the environment via animals predating on the larvae and defecating at other locations. If birds were to consume MAP infected larvae, it is possible that MAP could be distributed over large distances. The third is that other organisms that inhabit faecal material could also be contaminated with MAP, for example nematode larvae (Whittington et al 2001). These possibilities represent topics for future research.

In summary, MAP has been cultured from a wide range of wildlife, both ruminant and non-ruminant. In most cases these positive cultures are indicative of infection. A conclusive link between JD infection in wildlife and domestic stock has yet to be conclusively demonstrated.

However, wildlife may contribute to the geographic spread of MAP and could possibly represent a self sustaining reservoir of infection.

2.1.13. *Risk factor studies of John's disease on dairy farms*

The strength of risk factor studies as a means of investigating JD is that they typically involve many herds and are less influenced by between cow variation in the effect of the disease. The greatest difficulty in risk factor studies of JD is correct classification of herd-level infection status due to the long latent period of infection in individual cows. Although an association between the disease and a specific management practice is not indicative of causation, it may still provide valuable insight into the epidemiology of the disease, particularly if the association is consistently identified in several studies.

To date, eleven published studies have identified associations between JD and aspects of dairy farm management. Three of these included fewer than 50 herds, five studied between 86 and 315 herds while the studies by Wells and Wagner (2000), van Weering et al (2005), and Cetinkaya et al (1997) involved 1004, 1023 and 1450 herds respectively. The studies cover the United States of America, England, Norway, Denmark, The Netherlands and Scotland.

Three risk factors were identified in multiple studies. The most consistent of these was that large herds within the study population were more likely to have a positive association with JD (Jakobsen et al 2000; Wells and Wagner 2000; Daniels et al 2002; Koren et al 2005; van Weering et al 2005). It is consistent with the presence of an excreting cow being more likely in a large herd than in small herd, and the exposure of a greater number of susceptible individuals to an infected individual in a large herd.

Also a risk factor in multiple stuides was the use of Jersey cows, which were more strongly associated with JD than other breeds (Cetinkaya et al 1997; Jakobsen et al 2000). The link between the Jersey breed and JD has been suspected for over 50 years (Ringdal 1964) but its cause has yet to be clarified.

The third risk factor present in multiple studies was importing a high proportion of stock on to the farm, which was positively associated with JD in two studies (Wells and Wagner 2000; Tiwari 2006).

The importance of good hygiene during the neonatal period was demonstrated by a significant positive association between JD and group housing of neonatal cows (Wells and Wagner 2000) or pre-weaned calves (Tiwari 2006), having more than one cow in the maternity pen (Tiwari 2006), and the exposure of young calves to adult faeces (Obasanjo et al 1997). It was also demonstrated by a significant protective effect of cleaning of maternity pens (Johnson-Ifearulundu and Kaneene 1998) and calving in individual pens (Cetinkaya et al 1997).

Significance in multiple studies is strong evidence of a true association between these factors and JD, given the large differences in production systems studied, type of information collected and method of defining case and control herds. The methods by which the above studies definited case and control herds warrant further discussion.

The challenges of case definition and external validity are of particular importance in risk factor studies of JD. The most rigorous approach to case definition was that of Obasanjo et al (1997) who classified herds based on whole herd faecal culture. Unfortunately only herds enrolled in a JD control program were included, thus the study population was not representative of all infected herds. In contrast, Cetinkaya et al (1997) studied a random selection of English farms, but defined case herds as those in which cull cows with clinical signs of JD had been observed by the farmer, thus increasing the chance of false-negative herds in the control group. Random sampling approaches were also employed by Collins et al (1994) and by Johnson-Ifearulundu and Kaneene, (1998).

Efforts to avoid misclassification of negative herds are clearly evident in three studies. Johnson-Ifearulundu and Kaneene (1998) defined case herds as those having two or more cows reacting to the ELISA test. Herds with only one reactor were omitted from the study. The studies by Fredriksen et al (2004) and Wells and Wagner (2000) also took measures to reduce misclassification of negative herds. The former considered case herds as those with five or more reactors to the ELISA test or a single reactor if it had a very high titre. The latter considered herds with a single positive ELISA reactor as controls unless 5% or more of that herd's cull cows had shown signs of clinical JD. Misclassification of positive herds is more likely to occur, given the high specificity and low sensitivity of available diagnostic tests.

Farmer recall has been employed to support case definition (Wells and Wagner 2000; Naugle et al 2004) and to describe the frequency of JD (Cetinkaya et al 1994). This provides a low cost and logistically simple means of allocating infection status relative to testing a large number of herds. However, it subjects the study to variation in the record keeping ability between farmers, which is likely to be substantial, and introduces recall bias into the analysis. It would be interesting to compare the sensitivity of farmer recall relative to the serum ELISA for detection of JD. In the continued absence of a quick, highly sensitive, low cost ante-mortem test for JD case definition in risk factor studies will continue to be a challenge.

In summary, large herd size, the Jersey breed, and importing a high proportion of stock onto the farm have each been positively associated with JD in more than one study. Poor hygiene associated with calving has been linked to the disease by different variables in several studies. Attaining a high degree of internal and external validity in risk factor studies is challenging in the absence of a highly sensitive yet still specific diagnostic test.

Productivity losses attributable to Johne's disease

JD is a cause of productivity loss in dairy herds primarily due to reduced milk production and elevated culling rate in the infected population, but also because clinically affected cull cows have low slaughter value (Benedictus et al 1987). Links between JD and both poor fertility (Kopecky et al 1967) and mastitis (McNab et al 1991) have also been proposed.

Published estimates of productivity losses due to JD are from developed dairy industries only. Losses in less heavily developed countries may be even greater than available estimates as the prevalence may be higher (Gomes et al 2005), even if per cow productivity is lower.

Milk production is the net effect of a complex interaction of many factors, some operating at the herd level and some at the cow level. The reported effect of JD on milk production varies between studies due to variation in both study design and analysis, and in the production systems under consideration.

The general trend is well illustrated in the study by Benedictus et al (1987 who reported that the most significant effect of JD on milk production was in cows nearing their removal date, probably because disease in this group was comparatively advanced. Infected cull cows had a 16% decrease in production in their final lactation compared with their lactation 2 years previously, and a 6% decrease in milk production compared with the lactation immediately prior to their final lactation. No effect of JD was detected in infected cows during lactations prior to the two lactations before their removal. These results came from 18 Dutch farms, of which 11 were participating in a JD eradication program (Benedictus et al 1987).

When faecal shedding rather than parity was used as a proxy for severity of disease light, moderate, and heavy shedders produced 537kg, 1403kg and 1534kg of milk less than test negative herd mates in the two herds studied by Raizman et al (2007).

In contrast to the study by Benedictus et al (1987), both Gonda et al (2007) and van Leeuwen et al (2002) reported milk production loss associated with JD to be greatest in parity one cows. The loss remained significant in parity two cows but was insignificant in parity three, in the study by Gonda et al (2007. van Leeuwen et al (2002) reported a significant reduction in milk production due to JD in parity one cows, but a significant increase associated with JD infection in parity three cows. Small numbers of JD positive cows may have been responsible for the differences reported in van Leeuwen's study.

In the largest study conducted to date (974 dairy herds in the United States of America) JD positive herds produced 288kg of milk (4%, 7517kg vs 7803kg) less per cow than JD negative herds (Ott et al 1999). This result can be considered a robust estimate of JD induced milk loss, because it is supported by three other studies. A recent study of 232 DHIA herds throughout the United States of America reported a milk yield reduction due to JD of 304kg (Gonda et al 2007)

while an earlier study of 23 dairy herds in the United States of America (Nordlund et al 1996) and a third study of nine Canadian dairy herds (Hendrick et al 2005) reported reductions in milk production of 4% and 2%–6% (depending on the method of diagnosis), respectively.

The effect of JD on milk production varies between herds. Milk production was significantly reduced in only two of the 23 herds studied by Nordlund et al (1996). This variation may have biased results from small studies, especially those using herds enrolled in JD control programs, which are likely to have a higher prevalence. It represents an important epidemiological facet of JD and indicates that an 'overall' effect on productivity, calculated using a large number of herds, may fail to illustrate important subtleties in the effect of JD on milk production. There are many possible causes of this variation, for example herd-level or management related factors, cow-level genetic factors and, possibly, differences in virulence between strains of MAP. Herein lie opportunities for future research.

The effect of JD on replacement rate is less clear and published estimates are few. The disease was not associated with a significant increase in the number of replacement cows purchased nor the number of cows slaughtered in the study of 974 farms conducted by Ott et al (1999). However, cull cows of poor condition were 5.7 times more common from infected farms even though cows culled for the disease were of a similar age to those culled for other reasons (Whitlock et al 1985).

Evidence in support of a higher replacement rate in JD infected cows is present in two studies. In the study by Tiwari et al (2002) the odds of being culled during the 3 years after testing were 2.3 times greater in seropositive cows than in seronegative cows, while the hazard ratio of removal for faecal culture positive cows was 3.2 across nine high prevalence herds studied by Hendrick et al (2005). Yet the culling rate in a single Irish dairy herd experiencing heavy milk production losses due to JD did not differ from a local group of 25–30 herds until it implemented a control program for the disease (Barrett et al 2006).

A recent study reporting a significant decrease in life expectancy of 2.85 months in infected cows (Gonda et al 2007) should be interpreted with caution. Two reasons are likely to have contributed to the highly significant statistical difference. Firstly, the productive life of 47% of the cows studied was predicted using a regression equation. This may have reduced the between-cow variation to an unrealistic degree, such that the difference of 3 months appeared a significant deviation from the norm. Secondly, farmers were informed of the infection status of their cows, which may have resulted in infected cows being culled earlier than normal.

JD may reduce the life span of infected cows without increasing the herd-level replacement rate. The proportion of cows replaced annually is relatively fixed, while the reasons for removal are not. If a farmer involuntarily culls several JD cows during a season, their voluntary culling activity will be reduced to conserve the proportion of the herd replaced. In this way JD cows

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may not inflate the proportion of the herd replaced, but will still slow the rate at which the farmer can develop the herd through voluntary culling. This situation may explain the results of Barrett et al (2006), and why Ott et al (1999) reported a similar replacement rate in JD positive and negative herds, but Tiwari et al (2002) and Hendrick et al (2005) both report a higher risk of removal for infected cows.

The lost future income from culled JD cows may still be considerable, even though the herd-level culling rate is not elevated. Benedictus et al (1987) calculated the total financial loss associated with each clinical JD cow, including lost production and future income, reduced slaughter value, the cost of treatment and idle production factors. Lost future income represented the greatest proportion of this total loss (43%). Alternatively, cows infected with JD may be culled for unrelated reasons prior to showing clinical signs and in such cases it could be argued that the disease was of little financial impact.

JD was associated with mastitis in a 304 herd study in Canada at both the herd and individual animal level (Merkal et al 1975; McNab et al 1991). However no association was found between JD status and somatic cell count in two recent studies, one of which involved 232 dairy herds in the United States of America (Hendrick et al 2005; Gonda et al 2007). The proposed explanation for the JD-mastitis link was that JD weakened the immune system, making the host more susceptible to other infections. Evidence in its support remains inconclusive, and elevated rates of other disorders, which would be consistent with a generally weakened immune system, have not been reported in infected cows. One possible exception is infertility.

The link between JD and infertility has been made in two single herd studies (Kopecky et al 1967; Merkal et al 1975) but, overall, support for it is mixed. A recent study of 232 herds found a significantly higher pregnancy rate in JD positive cows (Gonda et al 2007) and infertility was not thought to be associated with the disease in the six New Zealand herds studied by de Lisle and Milestone (1989).

The potential for bias when studying the effects of JD on dairy cow productivity is great. In particular, bias may be introduced via definition of the eligible population, for example cull cows or entire herds. It may also be introduced during selection of the study population, and by selecting only a small number of study units. In addition, statistical approaches vary from analysis of variance (Buergelt and Duncan 1978) to comparatively complex mixed effects regression models (Gonda et al 2007) while in some cases the method was incompletely described (de Lisle 1989). Low diagnostic test sensitivity may bias the reported negative effect of JD. At the herd level, the effect of JD will be spuriously small if infection reduces production in false negatives.

In summary the reduction in milk production attributable to JD is most pronounced in cows approaching or experiencing clinical disease and is generally minor in sub-clinically affected

cows. Infected cows are at greater risk of being removed from the herd. This fact may not be evident in the herd-level culling rate due to the mix of voluntary and involuntary culling. The effect of JD on productivity varies between farms, being minor on most farms, but highly significant on a few.

2.1.14. Estimated financial losses attributable to Johne's disease

The benchmark figure for financial loss due to JD was published by Ott et al in 1999. It was derived from a regression model. The dependent variable in this model was the annual adjusted value of production, defined as the value of milk production plus the value of calves at birth minus the net replacement cost. Besides JD status, ten variables were included in the model representing herd size, geographic region of the farm, breed, and some management factors. The analysis used 974 herds for which the average herd size was 104 cows. The within herd prevalence of JD was unknown. The annual adjusted value of production relative to JD negative farms was estimated to be USD 97 (4.8%) less for every cow in the herd on JD positive farms after controlling for the effects of the other variables. The major component of this was reduced milk production (-288Kg of milk/lactation), corresponding to a loss of USD 83. This was extrapolated to a loss at the national level of USD 200–250 million annually (Ott et al 1999).

Losinger (2005) argues that additional revenue of this amount would not result from eradication of JD because the demand for milk is comparatively inelastic, thus the additional milk would cause over supply and consequent drop in price. In the current economic environment, global demand for dairy products is high and rising (Ma and Rae 2003), and it seems unlikely that additional production resulting from the control of JD would significantly devalue these commodities.

There is a surprising consistency between studies in cow-level estimates of financial loss due to JD. Ott et al (1999) discusses how estimates in their study and five other studies (Buergelt and Duncan 1978; Whitlock et al 1985; Chiodini and van Kruiningen 1986; Benedictus et al 1987; Abbas et al 1993; Meyer and Hall 1994), were between USD 20–27 per cow across all cows studied, provided the price of milk was held constant. Similarly, in New Zealand financial loss due to reduced milk production caused by JD was calculated to be NZD 26 per cow and NZD 42 per cow in two seasons on the worst affected of six farms studied, based on a milk production index devised by the authors (de Lisle 1989). On the least affected farm losses were thought to be about NZD 4 per cow. That is, for all cows in the herd, not just those infected with MAP. It is remarkable that given the limitations of, and differences between, the various studies, all come to such a consistent conclusion.

A comparatively minor national scale financial loss due to JD in the United Kingdom was estimated, £3 to £4 million, depending on whether the average scenario or worst-case scenario

regarding JD was considered (Bennett et al 1999). These estimates were derived from a simple spreadsheet model which may represent a weakness and explain the difference in estimates between this and other studies. Nonetheless, the estimates clearly suggest that JD in the average infected herd has less financial impact in the United Kingdom than in the United States of America. JD was ranked eleventh most important of the thirteen diseases of cattle considered by those authors.

Simulation of Johne's disease in dairy herds

To study JD from an epidemiological perspective is to be confronted with numerous unresolved issues. Uncertainty regarding the true infection status of individual cows and herds greatly hampers the understanding of prevalence and effects of the disease on productivity. If important epidemiological aspects of the disease are not known with certainty, then the question of how much effort ought be dedicated to the control of the disease is difficult to answer. One means of addressing this is to create a computer simulation model which incorporates uncertainty within its components. By exploring the behaviour of the model in response to changes in the input parameters, or changes in the uncertainty surrounding them, valuable insights may be obtained on where the greatest weaknesses exist in the understanding of the disease. By comparison, research in the field to address these weaknesses is difficult, expensive, time consuming and may fail to provide better information that what was known beforehand.

The first epidemiologic and economic simulation model of JD was developed by Walker in 1988 for Wisconsin, United States of America, dairy herds (Walker et al 1988). The strengths of this model were its novelty, stochastic nature and the many economic parameters considered. Weaknesses were its use of a single closed herd of 80 cows and fixed transmission probabilities. It lacked flexibility for adjusting replacement rates, herd size, and hygiene level. Furthermore, it was written in a specialized computer simulation language with which epidemiologists of the time struggled.

A more refined probabilistic model published by Collins and Morgan (1991) was able to simulate the impact of diagnostic testing and to simulate open herds. Here, a modified version of the Reed-Frost model (Abbey 1952) was used to obtain disease transmission characteristics. The unit of output was prevalence rather than the more limited financial values of Walker's model. The effective contact rate encompassed many aspects of disease transmission including host, pathogen, and environmental factors.

This model indicated that in the absence of control, the within-herd prevalence would increase to about 45% over a 50 year period, regardless of herd size (range 25 to 150 cows). The rate of increase was most strongly affected by changes in the cow-calf effective contact rate and by the number of cows purchased annually. The authors considered the most striking outcome of the

study to be the increase to a plateau of about 50% prevalence leading them to conclude that JD may be an epidemic in dairy cattle and could become a greater scourge for the dairy industry than was tuberculosis or brucellosis.

This model was extended to simulate control, either by test-and-cull methods, or by improvement of herd hygiene. Both of these control methods reduced the prevalence of JD from 10% to about 2% after 30 years when applied to an open herd. Prevalence declined to 2% after 20 years if the two methods were combined. The test sensitivity was assumed to be 70% and model output was sensitive to changes in this parameter. The authors noted that control of JD appeared more difficult in open herds, than in closed herds.

The model was then validated against a farm that was using both testing and improved herd hygiene to control JD. To best fit the real data, the simulated effective contact rate between susceptible individuals and the infective organism was 2.1 prior to attempting control and 0.8 in the presence of control (Collins and Morgan 1992).

Based on the above model these authors then published an economic model evaluating a test and cull program for JD in dairy herds in the United States of America. They concluded that, given the characteristics of diagnostic tests available for JD at the time (Se=0.50, Sp>0.98) and that JD caused at least a 6% reduction in milk production, a test and cull program should be profitable if the within-herd prevalence was greater than 5%. These results were comparatively insensitive to the cost of testing. It was noted that the financial benefit of a test-and-cull program would be greatest when within-herd prevalence is high and would become negative before within-herd prevalence reaches zero (Collins and Morgan 1991b). This last point indicates that a test-and-cull program should only be applied with support from additional control measures that restrain the effective contact rate below one when prevalence is low.

Ten years after this, a stochastic, dynamic simulation model 'JohneSSim' was developed to evaluate the effect on prevalence and associated economic loss of JD control programs at the herd-level (Groenendaal et al 2001, 2002a, 2003). The model was developed to assist in the creation of a national JD control program for The Netherlands. It was more complex than earlier efforts; in particular it incorporated six infection routes and simulated a population of herds, within which there were some key differences in management with respect to JD. Furthermore it was able to simulate changes in herd hygiene, diagnostic testing strategies and replacement rates. The simulated period was 20 years. The general behavior of the prevalence was similar to that in the model of Collins and Morgan (1991) in that, in the absence of control, within-herd prevalence increased to about 50%, but after 20 years rather than 40. In contrast to the earlier model, no test-and-cull program simulated by JohneSSim was economically attractive. The most outstanding result of the JohneSSim studies was that improvement of herd hygiene led to a greatly reduced prevalence, in stark contrast to test-and-cull programs. A similar theme was

evident in the model of Collins and Morgan (1991) when they varied the effective contact rate between infected cows and susceptible calves. These results suggest that the most effective means of JD control was improvement of the hygiene associated with calf management.

The JohneSSim model was subsequently adapted to represent the dairy production system in Pennsylvania, United States of America, to evaluate the Voluntary JD Herd Status Program (Groenendaal et al 2003). Very similar results were obtained to those from the use of JohneSSim in The Netherlands. Following this it was adapted to the New Zealand dairy production system, the results of which follow later in this thesis.

Recently another model has been described, PTB-Simherd (Kudahl et al 2007). It is a stochastic, dynamic and mechanistic simulation model for a single dairy herd covering a period of 10 years. Based on a Danish dairy herd simulation model 'SimherdIII', it reflects the complex feedback mechanisms between replacement, culling and feeding (Ostergaard et al 2000).

The aim of PTB-Simherd was to include further complexity than earlier models in an attempt to account for the effects of JD on indirect losses arising from an increased replacement rate such as more young cows in the herd which produce less milk, less feed consumed, and fewer age related diseases. Again the results were broadly similar to that of Collins and Morgan (1992) whereby a steady increase in prevalence was observed in the absence of control. The rate of increase and endpoint was greater in the PTB-Simherd model than in previously published models; it simulated a 90% within-herd infection rate after only 10 years. JD has been recognized as a problem for decades (Dunkin 1934) and active control has likely been minimal on most infected farms, but within-herd infection rates as high as 90% have not been reported. Validation against Danish dairy herds may help to further refine this model.

In summary, several broad trends are evident in JD simulation modeling studies. First, a steady increase in within-herd prevalence is predicted to reach about 50% in the absence of control. Secondly, test-and-cull based forms of control do not appear economically attractive, even in the face of increasing prevalence. Thirdly, improvement of the hygiene associated with calf managment may greatly reduce the prevalence. All the models were heavily influenced by the effective contact rate between infected cows and calves. The fact that this parameter is still known with such little accuracy, even in the United States of America where a large body of research on JD exists, is testament to the challenge of studying this disease. Clearly accurate information describing the importance of the various routes of infection would be of great value.

Farm level control of Johne's disease

The currently favoured approach to the farm-level control of JD is a case-by-case risk assessment (Weber et al 2005), then management changes and educational services to suit. For optimum control, a combination of available methods can be employed subject to cost

effectiveness. These efforts are directed at reducing the chance that calves younger than 6 months of age become infected (Sweeney 1996). The methods can be grouped into four categories: improvement of the hygiene associated with calf management, test-and-cull, replacement with JD-free heifers, and vaccination.

The aim of methods in the first category is to reduce the risk of transmission of MAP to calves, the goal is to reduce the infection reproduction ratio ($R_0 < 1$) to below one. The methods include provision of a clean calving area, removal of calves from their dams as quickly as possible, feeding the calf only colostrum from it's dam, and ensuring that calf raising areas are free from the faeces of adult cows. Faecal material may be from the cows directly, or from contaminated pasture, feed or bedding. OIE recommendations also include the feeding of pasteurised milk or milk replacer (Kennedy and Benedictus 2001).

The aim of the test-and-cull category is to identify and remove cows shedding MAP. The diagnostic test of choice and frequency of application depends on the balance between declining prevalence and increasing cost.

The third category, replacement practices, aims to minimise the risk of introducing JD on to the farm via the purchase of stock. This can be achieved by clarifying the infection status of vendor properties and by ensuring stock test negative for JD prior to purchase. Such biosecurity measures are particularly important for farms free from the disease.

The aim of the fourth category, vaccination, is to reduce the clinical incidence of disease among infected cows. As a result, infected cows are less likely to contaminate the environment and more likely to reach the end of their expected productive lifetime without showing clinical signs of the disease.

JD persists despite key methods for its management being published for over fifty years {Edwards, 1947 #674}. A pivotal reason for this persistence is the lack of compliance by farmers with biosecurity and management requirements (Muskens et al 1999; Wraight et al 2000). For example in Pennsylvania, eight of ten dairy herds enrolled in a three year control program maintained adequate management modifications while two did not (Hutchinson et al 1992). Even within the framework of an organised study, some farmers failed to implement the recommended changes. A study of 1004 dairy farmers from 20 states in the United States of America found that farmers familiar with JD did not manage their farms very differently from those unfamiliar with the disease (Wells and Wagner 2000). The most likely reason for this is that on most farms the disease was not considered of sufficient economic importance to warrant active control. Little motivation by cattle farmers to control diseases of minor economic consequence to them, such as *E. coli* 157 and Campylobacter has been reported (McKenna et al 2006). The OIE guideline for the control of JD notes that a significant gap remains between recommendations for control and their successful implementation on some farms. The guideline

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states that a program must not only be technically sound, but must also be communicated effectively and be affordable and practical (Kennedy and Benedictus 2001).

Adherence to the methods considered best-practice for the control of JD may not result in eradication in the medium to long term. The disease remained present at low levels after use of a test-and-cull program and stringent management practices for 20 years on one farm (Whitlock et al 2005c). The authors of this study suggested that calf to calf transmission might have facilitated this persistence.

In summary the preferred approach to the herd-level control of JD is by risk assessment, and based on this, selection of appropriate control methods. These methods are based on the improvement of hygiene associated with calf management, test-and-cull, purchase of test negative stock for replacements and vaccination. The persistence of JD is exacerbated by poor compliance by farmers with biosecruity and management techniques required for the control of the disease.

2.1.15. Diagnostic tests for Johne's disease

The two key characteristics that define the performance of a diagnostic test are its ability to detect diseased animals correctly (sensitivity) and at the same time give the correct answer if the animal in question is not diseased (specificity) (Gardner et al 2000). The performance of a new test can only be judged by comparison with an existing test. However, currently available tests do not have perfect sensitivity and specificity, thus no perfect definition of disease status (gold standard) is available. Consequently dependence (or correlation) between the new and existing test must be taken into account when evaluating a new test. Analytical methods to address the absence of a gold standard reference test (Hui and Walter 1980) and dependence between two tests (Dendukuri and Joseph 2001; Georgiadis et al 2003) have been developed. Challenges still exist in test evaluation such as contending with variation in test performance between subsets of tested individuals, parity, for instance. A review of literature pertaining to evaluation of diagnostic tests for JD demonstrates the importance of a clear description of the conditions under which a new test is evaluated and the characteristics of the 'gold standard' to which it is being compared.

The control of JD is hampered by the absence of a highly sensitive and highly specific *ante-mortem* diagnostic test for JD. A variable but often protracted period of the infection process occurs below the lower limit of detection of currently available diagnostic tests. Infection is difficult to detect in young animals (McDonald et al 1999). Cows are generally considered to be infected during calf-hood but are most likely to test positive when 2.5–4.5 years old (Nielsen and Ersboll 2006). At least some infected cows are infectious prior to being detectable by anti-body based methods (Milner et al 1987).

Test-and-cull methods reduce the prevalence to a threshold (Ringdal 1964). This threshold is based on the sensitivity of the test being used. Additional control techniques are required to reduce the prevalence further (Gay and Sherman 1992; Collins and Sockett 1993). Nevertheless diagnostic testing is an important method of disease control. The most appropriate form of testing depends on the desired outcome, for example confirmation of the infection status of an individual cow, a test-and-cull program, or showing absence of disease. A range of methods is available for detecting JD, or MAP itself. While none are infallible, this range enables a method applicable to the situation at hand to be selected.

Histopathology remains the most sensitive form of diagnosis and is generally considered the 'gold standard' (perfect definition) of infection status against which other tests are measured. However it is only applicable *post-mortem*. Application of the Ziehl-Nielsen stain to histopathological samples renders acid fast material, such as the cell wall of MAP a bright red colour. Further diagnostic evaluation, such as polymerase chain reaction (PCR) or demonstration of mycobactin dependence, is required to confirm that the acid fast organism identified is indeed MAP. While the presence of cell-wall-deficient MAP in cows with JD has been reported (Hulten et al 2000) this form was amongst cell wall competent MAP and unlikely to cause false negative staining results.

The Johnin intradermal skin test (Broerman and Fogle 1932) was an important means of detecting JD during early efforts to control the disease. It consists of testing the response of a recipient to a small amount of MAP derived antigen placed under the skin. After a set time the injection site is examined. Infected animals usually show a thickening of the skin fold to double the original thickness or more, but since such large swellings are not always present the nature of the swelling should also be noted (Rinjard and Vallee 1936). Subjective sensitivity and cross reactivity with other mycobacterial infections limit the effectiveness of this test method. In the United States of America it was recognised as unsatisfactory as early as the 1930s (Wight 1931).

The enzyme linked immunosorbent assay (ELISA) (Abbas et al 1983; Yokomizo et al 1983; Yokomizo 1986) may be applied to blood serum or milk (Nielsen 2000). This test measures humoral immune reactivity of the host to MAP specific antigen. It was greatly improved by inclusion of preabsorption techniques to remove cross reacting mycobacterial antigens (Yokomizo et al 1985). The key attractions of the ELISA method are the automation of its processing and comparatively low cost, in addition to sensitivity and specificity that are comparable with other methods (Collins and Sockett 1993). Two disadvantage are that it measures a humoral immune response which typically occurs later in the disease process and that it may cross react with antigen from mycobacteria other than MAP (Chiodini et al 1984a).

The ELISA test is reported to detect 20%–60% of faecal culture positive animals and have specificity of at least 99% (Sockett et al 1992; Collins et al 2005). Recently, sensitivity of the ELISA test was greatly improved in a study that tsted host reactivity to antigens present on the surface of MAP. Using this approach, coined the EVELISA, sensitivity was 96.6% in low faecal shedders and 100% in medium and heavy shedders, while by comparison, a commercially available ELISA detected only 13.7% of low shedders, 25% of medium shedders and 96.6% of high shedders (Eda et al 2005; Eda et al 2006).

The agar gel immuno diffusion (AGID) test (Sherman et al 1984) and complement fixation test (CFT) (Chandler 1956; Rankin 1958) are other examples of serum antibody tests that have largely been replaced by ELISA based methods.

Culture of MAP from the faecal or tissue samples is an attractive method of diagnosing JD because it is highly specific and measures levels of the organism directly, rather than changes in the immune behaviour of the host. Disadvantages include its high cost, long confirmation period (about 16 weeks) (Whitlock et al 2000), and reduction of sensitivity due to preparation and decontamination of samples prior to plating. A description of the culture methodology used at present is presented by Payeur (2005).

The specificity of the culture test when applied to faeces may be impaired by the 'transient infection' phenomenon whereby ingestion of MAP by adult cows may result in detection of the organism in faeces, but not represent infection (Sweeney 1992). Where transient infection is suspected, a combination of faecal culture and serum antibody testing may offer a more precise diagnosis. The importance of the pass through phenomenon in the epidemiology of JD is a point of contention at present.

Sensitivity of the faecal culture test was 38% on its first application to 954 cows tested twice annually over 4 years (Whitlock et al 2000). The pooling of individual faecal samples (Sergeant et al 2001) is a practical means of reducing the cost of this test, provided that dilution with non-infected samples does not reduce the concentration of MAP below detectable levels of 100 organisms per gram of faeces (Jorgensen 1982; El-Zaatari et al 1996).

Detection of a repetitive element thought to be specific to the DNA of MAP, IS900 (Collins and deLisle 1986; Green et al 1989) enabled polymerase chain reaction (PCR) to be used as a diagnostic test for JD. Subsequently, IS900-like elements were detected in mycobacteria other than MAP (Cousins et al 1999; Naser et al 1999),(Englund et al 2002). The sensitivity of this technique is conserved by the use of multiple genomic targets to ensure DNA is indeed that of MAP, a process described by Rajeev et al (2005).

The PCR method is faster than culture methods, maintains high specificity, and may be applied to materials not usually tested, for example semen (Herthnek et al 2006). The disadvantage if this technique is that a positive result does not indicate whether the organism was alive, nor if it

was capable of replicating. The PCR method is commonly used on positive culture samples to confirm the grown organism as MAP. The lower limit for detection of MAP by PCR has not been clearly identified. It was thought to be about 1000 CFU/ml in milk (Giese and Ahrens 2000) while Herthnek et al (2006) reported 100 CFU/ml in semen.

The ideal test for JD would detect cell mediated immune activity indicative of MAP infection, for example interferon γ (IFN-γ) (Billman-Jacobe 1992). Non-specific antigen reactivity (McDonald et al 1999; Stewart et al 2007) impairs the specificity of this test. At present the common *ante-mortem* tests are the serum ELISA, or faecal culture, or PCR depending on the application setting.

In summary, a highly sensitive and specific test for new and recent infection with JD remains elusive. However, the commonly used methods of ELISA, culture of the organism and PCR aid in the control and study of this disease. Recent research indicates that substantial improvements of the ELISA test sensitivity may be possible through the use of antigen found on the surface of MAP.

2.1.16. *Vaccines for Johne's disease*

The first vaccine for JD was developed in France in 1926 by Vallee and Rinjard (Vallee et al 1934). The subcutaneous injection of either live or killed MAP induce both cellular and humoral immune responses and reduce the number of animals that develop clinical disease, and the level of MAP excretion (Wilesmith 1982; Merkal 1984; Benedictus et al 1988; Kormendy 1994). Vaccination does not prevent infection, rather it enhances the host's ability to control the infection, as indicated by experimental studies (Larsen et al 1974; Nisbet et al 1982; Juste et al 1994). Vaccination may prevent a proportion of natural infections because infective doses used in the experimental studies were much larger than that likely to occur in the field.

In field trials vaccination of calves reduced the incidence of clinical cases of JD. The 231 closed herds studied by Wilesmith (1982) were free from clinical cases after an average of 4 years during which calves were vaccinated. This result is consistent with increasing herd immunity and declining environmental contamination. Further support for the use of vaccination, this time in combination with other management techniques and diagnostic testing was reported in a study of 500 French herds by Saint-Marc (1992.

Vaccines derived from CWD MAP may enhance rather than protect against infection, depending on the adjuvant. Lesions resulting from vaccination, with a CWD vaccine combined with an alum adjuvant, were more severe and had higher bacterial burden than non-vaccinated, challenged controls. In contrast, when this vaccine was combined with a different adjuvant, (QS21), performance was similar to its cell-wall-competent counterpart (Hines et al 2007). This suggests that the host immune response to CWD MAP may differ from its response to CWC

MAP. Further research into this topic could clarify these differences and provide a deeper understanding of the infection process.

Cattle vaccinated against JD may demonstrate cross reactivity with the tuberculin test for *Mycobacterium bovis* (Munday 1959) hence a positive tuberculin test may indicate infection with *M. bovis*, or vaccination against JD. Consequently the vaccine could only be used with careful planning where testing to control *M. bovis* is carried out, for example New Zealand.

2.1.17. Monensin

Monensin, derived from the actinomycete *Streptomyces cinnamonensis* (Haney and Hoehn 1967), is a broad spectrum antibiotic which is active against several gram-positive bacteria such as *Mycobacterium* spp. It is a polyether ionophore that modifies bacterial cell membrane permeability. Monensin is administered in the diet as a growth promotent in domestic animals (FDA 2007). It is commonly fed to breeding age heifers to promote growth and may also be fed to calves to assist in the prevention of coccidiosis (Fitzgerald and Mansfield 1973).

Monensin in the diet was found to reduce the severity of JD associated lesions in both experimentally infected mice (Brumbaugh et al 1992) and in naturally infected cattle (Brumbaugh et al 2000).

In subsequent research, Monensin reduced tissue colonization by MAP following oral challenge in calves. It also reduced faecal pass-through shedding of MAP. The daily dose of Monensin in this study was greater than would normal be given, as the aim was to prove the concept, rather than evaluate it as a form of control (Whitlock et al 2005a). The following year, in a randomized clinical trial, faecal shedding was marginally but statistically significantly reduced in cows receiving daily Monensin compared with herd and parity matched controls. When the treatments were reversed at the study's mid point, cows that began receiving Monensin showed a non-significant reduction in MAP organisms per cultured slope. There was also a borderline significant reduction in the ELISA test value of the treated group. The authors concluded that their study did not support a definitive effect of Monensin on ELISA or faecal culture test results, or faecal shedding (Hendrick et al 2006b).

Further evidence that there may be some prophylactic effect of treating with Monensin came from a cross sectional study of Canadian dairy herds. When 48 dairy herds with no history of JD were tested for presence of the disease, the use of Monensin was associated with a reduction in the odds of a cow testing positive by milk ELISA of 4.8 (Hendrick et al 2006a). The authors continued to be cautious, concluding that while the association was biologically plausible, it was more likely an indicator of better management.

The cause of the apparent prophylactic effect of Monensin on JD has yet to be clarified. Monensin may act directly by inhibiting growth of the mycobacteria, or it may enhance

phagocytic killing, or both. The drug could, conceivably, play a minor role in reducing the effects of infection with MAP but is unlikely to reduce the rate at which infections occur to any significant degree.

2.1.18. *Lactobacilli*

In vitro work suggests that there may be some inhibitory effect of some lactobacilli on MAP growth due to factors other than acid production. Irrespective of the mechanism of inhibition, the possibility of biotherapeutic application exists (Judge et al 2005).

National level programs to control Johne's disease

JD is perceived as a problem of sufficient importance to justify national or state level control measures in some countries. The rationale for such programs is to control diseases of economic or public health importance or diseases that may impact on international livestock trade. Eleven of 16 countries surveyed, by the International Dairy Federation in 2001, were taking measures to reduce the risk of importing animals with JD. These measures were instigated by the government in most cases and occasionally instigated voluntarily by the cattle industry (Kennedy et al 2001).

In general, programs to control JD aim to reduce the likelihood of infection of non-infected or low risk herds, and provide a pathway for farmers with infected herds to follow toward controlling the disease. From a consumer's perspective, the aim of such programs is to reduce the chance that MAP is present in dairy products.

Successful control of JD at the national level requires technical, legal and managerial capability in addition to clear achievable objectives and a strong, long-term commitment from both the affected animal industries and from the government. The source and coordination of funding must be transparent and agreed upon by all stakeholders to ensure sustained support for the program. In developed countries responsibility for funding of operational aspects is increasingly being transferred to the livestock industries from the government (Kennedy and Benedictus 2001).

At the operational level, two components are essential for a successful program. The first is an open and regular communication between farmers, veterinary practitioners, and other involved parties. The second, a good registration and identification system is required for cattle, herds, and veterinarians (Benedictus et al 2000). Where multiple species are infected with JD, coordination between control efforts in the affected species will be required (de Lisle 2002) to ensure optimum return on time and finances invested.

A brief summary of the history and current situation regarding the control of JD in several countries follows.

2.1.19. Australian National Johne's disease Control Program (NJDCP)

In 1996 the National JD Market Assurance Program for Cattle was launched, one of the first national scale JD control programs. In 1997 the Australian Animal Health Council advised the national cattle industry to fund operational aspects of the program by subsidizing farmer entry and assisting control programs in infected herds. The dairy industry was concerned about the high, long-term financial commitment and the general uncertainty about economic benefits. Consequently the proposal did not proceed and the government remained the principle funding body. Uptake of the program was very slow in areas where the disease was more common as farm owners did not want to reveal that their stock was infected. Uptake of control was also slow in low prevalence areas because the cost and additional management required was considered an inefficient use of money and time by farmers (Kennedy et al 2001). In 1999 the program was reviewed. An important recommended improvement was defining a clear pathway for farmers with infected herds to follow such that progress toward disease freedom in their herd could be demonstrated (Kennedy and Allworth 2000).

In its current form, the Australian program is designed to assist disease control in a nationally coordinated manner. It provides minimum standards and practices upon which the States and Territories formulate disease control programs to suit local circumstances. The rules apply to cattle, goats, deer, camelids, buffalo and bison and identify four zones; residual, control, protected and free, based on the prevalence of JD.

Where the disease is considered endemic (residual zone), no or minimal regulatory measures are enforced. In a control zone, the disease is notifiable and official control measures are applied to known infected and restricted herds. The protected zone contains 1% or fewer infected herds and the disease is managed more intensively and at the individual herd level in these areas using compliance and advisory programs. In the free zone there is no JD and a monitoring program is operating to continually provide evidence for freedom from disease. The number and type of herds in each zone are reported quarterly.

Vaccination against JD is generally discouraged though occasionally approved in the first two zones. It is not permitted in the protected or free zones. Restrictions on movement exist between zones (Animal Health Australia 2003).

The number of herds participating in the program has increased in a fairly linear fashion from about 180 in 1996 to 1000 in 2000 and by December 2003, 1623. In Victoria, the test and control program caused a marked decline in the number of clinical cases during 1993–2002 but failed to reduce the herd prevalence of JD significantly (Jubb and Galvin 2004).

2.1.20. The Dutch national voluntary Johne's disease control program

In 1942 a program for the organized control of JD in 138 dairy farms began based on annual testing. By 1979 it was clear that progress was less than acceptable and more effort was put into the early identification of MAP infected animals aged 4–18 months while subsidies increased. A further 9 years later only two of 115 participating herds were declared free of the disease.

A change of focus was required.

In 1983 a program was started that focused on the vaccination of calves and sometimes whole herds. While this strategy proved successful in reducing clinical JD and was cheaper than the subsidized test and slaughter program, infected herds persisted.

A further program was devised to being in 1998, which aimed to reduce the risk of consumer exposure to MAP, to minimize the economic loss associated with MAP and to eradicate the disease from farmed ruminants in the Netherlands. Incentives were added to increase participation in, and acceptance of, the program. It had two main components, firstly prevention of infection of JD free herds and, secondly, provision of assistance to known infected herds.

To retain the highest level of 'freedom', pooled faecal cultures from a herd had to be collected annually, at a subsidized cost. For known infected herds a tailor made control program based on risk assessment was developed jointly by the farmer, veterinarian and a JD expert. Funding was provided by the Commodity Board for Meat, the Commodity Board for Dairy and the Ministry of Agriculture, Nature and Fisheries (Benedictus et al 2000).

A further updated program was described in 2005 (Ebert et al 2005). Its overall structure was the same as its predecessor, but infection status in the new program was determined by an ELISA based program using individual milk or blood samples. It had three main components based on diagnostic screening, the first for herd classification, the second for surveillance and the third for the control of herds testing positive. The history of JD control at the national level in the Netherlands is a clear demonstration of the phenomenal tenacity of this disease.

2.1.21. United States national voluntary Johne's disease control program

JD was first reported in the United States of America in 1908 (USDA 2005b) and was present in at least 15 states by 1931 (Wight 1931). Some state veterinary authority coordinated JD control programs were implemented as early as the 1960s, however laws regarding the movement of cattle from infected areas were seldom observed due to lack of a rapid and sensitive diagnostic test.

In 1993 the Animal Health Association task force developed guidelines for a National Paratuberulosis Certification Program. Uptake of this program by States and individual herd

owners was poor. In 1998 a revised and more practical approach was launched, the US Voluntary JD Herd Status Program (Kennedy et al 2001).

The program has three components, education, management and herd testing. Education is provided by workshops or the veterinarian. Management is addressed through a risk assessment of the farmers' current practices from which a new management plan is devised utilising practices most likely to reduce the transmission of JD. The risk assessment is repeated in years two, four, six and ten after the initial assessment and an annual review conducted. Data collected in 2002 show that American dairy producers are becoming more aware of JD, insofar as 45.3% of producers surveyed were considered 'fairly knowledgeable' compared with just 17.7% in 1996 (USDA 2005a).

2.1.22. France

France was the first country to organize the control of JD in cattle in the 1920s by means of vaccination. The program was abandoned in the 1930s as it interfered with the eradication of tuberculosis (Vallee et al 1926).

In 1999, a national control program to reduce the incidence of clinical JD was proposed by the National Federation of the Groupements de Defense Sanitaire (regional farmer organizations responsible for animal health), based on regional control programs which had been operating for 5 to 10 years. It would be voluntary and implemented at the regional level (Kennedy et al 2001). No further information describing the implementation and subsequent success or failure of this program has been published.

2.1.23. Sweden

Prior to four clinically affected cows being detected in beef herds in 1993, JD had not been detected in Sweden. Trace-back investigation, diagnostic testing, and slaughterhouse investigation of 3200 faecal samples indicated infection on 51 beef farms, all of which were subsequently de-stocked.

In 1998 a voluntary state-funded program was started, aimed primarily at pedigree beef herds. It involved annual faecal sampling of all animals over 2 years of age. In 2001, 800 of the 1400 pedigree beef herds and 20 dairy herds were enrolled in the program (Kennedy et al 2001).

2.1.24. Switzerland

In Switzerland, the possibility of devising scientifically sound methods for the control and surveillance of JD has been investigated. None of the studied methods was regarded as cost

effective due to the low prevalence of JD (considered less than 5%) and poor performance of available diagnostic tests (Koren et al 2005).

2.1.25. Czech Republic

The Czech Republic imported 428 groups of cattle in the years from 1992–1998. JD was traced to at least 84 of these groups. A government subsidized program for the control of the disease, based on the faecal culture of all animals older than 18 months, was adopted in 1998. However in 2003 it had been adopted by only 25 to 30 of approximately 3500 commercial dairy herds (Judge et al 2005).

2.1.26. Japan

A subsidized test-and-cull surveillance and eradication program was initiated by the government in 1998 under the Livestock Infectious Disease Control Law. The program is based on serology supported by faecal culture and Johnin testing with compensation for slaughtered animals. A herd having four negative tests over 3 years is considered free of the disease (Kennedy et al 2001). No further literature is available describing the progress of this program.

2.1.27. Italy

In 2005 a pilot control program was implemented on 37 farms in the Lombardia region of Italy. The program was designed to be of low cost and easy to implement. It involved a farm-level risk assessment and diagnostic testing with an ELISA (Liandris et al 2005). While a reduction in the frequency of positive ELISA tests was observed over time, only a short follow up period had elapsed prior to publication of the article.

2.1.28. Israel

Israel introduced a subsidized voluntary program in 1993 for the control of JD in their 1083 dairy herds. The program entails a farm-level risk assessment, whole herd testing using an ELISA and confirmation of positive samples by faecal culture. The confidence that a herd is free of JD is expressed in its 'herd-safety' score (1–100%) (Koren et al 2005).

In summary, JD is considered by several countries to be an animal health issue worthy of control coordinated at the national level. Control programs at the state or national level aim to reduce the likelihood of importing animals infected with the disease, to protect disease free herds from infection and to provide a pathway by which farmers with infected herds may control the disease. Careful planning and a long term commitment by all parties involved are

required for a successful control program. The United States of America, Australia and the Netherlands have well established programs for the control of JD and feasibility studies for their implementation have been conducted by several other countries.

Johne's disease in New Zealand

JD was first reported in New Zealand in 1912, in dairy cattle (Stephens and Gill 1937) which at that time numbered about 0.65 million. No further diagnoses were made in the following 16 years, during which the cattle population doubled. Evidently the disease had spread geographically during that period as the number of infected herds increased steadily in the following years. In 1931 it became a notifiable disease under the Stock Act (1908). In 1937 it had still not been reported in the South Island.

In an effort to control the spread of JD, the Department of Agriculture decreed that imported cattle, and those to be moved from the North Island to the South Island, should be tested with the Johnin skin test beforehand. The disease continued to spread in cattle, largely due to the absence of restrictions on trade from infected properties, and due to the expansion of agriculture. In 1946 there were about 1.6 million milking cattle of which 514 were reported condemned due to JD (Chandler 1957). In 1982 an abattoir study reported signs consistent with JD in 4% of 945 ileal samples from culled adult dairy cows. These cows were from the Taranaki/Wanganui and Manawatu regions of New Zealand, in which JD was increasingly being considered a significant disease (Hebden and Nuttall 1982). The disease was detected by *ante-mortem* testing on 31% of farms tested in the Taranaki region around that time. The annual incidence of clinical cases was reported to range from 0% to 7.5% in six dairy herds believed to be representative of the spectrum of prevalence of clinical cases in 1985 (de Lisle 1989). In this study the milk production index for cows faecal culture positive for JD, ranged from 7 to 12% less than their test negative herd mates across three milking seasons.

JD remained a notifiable disease until 2000 however notifications recorded probably represent a small proportion of the total cases due to under-reporting. Under reporting was due to poor diagnostic test performance and non-reporting by some farmers and veterinarians (de Lisle 2002). At this time JD was considered widespread throughout New Zealand's approximately 12000 dairy herds but with a low incidence of clinical cases in the 3.9 million dairy cows.

JD was first reported in sheep in New Zealand in 1952 (Williamson and Salisbury 1952), but it may have been present as much as 30 years earlier (Armstrong 1956). Losses due to the disease appeared to be low and vaccination was identified as the only realistic means of control (Davidson 1970). The antibody response to vaccination in sheep was studied (Hilbink and West 1990) and a live vaccine in an oil-based adjuvant (Neoparasec, Rhone-Merieux Animal Health, Lower Hutt, New Zealand) is currently available. No further research has been conducted on JD

in New Zealand's approximately 39 million sheep, in contrast to research activity in Australia (Hussey and Morris 1998; Sergeant et al 2001; Sergeant and Baldock 2002; Lugton 2004; Reddacliff et al 2006).

Deer farming in New Zealand became established in the 1980s making it a recent venture relative to sheep, beef and dairy systems. In 2004 about 1.8 million deer were farmed, primarily for venison but also deer antler velvet. JD was first diagnosed in farmed deer in 1979 (Gumbrell 1986) and by 2001, 299 infected herds had been identified, representing approximately 6% of all herds in New Zealand. Many of these cases were reviewed by de Lisle et al (2003). Identification of infected herds was usually by detection of lesions in deer at slaughter. PCR on cultured colonies was required in many cases to differentiate between MAP infection and infection with *M. bovis*, as the lesions may appear similar.

MAP was isolated from wildlife present on infected deer farms in a preliminary survey in 2004. Culture positive species included feral cats, ferrets, hedgehogs, paradise shelducks and a single rabbit and black backed gull (Glossop, pers. comm.). The relative contribution of the culture positive species to the epidemiology of JD in New Zealand is unclear at this stage. While it is unlikely to be great in the dairy system, the role may be more prominent in the deer and/or sheep systems.

New Zealand researchers Collins and de Lisle (1986) were the first in the world to report on genetic variation within the DNA of MAP. Based on restriction endonuclease typing, they found that 23 field isolates, and two of the three reference strains studied were very similar despite some field isolates being from different countries and isolated many years previously. The remaining reference strain was found to be sufficiently different to be considered an atypical MAP variant, or a strain of another species of slow-growing mycobacteria. It was concluded that restriction endonuclease analysis was unable to provide a useful typing system for strains of MAP isolated from cattle, because the strains studied had such close genetic similarity. Subsequently, these authors studied MAP isolates from cattle, sheep and goats. From this they coined the 'C' strain, found in all cattle isolates and some isolates from sheep and goats, and the 'S' strain which was found only in sheep and a single goat. They reported a number of minor differences between the MAP isolates from cattle, and that isolates from sheep were particularly difficult to culture (Collins et al 1990).

The annual financial loss to the New Zealand dairy industry due to JD was estimated to be NZD 18.9 million in 1998 (Brett 1998). On the basis of this, it was considered unlikely that the benefits of control would outweigh the costs, given available control technology. Thus, significant market access issues would need to be the basis for control (Burton and Voges 2002). de Lisle (2002) recommended that any strategy for the control of JD should be developed jointly between the different livestock industries in New Zealand. On the strength of this, a pan-

industry consortium has been created to manage funds for JD research in this country and possibly overseas. The research will investigate food products for contamination with MAP and potential mechanisms to control JD across the broader agricultural farming sector (Meat and Wool New Zealand 2007).

In summary, JD was first reported in New Zealand in 1912. Today it is considered widespread amongst dairy herds and a disease of emerging importance in deer. It has also been reported in sheep and detected in several wildlife species. Control of the disease in the dairy industry is not considered cost effective, but much uncertainty is associated with the research underpinning this assumption. A pan-industry consortium has recently been created to address JD. An improved understanding of the epidemiology of JD in the New Zealand dairy herd would greatly assist in clarifying the importance of this disease, and consequently, determining what form of action ought to be taken against it.

Chapter 3.

A questionnaire based case-control study of clinical Johne's disease on New Zealand dairy farms

S Norton, C Heuer and R Jackson

Abstract

AIM: To investigate associations between farm management factors and patterns of clinical Johne's disease, *Mycobacterium avium* subspecies *paratuberculosis* (JD) on dairy farms in four major dairying regions in the North Island of New Zealand.

METHODS: A questionnaire-based case control study was conducted to identify associations between management practices and occurrence of clinical JD on dairy farms in the Waikato, Taranaki, Wellington-Manawatu-Wanganui and Wairarapa regions of New Zealand. The frequency of management practices on farms with no clinical cases of JD was compared to farms with a low incidence and to farms with a high incidence using multinomial logistic regression.

RESULTS: Of the 427 responding farmers, 47% had suspected clinical cases of JD in their herd in the preceding 5 years. Only 13% of all herds had an average annual incidence of >0.5 cases/100 cows during this period. Ninety percent of herd owners that had not observed clinical cases, and 20% of herd owners that had observed clinical cases, did not consider the disease a serious problem. Owners and veterinarians had a moderate level of agreement regarding the JD status of a farm. However, their perceptions were consistent for 86% of the high incidence group.

The presence of Jersey cows in the herd and the purchase of bulls were most strongly associated with the incidence of clinical JD. Grazing calves in the hospital paddock, purchase of heifers, larger herds and the use of induction were also positively associated with JD. Farmers that ensured heifers were at least 2 years old when mixed with adult stock were likely to observe fewer cases of clinical JD.

CONCLUSION: The annual incidence of clinical cases of Johne's disease was low and the disease was generally regarded as of little importance by farmers. Farmers that had a high proportion of Jersey cows or that purchased bulls from one, or from more than four, sources were most likely to report clinical cases of JD.

Management practices that could aid in the control of JD are the purchase of JD free bulls, ensuring that calves do not graze in the hospital paddock, and ensuring that young stock are at least two years old prior to contact with adult stock.

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Introduction

Johne's disease (JD) is a chronic enteric disease of cattle and other ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). It was first reported in New Zealand cattle in 1912 (Stephens and Gill 1937) and has since become widespread throughout the major dairying areas of New Zealand. The prevalence of infected herds and incidence of disease are unknown. In 1982 an abattoir study reported signs consistent with JD in 4% of 945 ileal samples (Hebden and Nuttall 1982). In 1989 the disease was detected by *ante-mortem* testing on 31% of farms tested in the Taranaki region (de Lisle 1989).

Key epidemiological aspects of JD remain unclear, for example the relative importance of the various transmission routes. The disease seems to be best controlled by the use of management practices that minimise the exposure of young stock to MAP and that ensure it is not introduced to the farm via stock purchase. Control may be supported by diagnostic testing. Studies in the USA (Hoe and Ruegg 2006), Scotland (Daniels et al 2002) and Australia (Kennedy et al 2005) indicate the adoption of such control measures is not commonplace.

Risk factors for JD within New Zealand's seasonal calving, year round out-door, pasture based dairy production system have not been studied. In other production systems risk factors include large herd size (Wells and Wagner 2000; Daniels et al 2002), failure to clean maternity pens (Johnson-Ifearulundu and Kaneene 1998) and contact between calves and adult faeces (Obasanjo et al 1997). In addition, the Jersey breed (Cetinkaya et al 1997; Jakobsen et al 2000), milking many non-homebred cows (Wells and Wagner 2000) and the presence of wildlife (Daniels et al 2002; Fredriksen 2004) have also been positively associated with JD.

The objectives of this study were fourfold. The first was to investigate associations between environmental and farm management factors and disease patterns of JD in four major dairying regions in the North Island of New Zealand. The second was to compare the veterinary practitioner estimates of the incidence of clinical JD with farmer's estimates. The third was to provide empirical guidance for designing future epidemiological studies of factors identified as most important for disease transmission. The fourth objective was to identify those risky management factors that best suited intervention points for disease control

Materials and methods

3.1.1. Study design

A questionnaire-based study of management practices and occurrence of clinical JD in the Waikato, Taranaki, Wellington-Manawatu-Wanganui and Wairarapa regions of New Zealand was conducted in 1999. Farmers willing to participate in the study were nominated by

veterinarians and mailed a questionnaire. They were not nominated in a random fashion because veterinarians were requested to preferentially select herds worst affected by JD. Farmers received a reminder letter and telephone call to encourage them to complete and return the questionnaire. Initially their farms were categorized according to veterinarians' perceptions as having 1) no history of clinical JD, 2) infection acquired within the previous 2 years, 3) <1% annual incidence for longer than 2 years and 4) >1% annual incidence for longer than 2 years. In some cases, practitioners had only recently become responsible for a herd's veterinary services and were unable to provide an assessment of farm status. Subsequently farms were re-categorised according to farmer's records. Categories three and four were combined as they each contained few farms.

The questionnaire covered farm demographics and management practices considered relevant to important transmission pathways for JD between and within herds.

Several terms used in the questionnaire are defined for clarity. Calves were defined as animals less than 4 months old. Induction is the practice of administering corticosteroid by injection(s) to late-calving cows to induce premature calving. A hospital paddock is a small paddock used for grazing sick animals, typically close to the dairy shed for close observation and easy access for milking and for treatment. Off-farm grazing applies to the practices of rearing of replacement heifers on other farms and sending milking cows to other farms after drying off until close to calving.

Data were collected on farmers' perceptions of how the importance of the disease ranked against a list of other diseases of dairy cattle. In addition, the clinical signs relied upon for diagnosis, confidence in making a correct diagnosis, actions taken to support a diagnosis, and management until disposal were also recorded.

3.1.2. Statistical analysis

Agreement between veterinarians and farmers on which JD category the farmer's property should be allocated to, was tested using the Kappa statistic (Fleiss 1981).

The dependent variable comprised three categories: 1) JD free: farms with no clinical evidence of JD, 2) low incidence: farms on which the annual incidence of suspected clinical cases of JD was <0.5 per 100 cow years and 3) high incidence: farms with >0.5 suspected clinical cases per 100 cow years. The annual incidence was an average of the preceding 5 years based on the farmer's records.

The frequency of categorical variables and the distribution of continuous variables describing management practices were evaluated at the univariate level. They were then screened for significant association with the dependent variable using the chi-square test for categorical

variables and analysis of variance for normally distributed continuous variables. Significant variables ($p<0.050$ and those of particular biological interest were screened for correlation then used in the multi-variate analysis. Where Pearson's rho value indicated a strong correlation ($p \geq 0.6$) between two variables either an aggregate variable was formed, or one was omitted.

A series of seven multinomial logistic regression models was constructed as a way for dealing with missing values. The first model included only variables of interest that had data from every farm. The seventh included all variables of interest. The five models in between represented a spectrum between these two extremes (Table 3.8).

For each variable, a single odds ratio was created by weighting each coefficient by the sample size of its model, then taking an average of the weighted coefficient values. For continuous variables the effect was reported as the change in odds given a change of one standard deviation. Biologically plausible interaction terms were examined. Ordinal and multinomial approaches to the logistic regression were compared using the difference in deviance between the two methods and a chi-square distribution.

Data were stored in Microsoft Access Version 7.0 (Microsoft, Redmond USA). Analysis and modelling was conducted in R (R Development Core Team, 2004) using the multinom package in the nnet library (Venables and Ripley 1994) and significance was declared at $p < 0.05$.

Results

3.1.3. Description of general management

Questionnaires were mailed to 664 farmers, of whom 427 (64%) responded with useable data. Respondents represented three percent of the dairy farms operating in New Zealand at that time. The average milking herd size was 236 cows (SD = 125, range 38 to 850) and most (398/427, 93%) calved in the spring while only 4/427 (1%) practised all-year-round calving. Both Friesian and Jersey cows were milked on most (265/427, 62%) farms, 102/427 (24%) of farms milked ≥95% Friesians, 47/427 (11%) milked ≥95% Jersey cows and 13/427 (3%) milked other breeds.

Calves were usually removed from the dam between 12 and 24 hours after birth on 244/425 (57%) farms, after less than 12 hours on 140/425 (33%) farms, and after more than 24 hours on 41/425 (10%) farms.

Nine per cent (39/427) of farms were closed, that is, they had not brought heifers, cows or bulls from other properties onto the farm in the preceding 5 years. Another 222/427 (52%) had not purchased adult milking cows in the previous 5 years and 107/427 (25%) had purchased ≤5 milking cows annually. Most farmers (253/378, 67%) had not purchased replacement heifers in the past 5 years and 83/378 (22%) had purchased, on average, up to 20 annually. The remaining 42/378 (11%) of farmers had purchased, on average, more than 20 heifers annually. In the

preceding 5 years, 92/403 (23%) farmers had not purchased bulls, 167/403 (41%) had purchased on average one or two bulls per year, 90/403 (23%) had purchased 3 to 4 and 54/403 (13%) had purchased more than four.

Most farmers (254/423, 60%) frequently sent stock off-farm for grazing, 84/423 (20%) did so occasionally and 85/423 (20%) farmers never did. Very few farmers grazed stock from other farms frequently (9/422, 2%) or occasionally (47/422, 11%), most farmers (366/422, 87%) never grazed stock other than their own.

Calves were grazed in the hospital paddock frequently on 23/414 (6%) farms and occasionally on 128/414 (31%) farms but never on 263/414 (63%) farms.

Slurry was applied to pasture that would later by grazed by cows only on 268/417 (64%) farms, and by cows and calves on 100/417 (24%) farms. Slurry was not applied to pasture on the remaining 12% of farms. The interval between application and grazing was most commonly 3 to 4 weeks (mean 3.9, median 3.0, inter quartile range 2–4, range 0–52).

Cows had been induced in most (320/409, 78%) herds during the previous 3 years. In these herds, the average annual percentage of cows induced was <5% of the herd in 93/409 (23%) herds, 5–10% in 141/409 (34%) herds and >10% in the remaining 86 (21%) herds.

Livestock other than cattle were kept on 129/371 (35%) farms. A few sheep (<30 in total), or a single goat were the most common other species and deer were rare. Wild rabbits were present on almost all farms (410/424, 99%).

3.1.4. Perceptions of Johne's disease and its diagnosis

JD was not considered a serious problem by 90% (203/225) of farmers that had no history of clinical disease on their farm and by 21% (42/202) of farmers that had identified clinical cases.

Most farmers (289/427, 68%) lacked confidence when diagnosing clinical JD based on its appearance alone, particularly those who believed the disease was absent from their farm (Table 3.2).

Of the 202 farmers who considered their farms to be infected, 37 (18%) reported that they never had any difficulty in deciding whether a cow had clinical disease, 132 (65%) had difficulty sometimes and 32 (16%) always considered diagnosis difficult. There was heavy reliance on gradual loss of condition and persistent diarrhoea as diagnostic criteria and less reliance on no response to worming or other treatment or a drop in milk production.

The most common course of action taken by farmers after they suspected a cow had JD (Table 3.3) was to have her checked be a veterinarian and sent to slaughter, usually as soon as possible. Between showing clinical signs and being sent to slaughter, it was most common for a JD cow

to be kept in the hospital paddock and very rare for her to be kept with younger stock (Table 3.4).

Only 16% of farmers who considered their herd to be free from clinical JD took special precautions to conserve this position.

Agreement between veterinarians' and farmer's estimates of the incidence of clinical JD was moderate (Kappa statistic 0.53, 95% CI 0.45–0.63). Veterinarians provided information for 146 of the 227 farms considered by the farmer to be free from disease and 141 of farms considered by the farmer to be infected. There was agreement between farmers and veterinarians for 112/146 (77%) farms on which the owner had not seen clinical cases and for 107/141 (76%) of farms on which the owner had seen clinical cases. There was agreement between farmers and veterinarians for 86% (38/44) of properties on which farmers considered the incidence of JD to be high and which had a veterinarian's estimate of incidence.

3.1.5.Incidence of Johne's disease

Clinical cases of JD had not been seen in 226 herds (53%, category 1) and were of low incidence in 145 herds (34%, category 2) and high incidence in 56 herds (13%, category 3). The distribution of average annual incidences per 100 cow years is shown in Figure 3.1. The median annual incidence of clinical cases was 0.31/100 cow years, (lower quartile = 0.15, upper quartile = 0.57).

3.1.6.Associations between Johne's disease and management practices

Of the 85 variables tested, 11 were significantly associated with the response variable at the bivariate level (Table 3.5). Two more variables were included for biological interest. The first was off-farm grazing, a potentially important means of reducing the exposure of young stock to MAP. The second was the purchase of milking cows, a means of re/introduction of JD.

Information describing the size and fit of the multivariate models is presented in Table 3.6. Models with more variables were better able to explain variation in the data, but omitted a greater proportion, up to 36%, of respondents. The first model included all 427 farms and three variables with no missing values; herd size, percentage of Jersey cows in the herd and percentage of cross-bred cows in the herd. The seventh model contained all 14 variables.

For eleven of the fourteen explanatory variables the strength of association with JD category was remarkably consistent across the seven models. The level of significance changed by less than one order of magnitude and did not alternate between significant and non-significant at the 5% level.

The remaining three variables fluctuated to a small degree. The age at which young stock first had regular contact with adult stock was significant in three of five models (p-value range <0.03–<0.24), the percentage of crossbreds in the herd was significant in six of seven models (p-value range <0.01–<0.08) and grazing of calves in the hospital paddock was significant in three of five models (p-value range <0.01–<0.06).

The weighted average effect of each variable is presented in Table 3.7. The risk factors most strongly associated with JD were the proportion of Jersey cows in the herd and the purchase of bulls. Relative to herds without Jersey cows, those with a high proportion of Jersey cows had a high chance of being in the low incidence category and a higher still chance of being in the high incidence category. Herds with at least 80% Jersey cows were 18.48 (95% CI 5.39–63.40) times more likely to be a high incidence herd than those without Jersey cows (Figure 3.2). Farmers that purchased bulls, from one farm or greater than four farms, were more likely to see clinical JD in their herd. Purchasing bulls from greater than four farms increased the odds of being a low incidence farm by 16.76 times (95% CI 2.75–102.17) and the odds of being a high incidence farm by 27.71 times (95% CI 2.41–302.11).

Five other risk factors had a positive association with JD. Farmers that annually induced up to five percent, or greater than ten percent of their herd were 4.13 (95% CI 1.24–13.72) and 4.65 (95% CI 1.39–15.6) times more likely to see a high incidence of clinical JD than those that did not use induction.

The grazing of calves in a hospital paddock approximated a dose-response relationship with JD category (Figure 3.3). Farmers that frequently grazed calves in the hospital paddock were 5.92 (95% CI 1.37–25.48) times more likely to be see a high incidence of clinical JD than those that did not graze calves in the hospital paddock.

Larger herds were more likely to be in the low incidence category than average sized herds. An increase in herd size of one standard deviation (125 cows) from the mean of 236 was associated with a 1.57 (95% CI 1.19–2.07) fold increase in the odds of being in the low incidence category.

Farmers that purchased a large percentage of their replacement heifers were more likely to see a high incidence of clinical JD. An increase of one standard deviation (19%) from the average percentage of heifers purchased (25%) was associated with a 2.47 (95% CI 1.21–5.03) fold increase in the odds of being in the high incidence category.

Having a high proportion of crossbred cows in the milking herd was associated with a 3.16 (95% CI 1.54–6.49) increase in the odds of being in the low incidence category relative to herds with purebred cows.

Only one variable had a negative association with JD. Farms on which calves were well matured before having regular contact with adult stock were less likely to be in the high incidence category. An increase in calf age at first regular contact with adult stock of one

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standard deviation (8 months) from the average of 24 months was associated with a 1.6 (95% CI 1.04–2.56) fold reduction in the odds of being in the high incidence category.

The increase in the odds of clinical JD was not linear across the three JD categories. The slope varied between variables and models, thus a multinomial model, which allows for a non-linear relationship was more appropriate (p<0.01) than an ordinal model.

Table 3.1 Summary of herd demography and management practice details recorded by the Johne's disease questionnaire

Questionnaire component	Information recorded
Herd demography	District, geographical location and land area of farm
	Milking herd size over the past 5 years
	Breed makeup of herd
	Number of cows culled annually for clinical Johne's disease in the preceding 5 years
	Number of cows induced annually in the preceding 3 seasons
Neonatal and early calfhood exposure	Average duration of calf-dam contact period following birth
	Calving area: main herd or separate calving mob
	Use of nurse cows
	Types of discarded milk (if any) fed to calves
Calf management	Type of calf rearing facility: individual or group rearing, housed or outdoor
	Number of calves per rearing group
	Calf age when weaned
	Age of calves when regular contact with adult cattle begins
Slurry management	Occurrence of slurry application to cow and/or calf pasture
	Frequency of slurry application
	Time interval from slurry application to first grazing (spell period)
Heifer replacements	Number of heifers bred and reared on-farm annually in the preceding 5 years
	Number of heifers purchased annually in the preceding 5 years
	Number of herds from which heifers were purchased in the preceding 5 years
	Number of heifers contract reared in the preceding 5 years
Purchase of adult cows	Number of adult cows purchased annually in the preceding 5 years
	Number of herds from which cows were purchased in the preceding 5 years
Bull purchases	Number of bulls purchased annually in the preceding 5 years
	Number of herds from which bulls were purchased in the preceding 5 years
Grazing practices	Frequency with which stock were sent off farm for grazing
	Frequency with which cattle from other farms were grazed
Non-dairy animals	Number of non-dairy cattle on-farm in the preceding 5 years
	Frequency with which non-dairy cattle were purchased
	Presence and number of sheep, deer, goats, rabbits in the past year

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Table 3.2. Frequency of responses by dairy farmers regarding levels of confidence when diagnosing clinical Johne's disease stratified by presence of the disease on the farm

Johne's disease	Number of farmers (%)		
	Had confidence	Lacked confidence	Total
Considered present	85 (42%)	116 (58%)	202
Considered absent	53 (23%)	173 (77%)	225
Total	138 (32%)	289 (68%)	427

Table 3.3. Frequency of four nominated courses of action taken by dairy farmers when a cow was suspected to have Johne's disease

Action	Frequency of action (%)		
	Sometimes	Always	Never
Have her checked by a veterinarian	90 (45%)	84 (42%)	28 (13%)
Treatment, e.g. copper or anthelmintic	62(31%)	42 (21%)	98 (48%)
Send to slaughter as soon as possible	71 (35%)	71 (35%)	60 (30%)
Disposal by other means, e.g. dog man or knacker	68 (34%)	11 (5%)	123 (61%)

Table 3.4. Frequency of three nominated courses of action by dairy farmers after deciding that a cow had Johne's disease

Action	Frequency of action (%)		
	Sometimes	Always	Never
Leave with milking herd until disposal	63 (31%)	22 (11%)	117 (58%)
Keep in hospital paddock until disposal	59 (29%)	110 (55%)	33 (16%)
Put her with young cattle until disposal	3 (1%)	1 (0.5%)	198 (98.5%)

Table 3.5. The association between Johne's disease category (low incidence, high incidence) and management factors significant at the bivariate level or of particular biological interest as an odds ratio, and their frequency of use across the studied New Zealand dairy farms

Risk factor	Mean (SD)	Category	Percentage of farms in category	Odds ratio (CI)	
				Low incidence	High incidence
Herd size n=427	236 (125)			1.36 (1.1–1.68)	1.05 (0.76–1.44)
Percentage Jersey cows		0 (Ref)	43		
n=427		1–20	24	2.16 (1.26–3.62)	1.69 (0.68–4.21)
		21–40	11	1.19 (0.57–2.46)	2.77 (1.04–7.36)
		41–80	9	2.52 (1.15–5.54)	4.5 (1.54–13.16)
		80–100	13	1.58 (0.75–3.30)	8.14 (3.5–18.95)
Percentage crossbred cows		0 (Ref)	43		
n=427		1–30	31	1.44 (0.87–2.38)	0.86 (0.43–1.70)
		>30	26	2.65 (1.57–4.47)	1.01 (0.47–2.15)
Percentage of milking herd purchased annually n=405		0 (Ref)	57		
		0.1–5	28	1.16 (0.70–1.91)	1.03 (0.53–2.01)
		>5	15	1.97 (1.08–3.60)	0.72 (0.26–2.01)
Percentage of heifers purchased annually n=378	25 (19)			1.12 (0.85–1.47)	1.49 (1.08–2.06)
Source of purchased bulls n=409		Not purchased (Ref)	25		
		1 Farm	26	1.77 (0.96–3.28)	1.96 (0.88–4.33)
		2-4 Farms	38	1.37 (0.78–2.40)	0.63 (0.27–1.48)
		>4 Farms	11	2.06 (0.92–4.58)	2.09 (0.75–5.82)
Slurry application n=395		Never (Ref)	37		
To cow grazing		4 Week spell	32	0.80 (0.47–1.36)	0.50 (0.24–1.03)
		>4 Week spell	7	0.64 (0.24–1.68)	0.67 (0.20–2.18)
To cow and calf grazing		4 Week spell	20	1.16 (0.65–2.09)	0.45 (0.18–1.12)
		>4 Week spell	4	1.20 (0.41–3.52)	0.31 (0.04–2.62)
Percentage of herd induced annually n=409		0% (Ref)	22		
		0.1–5%	23	1.72 (0.89–3.33)	2.44 (0.85–6.96)
		5.1–10%	34	2.60 (1.42–4.75)	3.07 (1.15–8.19)
		>10 %	21	1.61 (0.81–3.22)	4.26 (1.56–11.60)
Calving location n=422		With dry herd (Ref) Separate paddock	70	1.17 (0.73–1.86)	0.85 (0.46–1.59)
Calves grazed in the hospital paddock n=414		Never (Ref)	64		
		Sometimes	31	1.98 (1.25–3.15)	1.70 (0.88–3.29)

Risk factor	Mean (SD)	Category	Percentage of farms in category	Odds ratio (CI)	
				Low incidence	High incidence
		Frequently	5	1.27 (0.44–3.71)	4.53 (1.62–12.69)
Frequency of off farm grazing		Never (Ref)	20		
n=423		Sometimes	20	1.75 (0.91–3.37)	0.90 (0.33–2.44)
		Frequently	60	1.09 (0.63–1.89)	1.15 (0.54–2.43)
Calf group size n=407	15 (7.7)			1.16 (0.93–1.44)	1.25 (0.94–1.67)
Calves fed penicillin milk n=425		Yes	54	1.18 (0.78–1.80)	0.95 (0.53–1.70)
Age calves first contact adults (months) n=394	15.8 (8.0)			0.89 (0.71–1.1)	0.81 (0.60–1.09)

Table 3.6. Fit statistics and number of farms analysed (n) in seven multi-variate models describing the association between Johne's disease and up to fourteen dairy farm management practices

Model	1	2	3	4	5	6	7
n	427	377	353	298	284	275	267
AIC	799.0	726.19	661.7	538.1	544.4	515.0	512.3
Deviance	767.0	666.2	605.7	450.1	448.4	407.0	396.3
Pseudo R^2	0.07	0.20	0.27	0.46	0.46	0.51	0.52
df	16	30	28	44	48	54	58

Table 3.7. The weighted average association between Johne's disease category (low incidence, high incidence) and management factors as an odds ratio on the studied New Zealand dairy farms from seven multi-variate models

Risk factor	Mean (SD)	Category	Odds ratio (CI)	
			Low incidence	High incidence
Herd size	236 (125)[a]		1.57 (1.19–2.07)	1.33 (0.86–2.05)
Percentage Jersey		0 (Ref)		
		1-20	1.57 (0.81–3.05)	1.97 (0.61–6.34)
		21-40	1.05 (0.41–2.67)	3.03 (0.79–11.64)
		41-80	4.51 (1.56–13.01)	11.59 (2.57–52.28)
		80-100	2.81 (1.1–7.23)	18.48 (5.39–63.4)
Percentage Crossbred		0 (Ref)		
		1-30	1.24 (0.64–2.41)	0.76 (0.29–1.99)
		>30	3.16 (1.54–6.49)	1.54 (0.50–4.72)
Percentage of milking herd purchased/yr		0 (Ref)		
		0.1-5	1.02 (0.51–2.05)	0.71 (0.25–1.99)
		>5	1.96 (0.85–4.51)	0.35 (0.07–1.75)
Percentage of heifers purchased annually	25 (19)		1.02 (0.61–1.72)	2.47 (1.21–5.03)
Source of purchased bulls		Not purchased (Ref)		
		1 Farm	5.76 (1.53–21.72)	11.08 (1.49–82.53)
		2-4 Farms	2.57 (0.81–8.31)	1.48 (0.21–10.57)
		>4 Farms	16.76 (2.75–102.17)	27.71 (2.40–320.11)
Slurry application To cow grazing		Never (Ref)		
		4 Week spell	0.52 (0.19–1.44)	0.40 (0.08–1.92)
		>4 Week spell	0.51 (0.24–1.09)	0.58 (0.20–1.67)
To cow and calf grazing		4 Week spell	1.08 (0.33–3.58)	0.15 (0.01–1.58)
		>4 Week spell	0.88 (0.4–1.92)	0.29 (0.08–1.07)
Percentage of Inductions		0 % (Ref)		
		0.1-5 %	1.11 (0.55–2.25)	4.13 (1.24–13.72)
		5.1-10 %	1.42 (0.75–2.71)	2.36 (0.74–7.53)
		>10 %	0.89 (0.42–1.87)	4.65 (1.39–15.6)
Calving location (with dry herd)		Separate paddock (Ref)	1.46 (0.71–2.99)	0.82 (0.29–2.31)
Calves grazed in the hospital paddock		Never (Ref)		
		Sometimes	1.72 (0.76–3.9)	2.13 (0.71–6.38)
		Frequently	1.51 (0.43–5.24)	5.92 (1.37–25.48)
Off farm grazing		Never (Ref)		
		Sometimes	2.31 (0.87–6.12)	4.69 (0.79–27.82)
		Frequently	0.68 (0.31–1.49)	3.52 (0.83–15)
Calf group size	15 (7.7)		1.05 (0.77–1.43)	1.37 (0.87–2.15)
Calves fed penicillin milk		Yes	1.20 (0.63–2.28)	1.22 (0.44–3.38)

Risk factor	Mean (SD)	Category	Odds ratio (CI)	
			Low incidence	High incidence
Age calves first contact adults	15.8 (8.0)		1.01 (0.76–1.35)	0.62 (0.39–0.96)

[a] An increase in a continuous variable of one standard deviation from its mean was associated with the reported increase in odds of being a low-JD or high-JD herd.

Table 3.8. List of variables included (x) in each of the seven multivariate models

Variable	1	2	3	4	5	6	7
Per cent Jersey breed in herd	x	x	x	x	x	x	x
Per cent Crossbred in herd	x	x	x	x	x	x	x
Average herd size	x	x	x	x	x	x	x
Per cent of cows induced		x	x	x	x	x	x
Number of bull vendors used		x	x	x	x	x	x
Calves in hospital paddock			x	x	x	x	x
Calf age when adult contact becomes regular			x	x	x	x	x
Calving location (with dry herd)				x	x	x	x
Per cent of milking herd purchased annually				x	x	x	x
Off farm grazing					x	x	x
Slurry application					x	x	x
Average number of calves per group						x	x
Per cent of heifers purchased annually						x	x
Penicllin milk fed to calves							x
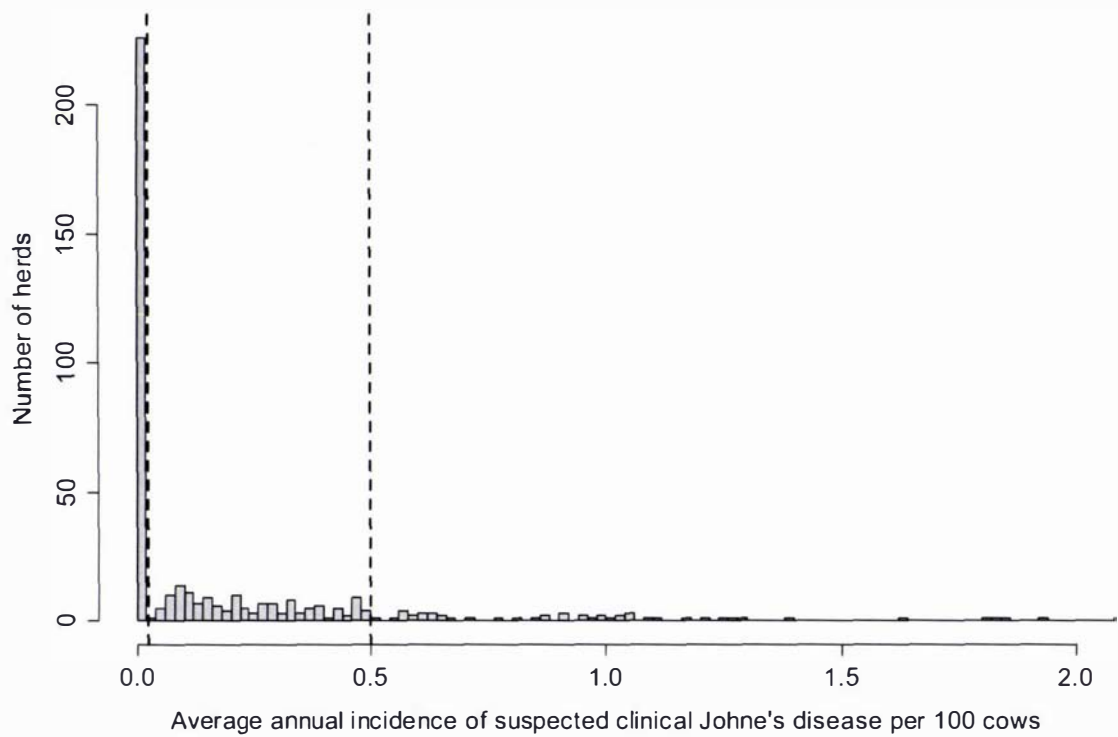
Figure 3.1. The average annual incidence of suspected clinical Johne's disease cases per 100 cows over five years, categories of infection are indicated by dashed lines: left = no cases, centre = low incidence (> 0 and < 0.5 cases), right = high incidence (≥ 0.5 cases)
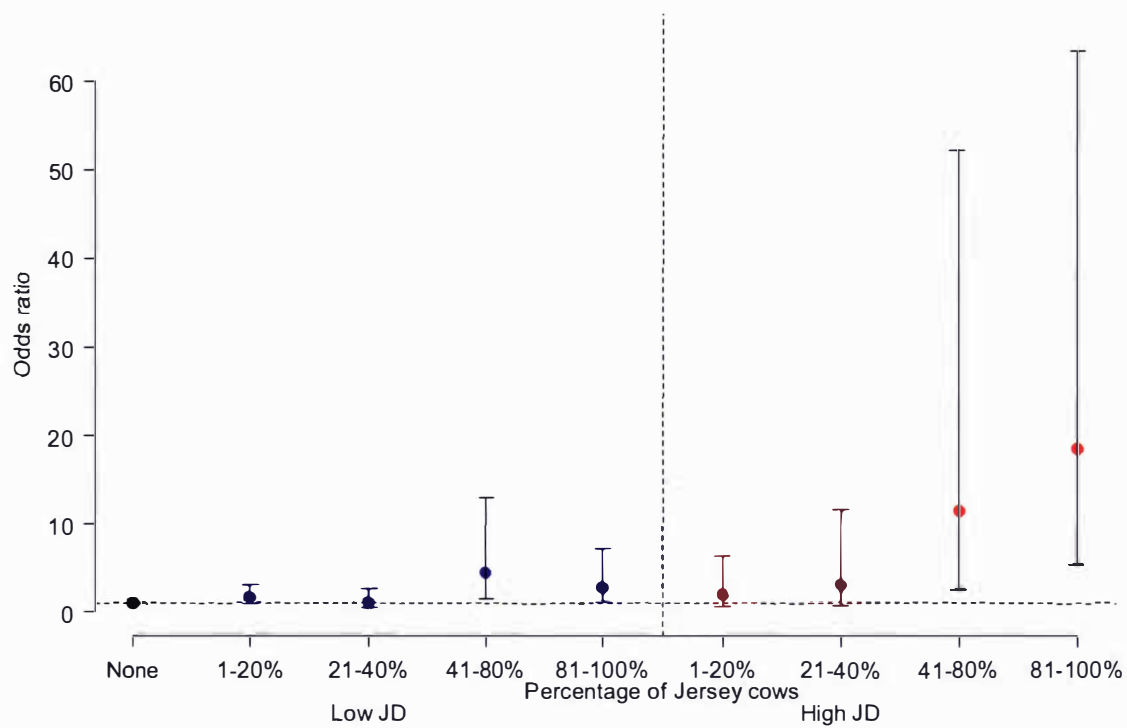
Figure 3.2. The odds of observing a low (low JD) or high (high JD) incidence of clinical Johne's disease on farms with the given percentage of Jersey cows relative to farms with no Jersey cows, as an odds ratio (point) and 95% confidence interval (bars)
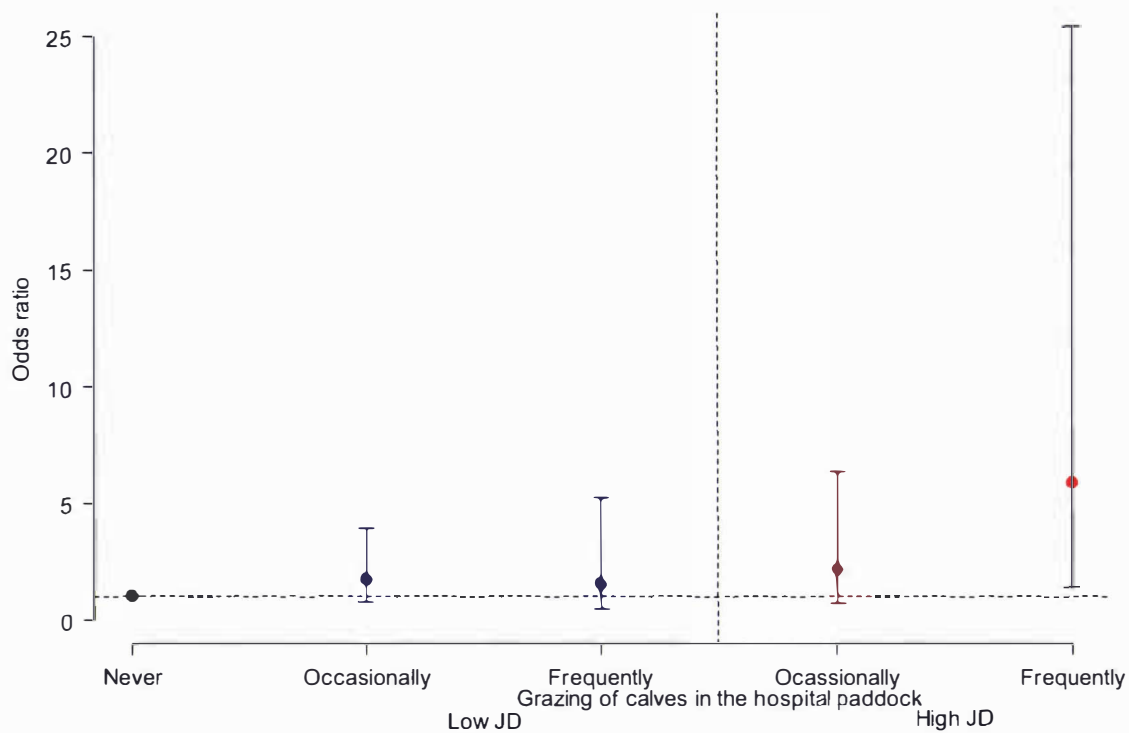
Figure 3.3. The odds of observing a low (low JD) or high (high JD) incidence of clinical Johne's disease on farms that occasionally or frequently graze calves in the hospital paddock relative to farms that never use this practice, as an odds ratio (point) and 95% confidence interval (bars)

Discussion

In this study we examined relationships between the occurrence of clinical JD and management practices using questionnaire data collected in 1999 from 427 New Zealand dairy farmers. We collected data from four major dairying areas and attempted to capture the spectrum of JD affected herds in New Zealand. Our aim was to identify risky management practices for the occurrence of clinical JD and to evaluate these as potential points of intervention for disease control. In addition, we aimed to identify issues that require further investigation at finer levels of detail than could be employed in this study.

National or state scale voluntary control programs for JD have been implemented in the USA (USDA 2005a), Australia {Frankin, 2005 #334} and The Netherlands {Frankin, 2005 #335}. The programs are generally based on first principles for a faeco-orally transmitted disease and the results of experimental studies which point to infection being most common in early life, rather than studies of the disease in its natural setting. Participation in these programs has generally been poor (McCaughan 1989; Wraight et al 2000; Raizman et al 2006). Only 9% of Wisconsin dairy producers that responded to a bio-security questionnaire were enrolled in the state JD program, while over 90% considered JD to be a problem (Hoe and Ruegg 2006). In Victoria, Australia only 8.3% of 470 responding dairy farmers implemented three or more of the six recommended practices for the control of Johne's disease (Wraight et al 2000). Participation in a JD control program in Minnesota was largely by high producing herds rather than those suffering losses due to the disease (Raizman et al 2006). In New Zealand, JD on dairy farms must be described before a suitable method of control can be determined.

The median annual incidence of clinical cases of JD in the 201 herds thought to be infected in this study was low (0.31 cases per 100 cow years, lower quartile=0.15, upper quartile=0.57) and the disease generally regarded as of little consequence. The true incidence is probably higher than this as farmers are more likely to underestimate the number of clinical cases over 5 years. The low incidence is likely to be a valid reflection of the pattern of disease in New Zealand since considerable effort was made to seek out and include known infected herds. An average annual incidence of 2.48% was reported in an earlier study of six New Zealand dairy herds selected as representative of the range of JD incidences in 1984/85 (de Lisle 1989). The disparity in incidences between the current study and that of de Lisle may be due to the small number of herds in the latter study. Clinical cases of JD had been suspected in the past five years on about half the farms in our study (47%), which may be indicative of the true situation, given the large number of herds studied and their wide geographic distribution, however our sample selection was non-random which invalidates this as a true measure of herd-level prevalence.

The behavior of farmers with respect to JD was generally of tolerance and minimal reaction. Few farmers took special precautions to reduce the risk of introducing the disease. Similar farmer behavior has been reported in Scotland (Daniels et al 2002) and the USA (Wells and Wagner 2000). The most common action taken by a farmer who suspected the presence of a cow with clinical JD was to remove her from the milking herd to a hospital paddock, with or without treatment for causes of wasting other than JD and, following a veterinarian's opinion, slaughter.

Multivariate analysis showed a strong dose-response relationship between JD and the proportion of Jersey cows in the milking herd. The link between Jersey cows and JD has repeatedly been described (Withers 1959; Cetinkaya et al 1997; Jakobsen et al 2000). Possible explanations include genetic susceptibility to infection, a predisposition to showing clinical signs (Cetinkaya et al 1997) and a greater antibody response to infection relative to other breeds (Jakobsen et al 2000).

As a method for controlling JD, further reducing the small proportion of Jersey cows in New Zealand's national herd holds little appeal. The once predominant Jersey breed has declined to only 15% of the national dairy herd primarily due to its lighter carcass weight, as the Friesian breed has become more popular. Breed choice by the farmer is a multi-factorial decision in which JD is unlikely to be of high importance. The declining proportion of high risk cows in the national herd may be linked to anecdotal evidence that clinical cases of JD are fewer today than during the 1970s.

The movement of cattle between properties via trade probably represents the most important means of disseminating JD between farms (Sweeney 1996). Almost all (91%) farmers had purchased stock in the previous 5 years and those purchasing bulls and/or heifers had a higher risk of seeing clinical JD.

Bulls had been purchased by most (75%) farmers and usually (65% of those purchasing bulls) from more than one source. Bulls are usually kept for a brief period, from October through to February, to mate with cows in which artificial insemination was unsuccessful. Such bulls are usually 2 or 3 years old, selected on good condition and sold after the mating season. They are generally not tested for JD and slaughtered before clinical signs are most likely to appear. While this association was strong (odds ratio of 27.71, 95% CI 2.40–320.11), a direct route of transmission of MAP from infected bulls to calves is not easily described. Indirect contact via environmental contamination would be most likely. But this route would seem of minor importance relative to infection via the dam. Conceivably it could be via sexual transmission (Ayele et al 2004). In the review by (Sweeney 1996) it was noted that bulls used for natural service may play a role in the transmission of JD. Alternatively this variable may be a proxy for some other factor, such as poor biosecurity, or large herd size.

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The purchase of bulls would be an attractive practice to change in order to control JD as it would apply to a high proportion of farmers and involves relatively few animals. Clarifying the JD status of vendor farms and testing bulls prior to purchase are realistic control options. However further evidence to confirm the association between bull purchase and JD should be collected beforehand.

The purchase of replacement heifers was positively associated with a high incidence of clinical JD. The average percentage purchased annually was 25%. Farmers that purchased 44% (one SD greater than the mean) were 2.47 (95% CI 1.21–5.03) times more likely to see a high incidence of clinical JD cases than farmers with average heifer purchase behavior. This association was considerably weaker than that for bull purchases and comparatively few (33%) farmers purchased heifers regularly. Consequently, changing this practice would probably not be a successful means for reducing the incidence of JD. Milking a high percentage of non-homebred cows was positively associated with JD herds in the study by Wells and Wagner (2000).

Increasing herd size was positively associated with JD in the current and previous studies (Withers 1959; Collins et al 1994; Wells and Wagner 2000; Daniels et al 2002; Hoe and Ruegg 2006). This is despite considerable differences in average herd size between studies. The larger herds within a population are at higher risk of observing clinical JD as they have more animals under observation thus are more likely to see the 'tip of the iceberg'. They are also likely to import a greater number of cows and consequently import infection. Furthermore, infected cows can potentially contact a greater number of susceptible individuals in large herds. As a method for controlling JD, reducing the average herd size is unrealistic. The average New Zealand dairy herd size has increased from 159 cows in 1990 to 315 in 2005 and the powerful economies of scale driving this trend arc unlikely to be influenced by JD.

The frequency with which calves were grazed in the hospital paddock showed a dose response relationship with JD category. Farms on which this practice was frequently used were 5.92 (95% CI 1.37–25.48) times more likely to be in the high incidence category than farms on which it was never used. Placing calving cows in a hospital paddock was previously identified as a risky practice in the United States of America (Merkal et al 1975). This environment is likely to be more heavily contaminated than other pasture with MAP as it is usually small and the place suspected clinical cows are kept. MAP may remain viable here for well over a year (Whittington et al 2004). A study of environmental MAP contamination on dairy farms, similar to that of Raizman and Wells (2005), would be a simple means of confirming this association and may identify other heavily contaminated areas from which calves should be excluded. Excluding calves from the hospital paddock is an attractive method for controlling JD as it entails only minor adjustments to farm management and the dose-response relationship indicates that it would be of most benefit on farms with a high incidence of clinical JD.

Herds in which 0.1 to 5% or greater than 10% of cows were induced had significantly higher odds (4.65, 95% CI 1.39–15.6) of being in the high JD incidence category. This association may be explained by poorly managed herds being likely to induce a high percentage of their herd and probably being more likely to have a high incidence of JD. However this practice was employed to some degree by most (78%) farmers in the previous 5 years.

The 38% of herds that ensured heifers were 2 years old before mixing them with adult stock were less likely to have a high incidence of JD. This may be attributable to a decline in susceptibility to infection with age (Payne and Rankin 1961a).

Potential methods for the control of JD must be easily implemented, of low cost and have a high probability of success. Examples of such methods identified in this study are ensuring purchased bulls are JD free, not grazing calves in the hospital paddock and ensuring heifers are well grown prior to being mixed with adult stock. These methods require minimal input from beyond the farm gate. The control of JD will be an enduring, if not unending, process and expensive, labor intensive approaches will not be sustainable.

The correct classification of infection status continues to be a challenge in the study of JD. In our study, farms were allocated into the three disease categories based on records kept by the farmer, as in previous studies (Cetinkaya et al 1997; Wells and Wagner 2000; Naugle et al 2004). As an instrument for measuring the incidence of JD in the preceding 5 years, the farmer's estimate was probably superior to that provided by veterinary records or diagnostic testing history. However, it may have introduced recall bias (Elwood 2007) into the analysis. It is highly likely that some herds in which clinical JD had not been seen were infected. But it is less likely that low incidence herds were misclassified as JD free and unlikely that high incidence herds were misclassified. Under these circumstances misclassification would lead to measures of association being biased towards the null. Large associations, such as those with odds ratios greater than three, or dose-response associations are unlikely to be solely due to bias (Woodward 2005).

It is also highly likely that many of the herds studied had been infected for a considerably longer period than the previous five years and that introduction of the disease may have occurred under different management techniques. We did not account for possible changes in management practices over time.

The external validity of this study is supported by the inclusion of dairy farms from four major dairying areas within New Zealand. It is not greatly compromised by the non-random selection of case farms nor by two differences observed when comparing the study population with the national population of dairy farms. These differences were that, firstly, the average herd size in the study was 236, compared with the national average in 2001/2002 of 271 (New Zealand Dairy Statistics 2002). Secondly, in the study population farms milking both Friesian and Jersey

cows were over represented at the expense of farms milking Friesian cows. Within the study population 62% of farms were comprised of both Jersey and Friesian cows while 24% contained only Friesian cows. Within the national population, these percentages were 23% and 54%, respectively (New Zealand Dairy Statistics 2002). External validity is maintained because the differences mentioned above are unlikely to strongly influence the association between exposure and the outcome (Elwood 2007).

Our approach to analyzing the data differed slightly from convention in order to report associations with maximum accuracy and precision. Missing or ambiguous data was present in 36% of questionnaires. Our analytical approach minimized the impact of this by averaging the results from a series of models. Estimates from models with the most data were the strongest by virtue of their weighting, but variables with less complete data could also be assessed. A high proportion (R^2=52%) of the variability in JD incidence in our data was accounted for in the largest model.

Comparing three levels of disease incidence enabled us to show trends in the data that were not apparent using a dichotomous classification. However, the nature of a case control study allows identification of associations but not causal relationships.

In conclusion, the estimated annual within-herd incidence of clinical cases of Johne's disease was low and the disease was considered of little importance by most surveyed farmers. There was a moderate level of agreement regarding the JD status of a farm between its owner and their veterinarian. The two risk factors most strongly associated with the annual incidence of clinical cases of JD were a high proportion of Jersey cows in the herd and the purchase of bulls from a single source or more than four sources.

The management practices best suited to change in order to control JD are the purchase of JD free bulls, ensuring calves do not graze in the hospital paddock and ensuring young stock are at least two years of age before mixing them with adult stock. Changes to these practices could be implemented with comparative ease and low cost, and they would benefit the majority of infected farm owners.

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Chapter 4.

The effect of Johne's disease on milk production and risk of removal in four New Zealand dairy herds

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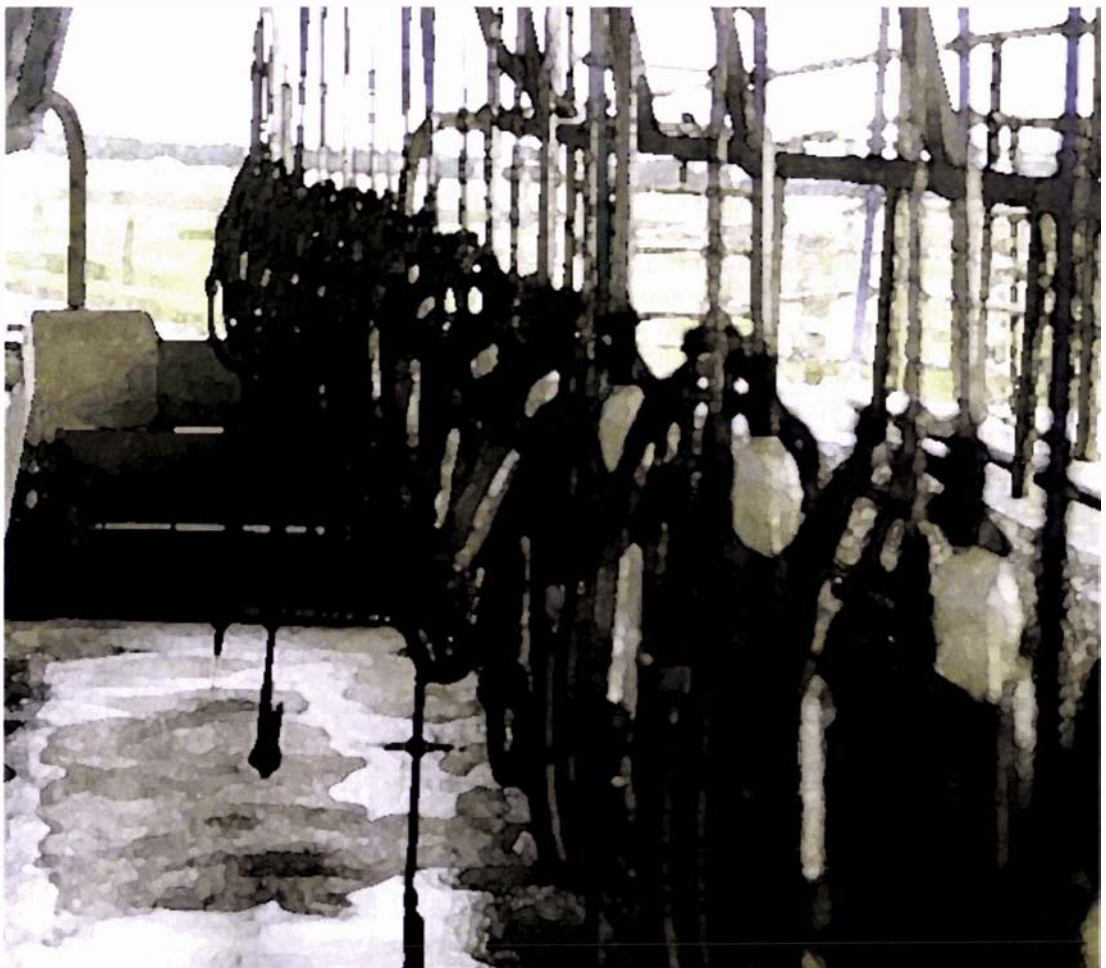
Abstract

AIM: To investigate the effect of sub-clinical Johne's disease (JD) on milk production and productive lifetime of New Zealand dairy cows, and secondly, to evaluate the effect of misclassification of disease status on productivity estimates.

METHODS: A longitudinal population study of dairy cows in four herds over four milking seasons was conducted. Cows were tested by serum ELISA and faecal culture for JD up to 11 times throughout the study. A general linear mixed model was used to quantify the effect of sub-clinical JD on milk production and a Cox proportional hazards model was used to quantify its effect on the time to removal. To accomplish the second objective, both models were applied to four subsets of the data in which cows had at least one, three, five, and seven tests for JD.

RESULTS: The within-herd true prevalence of JD ranged from 4.5% to 14.2%. For cows with at least one test for JD, the daily milksolids production for JD positive cows was 0.8% (95% CI -6.1%–4.5%, $P = 0.58$) less than that of JD negative cows. However, in one herd JD positive cows produced 14.9% (0.174kg of milksolids, $P<0.01$) less than JD negative herd mates daily. Relative to the reference herd, the annual hazard ratio of removal for JD positive cows was not significantly different from JD negative herdmates in two herds, but in the remaining herd it was 4.7 times ($P = 0.01$) and 1.4 times higher in cows older than 5 years and younger than 5 years, respectively. This herd also had significantly lower milk production in JD positive cows. The effect of JD on milk production and annual hazard of removal varied by only a small amount across the other subsets of data.

CONCLUSION: Johne's disease did not significantly influence milk production or annual hazard of removal in three of the four herds studied. However, the fourth herd experienced considerably lower milk production as well as a reduced productive lifetime due to JD. This suggests that control efforts would be most effective applied on a herd-by-herd basis. Differences between herds in the effect of JD on productivity may be linked to variation in virulence between different strains of the pathogen. Our results were insensitive to the effect of misclassification.

Introduction

Johne's disease (JD), caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is a chronic granulomatous enteritis endemic in ruminant species in many countries. The impact of JD on dairy cow productivity has been evaluated in several studies in the United States of America (Buergelt and Duncan 1978; Spangler et al 1992; Wilson et al 1993; Nordlund et al 1996; Johnson 2001; Gonda et al 2007; Raizman et al 2007), The Netherlands (Benedictus et al 1987), New Zealand (de Lisle 1989), Canada (McNab et al 1991; van Leeuwen et al 2002; Hendrick et al 2005), and Ireland (Barrett et al 2006).

At the individual cow level, the negative effect of JD on milk production is greatest when infected cows are close to or within the clinical stage of disease (Benedictus et al 1987; Raizman et al 2007). For example, cows with light, moderate, and heavy faecal excretion rates of MAP produced 537kg, 1403kg and 1534kg less milk per lactation than faecal culture negative cows (Raizman et al 2007).

Across all test positive cows, milk production is 300–500kg/lactation lower than for test negative cows. A study of 232 US dairy herds found that cows positive for JD by the ELISA and/or faecal culture test produced 304kg less milk per lactation than test negative cows (Gonda et al 2007). Similarly, ELISA positive cows produced 376kg less milk per lactation than ELISA negative cows in an earlier epidemiologic survey of 23 dairy herds (Nordlund et al 1996). Milk production was decreased by 548kg per lactation in faecal culture positive cows relative to test negative herd mates across nine high prevalence herds (Hendrick et al 2005).

The effect of JD on milk production varies between herds. It ranged from a decrease of 24% to an increase of 14% in the 23 herds studied by Nordlund et al (1996).

There is no clear consensus regarding the impact of JD on the productive lifetime of dairy cows. Measuring this impact is challenging as suspected sub-clinical infection is seldom confirmed at slaughter and these animals may be removed for other reasons associated with the disease, for example low production. JD did not significantly affect the number of cows replaced, or the number of cows slaughtered in the 974 herds studied by Ott et al (1999), or in the single, high prevalence Irish herd studied by Barrett (2006). In contrast, the hazard ratio of removal for faecal culture positive cows was 3.2 across nine high prevalence herds (Hendrick et al 2005) and in the study by Tiwari et al (2002), the odds of being culled during the 3 years after testing were 2.3 times greater in seropositive cows than in seronegative cows.

Variation between studies in the reported effect of JD on productivity may be due to misclassification of infection status which can be minimized by testing the study population repeatedly over time. It may also be due to differences in virulence between strains of MAP (Kunze et al 1991; Secott et al 2001).

Accurate estimates of the losses associated with JD would allow the financial impact of the disease to be calculated which in turn would influence decision making regarding the appropriate form of control. Estimates of loss are also vital inputs for simulation modeling (Groenendaal et al 2001; Kudahl et al 2007).

In New Zealand, clinical cases of JD had been suspected on about half of 427 herds in a cross sectional study but the incidence of clinical cases was low (see Chapter 3). Previously, the production index for faecal culture positive cows was, on average, 10% lower than for faecal culture positive cows across three seasons and six herds considered representative of the spectrum of within herd prevalence at the time (de Lisle 1989).

The aims of this study were to quantify the effects of JD on milk production and on the risk of removal for cows in herds that were typical of New Zealand's pasture based, seasonal calving dairy production system, and to explore the effects of misclassification of disease status on these estimates.

Materials and methods

4.1.1. Study herds

A longitudinal population study was conducted using a convenience sample of five dairy herds with a history of clinical Johne's disease in the Manawatu district of the North Island, New Zealand (40° 19' north and 175° 30' east). For the purpose of this study the herds were coded A, B, C, D and E. Herd managers agreed to participate and their herds were considered typical examples of New Zealand's seasonal calving, pasture based dairy production system. Table 4.1 lists the key features of the participant herds.

4.1.2. Data collection

The study spanned four milking seasons. It began late in the 1999/2000 season and concluded early in the 2002/2003 season. All herds utilised the production monitoring services offered by the Livestock Improvement Corporation (LIC). Individual animal event details such as birth date, calving dates, and removal reason and date for all animals that were present during the study period and milk volume, fat, and protein yields (derived from herd test events that were conducted on four occasions during each milking season) were retrieved in electronic format from LIC.

For each cow that was removed from the herd during the study period the herd manager selected one of 56 possible reasons for removal from a list of removal reasons provided by the herd recording services of LIC.

To detect JD in the study population, three herds were tested six times throughout the study. Herd D was tested four times but declined to participate on the last two scheduled visits. Herd E was tested three times but was then omitted from further study because JD was not detected. Testing occurred in early (October) and in late (May) lactation.

At the first herd visit, a sample of whole blood was taken by venipuncture of the caudal vein from all cows in the herd. On all subsequent herd visits a blood sample and a faecal sample were collected from all cows in the herd. In the largest of the five herds (herd D) faeces were collected from approximately 100 randomly selected cows because of budget constraints. Where previously sampled cows were no longer in herd D, they were replaced with herd mates such that the age distribution of the sampled fraction represented that of the entire herd. Herd managers were unaware of JD test results for the duration of the study.

Serology was conducted by Gribbles Veterinary Pathology Laboratory, Palmerston North, New Zealand, using the Johne's absorbed enzyme linked immuno sorbent assay (CSL ELISA, CSL Pty Ltd, Parkville, Victoria, Australia) and an in-house antigen prepared as described in Reichel et al. (1999). Culture of faeces was conducted by AgResearch Wallaceville. One gram of faeces was suspended in 40ml distilled water by shaking. The suspension was allowed to settle for 30 minutes and 5ml of the supernatant was transferred to a tube containing 35ml of 0.2% alkylbenzyl-dimethyl-ammonium chloride. After overnight decontamination the sediment was inoculated onto two tubes of Herrold's medium containing 3µg/ml mycobactin J, plus 50µg/ml amphotericin B, 200units/ml polymyxin B, 100µg/ml carbenicillin and 15µg/ml trimethoprim. Culture slopes were incubated at 37°C for four months.

4.1.3. Classification of Johne's disease status

A maximum of six ELISA and five faecal culture tests were possible per cow. Tests were interpreted in parallel such that any positive test defined a cow as infected (JD positive), while non-infected (JD negative) cows had no positive test at any time. Infection was assumed to have occurred during calf-hood and to persist for life.

The true within-herd prevalence was estimated using a previously published Bayesian model (see Method 3 in Chapter 5) which also estimated the performance of the ELISA and faecal culture tests. The sensitivity and specificity for the Johne's ELISA test was estimated at 26% (95% credible interval 11%–57%) and 98% (95% credible interval 96%–100%), respectively. The sensitivity and specificity for faecal culture was 30% (95% credible interval 14%–61%) and 99% (95% credible interval 99%–100%), respectively. These estimates were derived from a Bayesian model evaluating two diagnostic tests in four populations. The tests were considered dependent in only the infected subgroup of cows Covariance between the two tests in the infected subgroup was 0.24 (95% credible interval -0.28–0.56).

4.1.4.Statistical analyses

Statistical analyses were performed on production and demographic records for all cows that had been tested for JD. The number of ELISA and faecal culture tests varied between individuals.

True herd-level prevalence was estimated using the Bayesian model described above.

Analyses were conducted using the R statistical package (R Development Core team, 2004). The Linear and Nonlinear Mixed Effects Models Library (Pinheiro and Bates, 2000) and Survival Library (Lumley 2007), were used for the production and survival analyses. Significance was declared if $P < 0.05$.

Effect of Johne's disease on milk production

The effect of JD on daily milksolids production (the percentage fat plus the percentage protein multiplied by the total milk weight, kgMS) was first measured at the bivariate level using a two-sample t-test. It was then analysed as a function of days in milk (the interval from calving to the date of milk test, DIM) using a linear mixed model. Second and third order polynomials (Dohoo et al 2003) of days in milk were used to represent the shape of the lactation curve. A repeated measures error term with a first order auto-regressive correlation structure was included to adjust for correlation in repeated production measurements within cow (Diggle et al, 1994). The model was as follows:

$$Y_{ij} = \beta_0 + \beta_1 x_{1ij} + ... + \beta_9 x_{9ij} + Re_{ij} \qquad \qquad \text{Equation 1}$$

where Y_{ij} = daily milk solids production (kg) measurement i from cow j, $\beta_1 x_{1ij}$ to $\beta_9 x_{9ij}$ = the nine covariates: JD status, herd, breed, season, parity, JD status × herd, DIM, DIM^2, DIM^3, and Re_{ij} = residual error assumed to be correlated within cow in time. Model fit was assessed using the Akaike Information Criterion (AIC). {Burnham, 2003 #289}Plots of raw and normalized residuals were used to examine the homogeneity of variance of the error terms. The normal distribution assumption was evaluated by a normal probability plot of residuals.

Effect of Johne's disease on time to removal from the herd

For the survival analyses the outcome of interest was productive lifetime prior to removal from the herd. We defined productive lifetime as the interval from birth to the date of removal from the herd. Cows that died, survived past the end of the study or were sold for reasons not related to JD were treated as censored observations. Those cows that were present at the conclusion of the study were censored with the date of censoring equal to the date of the last milk test.

The frequency of removal events was compared between JD positive and JD negative cows using Pearson's chi-square test (Pearson 1941). Kaplan-Meier survival curves were used to describe the cumulative proportion of cows that remained in the herd as a function of age. Differences between categories within a variable were assessed using the log rank test. A Cox

proportional hazards model (Cox, 1972) (Equation 2) was developed to quantify the effect of JD status and other variables on the annual hazard of being removed from the herd. Variables with a significant log rank test were added first, then variables of biological interest followed by possible interaction terms and finally remaining variables to check for confounding. Herd was treated as a fixed effect.

$$h(t, X) = h_o(t) \exp(\beta_1 x_1 + ... + \beta_m x_m)$$ \hfill Equation 2

In Equation 2, $h(t,X)$ = the hazard function describing the instantaneous risk of removal occurring at time t, $h_0(t)$ = an arbitrary baseline hazard function, x_i = covariates: JD status, herd, breed and a time dependent covariate to compare the hazard of removal for JD positive cows less than five years old with that of JD positive cows older than five years.

The assumption of proportional hazards was evaluated by checking that the slope of a line fitted to the Schoenfeld residuals (Schoenfeld 1982) was approximately zero across all time points.

Sensitivity of results to misclassification of infection status
The likelihood that a cow's infection status is misclassified declines as the number of tests (ELISA and/or faecal culture) and duration of testing for that cow increases. Four data sets were derived from the complete data set. The first included all cows tested for JD and it comprised the main analysis. Three further subsets of data contained cows with at least three, five, or seven tests for JD respectively (Table 4.2). The models described in equations 1 and 2 were applied to each of these data subsets. An estimate of the sensitivity and specificity of JD detection for each subset (Table 4.2) was simulated using Equations 3 and 4 with a previously published Bayesian model (Method three in Chapter 5).

$$Se_{subset\ n} = 1 - (1 - Se_E)^a \times (1 - Se_F)^b - (positive\ covariance)^c$$ \hfill Equation 3

$$Sp_{subset\ n} = (Sp_E)^a \times (Sp_F)^b$$ \hfill Equation 4

Where:

n = the minimum number of tests for JD for cows in the subset: one, three, five or seven.

a = mean number of ELISA tests per cow in subset n.

b = mean number of faecal culture tests per cow in subset n.

c = average number of test pairs per cow (pair = simultaneous collection of serum ($_E$) and faecal ($_F$) samples).

The subsets represent increasing levels of confidence with which JD could be detected, thus providing a measure of the sensitivity of production parameter estimates to misclassification of JD status.

Results

4.1.5. Descriptive statistics

A total of 1541 cows took part in the study for which there were 5467 ELISA tests and 2444 faecal culture tests. Herd E was omitted after 225 ELISA tests and 167 faecal culture tests on 109 cows over 12 months failed to detect JD. A cow purchased by this herd had shown clinical signs of JD and was subsequently removed in the year prior to the study. Faecal culture results for herd C during the second sampling period could not be determined due to overgrowth of the samples.

After data screening, 1347 JD negative and 85 JD positive cows were available for milk production analysis, and 1373 JD negative cows and 87 JD positive cows were available for survival analysis.

JD positive cows were tested on average 7 times (median 7, range 1–11) and JD negative cows 5 times (median 4, range 1–11). The distribution of age at testing was similar for both JD positive and JD negative cows.

Eighty seven cows (6%) returned at least one positive test for JD during the study period. JD positive cows typically had their first positive reaction to a diagnostic test at 2, 5, or 6 years of age (Figure 4.1). The estimated true within-herd prevalence of JD was 4.5% (95% CI 2.6%–6.9%) in herd D, 5.2% (95% CI 3.2%–8.0%) in herd C, 8.1% (95% CI 5.0%–12.6%) in herd B, and 14.2% (95% CI 9.2%–20.6%) in herd A.

4.1.6. Milk production

Of 13047 milksolids measurements, 6.6% were from JD positive cows. A simple bivariate analysis showed that milksolids production by JD positive cows (1.27kg/day) was significantly higher than that of JD negative cows (1.16kg/day, $P < 0.01$). But after accounting for the effects of other variables, daily production of JD positive cows was 9 grams of milksolids, equivalent to 0.8% (95% CI -4.5%–6.1%, $P = 0.58$), less than the overall average for JD negative cows. The effect of JD varied between herds. In herd C, JD positive cows produced 0.174kg (95% CI 0.075Kg–0.273Kg, $P < 0.01$) less than JD negative herd mates, which equates to a reduction in milksolids production of 15.4% (95% CI 6.7%–24.2%). In contrast, production by JD positive cows in herd A was 6.2% (95% CI 0%–8.3%, $P = 0.05$) higher than JD negative herd mates (Table 4.3).

4.1.7.Hazard of removal from the herd

The average annual herd-level removal rate was 21%, 25%, 30%, and 26% for herds A, B, C and D during the study.

Failure to conceive was by far the most frequent reason for removal during the study. It was the reported reason for 83.3% of removal events in herd C and from 19.4% to 50.8% of removal events within the other herds. Mastitis and low production were the next most common reasons for removal.

JD was the recorded reason for only three (0.5%) of 558 removal events. Of these three, two cows had positive tests for JD, but the third had four negative tests.

During the study, 29/87 JD positive cows and 327/1373 JD negative cows were removed from their herds. Failure to conceive was the most commonly cited reason for both JD positive cows (17/29, 59%) and JD negative cows (158/298, 48%).

Log rank tests showed that JD positive cows were not removed from the herd significantly earlier than JD negative cows ($P = 0.90$, (Figure 4.2)) however differences in removal rate were evident between herds ($P < 0.01$) and between breeds ($P < 0.01$).

Multi-variable analysis (Table 4.4) showed significant differences between herds in the hazard of removal due to JD. In herd C, JD positive cows older than 5 years were 4.77 times ($P < 0.01$) more likely to be removed compared to the reference category. The effect was smaller but remained significant ($P < 0.01$) for JD cows up to 5 years of age (HR = 1.40) in herd C. The hazard ratio of removal was less than the baseline hazard for the other three herds. The R-square value for the model was 0.06 indicating that the data explained 6% of the total variation in the hazard of removal in the data.

4.1.8.Sensitivity of results to misclassification of infection status

Repeated testing greatly improved the sensitivity of detection of Johne's disease. Sensitivity was estimated at 0.80 (95% Bayesian credible interval 0.51–0.98) when analyzing all cows. It increased to 0.95 (95% Bayesian credible interval 0.75–1.00) by selecting only cows tested seven or more times but by doing this we excluded 73% of our study population from the analysis (Table 4.2).

The difference in daily milksolids production between the JD positive and the JD negative group changed only slightly as the sensitivity of detecting infection increased. When analyzing all 1432 cows, daily milksolids production by the JD positive group was 0.8% (95% CI -6.1%– 4.5%) less than the JD negative group (Table 5). When analyzing 372 cows with at least seven JD tests it was 1.6% lower (95% CI -7.7–4.6%). While the trend was for the difference in

productivity to be smallest when misclassification was most likely, the total difference across the subsets was only 1% change in daily production.

The annual hazard of removal for JD positive cows changed very little when analysing the different subsets of data. It remained significant in herd C and non-significant in the other herds. The coefficient for JD status ranged from 0.74 when all cows were analysed to 0.70 when only cows with seven or more tests were analysed and remained non-significant (Table 4.5).

Table 4.1. Johne's disease in four Manawatu dairy herds, May 2000–October 2002. Demographic, production, and testing details for the four study herds and the New Zealand average in 2002

Herd	A	B	C	D	New Zealand average
Median herd size [a]	101	115	259	571	262 [b]
Predominant breed	Friesian	Jersey	Friesian	Jersey	
Milksolids production (SD) [c]	1.19 (0.40)	1.20 (0.37)	1.15 (0.39)	1.17 (0.38)	1.01 [b]
Percentage of heifers in herd [d]	19%	22%	21%	17%	18.3 [e]
ELISA tests	566	635	1024	3014	
Fecal culture tests	377	533	441	826	

[a] Herd size on 1 December 1 averaged across 1999–2001.

[b] New Zealand Dairy Statistics for 2002 (Livestock Improvement Corporation 2007).

[c] Average daily milksolids production (1999–2001).

[d] An indication of the annual removal rate, averaged across 1999–2001.

[e] Average annual removal rate across 244 herds in 1999 and 2000 (Xu and Burton 2003).

Table 4.2. Johne's disease in four Manawatu dairy herds, May 2000–October 2002. Mean sensitivity and specificity of detecting cows with Johne's disease for subsets of cattle included in the sensitivity analyses

Group	Subset	Negative cows	Positive cows	Median sensitivity (95% credible interval [a])	Median specificity (95% credible interval [a])
≥ 1 tests	subset 1	1345	87	0.796 (0.513–0.979)	0.919 (0.855–0.978)
≥ 3 tests	subset 2	1073	78	0.860 (0.588–0.994)	0.903 (0.829–0.973)
≥ 5 tests	subset 3	682	68	0.906 (0.66–0.998)	0.891 (0.809–0.969)
≥ 7 tests	subset 4	327	45	0.949 (0.746–1.00)	0.878 (0.788–0.964)

[a] Bayesian credible interval.

Table 4.3. Johne's disease in four Manawatu dairy herds, May 2000–October 2002. A multivariable, mixed effects regression analysis showing the effect on daily milk production (kgMS) of Johne's disease status, herd, breed, season, parity and the interaction between Johne's disease status and herd

Explanatory variable	JD positive	JD negative	Coefficient (SE [a])	P-value
Intercept:			1.170 (0.009)	<0.01
DIM			-26.986 (0.208)	<0.01
DIM^2			-4.342 (0.205)	<0.01
DIM^3			0.730 (0.199)	<0.01
Johne's disease:				
Negative		1347	Ref	
Positive	85		-0.009 (0.031)	0.77
Herd:				
D	22	734	Ref	
A	24	116	0.240 (0.018)	<0.01
B	22	127	0.174 (0.014)	<0.01
C	17	370	-0.044 (0.013)	<0.01
Breed:				
Ayrshire	6	197	0.008 (0.012)	0.47
Friesian	35	509	0.127 (0.012)	<0.01
Jersey	44	641	Ref	
Season beginning:				
1999	62	807	Ref	
2000	72	1029	-0.017 (0.005)	<0.01
2001	65	1035	0.104 (0.006)	<0.01
2002	48	641	-0.079 (0.007)	<0.01
Parity:				
5+	47	566	Ref	
1	27	638	-0.308 (0.009)	<0.01
2	39	660	-0.188 (0.008)	<0.01
3	44	510	-0.076 (0.008)	<0.01
4	44	474	-0.012 (0.007)	0.09
JD × herd interaction:				
JD × herd D	22	734	Ref	
JD × herd A	24	116	0.087 (0.045)	0.05
JD × herd B	22	127	0.022 (0.046)	0.63
JD × herd C	17	370	-0.165 (0.05) [b]	<0.01

[a] Standard error, DF error = 407.

[b] Interpretation: in herd C, JD positive cows produced (-9–165)=174g less milk solids per day than negative cows (p < 0.01) after controlling for the effects of herd, breed, season, parity and lactation stage.

Table 4.4. Cox proportional hazards model showing the effect of Johne's disease, herd and breed on the annual hazard of being removed from the herd

Explanatory variable	JD positive	JD negative	Coefficient (SE[a])	P-value	Hazard ratio
Johne's disease					
Negative	0	1373	Ref		1.00
Positive	87	0	-0.297 (0.445)	0.50	0.74
Herd					
A	24	116	Ref		1.00
B	22	127	-0.139 (0.259)	0.59	0.87
C	19	384	0.534 (0.182)	<0.01	1.71
D	22	746	-0.631 (0.221)	<0.01	0.53
Breed					
Ayrshire	6	200	Ref		1.00
Friesian	35	519	0.067 (0.216)	0.76	1.07
Jersey	46	654	0.148 (0.182)	0.42	1.16
JD × herd interaction					
JD × herd A	24	116	Ref		1.00
JD × herd B	22	127	0.305 (0.576)	0.60	1.36
JD × herd C	19	384	1.323 (0.531)	0.01	3.76
JD × herd D	22	746	0.117 (0.732)	0.87	1.12
Time dependent covariate					
JD × age ≤5 years	87	1373	-1.225 (0.477)	0.01	0.29

[a] Standard error.

[b] Interpretation: in herd C, the mean annual hazard of removal for JD positive cows older than 5 years was 4.7 times ($P < 0.01$) that of the baseline hazard (JD negative cows older than 5 years in herd A). The comparable hazard of removal for JD positive cows in herd C less than 5 years old was 1.4 ($P = 0.01$) that of the baseline hazard.

Table 4.5. Effect of minimum number of tests for Johne's disease on difference in daily production and hazard of removal for test positive cows relative to test negative cows

Subset	Main analysis	Three	Five	Seven
JD test results	≥ 1	≥ 3	≥ 5	≥ 7
Daily milk production				
Per cent change due to JD [a]	-0.79 (-6.1–4.5)	-0.4 (-5.6–4.8)	-1.7 (-6.7–3.4)	-1.6 (-7.7–4.6)
P-value for JD status	0.77	0.88	0.52	0.61
AIC	-2367.459	-2307.798	-2020.059	-1236.241
Annual hazard of removal:				
Hazard due to JD	0.74	0.53	0.42	0.70
P-value for JD status	0.50	0.24	0.24	0.64
R-square	0.06	0.03	0.02	0.03

[a] Per cent change in daily milksolids production (kg)
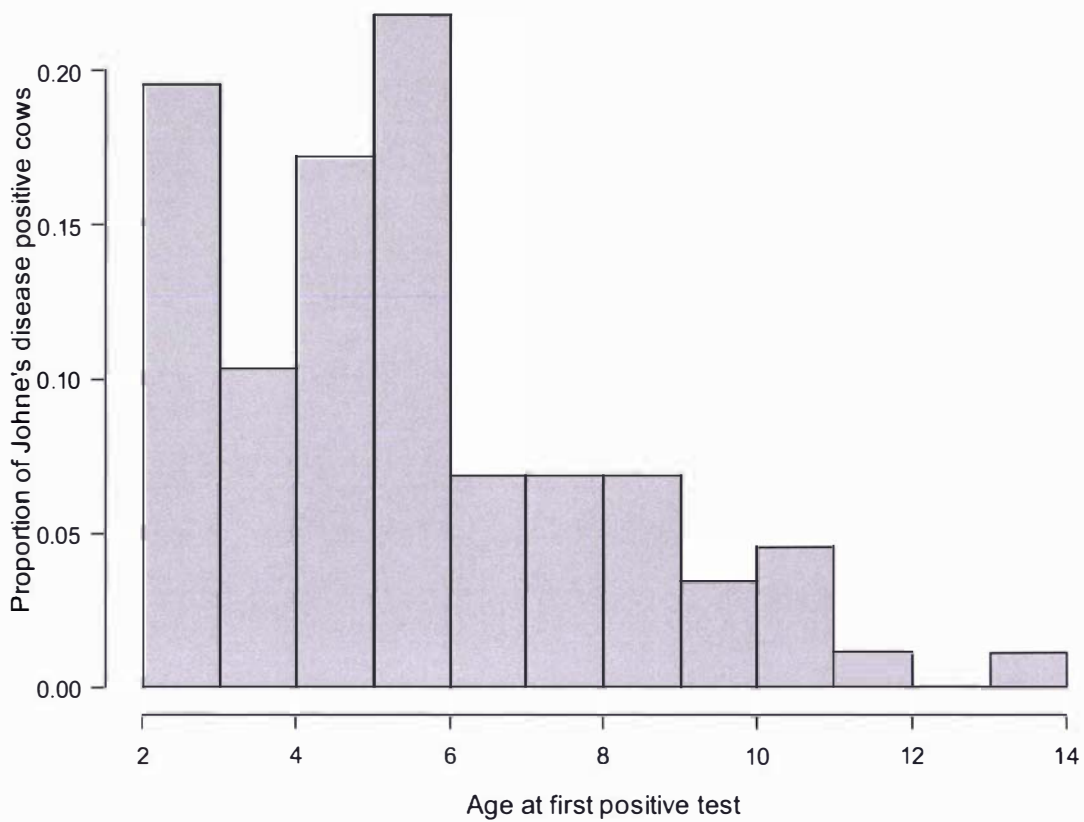
Figure 4.1. Johne's disease in four Manawatu dairy herds, May 2000–October 2002. Frequency histogram showing age (in years) at first positive ELISA or fecal culture test for 85 JD positive cows
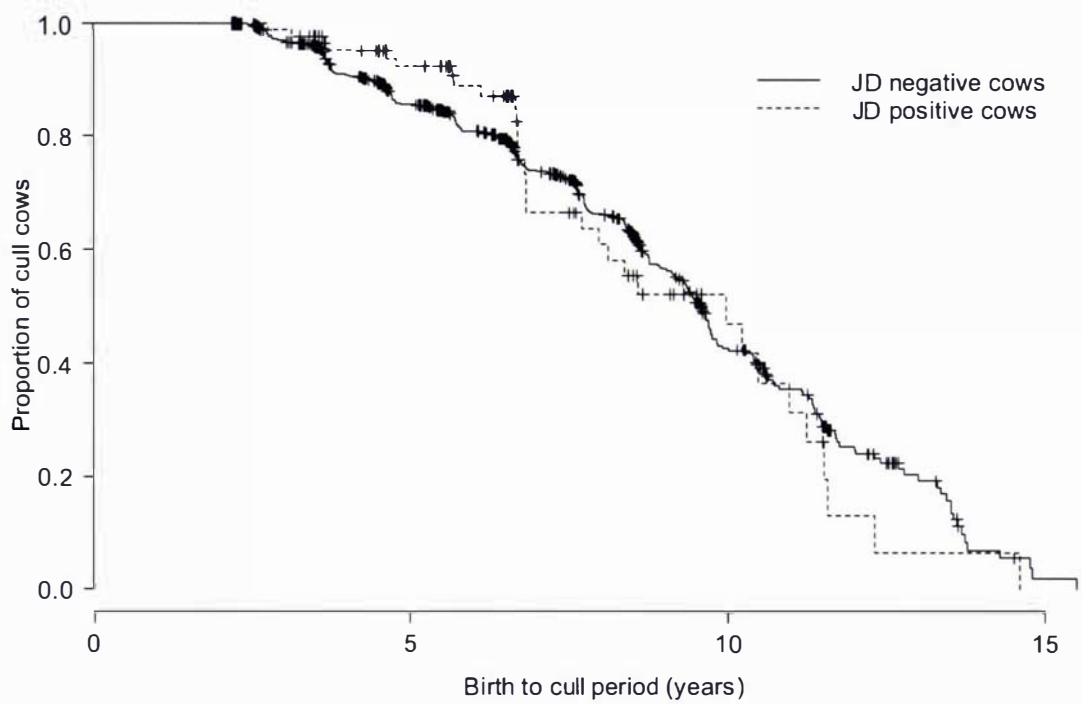
Figure 4.2. Johne's disease in four Manawatu dairy herds, May 2000–October 2002. Kaplan-Meier survival curves showing the survivorship as a function of age (in years), stratified by Johne's disease status

Discussion

This was a longitudinal population study to investigate the effect of Johne's disease on production and productive lifetime of dairy cows in four dairy herds selected as typical of the New Zealand dairy production system. Cows were tested for JD up to 11 times during the study. To maximize analytical power, we chose to include all cows in the analyses rather than a subset of cows with a high number of tests for JD. Reported differences are mostly due to the sub-clinical effects of disease as clinical cases were rare (0.5% of reported removal events).

The estimated true within-herd prevalence in our study ranged from 4.5% to 14.2%. Few previous studies have estimated the true prevalence of JD due to uncertainty regarding diagnostic test performance. Frequently, previously reported apparent prevalence has been close to the true prevalence in this study which suggests the disease was more prevalent in those herds than in the herds in this study. In 232 herds from six regions in Canada, the regional average within-herd sero-prevalence ranged from 4.6% in Ontario to 6.6% in Manitoba (Tiwari et al, 2006). In a cross sectional survey of 31 dairy herds in Wisconsin, USA the average seroprevalence was 10% (range 4.2%–28.6%) (Nordlund et al 1996). Prevalence was considerably higher (46.5%) in a study of five herds in Michigan, USA, based on faecal culture and ELISA (Johnson, 2001). There is little in the last study to explain the unusually high prevalence and the finding was not discussed by the authors. The average within-herd prevalence using faecal culture was 8.3% in an earlier study of six New Zealand herds considered to be a representative of the within herd prevalence at that time (de Lisle, 1989).

The overall effect of sub-clinical JD on milk production was negative but small (-0.8%), however the JD positive cows in herd C had 14.9% lower production than their herd mates, while JD positive cows in herd A had 6.2% higher production than herd mates. The financial loss due to decreased milksolids production per 270 day lactation would be NZD 244 for each test positive cow in herd C, given a value of NZD 5.50/KgMS. Large differences in the herd-level effect of JD were also reported by Nordlund et al. (1996). These authors found JD caused a significant reduction in milk production in two of the 23 herds studied, while higher production by JD positive cows relative to herd mates was also evident in one herd. The negative effect of JD on milk production varied from 7% to 12% in an earlier study of six New Zealand herds by de Lisle (1989). The finding of high losses in a small proportion of herds and no loss in the majority of herds suggests that the effect of JD depends heavily on herd level factors. Such factors could relate to herd management, or to differences in the virulence of MAP strains between herds.

The reported effect of JD on milk production varies considerably in the literature. In some instances it was large, for example 18.8% (Spangler et al, 1992) or 19.5%, (Benedictus, 1987) while in others it was non-significant (Johnson, 2001).

The magnitude of the effects reported by different studies may be influenced by the methods employed for selecting study units, defining disease status and statistical analysis. Three instances of possible bias were apparent. Firstly, studies of herds participating in JD eradication programs may report greater differences than those that exist across all JD positive herds if herds with a comparatively serious JD problem are more likely to join such a program (Benedictus et al, 1987; Wilson et al, 1993). Secondly, the primary aim of the study by Spangler et al (1992) was to evaluate the performance of diagnostic tests. Herds with high levels of JD are best suited to this purpose and a convenience sample of only two herds was analysed. Thirdly, the study by Ott et al (1999) classified herds with a single reactor to the ELISA test as negative unless greater than 5% of cows removed in the previous year from that herd had shown clinical signs of JD. This action was likely to misclassify infected herds as controls, and as a consequence bias any reduction in productivity due to JD toward the null.

The hazard of removal for individual cows was only significantly influenced by JD status in herd C. Furthermore, in herd C it was significantly greater for cows older than 5 years (HR = 4.76) than for cows younger than 5 years (HR = 1.40), possibly because more cows in the older age group were approaching the clinical phase of disease (Chiodini et al 1984a). The increased hazard of removal in this herd is consistent with a hazard ratio of removal of 3.2 in faecal culture positive cows reported by (Hendrick et al 2005).

JD was seldom the reported reason for removal possibly reflecting that farmers did not want their herd to be associated with JD. An annual incidence of clinical cases of 0.31 per 100 cows (see Chapter 3) suggests that each herd would have reported at least 5 clinical cases within the study period.

Johne's disease may increase the risk of removal for infected cows, but not the herd-level removal rate. The proportion of the herd replaced annually is relatively inelastic and this limits the culling decisions of farmers. Herds with higher rates of involuntary removal due to JD would have lower voluntary removal rates for other reasons. This would explain the apparent dichotomy between the similarity in removal rates between JD infected and test negative herds (Ott et al 1999; Barrett et al 2006), but the greater hazard of removal for JD positive cows, shown in this study and by Hendrick et al (2005). In this situation the presence of JD would slow development of the herd by reducing voluntary culling.

Herd C was experiencing a problem with infertility as well as JD. Failure to conceive was the reported reason for 83% of removal events in herd C, but it was also a common reason for removal from the other herds. While infertility was associated with JD in the single herd studied by Kopecky et al (1967) it was recently associated with a 1.4% higher pregnancy rate in the 232 herds studied by Gonda et al (2007). The evidence suggests that on average, infertility is not associated with JD infection.

It is interesting to speculate on reasons for the between-herd variation in the effect of JD identified in this study and the study by Nordlund et al (1996). Perhaps some strains of MAP are particularly virulent. It has been demonstrated that genetic variation exists in MAP recovered from bovine infections (Collins et al 1990) and that some genetic types are more frequently isolated than others (Pavlik et al 1995). A single genetic type may be found in distant geographic locations (Corn et al 2005). Secott et al (2001) showed that two strains of MAP, 5781 and 6594, differed in their ability to bind fibronectin depending on pH or the presence of an immunoglobulin, anti-FAP IgG. Fibronectin bound to MAP facilitates entry by MAP into the host (Secott et al 2004). While this suggests differences in the infectivity of MAP strains, the subject of pathogenicity remains to be thoroughly explored. Alternatively, poor management may be responsible for heavy losses due to JD.

It is conceivable but unlikely that a herd-level genetic resistance to JD exists, rather than a particularly virulent strain of the organism. Resistance of cows to JD has been studied and the heritability was considered low (about 0.1) (Koets et al 2000; Gonda et al 2006).

Sensitivity analyses showed that misclassification of JD status caused little change in the effect of JD on milk production and life span relative to their respective error bounds. This indicates that misclassification accounted for only a small amount of the total variation within the data. This finding may not be characteristic of other studies examining the effect of JD on productivity. An approach similar to ours, but of narrower scope was employed by Johnson (2001) to investigate misclassification of JD status but again no trend was observed due to the number of tests (one or two) or type (ELISA or faecal culture).

The consistency of our results across the subsets indicates that 'healthy worker' bias (McMichael 1976) was not introduced during the process of repeatedly testing cows. This bias arises if by selecting for analysis only cows tested repeatedly over some years, one also selected only high performing healthy cows which were less likely to have been removed from the herd. Investigation showed that JD positive and negative groups within a subset were alike in other respects, such as age distribution.

This study had three notable weaknesses. In particular, the small number of herds followed gives a narrow spectrum from which to generalize inferences about JD in New Zealand. These herds were not randomly selected; rather efforts were made to enroll herds that were considered typical of the 'average' New Zealand dairy herd. The financial and logistic requirement for studying many herds was prohibitive. A statistical weakness was the high AIC value and low R-square value which indicated that important information explaining milk production and life span was not included in our data. But the capability of our models was comparable with others describing such biological processes. The third weakness was that the manager of herd B was

informed of the JD test results for three cows. While unfortunate, the possible bias arising from this event was unlikely to influence our results.

We conclude that, overall, JD had little effect on productivity and productive lifetime of the cows studied, but that the effect varied substantially between herds. In one herd the losses were high. Accounting for misclassification of JD status did not change this conclusion. We suggest that further research should evaluate variation in virulence between strains of MAP. We also suggest that the herd-level effect of JD on milk production be evaluated in many herds and the results used to construct a distribution describing variation in the effect of JD across a population of herds.

4.1.9. Acknowledgements

Chapter 5.

Evaluation of diagnostic tests for Johne's disease in New Zealand dairy cows

S Norton, W Johnson, G Jones and C Heuer

Abstract

AIM: To quantify the sensitivity and specificity of the CSL serum ELISA and the faecal culture test, and estimate the prevalence of Johne's disease (JD) in New Zealand dairy herds using Bayesian statistical analysis.

METHODS: Cows in four New Zealand dairy herds were tested simultaneously by ELISA and faecal culture five times over three lactations. Test results were dichotomised. A latent-class regression model was developed that considered test sensitivity as a function of the covariates parity and lactation stage, and prevalence of JD as a function of the covariate herd. It was applied to a cross sectional subset of the data and the full, repeated measures, data set. Results from this model were compared with results from three other analytical methods using the cross sectional data. These methods were a frequentist pseudo gold-standard and two Bayesian latent class, 2 test, 4 population models; the first assumed tests to be independent, the second assumed tests to be dependent in the infected population. Results are presented for the complete (repeated measures) data set and the cross sectional subset.

RESULTS: Using the regression model, sensitivity of the ELISA test was clearly higher in older animals. There was also evidence that faecal culture sensitivity was higher in older animals. Both faecal culture and ELISA test sensitivity were lower in late lactation. Prevalence was lower and faecal culture sensitivity higher when analysing repeated measures data.

CONCLUSION: The regression model enables a more accurate diagnosis of Johne's disease to be made because it incorporates cow specific information in the diagnosis, such as age and lactation stage. The model also enables us to incorporate previous test results for an individual when diagnosing disease. The trends in results from the regression model support the current understanding of the disease process. A larger data set may have enabled the model to estimate test parameters with narrower margins of error.

Introduction

Johne's disease (JD), caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is a chronic enteritis of ruminant species. It is of particular importance in the dairy industry. In cattle, the disease is characterised by infection in the young calf by the faecal-oral route then a latent stage of some years. Progressive disease usually follows with clinical signs typically worsening diarrhoea, weight loss and reduced milk production (Chiodini et al 1984a).

The latent stage of infection is associated with poor diagnostic sensitivity when tests for JD are applied at the herd level. This has lead to the supposition that for every cow with clinical signs of JD, a further 15 to 20 have subclinical infection (Whitlock et al 2000).

Improvement and validation of existing diagnostic tests for JD has been the subject of considerable work (Milner et al 1987; Colgrove 1989; Milner et al 1990; Cox et al 1991; Billman-Jacobe 1992; Sockett et al 1992; Spangler et al 1992; Nielsen 2000, 2002; Eda et al 2006). This research has generally focussed on the more popular tests; the enzyme linked immunosorbent assay (ELISA) test for serum antibody and the culture of MAP from faecal material.

The evaluation of diagnostic test performance involves challenges that cannot easily be overcome by sampling methods or laboratory technology. The first is the absence of a gold-standard ie the true infection status of a tested individual remains unknown. Thorough post-mortem examination, during which tissues from a range of sites are tested for MAP by histopathology, culture and DNA detection techniques (PCR) provides the most conclusive diagnosis of infection status. It is rarely feasible to conduct post-mortem examinations of this detail on a large sample of trial animals in order to validate the results of a diagnostic method. Consequently misclassification rates of JD tests under field conditions are difficult to establish. A possible, but time consuming and expensive alternative, is to repeatedly test an animal for JD throughout its lifetime. This will allow infection status to be determined with greater certainty. In the absence of a gold standard, the performance of ELISA tests has previously been validated using faecal culture as the gold standard (Cox et al 1991; Sweeney et al 1995; Dargatz et al 2001a). Imperfect sensitivity of the faecal culture test will result in a spurious inflation of the estimate of ELISA sensitivity unless accounted for in the analysis.

A second challenge is dependence (correlation) between the results of diagnostic tests. Dependence is likely to occur between two tests if they measure a similar biological process (Gardner et al 2000) and may exist in the infected population, the non-infected population, or both. In the case of JD, dependence in the non-infected population can be considered negligible due to the high specificities of the commonly used tests.

Another challenge is that the sensitivity of tests for JD changes with host specific factors, such as the stage of disease and the stage of lactation (Nielsen et al 2002). In the early, pre-clinical stage of disease, neither antibodies nor faecal shedding of MAP are detectable. During more advanced and clinical stages of disease, cows develop high levels of antibody and shed large numbers of MAP in their faeces. Previously reported estimates of ELISA sensitivity range from 15.4% to 88.1% where increasing sensitivity was positively correlated to the progression of disease (Dargatz et al 2001b).

Probability models, based on Bayes' theorem (Bayes 1763), interpret the concept of probability as the degree of belief in, or the uncertainty about, a proposition. For example, the proposition that an individual is infected with a disease. In these models, probability is determined by sampling from a probability distribution with a Markov chain Monte Carlo algorithm (Gelfand and Smith 1990). 'Latent class' Bayesian models estimate the probability that an individual is infected, despite the true infection status of that individual remaining unknown, ie in the absence of a gold standard. There is a growing body of research in veterinary science describing the application of latent class models. First, Johnson et al (2001) presented a Bayesian version of the model developed by Hui and Walter (1980), in which the performance of two independent diagnostic tests was estimated in the absence of a gold-standard using two populations with different prevalence. Subsequently, two approaches were developed to adjust for dependence in the two-test model, one by Dendukuri and Joseph (2001) in which covariance parameters were included to adjust for dependence between two correlated tests, conditional on disease status, and another by Georgiadis et al (2003) . In the review by Branscum et al (2005) the approach by Dendukuri and Joseph was preferred over that of Georgiadis et al (2003) on the grounds of simplicity.

As yet there are no latent class statistical approaches that consider variation in test performance between covariates, or that can incorporate repeated testing of an individual when estimating diagnostic test performance and prevalence.

In this study, a novel Bayesian regression model, that incorporated cow-level covariates and used repeated measure data to estimate diagnostic test performance and prevalence, was compared to three existing analytical methods. The existing methods were a frequentist pseudo-gold-standard approach, and modified versions of two previously published Bayesian models. The original codes for the two latter models are available from the UC Davis website (www.epi.ucdavis.edu/diagnostictests/software) as 'two independent tests, two populations, no gold standard' and 'two dependent tests, two populations, no gold standard'.

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Materials and methods

5.1.1. Study herds

A longitudinal population study was conducted using a convenience sample of four dairy herds with a history of clinical Johne's disease in the Manawatu district of the North Island, New Zealand (40° 19' north and 175° 30' east). The herds were coded A, B, C and D. Herd managers consented to a request for participation and the herds were considered typical examples of New Zealand's seasonal calving, pasture based dairy production system.

5.1.2. Data collection

The data was collected from October 2000 to October 2002. Unique animal identification was provided by the production monitoring services offered by the Livestock Improvement Corporation (LIC).

Herds were tested for JD twice per milking season, once in early lactation (within two months of the start of the lactation period) and again in late lactation (within two months of the end of the lactation period). Three herds were tested five times and one herd was tested three times during the study.

At each herd visit, a sample of whole blood was taken by caudal venipuncture and a faecal sample collected. The simultaneous collection of a blood sample and faecal sample was defined as a single test-event. All milking cows in herds A, B and C were sampled at each visit. Herds A and B milked approximately 100 cows, while herd C milked approximately 250 cows. In herd D, which milked about 500 cows, samples were collected from a subset of approximately 100 cows. When removed from the herd, members of this subset were replaced with herd mates by random selection within age group, so that the age distribution of the sampled fraction continued to represent that of the entire herd.

Serology was conducted by a commercial veterinary laboratory using the Johne's absorbed EIA at a single cut-off value recommended by the kit (CSL ELISA, CSL Pty Ltd, Parkville, Victoria, Australia) and an in-house antigen. Culture of faeces was conducted by AgResearch Wallaceville. See Chapter two for a more detailed description of the serological and culture methods. Results were treated as dichotomous.

5.1.3.Data structure

The data had a hierarchical structure with three levels. The uppermost level was herd, then individual cow, and the lowest level was test-event repeated within cow.

A subset of the data was used for the first four test evaluation methods. It was cross-sectional and comprised the first test event for each cow, regardless of when the cow entered the study, thus omitted repeated tests of the same cow. The fifth method used the entire data set, thus incorporating repeated tests.

5.1.4.Methods for evaluating the performance of diagnostic tests

A summary of the five methods used is presented in Table 5.1. A detailed description of each method follows. Bayesian models were developed in Winbugs (Spiegelhalter et al 1996) while frequentist analysis was performed in the R statistical package (R Development Core team, 2004).

Method 1: Frequentist pseudo gold-standard
A frequentist approach was used to develop a pseudo gold-standard (Dohoo et al 2003). By this standard, any cow with a positive test at any time during the follow up period was considered infected with JD, regardless of previous or subsequent results. The true prevalence for each herd was defined as the proportion of cows that had a positive test of either type at any time during the study relative to the total number of cows present during the observation period in that herd.

Test sensitivity when applied once to each cow was estimated for the ELISA test and for the faecal culture test with reference to the pseudo gold-standard. Confidence intervals were calculated using standard methods based on binomial proportions. By definition of this method specificities were assumed to be perfect.

A point estimate and confidence interval for the covariance between ELISA and faecal culture test results within the infected population was obtained using the formula of Gardner et al (2000).

Method 2: Bayesian approach using four populations and two independent tests
The Bayesian form of the Hui-Walter (1980), model developed by Johnson et al (2001) (Equation 5), extended easily to the data. Two modifications were made to the model. First, the point mass component (Johnson et al 2001) was removed from zero, in the distribution describing prevalence in the low prevalence population. This component represented confidence that the prevalence in that population was zero, which was not the case in our study. The second modification was extension of the model from two to four populations. Each study herd was considered to be a population. The tests were assumed to be conditionally independent, that is, we assumed that

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there was no correlation between the two tests in their ability to detect infection, nor in their ability to detect freedom from infection.

The data consisted of a 2 x 2 contingency table for each herd (Table 5.2).

The cell counts corresponding to the ith row and jth column for population k were modelled with independent multinomial distributions where

$y_k \sim$ multinomial$(n_k, (p_{11k}, p_{12k}, p_{21k}, p_{22k}))$, and

$$p_{11k} = P_k(T_1+, T_2+) = pi_k \times Se_E \times Se_F + (1-pi_k) \times (1-Sp_E) \times (1-Sp_F)$$ (Equation 5)
$$p_{12k} = P_k(T_1+, T_2-) = pi_k \times Se_E \times (1-Se_F) + (1-pi_k) \times (1-Sp_E) \times Sp_F$$
$$p_{21k} = P_k(T_1-, T_2+) = pi_k \times (1-Se_E) \times Se_F + (1-pi_k) \times Sp_E \times (1-Sp_F)$$
$$p_{22k} = P_k(T_1-, T_2-) = pi_k \times (1-Se_E) \times (1-Se_F) + (1-pi_k) \times Sp_E \times Sp_F$$
$$k = 1, 2, 3, 4.$$

Where Se_E = ELISA sensitivity, Se_F = faecal culture sensitivity, Sp_E = ELISA specificity, Sp_F = faecal culture specificity, T_1 = ELISA, T_2 = faecal culture and pi = prevalence. Prior information for this model is presented in Table 5.3.

Method 3: Bayesian approach using four populations and two semi-dependent tests
For Method 3 the data were the same as in Method 2 but the model was extended from that in Method 2 by addition of the positive covariance parameter (rhoDp, Equation 7) (Dendukuri and Joseph 2001). The cell probabilities were then obtained under the assumption that the tests were conditionally independent in the non-infected population, but conditionally dependent in the infected population, that is, there was correlation between the two tests in their ability to detect infection (Equation 6).

$$p_{11}k = P_k(T1+, T2+) = pi_k \times (Se_E \times Se_F + covDp) + (1-pi_k) \times (1-Sp_E) \times (1-Sp_F)$$
$$p_{12}k = P_k(T1+, T2-) = pi_k \times (Se_E \times (1-Se_F) - covDp) + (1-pi_k) \times (1-Sp_E) \times Sp_F$$ (Equation 6)
$$p_{21}k = P_k(T1-, T2+) = pi_k \times ((1-Se_E) \times Se_F - covDp) + (1-pi_k) \times Sp_E \times (1-Sp_F)$$
$$p_{22}k = P_k(T1-, T2-) = pi_k \times ((1-Se_E) \times (1-Se_F) + covDp) + (1-pi_k) \times Sp_E \times Sp_F$$

To ensure a proper probability model, the covariance parameter must be constrained to values between zero and one (Equation 7). Thus we define

$$ls = (Se_E - 1) \times (1-Se_F), us = \min(Se_E, Se_F) - Se_E \times Se_F,$$ (Equation 7)
$$rhoDp = covDp / sqrt[Se_E \times (1 - Se_E) \times Se_F \times (1 - Se_F)]$$

Prior information was identical to that in the model assuming independent tests (Table 5.3) with the addition of a prior for the covariance parameter, with the uniform distribution dunif(ls, us).

Method 4: Bayesian approach to evaluating test performance as a function of covariates, using cross sectional data

A novel Bayesian approach referred to as the 'regression model' was used in Methods 4 and 5. It modelled the sensitivity of each test as a function of the covariates parity and lactation stage while the prevalence was modelled as a function of the covariates herd and breed. We assumed that the two tests were independent, based on the results of Methods 1 and 3. We also assumed that test specificity would not vary as a function of covariates.

Sensitivity of the ELISA test and the faecal culture test were modelled on the logit scale for each of the n test events (Equation 8). The covariate parity, categorised as 1, 2, 3, 4, and >4, was included as a proxy for stage of infection. The covariate lactation stage was categorised as either early or late. It was included to represent stress levels on the cow associated with parturition and the beginning of lactation. The sensitivity for each cow was modelled as

$$\text{logit}(Se_E[j]) \mathrel{<\!\!-} g(\text{parity}[j]) + g[6] \times x[j]$$
$$\text{logit}(Se_F[j]) \mathrel{<\!\!-} h(\text{parity}[j]) + h[6] \times x[j] \qquad \text{(Equation 8)}$$

where j denotes the jth cow in the data, parity$[j]$ its parity, and x$[j]$ its lactation stage. In this parameterisation, for each test, five coefficients are obtained one for the sensitivity at each parity level, in late lactation. A sixth coefficient is obtained for the effect of early lactation stage by the process of inducing a prior (Bedrick et al 1997). In this process a prior representing the effect of early lactation stage is applied to test sensitivity in parity one cows and the respective coefficient describes sensitivity in parity one cows in early lactation. The difference in sensitivity between early and late lactation is estimated for cows in parity one and it is assumed that this difference is the constant across all parity groups. Prior information describing the effect of parity and lactation stage on the test sensitivities is given in Table 5.4.

The prevalence was modelled on the logit scale for each cow and was a function of herd,

$$\text{logit}(pi[j]) \mathrel{<\!\!-} b(\text{herd}[j]) \qquad \text{(Equation 9)}$$

where herd$[j]$ identifies the herd of cow j and b is the associated fixed effect. Prior information regarding the prevalence in each herd is given in Table 5.4. Prior information on the within-herd prevalence was obtained before data collection commenced, based on the herd manager's perception of the annual incidence of clinical cases of JD and the assumption that for every clinical case observed, there were five more infected cows in the herd. Priors describing the change in ELISA test sensitivity across parity groups were set to be only slightly informative after considering the studies by Nielsen et al (2002) and Jubb et al (2004). Priors for faecal culture followed a similar pattern to this, but reflected our belief that faecal culture was more sensitive than the ELISA test. The prior for change in sensitivity due to lactation stage reflected

our belief that sensitivity would probably be slightly higher in early lactation, due to the higher stress level experienced by cows during this period.

Method 5: Bayesian approach to evaluating test performance as a function of covariates, using repeated measures data

In Method 5 the regression model (described in Method 4) was applied to the full repeated measures data set. Repeated tests on an individual were assumed to be independent. This assumption is discussed in detail in the discussion.

5.1.5.Assessment of model performance

To monitor the performance of the regression model, the estimated probability that an individual was infected, given its test results (predictive value positive), was monitored for a sample of 10 cows. This was compared with predictive value positive values calculated from a spreadsheet that used the regression model estimates for overall sensitivity, specificity and herd-level prevalence.

All models were run for 100,000 iterations after discarding an initial 5,000 as a burn in period.

Convergence to a single distribution was checked using three sets of starting values. The degree of autocorrelation was monitored using Gelmin-Rubin-Brooks plots (Brooks and Gelman 1998).

Posterior distributions of interest were described using the median and 95% credible intervals. Sensitivity, specificity, prevalence and covariance values were reported as percentages.

To ensure the regression model (Method 4 and 5) was stable, a sensitivity analysis was conducted. The model was re-run after a single prior was changed within biologically sensible limits and such that the prior retained a sensible distribution. This process was repeated for four priors in total, two for diagnostic test sensitivity, one for specificity and one for Johne's disease prevalence.

Results

In all, 779 cows were tested. For these cows there were 2046 test events, that is, when both tests were used on the same animal simultaneously. Two hundred and fifty cows had one test event, 146 had two test events, 151 had three test events, 109 had four test events and 123 had five test events. In herds A, B, C and D there were 131, 142, 235, and 271 cows tested. Sixty five cows had at least one positive test, while 98/2046 test events were positive for one or both tests. Descriptive statistics for the study herds and diagnostic testing history are presented in Table 5.5.

5.1.6. Overall Sensitivity of the ELISA test

Point estimates of the overall sensitivity of the ELISA test (Figure 5.1) ranged from 26.0% (Method 3; Table 5.6) to 41.4% (Method 4; Table 5.7). Estimates by Methods 4 and 5, which used the regression model, were similar. However, the error bounds for all methods were wide.

5.1.7. Overall Sensitivity of the faecal culture test

The point estimates for the overall sensitivity of the faecal culture test (Figure 5.2) ranged from 29.3% (Method 3; Table 5.6) to 74.1% (Method 5; Table 5.7). With Method 5, sensitivity was clearly higher than when estimated by the other methods, but again the error bounds for all methods were wide.

5.1.8. Specificity of the ELISA test

Estimates of the specificity of the ELISA test ranged from 97.7% to 99.5%. The regression model (Methods 4 and 5) had higher point estimates and narrower error bounds than the simpler Bayesian models (Methods 2 and 3).

5.1.9. Specificity of the faecal culture test

Estimates of the specificity of the faecal culture test ranged from 98.5% (Method 4; Table 5.7) to 99.8% (Methods 2 and 3; Table 5.6). The results from the regression model using cross sectional and repeated measures data were very similar. They were also slightly lower and had wider error bounds than the simpler Bayesian approaches.

5.1.10. ELISA sensitivity within levels of covariates

The general trend was for estimates of ELISA sensitivity to be lowest in parities one and two, and higher in parities three and above (Table 5.7). This trend was shown by both Methods 4 and 5.

Early lactation was associated with increased sensitivity of the ELISA test, relative to sensitivity in late lactation, by a consistent magnitude using Methods 4 (increase of 31.6%) and 5 (increase of 28.1%; Table 5.7).

5.1.11. Faecal culture sensitivity within levels of covariates

The sensitivity of the faecal culture test estimated by Method 5 was highest in parities greater than three, but also high in parity one. When estimated by Method 4, no trend was evident across

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parity groups. The error bounds associated with the estimates by both methods were wide (Table 5.7).

Early lactation was associated with an increase in faecal culture sensitivity, relative to sensitivity in late lactation, in both Methods 4 (increase of 31.9%) and 5 (increase of 18.1%; Table 5.7).

5.1.12. Prevalence

Estimates of the true herd-level prevalence varied among the five methods (Figure 5.3). The ranges for each herd obtained from the five analytical methods were: for herd A 4.1%–28.7%, for herd B 6.8%–14.8%, for Herd C 3.2%–6.2% and for herd D 2.3%–7.0%.

For three of the four herds point estimates of prevalence by the regression model using cross sectional and repeated measures data were similar. But in herd A the prevalence was lower when estimated with repeated measures data. Regression model point estimates of prevalence were generally slightly lower than estimates by the other methods. Error bounds for each herd-level prevalence were noticeably wider when estimated using Method 3 than when estimated by the other methods.

5.1.13. Covariance between the ELISA and faecal culture test in the infected population

Covariance between the ELISA and faecal culture test in the positive population was estimated to be very small, put positive using the pseudo gold-standard method (0.011, 95% confidence interval 0.005–0.020) and not significantly different from zero using Method 3 (0.240, 95% credible interval -0.285–0.556) (Table 5.6). This suggests that there was little dependence between the two tests.

5.1.14. Sensitivity analysis

Additional information provided by repeated measurements substantially changed the probability that an individual cow was infected (Table 5.8). Cows with subsequent positive tests (for example cows 3 and 6) were much more likely to be infected. The probability that cows were infected after multiple negative tests was always lower than when only the single negative test event was considered. The probability of infection was also influenced by lactation stage and parity at testing and the within-herd prevalence which explains the variation in probability of infection between cows with a similar pattern of test results.

The model remained stable in response to changes in prior values. Only the value for which the prior was altered showed any change in response to the new prior. The greatest change was a

decline of 6% to the sensitivity of faecal culture for cows in parities greater than four of 34.3. This change and the others resulting from the sensitivity analyses were very small in comparison to the error bounds surrounding the point estimates.

All Markov chains converged consistently to the same distributions when initiated from different starting values. Autocorrelation had generally disappeared completely after a lag of between 10 and 20 iterations and there was no evidence of poor mixing.

Table 5.1. Description of statistical methods used to evaluate the performance of the ELISA and faecal culture tests for Johne's disease

Description	Method				
	1	2	3	4	5
Statistical approach	Frequentist	Bayesian model 1	Bayesian model 2	Bayesian model 3[a]	Bayesian model 3
Data	Cross sectional	Cross sectional	Cross sectional	Cross sectional	Repeated measures
Data set size (test events)	779	779	779	779	2046
Assumption of dependence between tests	Independent	Independent	Semi-dependent	Independent	Independent
Sensitivity modelled as function of covariates	No	No	No	Yes	Yes

[a] WinBugs code included in Appendix one.

Table 5.2. Cross tabulated results for an ELISA (E) test and faecal culture test (FC) applied to 779 dairy cows from four New Zealand dairy herds

herd A

	FC +	FC -
E +	0	13
E -	7	111

131

herd B

	FC +	FC -
E +	4	1
E -	5	132

142

herd C

	FC +	FC -
E +	3	0
E -	9	223

235

herd D

	FC +	FC -
E +	3	0
E -	5	263

271

Table 5.3. Prior information describing the sensitivity and specificity of the ELISA and faecal culture tests and the prevalence in the four populations

Parameter	Prior estimate	95th Percentile	Distribution
ELISA sensitivity	0.25	<0.80	dbeta (1.40, 2.10)
ELISA specificity	0.95	>0.65	dbeta (8.50, 1.40)
Faecal culture sensitivity	0.40	<0.80	dbeta (2.06, 2.60)
Faecal culture specificity	0.98	>0.70	dbeta (9.20, 1.17)
Herd-level true prevalence			
Herd A	0.15	<0.60	dbeta (1.90, 4.61)
Herd B	0.15	<0.60	dbeta (1.90, 4.61)
Herd C	0.05	<0.50	dbeta (1.36, 5.12)
Herd D	0.05	<0.50	dbeta (1.36, 5.12)

Table 5.4. Prior information for the sensitivity of the ELISA test and faecal culture test for Johne's disease, at each parity and for early and late lactation, and herd level prevalence (Methods 4 and 5)

Parameter	Prior estimate	95th Percentile	Distribution
ELISA sensitivity in late lactation:			
Parity 1	0.20	<0.75	dbeta (1.38, 2.51)
Parity 2	0.30	<0.85	dbeta (1.33, 1.78)
Parity 3	0.50	<0.85	dbeta (2.23, 2.23)
Parity 4	0.50	<0.85	dbeta (2.23, 2.23)
Parity >4	0.40	<0.85	dbeta (1.62, 1.94)
ELISA sensitivity in parity 1, early lactation	0.23	<0.75	dbeta (1.48, 2.59)
Faecal culture sensitivity in late lactation:			
Parity 1	0.25	<0.80	dbeta (1.38, 2.15)
Parity 2	0.35	<0.85	dbeta (1.46, 1.85)
Parity 3	0.55	<0.90	dbeta (1.75, 1.62)
Parity 4	0.55	<0.90	dbeta (1.75, 1.62)
Parity >4	0.45	<0.85	dbeta (1.87, 2.06)
Faecal culture sensitivity in parity 1, early lactation	0.28	<0.80	dbeta (1.47, 2.21)
Herd-level prevalence			
Herd A	0.15	<0.60	dbeta (1.55, 4.12)
Herd B	0.15	<0.60	dbeta (1.55, 4.12)
Herd C	0.05	<0.50	dbeta (1.25, 4.88)
Herd D	0.05	<0.50	dbeta (1.25, 4.88)

Table 5.5. Descriptive information for study farms and diagnostic testing

Farm	A	B	C	D
Cows sampled	131	142	235	271
Predominant breed	Friesian	Jersey	Fricsian	Jersey
Test events				
Early lactation (00/01)	100	110	109	160
Late lactation (00/01)	65	105	94	152
Early lactation (01/02)	104	107	175	176
Late lactation (01/02)	92	101	0	146
Early lactation (02/03)	77	88	0	85
Total	438	511	378	719

Table 5.6. Estimates of ELISA and faecal culture test performance for Johne's disease and estimates of prevalence of infection using a pseudo gold-standard approach (Method 1), Bayesian model assuming independent tests (Method 2) and Bayesian method assuming semi-dependent tests (Method 3)

	Estimate and 95% error bound		
Parameter	Method 1 [a]	Method 2 [b]	Method 3 [b]
ELISA Sensitivity	30.4 (20.2–40.5)	35.4 (19.7–55.0)	26.0 (11.3–56.8)
Faecal culture Sensitivity	45.6 (34.6–56.6)	34.1 (18.3–59.6)	29.3 (14.4–60.8)
ELISA Specificity	1 [c]	98.3 (96.5–99.7)	97.7 (95.7–99.4)
Faecal culture Specificity	1 [c]	99.8 (99.1–1.0)	99.8 (99.0–99.9)
Covariance (Inf) [d]	0.011 (0.005–0.020)	N/A	0.240 (-0.285–0.556)
Herd-level prevalence			
Herd A	19.1 (12.4 25.8)	15.3 (10.2–21.7)	28.7 (13.3–53.6)
Herd B	14.8 (8.9–20.6)	8.4 (5.0–13.1)	13.4 (5.1–30.8)
Herd C	6.0 (2.9–9.0)	5.3 (3.2–8.4)	6.2 (1.8–16.0)
Herd D	7.0 (4.0–10.0)	4.2 (2.4–6.6)	4.4 (1.3–12.0)

[a] Confidence intervals are reported for Method 1.

[b] Credible intervals are reported for Method 2 and 3.

[c] Specificity of diagnostic tests was assumed to be one to facilitate definition of pseudo gold-standard.

[d] Covariance between the ELISA test and faecal culture test in the infected population.

Table 5.7. Estimates of overall and co-variate level sensitivity and specificity for ELISA and faecal culture, and herd level prevalence, using a Bayesian model and either a single simultaneous application of both tests to 779 dairy cows (Method 4) or repeated applications of both tests (Method 5)

	Estimate (95% credible interval)	
Parameter	Method 4	Method 5
ELISA		
Overall sensitivity	41.4 (25.5–65)	38.2 (26.5–53.5)
Overall specificity	99.7 (98.9–99.9)	99.1 (98.6–99.4)
Sensitivity by parity (late lactation)		
Parity one	22.9 (5.7–54.3)	25.8 (9.5–50.7)
Parity two	26.5 (4.8–69.6)	24.7 (4 .0–66.9)
Parity three	48.3 (17.4–83.1)	44.4 (20.5–74)
Parity four	33.6 (7.1–76.9)	51.5 (27.5–79.3)
Parity >4	60.3 (25.2–90.6)	35.6 (13.4–65)
Sensitivity in lactation stage [a]		
Late lactation	22.9 (5.7–54.3)	25.8 (9.5–50.7)
Early lactation	54.5 (23.5–88.4)	53.9 (31.7–76.2)
Faecal culture		
Overall sensitivity	40 (26.7–55.4)	74.6 (61.5–85.3)
Overall specificity	98 (96.2–99.6)	98.5 (97.7–99.0)
Sensitivity by parity (late lactation)		
Parity one	19.8 (4.7–51.9)	59.2 (30.7–84.2)
Parity two	45.5 (12–84.6)	46.6 (11.6–89.9)
Parity three	74.5 (35.6–95.7)	52.2 (24.3–83.9)
Parity four	32.2 (4.9–78.5)	68.4 (41.2–88.8)
Parity >4	40.2 (12.9–74.2)	74.9 (39.4–95.6)
Sensitivity in lactation stage [b]		
Late lactation	19.8 (4.7–51.9)	59.2 (30.7–84.2)
Early lactation	51.7 (21.2–81.8)	77.3 (52.4–92.8)
Herd-level true prevalence		
Herd A	21.7 (11.5–36.4)	3.9 (1.0–8.9)
Herd B	9.6 (3.6–20.1)	6.7 (3.2–11.9)
Herd C	4.9 (1.3–11.7)	3.2 (1.2–6.3)
Herd D	2.8 (0.8–6.8)	2.3 (0.9–4.7)

[a] ELISA test sensitivity in early lactation was 31.6% higher (54.5 - 22.9 = 31.6) than in late lactation, using Method 4, and 28.1% higher (53.9 - 25.8 = 28.1) higher using Method 5.

[b] Faecal culture sensitivity in early lactation was 31.9% higher (51.7 - 19.8 = 31.7) than in late lactation using Method 4 and 18.1% (77.3 - 59.2 = 18.1) using Method 5.

Table 5.8. The probability that individual cows with varying test result combinations were infected with Johne's disease estimated by the regression model using longitudinal data, and cross sectional data, compared with this same probability calculated in a spreadsheet

				Predictive value positive		
Cow	Herd prevalence	First test event result [a]	Subsequent test event results [b]	Repeated measures	Cross section	Spreadsheet
1	4.1	- +	- - / - - / - - / - -	0.00	0.67	0.00
2	4.1	+ -	- - / - - / - - / - -	0.00	0.89	0.00
3	6.8	- -	- - / - + / - - / - +	0.32	0.04	0.27
4	6.8	- -	- - / - - / - - / - - / - -	0.00	0.07	0.00
5	3.2	- +	- -	0.14	0.47	0.14
6	2.3	- +	- +	0.93	0.26	0.96
7	6.8	- -	- - / - - / - +	0.12	0.06	0.01
8	2.3	- -	- - / - -	0.00	0.01	0.00
9	2.3	- -	- -	0.00	0.01	0.00
10	2.3	- -	- - / - - / - -	0.00	0.01	0.00

[a] The ELISA result precedes the faecal culture result where + = positive, - = negative.

[b] The ELISA result precedes the faecal culture result where + = positive, - = negative, and / represents a 6 month period between testing.
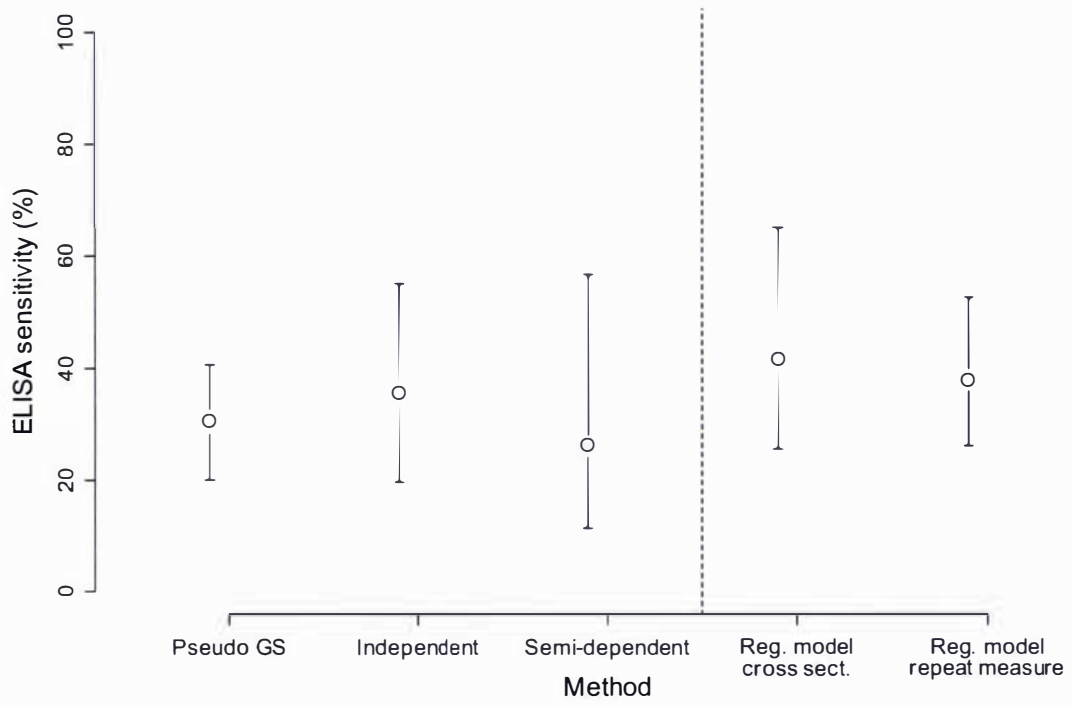
Figure 5.1. Sensitivity of the serum ELISA test for Johne's disease in four New Zealand dairy herds estimated by five methods
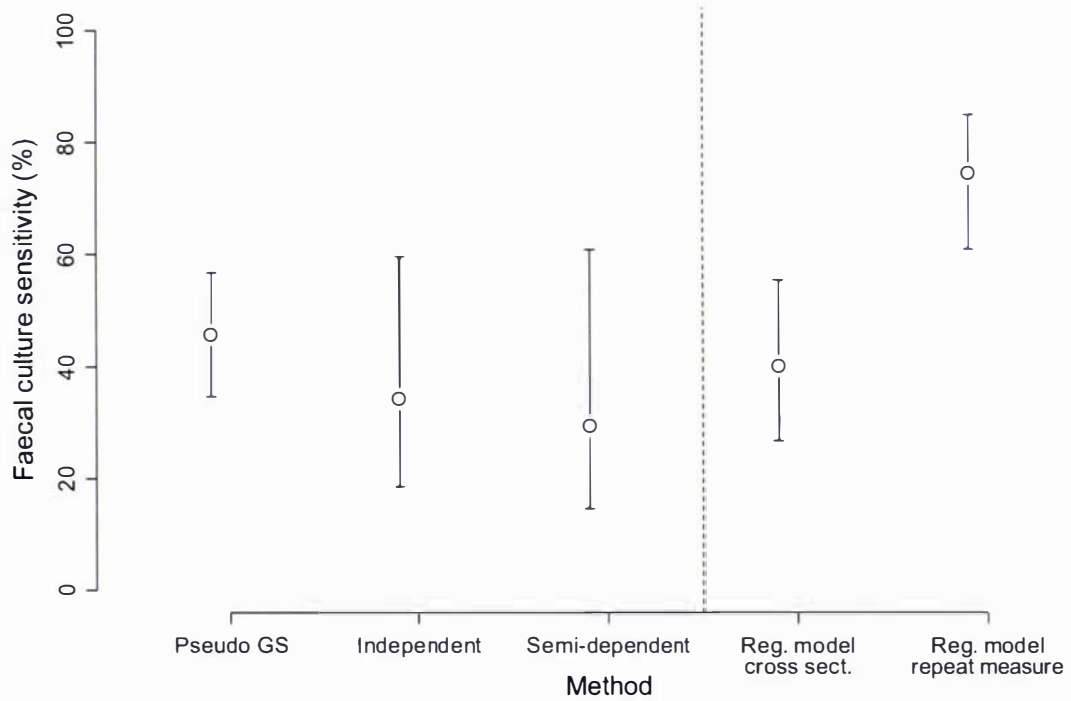
Figure 5.2. Sensitivity of the faecal culture test for Johne's disease in four New Zealand dairy herds estimated by five methods
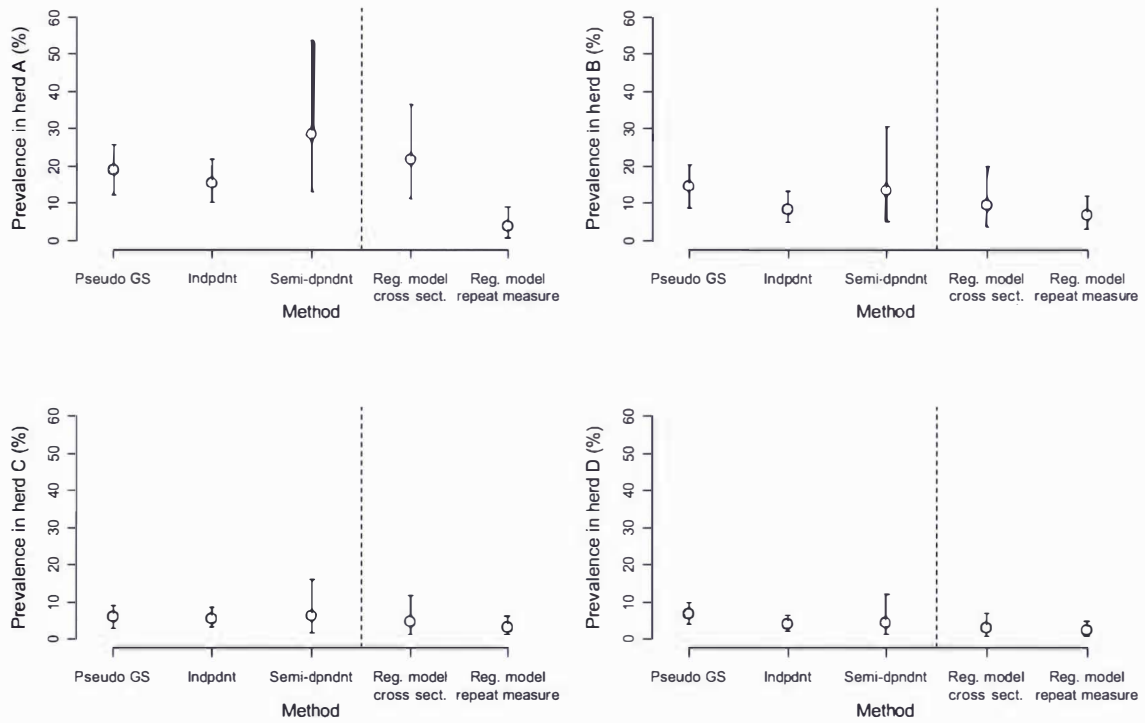
Figure 5.3. Estimates by five methods of the prevalence of Johne's disease in four New Zealand dairy herds

Discussion

In this study we estimated the sensitivity and specificity of the ELISA and faecal culture tests for Johne's disease in dairy cattle. A novel Bayesian regression model approach to diagnostic test evaluation was compared with three established analytical methods. Also, estimation using cross sectional data and repeated measures data was compared. The regression model represented a culmination of efforts to take into consideration the analytical challenges. Specifically, these challenges were the absence of a true gold-standard, the influence of other covariates on the sensitivity and prevalence and the use of repeated measures testing data. The strengths and weaknesses of the various methods are discussed.

Whitlock et al (2000) concluded that only repeated testing of cattle would provide an estimate of infection rate that approached the true infection rate. In this study the pseudo gold-standard method (Method 1) utilised up to ten tests (five ELISA and five faecal culture) per cow over 4 years. Yet this approach to classification of disease status may still be biased. For example, a cow with a single positive ELISA test and multiple negative tests is not easily classified. Also, the assumption that infection occurs when young and persists for life does not account for elimination of infection in the host, which may occasionally be seen in experimental infection studies (Stewart et al 2007). Selection of only cows tested at multiple time points for analysis would have maximised our confidence in gold-standard infection status, but was unattractive for two reasons. Firstly, positive tests only occurred in a small proportion (65/779) of cows and selection of only repeatedly tested cows would further reduce the number of positive testing animals. Secondly, selecting only cows tested over a long period would bias the analysis towards older cows that remained in the herd for relatively long periods. Thus it would not be representative of the age distribution or average health of all dairy cows. A gold standard should be representative of the population to which the test is applied. We considered inclusion of all cows tested for JD with their infection status defined using all available testing information to be the most appropriate approach. While with this approach the likelihood of misclassification varies between cows depending on the number of times they were tested, it maximises analytical power by including all cows tested for JD and maximises the likelihood that the gold-standard infection status of an individual is assigned correctly.

The strength of the simpler Bayesian approaches, Methods 2 and 3, was that they did not require the true infection status to be known or assumed. Also, they were robust, as evidenced by their previously published applications (Branscum et al 2005). A further strength of Method 3 was that it provided an estimate of the dependence between the two tests in the infected population. The

semi-dependent model provided a parsimonious means of assessing the covariance in the positive population when covariance in the negative population was negligible (Kostoulas et al 2006).

There were three weaknesses of Methods 2 and 3. The first was that they rely on informative priors to overcome being weakly and strongly non-identifiable, respectively. The second weakness was that they offered no scope for investigating the subtleties of test performance within levels of covariates. The third, that they were unable to use repeated measures data.

The strength of the regression model (Methods 4 and 5) was that it provided estimates of test performance and prevalence for the population overall and for covariate specific sub-groups. Also, it did not require the true infection status to be known or assumed, and it was capable of analysing repeated measures data. Hence it was a more powerful analytical approach than Methods 1 to 3. The regression model did not account for dependence between the diagnostic tests, nor did it include a random effect to account for correlation between repeated tests on an individual cow. This approach warrants further discussion.

Analysis of the performance of diagnostic tests with moderate accuracy and measuring the same biological response should adjust for dependence between the tests (Georgiadis et al 2003). Dependence between the ELISA and faecal culture test in this study was estimated by two methods and found to be negligible. Similarly only slight (less than 0.2) dependence in the infected population was reported in a comparison of five diagnostic tests for JD by Gardner et al (2000). In this study all point estimates of dependence and their upper error bounds were less than 0.15 and 0.19 respectively. While that study did not include faecal culture, it did compare tests that were more likely to be dependent than faecal culture and ELISA, for example two ELISA tests. Consequently we concluded that the dependence parameterisation was unnecessary. Given the high specificity of tests for JD, dependence in the non-infected population was considered negligible. In some situations the assumption of independence is unlikely to be valid (Vacek 1985; Brenner 1996; Torrance-Rynard and Walter 1997).

The assumption that repeated measurements on the same individual are independent may not be valid (Gardner et al 2000). Omission of a random effect to account for correlation of repeated test results within cow is recognised as a simplification of the true situation. Given that testing occurred at six monthly intervals, independence between test results for an individual cow is not improbable. Inclusion of a random effect impaired model convergence, but could possibly be supported by a larger number of observations for each individual, or a greater proportion of positive test results.

The wide error bounds associated with most point estimates, particularly within levels of covariates were probably due to the small proportion of positive test results (4.8%). Nevertheless, trends in the results were consistent with the current biological understanding of the disease.

Increasing parity was associated with a trend of increasing sensitivity in the ELISA test. The ELISA trend was consistent with previous findings (Nielsen et al 2002; Jubb et al 2004). It was also consistent with the understanding that animals in the early stages of infection, even if shedding the organism, cannot necessarily be identified by ELISA because months or even years may elapse before sero-conversion (Milner et al 1987).

Sensitivity of the faecal culture test was highest in parities greater than three which was consistent with widely accepted view that excretion rates are highest in older cows as they are more likely to be approaching the clinical stage of disease (Chiodini 1996). It is possible that there was insufficient information in the cross sectional data set to demonstrate this trend.

The sensitivity of both tests was higher in early lactation. The prior described ELISA and faecal culture sensitivity as 3% higher in early lactation. The posterior estimate (Method 5) described ELISA sensitivity and faecal culture sensitivity as 28.1% and 18.1% higher in early lactation. This was consistent with our prior belief that the stress of calving and peak milk production would make JD infected cows more likely to test positive. However this result conflicted with the finding by Nielsen et al (2000) that a positive serum ELISA result was more likely at the end than in early lactation. The wide error bounds indicate our point estimates should be interpreted with caution, but the increase in sensitivity was consistent for both ELISA and faecal culture, and when using cross sectional, and repeated measures data.

Estimates of prevalence were slightly lower when using repeated measures data than when using cross sectional data, which seems counter-intuitive. This result is statistically sound for two reasons. The first is that when an individual cow had a single positive test and multiple negative tests, and given low prevalence, it was probably not infected. Even in the presence of high specificity. The second reason is that the probability of freedom from infection was greater given multiple negative tests over time, than given a single negative test event. Examples of these events are given in Table 5.8. The majority of cows in this study were test negative and all had a lower probability of infection considering multiple test events than a single test event, leading to a lower herd-level prevalence estimate.

The decline in prevalence when using repeated measures data was especially evident in herd A. Cows testing positive in this herd usually did so only once and also had multiple negative test results. Consequently the probability that they were infected was high using cross sectional data, but low using repeated measures data. These cases may be due to temporarily elevated antibody

levels in infected cows, possibly due to transient infection. Alternatively they may be due to cross reaction with antigen from sources other than MAP, thus genuinely false positive results. It is conceivable that an organism present on farm A but not on the other three farms caused cross reaction of the test (Norby et al 2005).

The final form of the regression model performed well. There was little autocorrelation between subsequent iterations during the Gibbs sampling process and posterior distributions remained the same when sampling began from different starting values. However the small number of positive tests resulted in wide error bounds surrounding the point estimates. A potential remedy for this would be to adapt the model to Australian data describing up to seven annual test events for cows in over 500 herds in Victoria (Jubb et al 2004).

In conclusion, the regression model enables a more accurate diagnosis of Johne's disease to be made because it incorporates information about the individual, such as age, lactation stage, and previous test results. Results from this model were consistent with the current understanding of the disease process and previous research. One of the key findings in this study was that prevalence estimates may actually be lower following repeated testing of a population, compared with when the population is tested in a cross sectional manner. The regression model described in this study increases the likelihood of a correct diagnosis of Johne's disease being made and contributes to a cost-efficient approach to large scale disease control in which diagnostic testing plays a role.

5.1.15. Acknowledgements

Chapter 6.

A simulation study of Johne's disease and control strategies for New Zealand dairy herds

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Abstract

AIM: To evaluate the epidemiology and economic impact of Johne's disease (JD) in New Zealand dairy herds, and the effect of control options on these features of the disease, using simulation.

METHODS: An existing stochastic and dynamic simulation model for JD 'JohneSSim' was adapted to represent the disease in the average infected New Zealand dairy herd. Test-and-Cull based control strategies were compared with strategies of Vaccination, Genetic Resistance, and Improved Calf Management. Sensitivity analysis was conducted.

RESULTS: During the 20 year simulation period, true within-herd prevalence varied between 13% and 16% in the absence of control. The simulated prevalence declined to less than one percent using the Improved Calf Management strategy. Test-and-Cull based strategies were unattractive due to high cost and diagnostic limitations of the tests. The Vaccination strategy was unable to reduce the prevalence below 9%, but was comparatively inexpensive. The model was most sensitive to the infectiousness of pooled milk/colostrum, to the rate of faecal-oral contact and to having an open herd.

CONCLUSION: Simulation indicates JD would be most effectively controlled by reducing the contact rate between susceptible calves and the infectious organism. A strategy representing improved hygiene associated with calf management reduced the prevalence to very low levels. Control strategies for which a cost was estimated could not be implemented for less than the cost of the disease. To improve the epidemiological understanding of JD the effective contact rate between calves and the infective organism should be studied more thoroughly.

Introduction

Johne's disease (JD), caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is a chronic enteritis affecting ruminants worldwide. In cattle, it is characterized by faecal-oral infection of calves, a latent period of about two to five years during which the disease is most likely undetectable, then progressive wasting and eventual death. Infected cows may shed the organism in faeces for years prior to showing clinical signs of disease. It is a cause of financial loss to dairy producers as infected cows have lower milk production, a higher removal rate (Hendrick et al 2005) and reduced slaughter value (Benedictus et al 1987; Nordlund et al 1996; Ott et al 1999).

Coordinated control programs for JD have been implemented by major dairy producing countries such as the United States of America, Australia, and the Netherlands. Control programs aim to reduce the prevalence and economic loss associated with the disease. They also act as a marketing tool, providing a potential marketing advantage for dairy exports from these countries.

Estimating the effect of JD across a population of herds, for example at the national scale, is fraught with problems. Variation in its effect between individuals (Rankin 1958) and between herds (Nordlund et al 1996) and uncertainty associated with the importance of some epidemiological aspects of the disease are but a few of the challenges.

Computer simulation models are well suited to exploring such problems. They provide useful insights into the behavior of the disease at the population level by combining probability distributions and data, while avoiding the biological challenges inherent in other types of study. The first epidemiologic and economic simulation model of JD was developed by Walker in 1988 for Wisconsin dairy herds. A more refined model published by Collins and Morgan (1991) was able to simulate the impact of diagnostic testing and the effect of open versus closed herds. The results of the latter study were used to develop an economic decision analysis model for test and cull programs (Collins and Morgan 1991b). More recently a very complex model of the effect of JD in a single Danish herd, 'PTB-Simherd', was devised (Kudahl et al 2007) based on a Danish dairy herd simulation model 'SimHerdIII' (Ostergaard et al 2000).

JohneSSim is a stochastic model of a dynamic population of dairy herds, simulating JD and strategies for its control, over a 20 year period. Initially developed in the Netherlands (Groenendaal et al 2002b; Weber 2004) it was later adapted for use in the United States of America (Groenendaal and Galligan 1999).

JD was first reported in New Zealand in 1912 (Stephens and Gill 1937) and has since become widespread in the national dairy herd. However the typical within-herd prevalence is probably low to moderate, based on a median annual within-herd incidence of 0.31 clinical cases per 100

cow years across 201 herds (see Chapter 3). The effect of JD on New Zealand dairy cow productivity was first studied in six herds in 1989 (de Lisle 1989) and more recently in a longitudinal study of four herds (see Chapter 4). A questionnaire based case-control study of JD in 427 New Zealand dairy herds has aided our understanding of its epidemiology (see Chapter 3).

The New Zealand dairy production system is unique and generally of lower intensity than those of other developed countries (Holmes 2002). A seasonal calving pattern utilizes the high pasture growth rate during spring. Cows are not housed and receive a pasture diet with pasture based supplements.

The aim of this study was, firstly, to adapt JohneSSim to simulate JD in New Zealand dairy herds, and second, to simulate the effect of control strategies for the disease.

Materials and methods

The JohneSSim model has been thoroughly described (Groenendaal et al, 2002; Groenendaal et al, 2003; Weber et al, 2004) and the following will highlight key components of the model and describe model inputs regarding the average New Zealand dairy herd, the spread of JD within the herd, and the economic impact of the disease. JohneSSim is written in the Visual Basic programming language (http://msdn2.microsoft.com/en-nz/vbasic/default(en-us).aspx) and linked to Excel (Microsoft, Redmond, Washington, United States of America, 2008) spreadsheets. Probability distributions describe variation in key parameters such as replacement, infection with JD, pathogenesis, mortality, and various control options. By simulating a population of herds containing sub-populations to represent differences in key management parameters, insight can be obtained into the effect of a control strategy at the national level. The model simulates a population of herds and results are presented as the average of this population and the range of values within which the central most 80% of the population lay.

6.1.1. The average New Zealand dairy herd in JohneSSim

The simulated average New Zealand dairy herd contained 280 cows, with an annual increase in herd size of 3% and in which the annual replacement rate was 23%. Cows first calved at two years of age. Calving was strictly seasonal, once per year in spring, hence the calving interval was 12 months. Forty nine percent of calves born were female. The calf mortality rate was 8% in heifers and 5% in older cows.

JohneSSim considers both voluntary and involuntary culling. Voluntary culling is the practice of selectively replacing old or poor producing stock with young stock, typically at the end of the milking season. Involuntary culling is the unavoidable removal of stock, for example due to injury or sickness. Involuntary culling for reasons other than production had top priority. For

voluntary culling a separate culling model (Groenendaal et al, 2004) was used to calculate the production potential of each cow, compared to a replacement heifer, based on the retention pay-off (RPO) which describes expected future profit from a given animal. Data indicated that about 10% of all culling was voluntary (Xu and Burton 2003). The percentage of cows in each lactation culled involuntarily (Table 6.1) was based on data described in Heuer et al. (2005).

6.1.2.Johne's disease in New Zealand dairy herds in JohneSSim

An expert group meeting was arranged to estimate key epidemiological parameters for which no information specific for New Zealand was available. Twelve people attended including the designer of the JohneSSim model, epidemiologists, experienced field veterinarians, and experts in the dairy industry. The group believed that approximately 70% of New Zealand dairy herds had JD, even though no formal prevalence studies had been conducted. The within-herd test prevalence using an ELISA test was between 2% and 4% based on a recent longitudinal study of four dairy herds (see Chapter 4).

The spread of Johne's disease within a New Zealand dairy herd is represented by six infection states, from susceptible calves through to clinically affected cows (Table 6.2). The highly infectious stage was most likely to commence in the third lactation but could occur in the first lactation, or may never occur. The interval between becoming highly infectious and becoming clinical was most frequently 1 year with a minimum of 0.5 years and maximum of 1.5 years (Chiodini et al 1984a).

The estimated probability of foetal infection depended on the infection status of the dam. It was 0.035, 0.07, and 0.22 for infected dams more than 12 months, 7–12 months, and less than 7 months from showing clinical signs of JD. The probability of foetal infection when the dam was clinically affected by JD was 0.5. These estimates were a concensus provided by the expert group and consistent with published data (Doyle 1958; Seitz et al 1989; Sweeney et al 1992). No data specific to New Zealand was available.

For the probability of neonatal infection (infection of the calf between parturition and removal from its dam) the Dutch estimates were retained as no data specific to New Zealand was available (Table 6.3).

The number of faecal-oral contacts increased with the number of MAP shedding cows in a herd and with the number of direct and indirect contacts (parameter k) between a calf and infectious faeces (Table 6.4). To represent this dynamic, the susceptible period for a calf was divided into three stages. The first stage was from birth to weaning (3 months old), when calves were kept on the farm but had little chance of being exposed to infectious faeces. The second stage, from 3 to 9 months of age, had two categories, each containing 50% of herds: either the calves were kept on farm and grazed pasture about 2 to 4 weeks ahead of the milking herd in a rotational

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grazing system with access to infectious faeces, or calves were sent off-farm for grazing, where they only had a small chance of being exposed to infectious faeces.

The third stage spanned from nine to 12 months of age. During this time, the calves were assumed to be grazed off-farm without contact to the adult herd, thus had little chance of being exposed to infectious faeces. The expert group agreed that off-farm grazing was utilized by almost all farmers. The expert group considered that one faecal-oral contact occurred for every two calves during stages one and three. During stage two, calves grazing off-farm were assumed to have only one contact due to a a low risk of exposure to potentially contaminated pasture at this age compared to stage three. Calves remaining on farm during stage two were assumed to have 35 contacts (Table 6.4).

The probability that pooled colostrum, waste milk or bulk milk (subsequently referred to as pooled milk) was infectious to calves was assumed to increase by 2% for every highly infectious or clinical cow contributing to the pool. If infectious, pooled milk was assumed to infect 40% of the calves to which it was fed. The distribution representing the probability of infection of calves was assumed to represent variability due to natural resistance and/or increasing age.

All herds were assumed to be closed. Where open herds were simulated in the sensitivity analysis, the probability of introducing infection into the herd via purchase was dependent on the number of cattle purchased and the prevalence in the population from which they came.

Prevalence in the results refers to the true within-herd prevalence in the average infected dairy herd.

6.1.3. The risk profile for calf management

The four risk profiles represent key divisions between herds in calf management with respect to JD. They characterise variation in important management practices between herds. The expert group decided that the scenarios should reflect differences in the neonatal contact period between the calf and its dam, and differences in the use of off-farm grazing of calves three to nine months of age (Table 6.5). Each herd is allocated to one of the 'risk profiles'.

6.1.4. Performance of diagnostic tests for Johne's disease

The sensitivity of the ELISA test and faecal culture test varied between 0% and 99% depending on the infection state of the tested animal, while the specificity was 99% and 100% respectively. These figures were discussed and summarized by the expert group on the basis of the available literature and data collected by the author (see Chapters 3 and 4).

6.1.5.Economics

In JohneSSim all financial values were discounted to account for inflation occurring over the 20 year simulation period. The benefit of a control strategy was calculated as the amount by which it reduced the financial loss due to JD. The cost of a strategy was calculated as the total expenditure attributable to control option(s).

Milk production loss due to JD was estimated following a review of the literature and results of the author's study in New Zealand (see Chapter 4). The financial impact of reduced slaughter value, premature culling and diagnostic testing were agreed on by the expert group (Table 6.7). It was assumed that no treatment of cows with JD was attempted.

The economic attractiveness of a control strategy to the average infected dairy herd was evaluated using the Net Present Value (NPV) of the strategy. The NPV was defined as the benefit of a strategy minus its cost. It represents the value of using a control strategy for 20 years, on the day it was implemented.

The NPV for strategies for which the cost was not estimated represents the benefit only. This value provides an upper limit for the cost of implementing the strategy, above which it would not be economically attractive.

6.1.6.Control Strategies

Eight control strategies were selected for simulation by the expert group on the basis of their perceived potential to provide an economic benefit to infected herds (Table 6.8). Some strategies require further definition for clarity. Diagnostic testing, by either ELISA or faecal culture (FC), represents annual testing of cattle 2 years or older, where cattle that test positive are sent to slaughter. In strategy seven, progeny removal (PR), all progeny of a clinical case of JD are removed from the herd.

The Vaccination strategy was modeled as an increase, from 5 to 7 years, in the average period between when a cow became infected and when it entered the highly infectious stage of disease. This was based on estimates by Groenendaal and Galligan (2003).

Genetic Resistance was modeled as an annual decrease of 1% in the probability that a calf became infected, and an annual decrease of 1% in the probability that a resistant cow became highly infectious. It was assumed that the first cohort of resistant calves were born in year one, thus ignoring a preparation period for the elements of resistance.

The Improved Calf Management strategy (ICM) represented three improvements in the hygiene associated with calf management. The first was a reduction in the probability of neonatal infection of 90%. In practice this relates to ensuring calving occurs in an area free of faecal contamination and that the calf spends only a short period with the dam (less than 12 hours).

The second improvement was elimination of infection via the feeding of pooled milk. In practice this would equate to using milk replacer or pasteurization of milk for calves. The third improvement was reduction of the faecal-oral contact rate in calves 3–9 months of age by 98%. In practice this would be achieved by grazing young stock off-farm between weaning and entering the milking herd.

The cost of Test-and-Cull based strategies, Vaccination, and PR could be estimated with reasonable certainty. Costs for the strategies of ICM and Genetic Resistance were considered too uncertain to estimate.

6.1.7. Sensitivity Analysis

Uncertainty was inherent in many input parameters. Five parameters that had been either points of debate or were of biological interest were selected for sensitivity analysis.

In sensitivity analyses one to four, the 'No-Control' scenario was used as the default situation and an epidemiological component of the disease was changed within biologically sensible limits. In the fifth sensitivity analysis the ICM strategy was used as the default situation and a single parameter within this strategy was changed. The following scenarios were evaluated in the sensitivity analysis.

Faecal-oral contact rate in calves 3-9 months of age
The faecal-oral contact rate for calves 3-9 months old not sent off-farm to graze was 35 in the default situation. This value was increased to 60, similar to that used during simulation of Dutch dairy herds.

Rate of foetal infection
In the default situation we assumed the probability of foetal infection to be 3.5% for infected cows at least one year before becoming clinical, 7% when six to twelve months before becoming clinical, 22% when less than 6 months before becoming clinical and 50% for clinically affected cows. In the sensitivity analysis these values were increased to 7%, 25%, 50% and 75% respectively.

Highly infectious pooled milk
In the default situation there was a 2% probability that an infectious cow contributing milk or colostrum to the pool rendered the pool infectious. This value was increased to 25%. Also, given that a pool was made infectious, it infected 40% of the calves to which it was fed. This value was increased to 75%.

Frequency of re-introduction of Johne's disease into herds
In the default situation we assumed that 100% of herds remain closed, excluding the purchase of bulls. This value was reduced to 9% on the basis of a recent study (see Chapter 3). Of the open herds, 20% bought 2–6 animals per year, 18% bought 8–12, 13% bought 14–18, 19% bought

20–28, 15% bought 30–46 and the remaining 15% bought 48–100. Fifty percent of purchased stock were cows older than two years, 25% were heifers and 25% were calves younger than one year.

Partial effectiveness of the Improved Calf Management strategy

Here we examine the possibility that, given that all calves were removed after only a short period with the dam, the reduction in neonatal infection rate was only 50%, rather than 90% as in the default situation.

Results

6.1.8. Epidemiology

In the absence of control, the simulated true within-herd prevalence of JD in the average infected dairy herd in New Zealand varied only slightly, ranging from 13% to 16% over the 20 year period (Figure 6.1). JD was not eradicated by any control strategy, however some strategies reduced the prevalence to a very low level. The control strategies can be divided into two groups, those involving a Test-and-Cull approach (Figure 6.1) and those relying on other means (Figure 6.2).

The ICM strategy resulted in the lowest prevalence (0.2%) when evaluating individual strategies (Figure 2). Combining the ICM and FC strategies increased the speed at which prevalence declined and resulted in a slightly lower final prevalence of 0.05% (Figure 1). Combining the ICM and Vaccination strategies provided an almost identical reduction in prevalence to ICM used on its own (Figure 2).

Test-and-Cull based control strategies reduced the prevalence initially but their effectiveness waned with declining prevalence. The ELISA strategy did not reduce the prevalence below 12% and the lower limit of prevalence reduction by FC was 5%.

Under the Vaccination strategy prevalence declined slowly from 15% to 10% over the 20 year period. This strategy appeared to be reaching a lower limit of effectiveness at about 9% prevalence.

The Genetic Resistance strategy reduced the prevalence to a similar extent as the ELISA strategy during the first 12 years. However at this point it reached a lower limit such that in the last 8 years, the prevalence remained between 10% and 11% in the average infected herd.

Removing the progeny of JD cows had very little effect on the prevalence.

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6.1.9. Economic impact of disease

In the absence of control, the simulated annual loss due to JD in the average infected dairy herd increased from NZD 1,813 in the present day to NZD 5,864 in year 20 despite a reasonably constant prevalence. This is primarily due to inflation and increasing herd size, thus more infected cows. The range of losses for the central most 80% of herds simulated was from NZD 0 to NZD 5,585 in year one and from NZD 0 to NZD 20,713 in year 20.

The benefit of control (Table 6.9) was greatest when using the ICM strategy, either on its own (NZD 24,295), or in combination with the FC strategy (NZD 38,110). In contrast, the benefit associated with the PR strategy was small (NZD 1,461). The respective benefits of using the ELISA strategy (NZD 14,787) and Vaccination (NZD 15,838) were similar.

The annual loss in year 20 was negligible after using a combination of ICM and FC strategies (NZD 11), or when ICM was used alone (NZD 102).

The simulated cost of control was high for Test-and-Cull strategies, particularly those involving FC (Table 6.9). The discounted cost of using the FC strategy for 20 years was NZD 206,086. The ELISA strategy (NZD 8,801) cost less than half, and the Vaccination strategy (NZD 19,789) about one tenth, of the FC strategy. The cost of the PR strategy (NZD 14,293) was due to lost milk production, and replacement of prematurely culled infected cows and their offspring.

No strategy for which all costs could be calculated was economically attractive to the average infected herd, as all had a negative NPV. Vaccination represented the smallest loss (NZD 3,950) while the FC strategy represented the greatest loss (NZD 172,465).

The ICM and Genetic Resistance strategies would be economically attractive provided they could be implemented for 20 years for less than their respective benefits. This is most likely for ICM, for which the benefit was NZD 24,295.

6.1.10. Comparison of control strategy efficiency

The overall attractiveness of each control strategy, based on its ability to reduce the prevalence and its NPV was compared (Figure 6.3). Only strategies for which a cost was estimated were plotted. Strategies located toward the bottom right corner of the plot were more attractive than those above and/or to the left of them. The large negative NPV associated with the FC and ELISA strategies is clearly evident.

The vertical bars in Figure 6.3, representing the central most 80% of the population of herds, indicate that the prevalence remained above 20% in a proportion of herds regardless of the control strategy. This was particularly evident for the PR and ELISA strategies.

The range in NPV for the central most 80% of the population of herds (horizontal bars) show that the vaccination strategy would be economically attractive to a proportion of herds, but that none of the other strategies, for which a cost was estimated, would be economically attractive.

6.1.11. Sensitivity Analysis

The model, under the No-Control scenario, was most sensitive to a change in the infectivity of pooled milk (Figure 6.4). Assuming pooled milk was highly infectious caused the prevalence to rise throughout the 20 year period to a plateau at 43%.

The No-Control scenario was moderately sensitive to the assumption that only 9% rather than 100% of herds were closed. This change caused the prevalence to increase steadily to 25% at the end of the simulation period. The No-Control scenario was also moderately sensitive to the number of effective faecal-oral contacts by calves. Again the prevalence increased steadily to 25%. The steady increase in prevalence under both the open herds assumption and the high faecal-oral contact assumption showed no signs of tapering off at the end of the simulation period.

Doubling the probability of foetal infection caused the simulated prevalence to change very little relative to the default No-Control scenario.

Simulating a partially effective ICM strategy only slightly slowed the rate at which prevalence declined relative to the default ICM strategy ('Management partly effective' in Figure 4). After 20 years the prevalence was 1%, rather than 0.2% in the default ICM strategy.

Table 6.1. Lactation-specific involuntary culling percentages

Lactation	1	2	3	4	5	6	7	8	9	10	11	12
% Involuntary culling	15.6	16.4	15.1	16.3	17.7	20.7	24.4	28.8	34.9	40.3	46.1	46.8

Table 6.2. The six infection statuses for individual cows simulated in JohneSSim

Infection status	Definition
Susceptible calves	Calves up to two years of age
Resistant cows	Non-infected cows (older than two years)
Latently infected cows	Infected but not shedding MAP
Slightly infectious	Infected and shedding MAP two months after calving
Highly infectious	Infected and shedding MAP continuously
Clinical	Infected with clinical signs of disease and shedding MAP continuously

Table 6.3. Probability of infection during the neonatal period for a calf born in a herd with standard calf hygiene

Dam infection status	Herd infection state		
	No infectious cows	One or more cows shedding low levels of MAP	One or more cows shedding high levels of MAP
Not infectious	0.00	0.025	0.10
Shedding low levels of MAP			
Two calvings before becoming highly infectious	N.A.	0.20	0.50
One calving before becoming highly infectious	N.A.	0.50	0.50
Shedding high levels of MAP or clinical	N.A.	N.A.	0.95

Table 6.4. Estimated faecal-oral contacts for three age groups of calves (parameter k)

	0–3 months	6–9 months	9–12 months
Herds not grazing young stock off-farm	0.5	35	0.5
Herds grazing young stock off-farm	0.5	1	0.5

Table 6.5. Risk-profiles used to model Johne's disease representing key divisions in current calf management and hygiene practices in New Zealand dairy herds

	+ Calf-dam separation within 12 hours	+ Calves grazed off-farm when 3–9 months of age;	Percentage of herds
	– Calf-dam separation >12 hours	– Calves 3-9 months of age grazed on-farm	
Profile			
A	–	–	17 %
B	+	–	33 %
C	–	+	17 %
D	+	+	33 %

Table 6.6. Estimates of diagnostic test performance in New Zealand used in JohneSSim

	ELISA		Faecal culture	
Infection state	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Latent	1	–	0	–
Shedding low levels of MAP	10	–	40	–
Shedding high levels of MAP	60	–	95	–
Clinically affected	80	–	99	–
Uninfected	–	99	–	100

Table 6.7. Loss caused by Johne's disease and costs associated with its control used in JohneSSim

Category	Description of loss or cost	Value
Milk production loss	Average milk production per lactation	311kgMS/lactation [a]
	Reduction depends on infection state:	
	Shedding low levels of MAP, 6 months to 1 year before high shedding	0%
	Shedding low levels of MAP, up to 6 months before high shedding	5%
	Shedding high levels of MAP	10%
	Clinically affected cow	15%
Reduction in slaughter value	Standard slaughter value (per cow):	NZD 300
	Reduction depends on infection state:	
	Shedding low levels of MAP	5%
	Shedding high levels of MAP	10%
	Clinically affected cow	~100%
Costs of testing	Visit by veterinarian	NZD 50/visit
	Veterinarian's testing costs	NZD 6/test
	ELISA test	NZD 16.50/test
	Faecal test	NZD 38.50/test
	Sample delivery	NZD 10/delivery
Vaccination		NZD 10/calf

[a] Milksolids: the percentage of fat plus the percentage of protein multiplied by the total milk weight.

Table 6.8. Control strategies for Johne's disease in New Zealand dairy herds evaluated by simulation with JohneSSim

Strategy	Test-and-cull method	Calf Management	Other
Annual ELISA	Annual ELISA, with confirmation by faecal culture test	Standard	None
Annual FC	Annual faecal culture test	Standard	None
Improved calf management (ICM)	None	Improved	None
ICM and FC	Faecal (once a year, all animals <2 yrs)	Improved	None
Vaccination	None	Standard	Vaccination
ICM and Vaccination	None	Improved	Vaccination
Progeny removal (PR)	None	Remove progeny of clinical cows	None
Genetic resistance	None	Standard	Genetic resistance

Table 6.9. Simulated financial output from JohneSSim for application of control strategies to the average Johne's disease infected New Zealand dairy herd over a 20 year period relative to the No-Control situation (NZD × 1000)

Strategy	Discounted loss	Discounted costs	Discounted benefits	NPV
No-Control	48 (0–154)[a]	N/A	N/A	N/A
Annual ELISA	30 (0–98)	84 (69–112)	15 (0–46)	-69 (-78– -60)
Annual FC	11 (0–35)	206 (180–256)	33 (0–106)	-172 (-191– -141)
ICM[b]	21 (0–62)	unknown	24 (0–84)	unknown
ICM and FC[c]	7 (0–20)	198 (180–228)	38 (0–123)	-159 (-184– -97)
Vaccination	33 (0–97)	20 (19–24)	16 (0–60)	-4 (-20–39)
ICM and vaccination[c]	20 (0–58)	20 (19–21)	29 (0–102)	9 (-20–81)
PR[d]	47 (0–150)	14 (0–52)	1 (-3–9)	-13 (-49–0)
Genetic resistance	47 (0–153)	unknown	1 (-3–7)	unknown

[a] Range containing the central most 80% of the simulated population of herds.

[b] ICM = Improved calf management.

[c] The costs and NPV for these strategies does not include the ICM component.

[d] PR = Progeny of clinical JD cases removed from the herd.
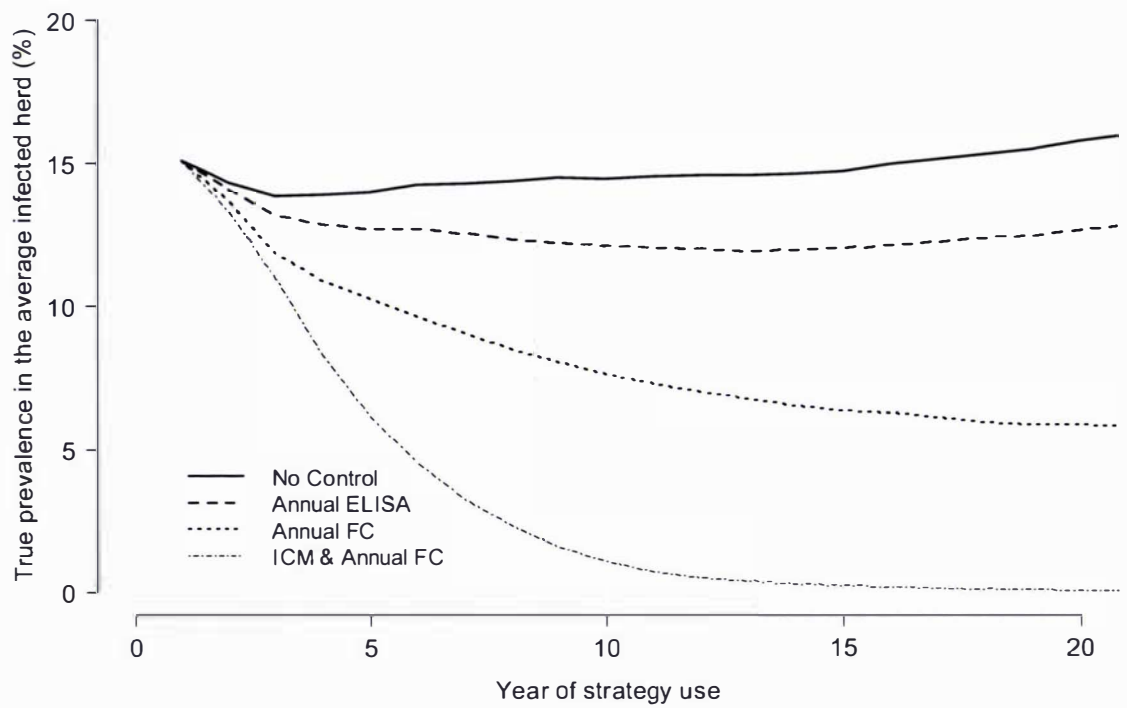
Figure 6.1. True prevalence of Johne's disease on the average infected New Zealand dairy over twenty years using control strategies based on test-and-cull methods simulated using JohneSSim
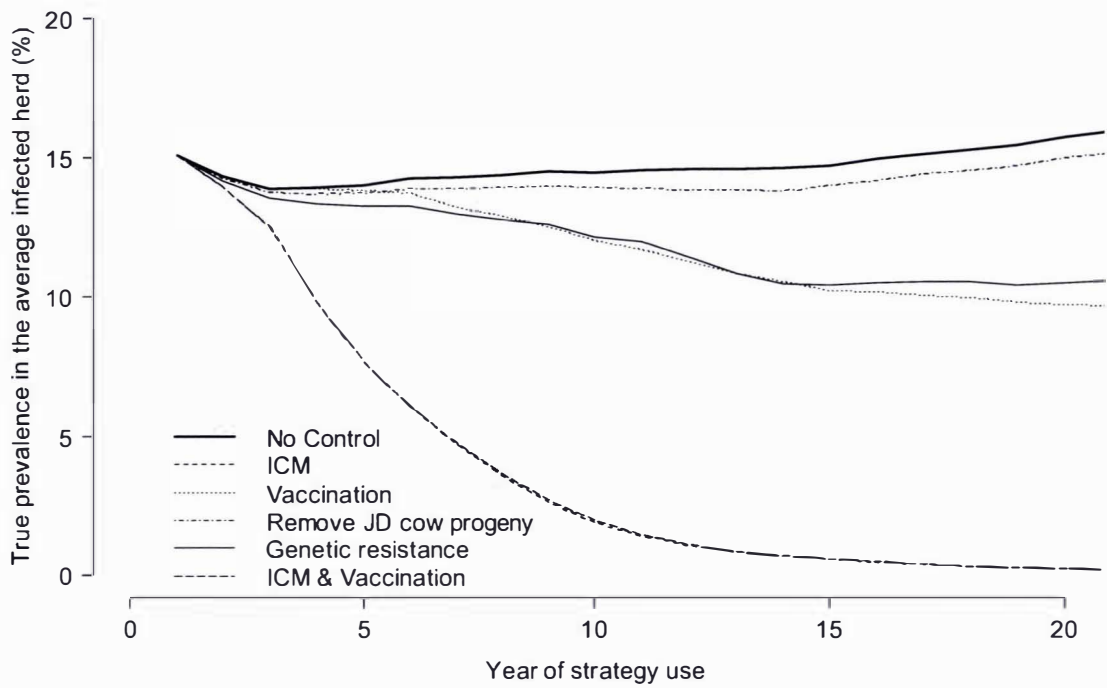
Figure 6.2. True prevalence of Johne's disease on the average infected New Zealand dairy over twenty years using control strategies other than test-and-cull methods simulated using JohneSSim
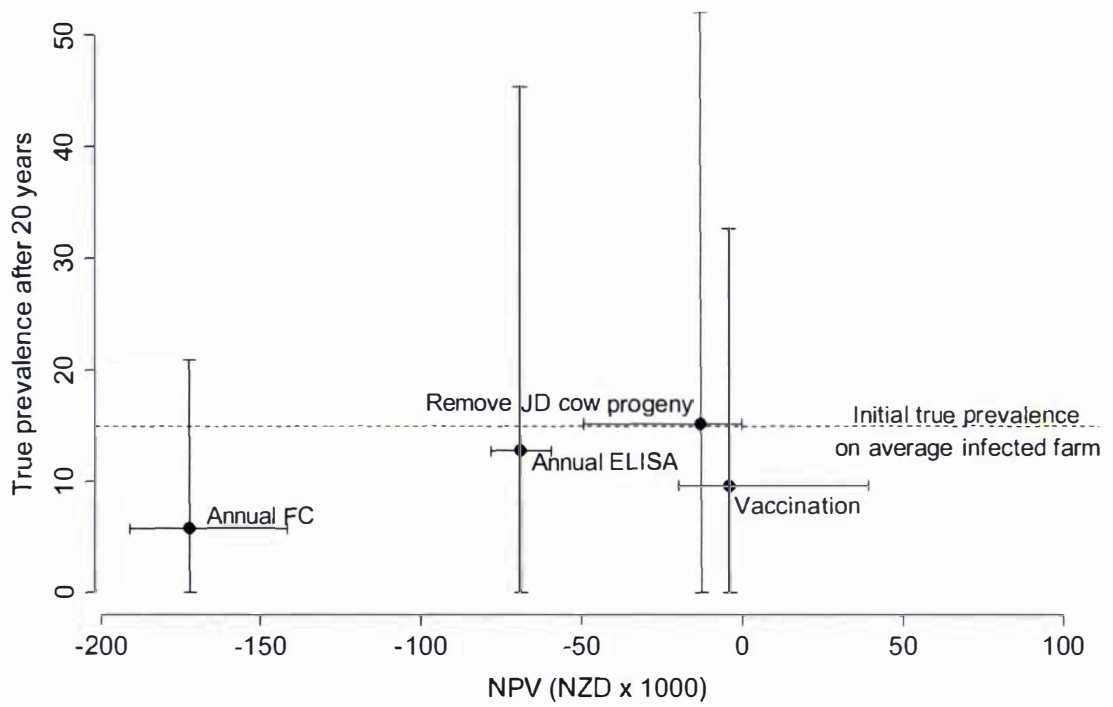
Figure 6.3. A comparison of the cost-effectiveness of control strategies for Johne's disease in the average New Zealand dairy herd and the central most 80% of the population of herds (bars) simulated with JohneSSim using strategies for which costs were estimated
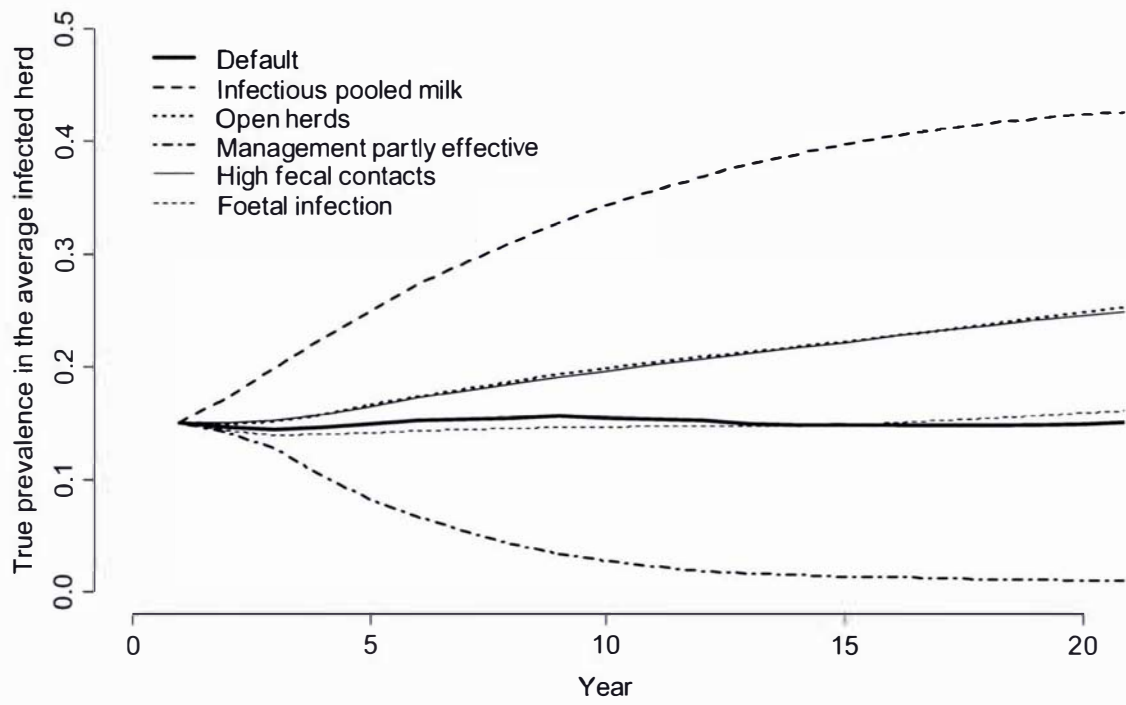
Figure 6.4. A sensitivity analysis of the true prevalence of Johne's disease in the average infected dairy herd under the No-Control strategy simulated using JohneSSim

Discussion

Simulation provides a complimentary method to expensive intervention studies, for evaluating JD and its control in New Zealand dairy herds. Data from recent studies and expert opinion were used to adapt the Dutch model, JohneSSim, to the dairy production system in New Zealand. Sensitivity analyses were conducted to identify input parameters which strongly influenced model output.

The most prominent difference between this and earlier simulation studies of JD was the almost constant prevalence during the 20 year simulation period in the absence of disease control. In this study it remained about 15% whereas it increased from 22% to about 40% when simulating Dutch herds (Groenendaal et al 2003) or medium sized dairy herds in the United States of America with JohneSSim (Groenendaal and Galligan 2003). A different, earlier model developed in the United States of America predicted an increase from zero to about 40% over 30 years (Collins and Morgan 1991a). Recently a much greater increase in prevalence, from 25% to about 90% over only 10 years, was simulated in a Danish dairy herd (Kudahl et al 2007). We believe that prevalence would remain fairly constant in the absence of measures to control JD, else one would expect a high prevalence of infected animals in general because the disease has been present for a long time. We are therefore confident that the presented model output is credible, yet we concede that our results are difficult to validate. The JohneSSim model has previously been validated against 21 dairy herds in The Netherlands (Groenendaal et al 2002b) while, in a related study, R_0 for the model was estimated using a generalized linear model (van Roermund et al 1999).

JD has been present for at least 90 years in New Zealand and while widespread amongst dairy herds, evidence suggests that the within-herd prevalence is generally low. This situation indicates a near equilibrium state in the epidemic cycle as discussed by Frost (1976). Experts were of the opinion that the within-herd prevalence in the average infected herd was 15% which concurs with our estimates in four herds studied over four seasons (see Chapter 4). The following arguments provide anecdotal evidence that the prevalence is reasonably static over time in most herds.

Firstly, between the 1940s and 1980s, JD was not noted as a major problem, thus was probably present within-herds at a low prevalence. In 1931, the disease became notifiable, but no coordinated control effort was initiated. In 1946 there were about 1.6 million milking cattle of which 514 were reported condemned due to the disease (Chandler 1957). Thirty six years later an abattoir study reported that 4% of 945 terminal ilea examined showed signs consistent with JD (Hebden and Nuttall 1982). These figures suggest that the within-herd prevalence was not increasing at a high rate during this period.

Secondly, more evidence for a generally static prevalence between the 1980s and 2000 was provided by de Lisle and Milestone (de Lisle 1989) who reported that during a 3-year study, the annual incidence of clinical cases ranged from 0.6 to 2.8/100 cow years in six dairy herds believed to be representative of the spectrum of prevalence of clinical cases at that time (de Lisle 1989). Fourteen years later the median annual incidence of clinical cases in 201 herds was 0.31/100 cow years (lower quartile 0.15, upper quartile 0.57) (see Chapter 3). Furthermore, in a four year study of four herds considered representative of the 'average' New Zealand dairy herd JD was the recorded reason for only three of 558 removal events (see Chapter 4). The duration of infection and the rate and direction of change in prevalence and incidence in all these herds was not known with certainty.

The economic values from JohneSSim should be interpreted as indicative, rather than definitive, with emphasis being on the relative differences in financial parameters between the strategies, due to uncertainty within the model. Nevertheless, the simulated financial loss, due to JD, in the average infected herd in year one was consistent with previous estimates of loss of milk production due to JD in infected herds in New Zealand, which ranged from NZD 500 to NZD 6,651 depending on incidence and seasonal variation (de Lisle 1989). On farms with minor losses due to JD there is little financial incentive to implement control and it is difficult to find a method of control that is cheap enough to be financially attractive.

By far the most effective strategy for reducing the prevalence of JD was ICM, under which the prevalence declined from 15% to 0.2% in the average infected herd after 20 years. If it could be implemented and maintained cheaply (the model indicated for less than NZD 24,295) then the owners of average infected herds may benefit financially from its use. Most financial gains would occur in years 10 to 20.

Controlling JD by Test-and-Cull strategies was unattractive for two reasons. Firstly, the high cost. The discounted cost of a Test-and-Cull strategy over 20 years using a faecal culture test (NZD 206,086) or the ELISA test (NZD 83,801) as much greater than for Vaccination. Secondly, a threshold was reached below which they would not drive the prevalence due to poor test sensitivity in subclinically infected animals. The likelihood of a highly sensitive test for JD being developed in the near future must be regarded as low, however a promising attempt has been reported (Eda et al 2006). Tests should be of low cost to maximize their financial attractiveness as a tool for control (Collins and Morgan 1991b).

Vaccination was relatively inexpensive and financially attractive to a proportion of the simulated herds (NPV was positive from some herds). But it was unable to decrease the prevalence below 9%. It is unlikely to be an attractive means of JD control for three reasons. Firstly, the reduction in prevalence relative to the No-Control scenario was small compared to the ICM strategy. It is considered that vaccination does not prevent infection (Larsen et al

1974), rather it reduces the probability that an infected cow sheds the organism or develops clinical disease. Secondly, vaccines for JD sensitize recipients to the tuberculin test (Munday 1959). This could interfere with the national program for the control of bovine tuberculosis in New Zealand. Thirdly the animal welfare issue associated with injection site lesions and the danger of accidental self-vaccination must also be addressed.

The prevalence of JD declined to a plateau of about 10% under the Genetic Resistance strategy. This is consistent with the Reed-Frost model (Abbey 1952) which demonstrates that the prevalence of a disease decreases as more resistant animals are present and the number of susceptible individuals is reduced. A cost for the Genetic Resistance strategy could not be estimated. The financial investment required to create a form of control for JD based on genetically resistant cows and having it available for farmers is likely to be substantial. By assuming that the first generation of resistant calves were born in year one of the simulation period we ignored the potentially protracted period for preparation of this strategy. However, resistance to JD is heritable to some degree (Veazey et al 1995; Koets et al 2000) and it may be possible to breed for resistance and improved milk yield simultaneously (Mortensen et al 2004). Hence, such a strategy may be attractive to the dairy industry. Attractiveness to farmers would be increased if the cost of the strategy was met by the industry.

The conclusion that economic loss in the absence of control was relatively low, thus control strategies would only have minor financial benefit, was similar to an earlier evaluation of JD in New Zealand (Brett 1998).

Our simulation indicated that in spite of control, the prevalence remained higher than 20% in a proportion of herds irrespective of the control strategy. Such herds represent a reservoir of re-infection for herds that had become free of the disease. Consequently, action taken to ensure that JD did not disseminate from this reservoir, and tools to identify JD free herds for the provision of replacement stock would be required.

The model outputs were highly sensitive to the infectivity of pooled milk. A high chance that the pool became highly infective caused the final prevalence to almost triple, from 15% to 43%. The pattern of prevalence increase under this assumption was at odds with our understanding of the disease in New Zealand and it would be of value to study the infectivity of pooled milk even though it is a challenging task. The likely concentration of MAP in pooled milk is probably at least two orders of magnitude lower than that required to consistently initiate infection. Previous research has shown that the milk of faecal culture positive cows may contain 'a few' infective organisms – probably less than 100 CFU/mL (Giese and Ahrens 2000). The pool to which at least six cows with culture positive milk were contributing was reported to contain 12 +/-12 CFU/100ml (Herman et al 2005). This is a very low concentration of organisms, given that 375 CFU/100ml (1.5×10^6 CFU/ml) when administered via 40mls of milk replacer, is the minimum

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infective dose required for consistent experimental infection (detectable after three weeks) (Sweeney et al 2006a). It remains to be shown that infection in calves is relatively common via consumption of pooled milk. Research to demonstrate this is problematic in the absence of a means of detecting the organism more sensitive than those currently available.

A change in the effective contact rate between susceptible calves three to nine months of age and MAP had moderate impact on the prevalence of JD. No data from New Zealand was available for which to estimate a contact rate. Estimates were uncertain, particularly for calves raised on-farm, due to considerable variation in the cow-calf management between dairy herds. In calves raised off-farm it was considered low, because dairy cows are generally not present on farms rearing calves. Research into this parameter would help to define the role of off-farm grazing in limiting the infection rate, especially since it was recently shown that the number of calves excreting MAP may be greater than previously believed (Bolton et al 2005; Weber et al 2005).

The simulated prevalence was also moderately sensitive to a change in the proporion of open herds. Changing from the simplistic assumption of 100% to the more realistic situation of 9% closed herds caused the prevalence to increase slowly but steadily throughout the simulation period. Previous models of JD showed that the presence of open herds clearly increased the rate at which prevalence increased (Collins and Morgan 1991a). This result demonstrates the importance of minimising the number of stock purchased. A high proportion of dairy farmers in New Zealand purchase stock, thus many herds stand to benefit from a reduction in purchase frequency.

The effectiveness of the ICM strategy was only slightly sensitive to the assumption that it reduced neonatal infection by only 50%, rather than 90%. Improved calf management would probably be more difficult to consistently achieve in practice than the other two simulated management changes of using milk replacer and off-farm grazing. The time and means with which to provide optimally hygienic calving conditions for every cow in the herd are limited. All cows calve in a short period of six to eight weeks, often unobserved by the farmer and in wet and muddy conditions. In contrast the other management changes would be comparatively easy to implement. A pasteurisation system is not labour intensive, once operational. Similarly, grazing young stock off-farm is common practice on many farms already. The sensitivity analysis supported ICM as a robust means of controlling JD in addition to being the most effective of the strategies simulated.

There was little difference in the simulated effect of control options in the New Zealand dairy system relative to the Dutch and American dairy systems (Groenendaal and Galligan 2003; Groenendaal et al 2003). Reducing the effective contact rate between susceptible calves and MAP via hygienic calf management was the most effective strategy for reducing the prevalence

of JD in these studies and in the studies by Collins and Morgan (1991), Antagnoli et al (2005) and Kudahl et al (2007). The greatest divergence between these earlier studies and ours was that our No-Control scenario produced a fairly constant prevalence over time, which is consistent with our understanding of this disease in New Zealand.

In conclusion, improvement of the hygiene associated with calf management appears capable of reducing the prevalence of JD in the long term. Although we did not estimate the cost of this control strategy, it is most likely to be cost effective in herds with a severe JD problem. In contrast, the Test-and-Cull strategies and Vaccination were not economically feasible in the absence of low-cost and accurate tests, or more effective vaccines. Methods that reduce the effective contact rate between susceptible calves and MAP have the greatest potential to reduce the prevalence of JD. Consequently, the epidemiological understanding of JD would be best improved by studying factors that influence the effective contact rate.

6.1.12. Acknowledgements

Chapter 7.

General discussion
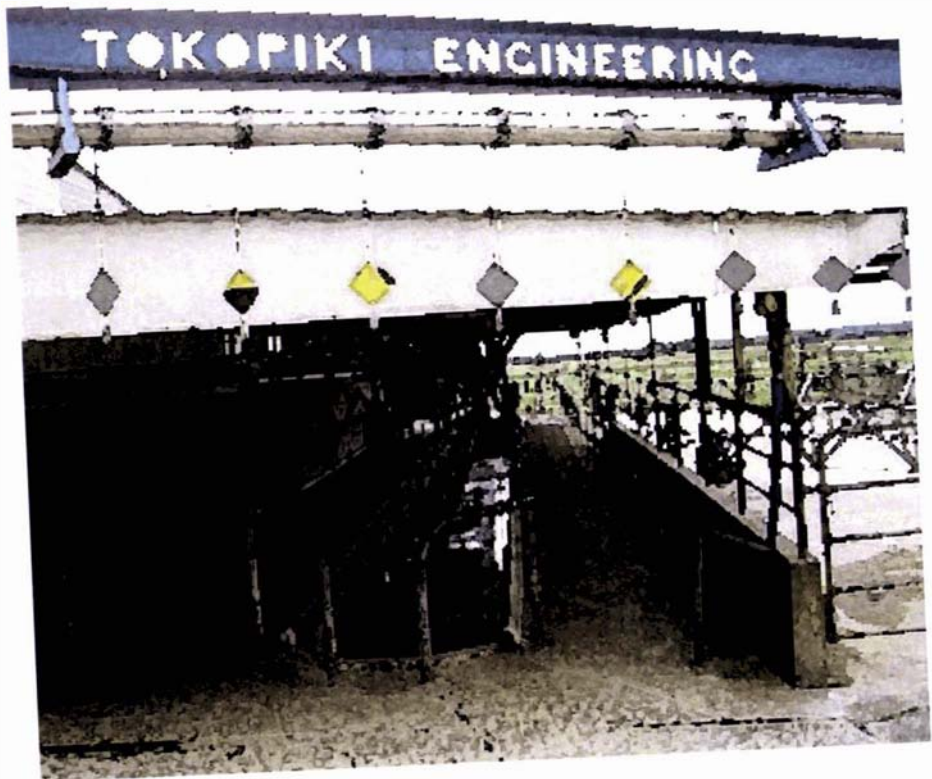
A review of the studies in this thesis

The objective of the work described in this thesis was to provide insight into the epidemiology of Johne's disease (JD) in the New Zealand dairy production system, and based on this, discuss possibilities for disease control.

Data collection for this thesis was a large undertaking. In Chapter three a questionnaire was administered to 664 farm owners from major dairying areas of New Zealand with a response rate of 64%. Respondents provided information on herd-level infection status and details of farm management practices. In Chapter four herds infected with JD were followed through four milking seasons, providing a rare opportunity to describe the impact of this disease on dairy cow productivity over a long period.

The findings from the studies presented in this thesis deepen the understanding of JD in the New Zealand dairy production system, provide a clearer direction for future epidemiological research and often support the findings of JD studies overseas.

Chapter three describes the within-herd incidence of clinical cases of JD, and management and demographic characteristics of 427 herds from major dairying areas of New Zealand. It represents the first risk-factor study of JD in a seasonal calving, pasture based, dairy production system. The median annual incidence of 0.31 clinical cases/100 cow years is the strongest evidence to date that clinical JD is a relatively minor cause of wastage in affected herds. JD was also considered a minor cause of wastage in dairy herds in Victoria, Australia which has a comparable production system (Hill 1989; Stoneham et al 1994). The risk factors most strongly associated with JD, besides the Jersey breed, could be broadly described as being related to management of calves and farm-level biosecurity. This is consistent with the general understanding of its epidemiology. Our analytical approach allowed us to identify dose-response relationships between risk factors and JD status, supporting a claim for causation, rather than simply identifying association. The main weakness of this study was that herd infection status was based on farmer records which exposed the analysis to recall bias, however the effect of this was believed to be relatively small.

Chapter four describes of a longitudinal study of four herds in which JD was present over four milking seasons. The effect of JD on dairy cow productivity, that is milk production and the risk of removal, was evaluated. The effect of misclassification of infection status on productivity estimates was also studied. The strengths of this study were its duration and repeated testing for JD of individual cows to minimise the chance of misclassification of infection status. Also, our selection criteria was for herds 'typical' of Johne's infected New Zealand dairy herds, rather than for herds with an unusually serious Johne's problem, or herds on a control strategy, which are also likely to be suffering unusually heavy losses due to the disease (Benedictus et al 1987).

The results showed that this disease could significantly impair productivity, but that its effect varied between herds. Between herd variation was also noted by (Nordlund et al 1996) and when considered in view of the genetic variation between strains of MAP (Pavlik et al 1999; Whittington et al 2000) it raises for further study the possibility of differences in the virulence of MAP strains found in cattle. Misclassification had a very minor effect on the estimated effect of JD on productivity indicating that to best illustrate the effects of the disease future research should study as many herds as possible rather than intensively studying a few. The greatest weakness of this study was the small number of herds followed. The high cost of testing prohibited the study of a large number of herds.

Data collected in Chapter four were analyzed in Chapter five. The repeated application over time of both the ELISA and faecal culture tests for JD to cows from four herds provided a data set with which to critically evaluate the performance of these tests. The greatest strength of Chapter five was the development of a novel 'regression model' method for the estimation of diagnostic test performance. The regression model incorporates into the probability that an individual cow is infected, information about its age, lactation stage, and previous test results. This method was compared with previously published methods. It revealed that the sensitivity of tests (ELISA, faecal culture) increased with age and with lactational stress to higher levels than previously believed, but consistent with the biology of JD. It also showed that prevalence estimates may actually be lower following repeated testing of a population, compared with when the population is tested in a cross sectional manner.

In Chapter six key results from Chapters three and four were combined with systematically collected expert opinion in a computer simulation model. The model was designed to simulate the effect of JD on the average JD positive farm in New Zealand and the effect of control strategies on the prevalence and economic impact of the disease. This study represents the first time that the disease has been simulated in a pasture based dairy production system. Furthermore, the results were quite different from those of earlier studies, when predicting the change in prevalence over time, in the absence of control. Differences in the rate at which prevalence changes impact on the financial attractiveness of potential control strategies. Reduction of the effective contact rate, achieved by hygienic management of calves was identified as the most effective means of reducing the prevalence of JD, but uncertainty surrounding its cost meant that we were unable to compare its economic attractiveness with that of other control strategies. Control via test-and-cull methods appeared untenable due to high cost. Results from this study and at least one of the earlier models (Collins and Morgan 1991a) were sensitive to changes in the effective contact rate between susceptible calves and MAP. Future research should define the effective contact rate more accurately and validate the simulated output against field data. This would improve both the epidemiological understanding

of JD and, as a consequence, related simulation models. The limitations of the simulation study were primarily due to uncertainty regarding many input parameters, and to the challenges inherent validating the results.

Further application of methodology and results

During the course of this project there were two instances in which novel analytical techniques were developed. These techniques might be profitably used in futures studies of the epidemiology of JD.

The first was the Bayesian regression model that evaluated diagnostic tests for JD in the absence of a gold standard while accounting for the effect of covariates and repeated testing. The strength of this approach was that it allowed test sensitivity to be estimated for levels within covariates, for example in cows at different parity levels. A further strength of this approach is that it provides a means of analysing repeat measure data which is not possible with the existing Hui-Walter type models (Dendukuri and Joseph 2001). The covariate model described in this thesis could be applied to other data describing JD in dairy cows, but more importantly, it could also be applied to data describing diagnostic tests for other diseases.

The second novel technique used in this thesis was the method of extending the logistic regression analysis to adjust for missing values. The adjustment comprised taking a weighted average of several models, where weighting was by the number of individuals analysed. In this way the resulting coefficients were most strongly influenced by models with complete or nearly complete data, but coefficients could also be obtained for variables with more missing data. While this approach entails more work than applying a single model, it provides a greater understanding of the data. In the event that it is used in the future, this method should also include a description of the amount of data missing from each variable in the model.

A bank of 5467 serum samples from 1541 cows has been stored for future reference. It contains sera from the full spectrum of JD infection, from very mildly affected (e.g. a single positive test over 4 years) through to serum from animals that were consistently test positive. The serum bank is the culmination of a large amount of work and the spectrum of infection states in the sampled cows is of particular value for two reasons. It facilitates the study of the immune behaviour of cows across the spectrum of subclinical disease. It is also an excellent resource with which to validate new diagnostic tests. Validation against field-collected data rather than against a group of heavily infected cows will provide much more informative insights into how newly developed tests may perform in the 'real world'.

Key deficiencies in the epidemiological understanding of Johne's disease

Significant gaps in our understanding of JD persist after over a hundred years of study (Chiodini 2005). It is a disease of probabilities. We are reasonably confident of some aspects but can only guess at others.

Pivotal to our understanding of the disease is the effective contact rate between susceptible individuals and the infectious organism, or, in other words, factors pertaining to hygienic calf management most importantly, but also factors such as the infection rate in adult cattle. The effective contact rate depends on the number of organisms required to initiate infection, which, under field conditions, remains unclear. The effective contact rate could be better understood by investigating transmission via individual management components. In particular, the probability of infection via pooled milk and colostrum, and the probability of infection via other calves excreting MAP. These topics are notoriously difficult to study. The concentration of MAP in colostrum is generally below the limit of detection by culture, while the infection of calves by natural means is difficult to achieve. Calves have been successfully infected by being kept in pens with other experimentally infected calves (Stuart 1965) and this method could used to further study MAP excretion by calves. Recent research indicates the proportion of infected calves shedding MAP may be surprisingly high (Bolton et al 2005; Weber et al 2005). At present, the study of this subject is limited by the poor performance of diagnostic tests and the necessity of ensuring that infection occurs only by the route of interest and not by other means.

The rate of change in both the prevalence and incidence, within and across New Zealand dairy herds represents another significant knowledge gap. Available data is inadequate but suggests a slowly spreading disease which in most herds exists at equilibrium with few clinical cases and little economic effect. The answer to this question may be influenced by changes in dairy production in the future, as more intensive production and a greater population of dairy cows is likely to result in more JD. Answers to help clear the waters with respect to the incidence and prevalence of disease could probably be obtained more easily than answers regarding the effective contact rate.

Further study of the genetic variation between strains of MAP may shed light on between herd variation in the effect of JD, and the roles of wildlife and domestic species in its epidemiology.

Between herd variation is evident in the longitudinal study in this thesis and elsewhere (de Lisle 1989; Nordlund et al 1996) but in general, it is seldom described. It may be due to particularly virulent strains of the organism, or to differences in management practices, or both. Characterisation of the RFLP (Chiodini 1990) types of MAP from farms with severe JD and from farms on which the disease has only mild effects may demonstrate genetic trends. These

trends could subsequently be used to identify farms on which the disease is more likely to become a serious problem.

JD is present in farmed deer (de Lisle et al 2003) and sheep (Davidson 1970) in New Zealand, however epidemiological links between these species and cattle remain unclear. Differences between the strains of MAP commonly infecting cattle and sheep have been reported (Collins and deLisle 1986) indicating that an epidemiological link between these species is unlikely, but the possibility of a link between deer and cattle remains.

A highly sensitive diagnostic test would greatly aid in the control of JD, and its epidemiological investigation. One attractive possibility achieves sensitivity in excess of 90% by using antigen extracted from the surface of MAP by ethanol (Eda et al 2006), however it remains to be evaluated in the field. The variable but often long latent period following infection, during which MAP is present in low numbers with little or no replication (Chiodini 1996) represents a challenge to the sensitivity of any diagnostic test. Cross reactivity with other mycobacterial species represents a challenge to test specificity. Considerable improvements in test performance have been achieved over the years and while the chances of developing the 'perfect' test are slim, existing methods could well be improved further yet.

In summary, some important aspects of the epidemiology of JD in dairy cattle and other species remain uncertain. Future research will definitely diminish this uncertainty and may, with new technologies, lead to 'eureka' moments that greatly improve our understanding of the disease.

The case for a coordinated national control program for Johne's disease in New Zealand

A nationally coordinated control approach to controlling JD in New Zealand dairy herds is not justified based on the evidence presented in this thesis and a critical review of the literature. The following discussion addresses the reasons for this, while focusing on the scale of the strategy, rather than the particular type of control employed.

On most infected farms, the financial loss associated with JD is unlikely to warrant a concerted control effort. The results from Chapters three and four indicate that, for typical infected herds, some may experience significant losses, but most do not, and the incidence of clinical cases is usually low. The major impediments to dairy productivity in New Zealand are more likely to be infertility, mastitis or lameness. A farmer is likely to focus on these issues to improve productivity and herd health. Were a strategy not funded by the government, market forces must eventually drive its success, with participants gaining a competitive advantage from their investment. Absence of this competitive advantage probably explains the low levels of participation in existing JD control strategys (McCaughan 1989; Wraight et al 2000; Raizman et al 2006).

Control of JD at the national level is not attractive from a prevalence perspective. JD is considered widespread amongst New Zealand dairy herds and has been for some decades. A control strategy is unlikely to reduce the proportion of infected herds significantly, based on control efforts in The Netherlands (Benedictus et al 2000). Furthermore, it would require a source of disease free replacement animals and creation of this source would be a significant challenge.

The use of control strategies to enhance market share must not be detrimental to the overall demand for dairy products. In a global economy, in which the major dairy producing nations all have herds infected with JD, actively advertising the presence of this disease is likely to be detrimental to consumer perception of dairy products, particularly if negative speculation regarding the possible link between human Crohn's disease and MAP were to emerge.

A control strategy for JD in dairy cattle would require consideration of other susceptible farmed and wild species. Consequently it would be a much more complex program than for a single species in isolation. The control of bovine tuberculosis (*M. bovis*, closely related to *M. avium* subsp. *paratuberculosis*) in New Zealand involves cattle, farmed deer and wildlife species, primarily possums, ferrets and wild deer (Morris et al 1994). In 2005 NZD 87 million (Animal Health Board, 2006) was spent maintaining this strategy and while it has successfully reduced the number of infected farms to a very low level, bovine tuberculosis has continued to spread geographically. This strategy and efforts to control *M. bovis* in the United Kingdom (Krebs 1997) and other countries (de Kantor and Ritacco 1994) demonstrate the difficulty in controlling mycobacterial disease when multiple susceptible species must be considered.

The unknown form of a possible strategy limits the discussion of methods for ensuring high subscription rates, securing funding, deciding on subsidies, providing a transparent means of monitoring success and devising a clear pathway down which farmers can move to freedom from the disease. These are all challenges in their own right that should not be underestimated.

Human health grounds are the single and most powerful unknown regarding the justification of JD control. If the tentative link between human Crohn's disease and MAP (Chiodini et al 1984b; Hermon-Taylor 2000) were confirmed to the satisfaction of the wider scientific community, then findings of MAP in pasteurised dairy products (Grant et al 2001; Grant 2002; Lund et al 2002) would dictate that a renewed and considerably more active approach to the control of JD is in order. As it stands, the link remains unproven however the impact of negative speculation in the public arena should not be underestimated.

How Johne's disease could best be controlled in New Zealand dairy herds

A strategy for the control of JD in New Zealand is proposed based on a review of strategies in other countries and the results in this thesis. In this strategy a series of articles in dairy farmer oriented publications would help raise awareness of the disease, and list veterinary practices where help could be obtained in its control. The listed veterinary practices would represent a small proportion of those in dairy farming areas of New Zealand. A small number of staff from these practices would be trained to administer a farm-level risk assessment for JD and recommend alterations to management based on this assessment. The farmer is left to implement the recommended changes. The cost to the farmer of the veterinarians time could be subisidised. Periodically, refresher articles would be published in dairy farmer oriented publications to maintain awareness of the disease.

This strategy is simple, of low cost and easy to implement. It would select for farmers who actively want to control JD. Money and effort would not be invested in encouraging uptake of control by hitherto disinterested farmers, or farmers with herds only mildly affected by the disease. Furthermore, there may be other advantages related to improving the hygiene associated with young stock, such as reduced mastitis transmission and lower rates of gastrointestinal infection. Consequently the return on investment for implementing changes to calf rearing systems may be larger than due to JD alone.

A weakness of this approach is that it does little to control the disease, hence a significant reduction in the prevalence of infected herds is unlikely. But in its defence, the more elaborate and expensive approaches used overseas also struggle to reduce the prevalence of infected herds. Also there is no form of monitoring to ensure farmers act on veterinarian's recommendations. The main attractions of this approach are that it stimulates awareness of JD and presents farmers with a means of reducing the associated economic losses.

Predicting the future impact of Johne's disease in New Zealand

In New Zealand, the last three decades have seen a slow but steady decline in the number of dairy farms, but a rapid increase in herd size. In addition, the dairy sector is currently profiting from unprecedented growth in the global demand for dairy products, complimented by a limited supply. The economic attractiveness of dairy farming continues to grow in view of poor returns for sheep farming. In the next few years this is likely to result in a rise in dairy cow numbers, probably in large herds. Consequently, an increase in the gross amount of JD may be observed. The prevalence of infected herds will also rise, as increased trade of dairy cows disseminates the disease to new and growing herds. The most consistent risk factor for JD across existing studies

is large herd size (Jakobsen et al 2000; Wells and Wagner 2000; Daniels et al 2002; van Weering et al 2005).

Changes in the within-herd prevalence are more difficult to predict. Given the scant available evidence, it could be said that if within-herd JD had not become a matter of great national concern in its first ninety years it is unlikely to become so in the next few years.

In the longer term, for example 20 years, the disease will, most likely, continue it's slow but steady spread. Infertility and mastitis will continue to rank highest as animal health issues, while JD will occupy a somewhat lower position in the ranking and may well be further overshadowed by other factors.

Rising pressure to limit the environmental impact of dairy farming is one of these factors. Growing public awareness of environmental degradation is likely to increasingly conflict with a growing dairy industry and with growth in dairy related income. Water consumption is an example that presently affects dairy farms on the Canterbury plains in the South Island (Bidwell 2006). Other growing issues include green house gas emissions and pollution via high nitrogen levels in water draining from dairy farms (Ledgard et al 1998). Constraints in response to these issues are most likely to be imposed by regulatory bodies, and failing this, may be imposed by the physical environment itself if predictions of climatic instability prove accurate.

Another factor is increasing production costs, energy in particular. The cost of energy invested in producing and collecting 1.4 billion tonnes of milksolids annually (Ministry of Agriculture and Fisheries, 2007), from all corners of New Zealand, will continue to climb, decreasing profitability. A similar situation is evident in the United States of America, where dairy production is becoming more expensive due to high grain prices in response to demand for materials to produce bio-fuel (Aware 2007). JD, which on most farms is not of great economic importance, will be relegated further down the farmer's priorities as maintaining profitable production becomes a greater challenge.

In conclusion, the studies described in this thesis greatly improve the understanding of JD in the New Zealand dairy herd. Perhaps the only certainty in the years ahead is that the disease will persist in some capacity. Growth in the dairy industry will result in growth in the total loss in productivity but herd-level losses are unlikely to change greatly. JD will remain of minor concern next to the major animal health issues such as infertility and mastitis, particularly if other environmental issues become more prominent. The caveat to this prediction is conclusive proof of the Crohn's-Johne's link which would lead to great changes in the way JD was perceived and to initiation of a much more aggressive approach to its control.

The epidemiological insights provided in this thesis, together with related research, provide exciting reasons to study JD further. Clarification of the effect of genetic strain on the virulence of MAP may help explain differences in the effect of the disease between herds, improving our

understanding of JD. The challenge of utilising this information to further refine the cost effectiveness of disease control programs is quite achievable.

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Appendix one

Bayesian regression model for estimating diagnostic test performance
parameters and prevalence of disease

```
model;
#          Sensitivities
{  for (j in 1:n) {
           logit(Se[1,j]) <-  g[parity[j]] + g[6]*x[j]
           logit(Se[2,j]) <-  h[parity[j]] + h[6]*x[j] }

# In late lactation, Se (ELISA) for each parity:
st1[1] ~ dbeta(1.38,2.51)        # Parity 1, mode 0.20, 95% sure <0.75
st1[2] ~ dbeta(1.33,1.78)             #2 mode 0.30, 95% sure <0.85
st1[3] ~ dbeta(2.23, 2.23)            #3 mode 0.50, 95% sure <0.85
st1[4] ~ dbeta(2.23, 2.23)            #4 mode 0.50, 95% sure <0.85
st1[5] ~ dbeta(1.62, 1.94)            #5+  mode 0.40, 95% sure <0.85
st1[6] ~ dbeta(1.48, 2.59)        # Parity 1, early Lactation, mode 0.23, 95% sure <0.75
# In late lactation, Se (faecal culture) for each parity:
st2[1] ~ dbeta(1.38, 2.15)            # parity 1, mode 0.25, 95% sure <0.80
st2[2] ~ dbeta(1.46, 1.85)            # mode 0.35, 95% sure <0.85
st2[3] ~ dbeta(1.75, 1.62)            # mode 0.55, 95% sure <0.90
st2[4] ~ dbeta(1.75, 1.62)            # mode 0.55, 95% sure <0.90
st2[5] ~ dbeta(1.87, 2.06)            # mode 0.45, 95% sure <0.85
st2[6] ~ dbeta(1.47, 2.21)        # Parity 1, early lactation, mode 0.28, 95% sure <0.80

#  Induces priors on the regression coefficients for both tests
for (i in 1:5){ g[i] <- logit(st1[i])  h[i] <- logit(st2[i]) }
    g[6] <- logit(st1[6]) - g[1]  h[6] <- logit(st2[6]) - h[1]

cow[1] <- z[24]        # (ELISA, FC) + -       - -       - -
cow[2] <- z[105]       #         + -      - -       - -       - -
cow[3] <- z[151]       #         - +      - -       - -       - -       - -
cow[4] <- z[245]       #         + -      - -       - -       - -       - -
cow[5] <- z[376]       #         - -      - -      - +      - -      - +
cow[6] <- z[674]       #         + -      - -       - -       - -       - -
cow[7] <- z[533]       #         - +      - -
cow[8] <- z[206]       #         - +      - +
cow[9] <- z[726]       #         - -      - -       - -       - +
cow[10] <- z[15]       #         - -
cow[11] <- z[23]       #         - -      - -       - -
cow[12] <- z[51]       #         - -      - -       - -       - -       - -       - -
cow[13] <- z[68]       #         - -      - -       - -       - -       - -

#          Specificities
Sp[1] ~ dbeta(8.5, 1.4)          #  mode 0.95, 95% sure >0.65
Sp[2] ~ dbeta(9.2,1.17)          # mode 0.98, 95% sure >0.70

#          Prevalence
for (i in 1:ncid){ z[i] ~ dbern(pi[i])
    logit(pi[i]) <- b[hid[off[i]]]              }
for (i in 1:n)  {
    y[i,1:4] ~ dmulti(p[i,1:4],1)

p[i,1] <- z[cid[i]]*Se[1,i] * Se[2,i] +  (1-z[cid[i]]) *  (1-Sp[1]) * (1-Sp[2])
p[i,2] <- z[cid[i]]*Se[1,i] * (1-Se[2,i]) + (1-z[cid[i]])*(1-Sp[1])*Sp[2]
p[i,3] <- z[cid[i]]*(1-Se[1,i])*Se[2,i] + (1-z[cid[i]])*Sp[1]*(1-Sp[2])
p[i,4] <- z[cid[i]]*(1-Se[1,i])*(1-Se[2,i]) + (1- z[cid[i]])*Sp[1]*Sp[2]          }

pt[1] ~ dbeta(1.55, 4.12)  # True prevalence for herd B, mode: 0.15, 95% sure <0.60
pt[2] ~ dbeta(1.55, 4.12)  # True prevalence for herd H, mode: 0.15, 95% sure <0.60
pt[3] ~ dbeta(1.25, 4.88)  # True prevalence for herd M, mode: 0.06, 95% sure <0.50
pt[4] ~ dbeta(1.25, 4.88)  # True prevalence for herd T, mode: 0.06, 95% sure <0.50

for (i in 1:4){ b[i] <- logit(pt[i])   }
```