This is to certify that the
dissertation entitled

CONTROL STRATEGIES FOR JOHNE'S DISEASE IN DAIRY CATTLE

presented by

ROXANNE BEE PILLARS

has been accepted towards fulfillment
of the requirements for the

Ph.D degree in Large Animal Clinical Science (Epidemiology)

Major Professor's Signature

9-15-08

Date

CONTROL STRATEGIES FOR JOHNE'S DISEASE IN DAIRY CATTLE

By

Roxanne Bee Pillars

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Large Animal Clinical Sciences

2008

ABSTRACT

CONTROL STRATEGIES FOR JOHNE'S DISEASE IN DAIRY CATTLE

By

Roxanne Bee Pillars

A five-year longitudinal study was performed to better understand Johne's disease (JD) control in dairy cattle in terms of its impact on the environmental reservoir for the disease, the disease burden within the herd, and the cost-effectiveness of control programs. The objectives of this study were to: (1) describe the distribution *Mycobacterium avium paratuberculosis* (MAP) in the environment of infected dairy farms over time, and observe how that distribution changes as herd prevalence changes; (2) evaluate the effectiveness of management practices in reducing the JD burden within the herd; and (3) determine if the management practices implemented to control JD were cost-effective.

Seven dairy herds infected with JD participated in this study. Upon study enrollment, each herd implemented a JD control program designed specifically for that farm, based on a JD risk assessment and the operation's goals and capabilities. The risk assessment was repeated annually, and the control program modified as necessary. Within herd JD prevalence was monitored annually by fecal culture and/or serum ELISA testing of all adult cows. Every six months, samples of feed, water, and bedding were collected and cultured for MAP, from the pre-weaned calf, weaned calf, lactating cow, and maternity areas, as well as the primary manure storage area and pasture when appropriate. A questionnaire was developed and administered to each producer and/or

herd manager yearly, to collect information on the costs incurred as a direct result of the JD control program. Based on the data collected, descriptive statistics were generated. Logistic regression was used to assess the effectiveness of management changes in preventing infection with MAP, and the net present value (NPV) of the each farm's JD control program was calculated.

Environmental contamination with MAP was consistent over time. When herd prevalence was >2%, MAP was cultured from the lactating cow floor and/or manure storage 75% of the time. When herd prevalence was $\leq$2%, MAP was never cultured from any area sampled.

Management practices associated with neonatal calf care were found to have the greatest impact on cows subsequently testing positive for JD as adults. Specifically, those factors were: exposure to adult cows other than dam at birth (OR = 1.09, 95% CI: 1.06 – 1.13), and feeding colostrum from one cow to multiple calves (OR = 1.10, 95% CI: 1.09 – 1.12). When designing JD control programs, implementing management practices that minimize the exposure of newborn calves to *Mycobacterium avium paratuberculosis* being shed by infected adult cows should take priority.

The NPV for the JD control program varied greatly across the herds. When calculated across all cows in the herd, the costs of the JD control programs implemented on these herds averaged $30/cow/year with a median of $24/cow/year. The annual losses due to JD averaged $79/cow/year with a median of $66/cow/year. Investing in a JD control program can be cost effective, and doing something to control JD was always a better economical decision than doing nothing.

ACKNOWLEDGEMENTS

I would like to thank my graduate committee members, Drs. John Kaneene (Center for Comparative Epidemiology), Daniel Grooms (Department of Large Animal Clinical Sciences), Joseph Gardiner (Department of Epidemiology), and Christopher Wolf (Department of Agricultural, Food and Resource Economics), for their guidance and assistance in completing this project. A special, sincere thank you is extended to my major advisors, Drs. John Kaneene and Dan Grooms. Thank you for always having open doors, open minds, and an endless reserve of patience.

Another special thank you is sent to Dr. Joseph Woltanski, USDA-APHIS, Veterinary Services, East Lansing, MI, for always being willing to get down and dirty while collecting samples, and still being able to write legibly. I would also like to thank Joseph Hattey, Diagnostic Center for Population and Animal Health, Michigan State University; and Tom Martin, Geagley Laboratory, Michigan Department of Agriculture; and their respective assistants; for processing and testing the thousands of fecal and serum samples collected over the course of this study. The data analysis is only as good as the test results on which it is based.

This project was funded in a large part by a grant from the USDA/APHIS/VS-Johne's Disease Program, with additional support provided by the Michigan Department of Agriculture, and the Center for Comparative Epidemiology, Michigan State University.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Johne's disease (JD) is ranked as one of the top three health issues affecting dairy cattle in the US (Wells, et al, 1998), and is predicted to be the most economically important infectious disease in the dairy industry unless control practices are implemented (Collins, 2003). Johne's disease is prevalent worldwide, and is becoming increasingly so in the US. Based on a national survey in 1996, it was estimated that JD cost the US dairy industry between $200 – 250 million annually (Ott, et al., 1999). Since that time, the estimated reported prevalence of JD infected dairy herds in the US has tripled (USDA, 1997; USDA, 2008). It is likely the economic burden of JD has increased too.

Aside from the substantial cost of JD to both the herd and economic health of dairy farms, there is also a potential human health risk. In recent years, there has been emerging evidence linking *Mycobacterium avium paratuberculosis* (MAP), the causative agent of JD, to Crohn's disease in people (Feller, et al. 2007). If this link is ever proven, or perceived, to be causal, the economic damage to the dairy and meat markets, both foreign and domestic, not to mention the loss of consumer confidence, could be unimaginable (Hansen and Rossiter, 1999). Consequently, understanding JD, along with its management and control, has become a priority for the US livestock industry (Linnabary, et al., 2001).

Johne's disease is a chronic disease, characterized by an untreatable, slowly progressive, granulamatous enteritis (Sweeney, 1996). Cows generally become infected as young calves, but do not develop clinical signs of the disease until they become adults,

two to five years later (Sweeney, 1996; Collins, 2003). Due to the long incubation period and subclinical stage of the disease, infected cattle are difficult to identify using currently available diagnostic tests, yet can become infectious at any time, potentially spreading the infection to susceptible herdmates (Whitlock 1992; Whitlock and Buergelt, 1996). As a result, testing and culling positive cattle, in and of itself, is relatively ineffective in controlling JD. Instead, control of JD must focus on implementing farm management practices that minimize the transmission of MAP to susceptible animals (Collins, 2003; McKenna, et al., 2006).

Recommended farm practices to control JD are all based on what is currently known about MAP infection and its pathogenesis. Validation of these control practices in the field is limited, because of the time and expense it would require to collect the data (Groenendaal and Galligan, 2003). Instead, farm practices to control JD have been simulated, using existing theory for control, expert opinion, and the limited field data available (Collins and Morgan, 1992; Groenendaal, et al., 2002; Dorshorst, et al., 2006; Kudahl, et al., 2007). Field validation of these control practices is still warranted.

Equally important as determining which farm practices are effective in limiting the transmission of JD, is estimating what it costs to implement those practices. Voluntary, widespread adoption of JD control programs will only occur if they are proven to be cost-effective. Numerous studies have attempted to quantify the costs of JD in terms of lost milk production and herd performance (Ott, et al., 1999), but studies on the cost to control the disease are lacking. Only when both sides of the equation are known (what the disease is costing the farm in lost production, and what it will cost to

implement changes to control the disease) can producers make sound economic decisions regarding JD control.

In summary, the control of JD is dependent on the implementation of farm practices that minimize the transmission of the disease and are cost-effective. Further farm-based studies are necessary to validate the effectiveness of practices recommended for JD control. Above all, there is a need to quantify the costs of implementing these control practices.

This dissertation documents a five-year observational study of JD control practices implemented on seven commercial dairy herds in Michigan. The objective of this study was to answer the following four questions:

1) What is the extent of the MAP infectious burden in the environment of infected farms in relation to the JD burden in the herd, and does it change over time?

2) Do farm management practices designed to limit the transmission of MAP infection actually decrease the JD burden in a herd over time?

3) What specific management practices are the most effective in decreasing the JD burden in a herd?

4) Are management practices to control JD cost effective?

CHAPTER 1

LITERATURE REVIEW

1.1. Introduction

Johne's disease (JD) is a chronic enteric disease caused by *Mycobacterium avium paratuberculosis* (MAP). Although recognized primarily in cattle and other ruminant species, JD has been diagnosed in many other species of domestic and wild animals (Thoen, et al., 1975; Chiodini, and VanKruiningen, 1983; Williams, et al., 1983; Stehman, 1996; Buergelt and Ginn, 2000; Beard, et al., 2001; de Lisle, et al., 2002; Daniels, et al., 2003a; Daniels, et al., 2003b; Corn, et al., 2005; Davidson, et al., 2004; Palmer, et al., 2005; Raizman, et al., 2005). It is prevalent worldwide, and has been predicted to be "the single most economically important single etiology infectious disease of dairy cattle" unless control measures are instituted (Collins, 2003).

Johne's disease is not a new disease. It was first diagnosed in a cow in Germany in 1895 by Drs. Johne and Frothingham (Olsen, et al., 2002). The first report of JD in the US occurred in Pennsylvania in 1908 (Pearson, 1908). Since that time it has spread across the country, and prevalence is increasing. In 1996, the National Animal Health Monitoring Service (NAHMS) estimated that 21.6% of dairy herds were infected with JD (USDA, 1997). In 2007, that estimate increased to 68% of dairy herds infected (USDA, 2008). Other regional estimates of the prevalence of MAP infected dairy herds range from 50 – 96% (Collins, et al., 1994; Thorne and Hardin, 1997; Johnson-Ifearulundu and Kaneene, 1998; Hirst, et al., 2004; Keller, et al., 2004; Berghaus, et al. 2006; Lombard, et al., 2006). In fact, JD is ranked as one of the top three health issues affecting dairy cattle (Wells, et al., 1998). The most recent national survey of beef herds estimated that 8% were infected with JD (Dargatz, et al., 2001), although other estimates vary from 4 – 76% depending on the method used to detect the disease, study design, and region of country

(Thorne and Hardin, 1997; Hill, et al., 2003; Pence, et al., 2003; Keller, et al., 2004; Roussel, et al., 2005). Economic losses to the US cattle industry due to JD have been estimated to exceed $1.5 billion annually (Stabel, 1998; Harris and Barletta, 2001). Because of its substantial economic impact, and the potential public health issues should MAP be definitively linked to Crohn's disease in people, understanding JD along with its management and control has become a priority for the US livestock industry (Linnabary, et al., 2001).

The purpose of this paper is to review what is currently known about JD: the causative agent; pathogenesis as it relates to disease transmission; epidemiology in terms of environmental reservoirs, risk factors for transmission, and recommended control practices; and the economics of the disease and its control. Particular emphasis will be given to management, control, and economics of JD on dairy farms.

1.2. *Mycobacterium avium paratuberculosis*

Mycobacterium avium paratuberculosis (MAP) is a gram-positive, acid-fast bacterium (Stabel, 1998; Harris and Barletta, 2001; Whittington and Sergeant, 2001; Olsen, et al., 2002; Tiwari, et al., 2006). It possesses a thick waxy cell wall made up of approximately 60% lipids (Rowe and Grant, 2006). This cell wall provides MAP with a survival advantage, both inside and outside of the host. It is sticky which leads to clumping of the bacteria (Klijn, 2001; Grant, 2003) and provides it with increased resistance to disinfectants (Whan, et al., 2001) and physical processes such as pasteurization (Grant, et al.1996; Grant, et al., 1998; Ellingson, et al., 2005). However, the cell wall also restricts the uptake of nutrients, making MAP the slowest growing of

the cultivable mycobacteria (Rowe and Grant, 2006), with a generation time, under optimal growth conditions, exceeding 20 hours (Lambrecht, et al., 1988). MAP is differentiated from other members of the *M. avium* complex phenotypically by its dependency on mycobactin, an iron-binding siderophore necessary for growth; and genotypically by multiple copies of an insertion element, IS900 (Harris and Barletta, 2001; Olsen, et al., 2002; Tiwari, et al., 2006, Rowe and Grant, 2006).

Similar to other pathogenic mycobacteria (*M. tuberculosis, M. bovis, M. leprae*), MAP is an obligate intracellular pathogen, surviving and multiplying within macrophages (Rastogi and David, 1988). Viable mycobacteria have been identified in three different sites inside the macrophage: phagosome, phagolysosome, and the cytoplasm (Rastogi and David , 1988), suggesting multiple mechanisms for evading the killing mechanisms of the macrophage. Most of these mechanisms appear to center around the bacterial cell wall (Ryter, et al, 1984; Rastogi and David, 1988; Frehel, et al., 1989). The lipid-rich cell wall of MAP is difficult to permeate (Rastogi and David, 1988, Chiodini 1996). Also common among intracellular mycobacteria, including MAP, is the ability to inhibit the fusion between phagosomes and lysosomes, thereby avoiding degradation by lysosomal enzymes (Ryter, et al., 1984; Frehal, et al., 1986; Frehal, et al., 1989). In other instances, mycobacteria develop a protective capsule composed of mycosides which allow the bacterium to survive in the hostile environment of the phagolysosome (Ryter, et al., 1984; Rastogi and David, 1988, Frehel 1989). While all mycobacteria, in general, have the ability to employ each or all the above evasion mechanisms, recent work suggests there are important strain differences, and some strains of MAP are more capable than others

of surviving inside the macrophage; hence making them more pathogenic (Gollnick, et al., 2007).

As an obligate intracellular pathogen of animals, MAP does not replicate outside the host in the environment (Whittington and Sergeant, 2001). It has been suggested that in addition to its cell wall, survivability of MAP is aided by recently identified gene sequences that, in the absence of adequate nutrients, allow it to enter a dormant, "viable-noncultivable state" and revert to a vegetative form when conditions again become favorable (Whittington, et al., 2004; Greig, 2005). Regardless, survival time of MAP in the environment, even with the ability to become dormant, is finite in the absence of an animal host.

1.3. Transmission of Johne's disease

In the domestic livestock industry, transmission of MAP from an infected to an uninfected herd almost always occurs through the purchase or introduction of infected animals (Sweeney, 1996; Step, et al., 2000; Whittington and Sergeant, 2001). The more important aspect of JD is how it is maintained, or spreads within a population or herd.

The primary route of MAP infection is fecal-oral, with ingestion of the bacterium occurring via exposure to contaminated feedstuffs or environment (Sweeney, 1996; Step, et al., 2000; Whittington and Sergeant, 2001; Olsen, et al., 2002; Greig, 2005). Young animals are more susceptible to MAP infection than older animals (Hagan, 1938; Rankin, et al., 1961; Larsen, et al., 1975; Sweeney, 1996; Whittington and Sergeant, 2001). For example, in an observational study, 13 of 26 (57%) of calves born and raised on an infected farm either died of JD or had MAP lesions; while only one of six (17%) calves

introduced into the herd when less than one year old, and none of six heifers introduced at greater than one year of age, had evidence of JD (Hagan, 1938). Also, when cattle were experimentally challenged with the same dose of MAP, the tissues of calves exposed at one month of age contained more MAP and pathologic lesions than did those of calves exposed at nine months of age or adult cows (Larsen, et al., 1975). However, that does not mean adult cattle are immune to MAP infection. Evidence suggests adult cows can become infected if repeatedly exposed to high doses of MAP (Rankin, 1962, Sweeney, 1996; Kovich, et al., 2006), but the extended incubation period characteristic of MAP makes it unlikely the disease will manifest itself during their productive lifetime (Sweeney, 1996; Whittington and Sergeant, 2001). The mechanism for increasing resistance to MAP infection with age is unknown. It has been hypothesized to be due do to the incomplete development of the immune system in young ruminants and/or easier access to the intestinal mucosa due to the "open gut" during the first 24 hours of life, which allows the absorption of macromolecules such as colostral immunoglobulins, and perhaps MAP (Sweeney, 1996; Olsen, et al., 2002). In addition, JD transmission is facilitated by the fact that shedding of MAP by infected cows is precipitated by parturition (Harris and Barletta, 2001; Greig, 2005); thereby increasing the probability of exposing and infecting the next generation of herd replacements.

While fecal-oral transmission is the most common, MAP infection can also be spread directly from dam to calf through *in-utero* infection or from contaminated colostrum and milk. Transplacental infection has been reported in multiple studies. The incidence of fetal infection occurring in cows in the clinical stages of JD ranges from 20-40% (Pearson and McClelland, 1955; Lawrence, 1956; McQueen and Russel, 1979;

Seitz, et al., 1989). In asymptomatic, MAP infected cows, transplacental infection occurred in only 8.6% of fetuses, and all occurred in cows classified as heavy fecal shedders (Sweeney, et al., 1992a). Collectively, in a recent meta-analysis, it was estimated that *in-utero* MAP infection occurred in 9% of fetuses from subclinically infected cows and in 39% of fetuses from clinically infected cows (Whittington and Windsor, 2007).

Johne's disease can also be transmitted from dam to offspring through colostrum and milk. In one study, MAP was isolated from the colostrum of subclinically infected cows, as identified by fecal culture; 36% from cows classified as heavy shedders and 9% from light shedders. Isolation of MAP from colostrum was nearly three times of that in milk (Streeter, et al., 1995), and may again be due to the propensity for infected cows to shed MAP at parturition. MAP has been isolated in the milk of up to 35% of cows with clinical JD (Taylor, et al., 1981), 19% of asymptomatic heavy shedders, and 3% of asymptomatic light shedders (Sweeney, et al., 1992b). Thus, evidence suggests that as JD advances, the more disseminated the infection becomes, and the more likely it is for infected dams to pass MAP to their offspring, rather it be *in-utero*, directly in colostrum or milk, or from fecal contamination of the environment.

Other potential routes of infection include: semen from infected bulls, embryo transfer, wildlife reservoirs, and fomites. MAP has been isolated from the semen and accessory sex organs of naturally infected bulls, providing the potential for infecting the uterine environment of cows (Ayele, et al. 2004). However, following experimental inoculation into the uterus near the time of insemination, MAP was not cultured in the uterus or any extra-uterine organs beyond three or four weeks; leading to the conclusion

that MAP in the semen of bulls is more likely to be destroyed in the uterus rather than establishing a systemic infection in the cow (Merkal, et al., 1982).

Regarding embryo transfer, *in-utero* infection of an embryo from an uninfected cow placed into a MAP infected recipient has been documented (Manning, et al., 2003). However the reverse, infection of the recipient after implanting an embryo from an MAP infected cow, has not been proven. MAP has been isolated from the uterine horns of naturally-infected donor cows as well as embryos collected from them (Kruip, et al., 2003; Bielanski, et al. 2006). Yet, when embryos were washed according to the procedure established by the International Embryo Transfer Society and placed in uninfected recipients, none of the recipients, or the resulting calves, developed MAP infection over a period of five years (Bielanski, et al., 2006). It was concluded, therefore, that the risk of embryo transfer transmitting MAP infection from an infected donor to the recipient or the calf is very small (Sweeney, 1996, Kruip, et al., 2003; Bielanski, et al., 2006).

Aside from domestic ruminants such as cattle, sheep and goats, MAP has been cultured from a variety of other domestic and wild animals including: swine (Thoen, et al., 1975), South American camelids (Belknap, et al., 1994; Stehman, 1996); multiple species of deer (Chiodini and VanKruiningen, 1983; Williams, et al., 1983; Davidson, et al., 2004; Raizman, et al., 2005), bighorn sheep (Williams, et al., 1983), Rocky Mountain goats (Williams, et al., 1983), elk (Williams, et al., 1983), bison (Buergelt and Ginn, 2000), rabbits (Daniels, et al., 2003a; Raizman, et al., 2005); feral cats (Palmer, et al, 2005) as well as a variety of wild birds (Beard, et al., 2001; Daniels, et al., 2003b; Corn, et al, 2005) and non-ruminant wildlife, both predator and prey (Beard, et al., 2001;

deLisle, et al., 2002; Daniels, et al., 2003b; Corn, et a., 2005). Many of these animals are dead end hosts; meaning that although they are infected, they do not excrete MAP at sufficient levels to be infectious, or their feces (such as that of foxes or stoats that eat infected rabbits) are repulsive causing avoidance by grazing ruminants (Grieg, 2005). It has been demonstrated that MAP can cross the wildlife - domestic species barrier (Williams, et al., 1983, Cetinkaya, et al., 1997; Daniels, et al., 2001; Judge, et al., 2005). However, the volume of MAP shed by infected wildlife is several times lower than that of infected sheep or cattle (Daniels, et al., 2003a; Corn, et al., 2005), and the pelleted nature of the feces of many of these species makes widespread dissemination of MAP into the environment unlikely (Sweeney, 1996). Moreover, the confined housing systems commonly used on many livestock, particularly dairy, operations limits the potential for domestic livestock to commingle with MAP infected wildlife or graze the same pasture. While the contamination of stored feedstuffs by infected wildlife on these operations remains a possibility (Beard, et al., 2001; Daniels, et al., 2003b; Palmer, et al., 2005), transmission of MAP by this route is negligible compared to the contamination of the environment by infected domestic ruminants (Corn, et al., 2005).

A fomite is an object that serves to transfer infectious organisms from one individual to another. MAP is an organism that can readily adhere to objects such as boots, clothing, feeding equipment, vehicles, even other animals, and be transported to different areas within a herd or between herds (Johnson-Ifearulundu and Kaneene, 1998; Grieg, 2005; McKenna, et al., 2006). Observations supporting this have been made during ongoing JD research at Michigan State University. MAP was isolated from the boots of four out of four different people, after walking through the holding pen on an

infected dairy farm (Grooms, unpublished 2008). Failure to wash and disinfect the boots before feeding calves could transport MAP to a population of highly susceptible animals. In another instance, MAP was isolated from skin swabs of the brisket, hock, and/or teats on 7 out of 10 cows housed in the close-up dry cow and maternity pens on an infected dairy farm. Concurrent fecal culture on all ten cows was negative, suggesting these cows were either not infected with MAP or were not actively shedding the bacterium at detectable levels at the time (Bolton, et al., unpublished 2006). Even though these periparturient cows may not have been infected, they potentially could infect their calves with MAP simply because they were carrying the bacterium on their bodies in areas commonly nuzzled by newborn calves. Transmission of MAP between cows by veterinary procedures such as rectal palpation has been suggested, but the ability of MAP to penetrate the rectal mucosa compared to the mucosa of the ileum remains unknown (Sweeney, 1996). While these and other breaks in biosecurity represent potential routes of MAP transmission, they are rarely implicated because they can almost always be traced back to an infected animal in the herd (Sweeney, 1996).

1.4. Pathogenesis of Johne's disease

Infection is defined as the invasion and colonization of pathogens in an organism. Disease is defined as the abnormal functioning of an organism. Disease can result from infection. However, often disease is not directly due to the pathogen, but is rather the result of the body's attempt to rid itself of the infecting pathogen. Such is the case with JD. The granulamatous lesions characteristic of JD are the result of the immune system's battle to contain and rid itself of the infecting MAP (Chiodini, 1996; Coussens, 2004).

Unfortunately, as happens in many battles, that which is being protected also sustains damage, and has to deal with unintended consequences. While an in depth discussion of the molecular immunology of MAP is beyond the scope of this paper, a brief description of what occurs at the cellular level is provided, as it the basis for understanding the clinical manifestations of JD.

Immunology of MAP infection

MAP gains entry through the intestinal mucosa, primarily in the ileal region of the small intestine (Gilmour, et al., 1965; Momotani, et al., 1988; Chiodini, 1996; Sweeney, et al., 2006b). There are three potential mechanisms by which MAP can penetrate the mucosal barrier: (1) paracellular route in which MAP passes between enterocytes despite tight junctions and the intact bacterium reaches the underlying lamina propria; (2) transcellular route in which the MAP is taken up by enterocytes by endocytosis and broken down with antigens being processed and presented on the basolateral cell surface in association with class II molecules that activate the intraepithelial, or lamina propria, lymphocytes; and (3) M cell route in which MAP is transported intact through the these specialized cells overlying the Peyer's patches to be presented to the underlying immune cells. The route by which MAP is transported across the intestinal mucosa plays an important role in determining the type of immune response mounted by the host (Chiodini, 1996). Evidence suggests that the primary portal of entry for MAP is through the M cells overlying the Peyer's patches in the ileum (Momotani, et al., 1988); and therefore, the following discussion will focus on the immune response resulting from this route of infection. However, other routes such as the paracellular and transcellular routes,

cannot be excluded due to the occurrence of lesions in areas outside of the ileum, such as the colon, that are devoid of M cells (Chiodini, 1996).

Unlike the surrounding enterocytes, M cells lack brush border microvilli, and they do not produce digestive enzymes or mucous; thus they provide an easily accessible surface for microorganisms such as MAP (Featherstone, 1997). MAP is transported across the M cell by transcytosis and deposited intact on the basolateral side of the cell. There it is phagocytized by resident macrophages or dendritic cells within the lamina propria (Chiodini, 1996; Stabel, 2000; Storset, 2003). At this point, MAP either, evades the macrophage's killing mechanisms and multiplies, or it is processed and presented to T-lymphocytes, thus starting the immune process.

The immune response to MAP is typical, and similar to the responses documented for other pathogenic mycobacteria (Coussens, 2004). It is paradoxical in nature; starting predominately as a cell-mediated response and transitioning in the latter stages of the infection to a humoral response. At the very end stages of the disease, immune anergy has been reported and there is no detectable immune response, either cell-mediated or humoral, allowing the infection to disseminate unchecked throughout the body (Chiodini, 1996; Stabel, 2000).

The immune response to MAP is carefully choreographed by cross-talk between immune cells using a series of complex cytokine signals (Stabel, 2000; Coussens, 2004). Upon initial insult, MAP infected macrophages send a signal to the underlying Peyer's patches, activating T-lymphocytes. These lymphocytes bind to the infected macrophages and either process the bacterial antigens for further immune processing (CD4+ cells), or kill the infected macrophages (CD8+ cells), releasing viable MAP into the surrounding

tissues where the immune process begins again (Stabel, 2000; Storset, 2003). At the same time these T-cells are releasing cytokine signals, the primary one being gamma interferon (IFNγ) (Zubrick, et al., 1988; Waters, et al., 2003; Buza, et al., 2004; Khalifeh and Stabel 2004). IFNγ recruits blood monocytes to the infection site where they become activated macrophages to aid in controlling the spread of MAP (Zubrick and Czuprynski, 1987). IFNγ also promotes CD4+ activity, and as the process repeats, more and more macrophages migrate to the infection site, causing the infected tissue to become inflamed. Gradually, this inflammation hinders function. At some point, perhaps due to tissue damage caused by the ongoing proliferative cellular immune response (Coussens, 2004), a signal is sent suppressing the release of IFNγ. This slows the recruitment and influx of additional inflammatory cells into the infection site, and stimulates B-lymphocytes, which turn into plasma cells and produce antibodies (Stabel, 2000; Storset, 2003; Khalifeh and Stabel, 2004).

Antibodies to MAP resulting from a natural infection do not protect the host from disease, and are ineffective in controlling the spread of the infection (Chiodini, 1996; Stabel, 2000, Coussens, 2004). This is because, by the time MAP antibodies are produced, the infection has become too well established, with the bacteria safely ensconced inside macrophages where they cannot be killed by the antibodies. In fact, the detection of MAP antibodies has been associated with the fecal shedding (Perez, et al., 1997; Storset, et al., 2001), and the onset of clinical disease. It is possible for there to be overlap in the cell-mediated and humoral immune responses (Chiodini, 1996). As the infection spreads, new foci of infection are formed within the intestinal wall. The earliest lesions may reach the humoral stage of the immune response, while the newer ones are

still in the cell-mediated stage (Storset, 2003). Over time, it is believed the constant exposure to MAP and its antigens overwhelms the immune system, resulting in complete anergy and the rapid dissemination of MAP throughout the body (Chiodini, 1996; Stabel, 2000).

The immune response to MAP is often successful in controlling the infection. In endemically infected herds, it is likely most, if not all, animals would ingest or be otherwise exposed to MAP. Yet, usually only a small proportion of the herd is found infected (Chiodini, 1996), and only 10-15% of the infected animals develop clinical JD (Olsen, et al., 2002; Tiwari, et al., 2006).

The ability of the immune system to completely eliminate the infection is not clear (Olsen, et al., 2002). In the case of *M. tuberculosis,* another intracellular mycobacteria that elicits an immune response similar to MAP, 95% of the exposed individuals are successful in eliminating the infection (Ellner, 1989). Evidence for the successful elimination of a MAP infection, is the observation that some animals identified as infected are later found to be MAP free (Chiodini, 1996). Others theorize that this phenomenon is the result of transient "pass through" of MAP in uninfected animals. In highly contaminated environments, animals ingest MAP; and it transits the gastrointestinal tract and exits in the feces where it can occasionally be detected by culture, but the animal itself does not become infected (Sweeney, et al., 1992c).

In summary, the immune response to MAP and its outcome depends on many variables including: the number of exposures (Chiodini, 1996, Tiwari, 2006), the size of the infecting dose (Chiodini, 1996; Olsen, et al., 2002), the pathogenicity of the infecting strain (Miltner, et al., 2005; Gollnick, et al., 2007), the number of infectious foci

(Chiodini, 1996), age of the host at exposure (Sweeney, 1996; Olsen, et al., 2002;) the immune status and capability of the host (Chiodini, 1996; Whittington and Sergeant, 2001; Olsen, et al., 2002; Tiwari, et al., 2006); and the host's genetic susceptibility (Koets, et al., 2000; Olsen, et al., 2002; Coussens, 2004).

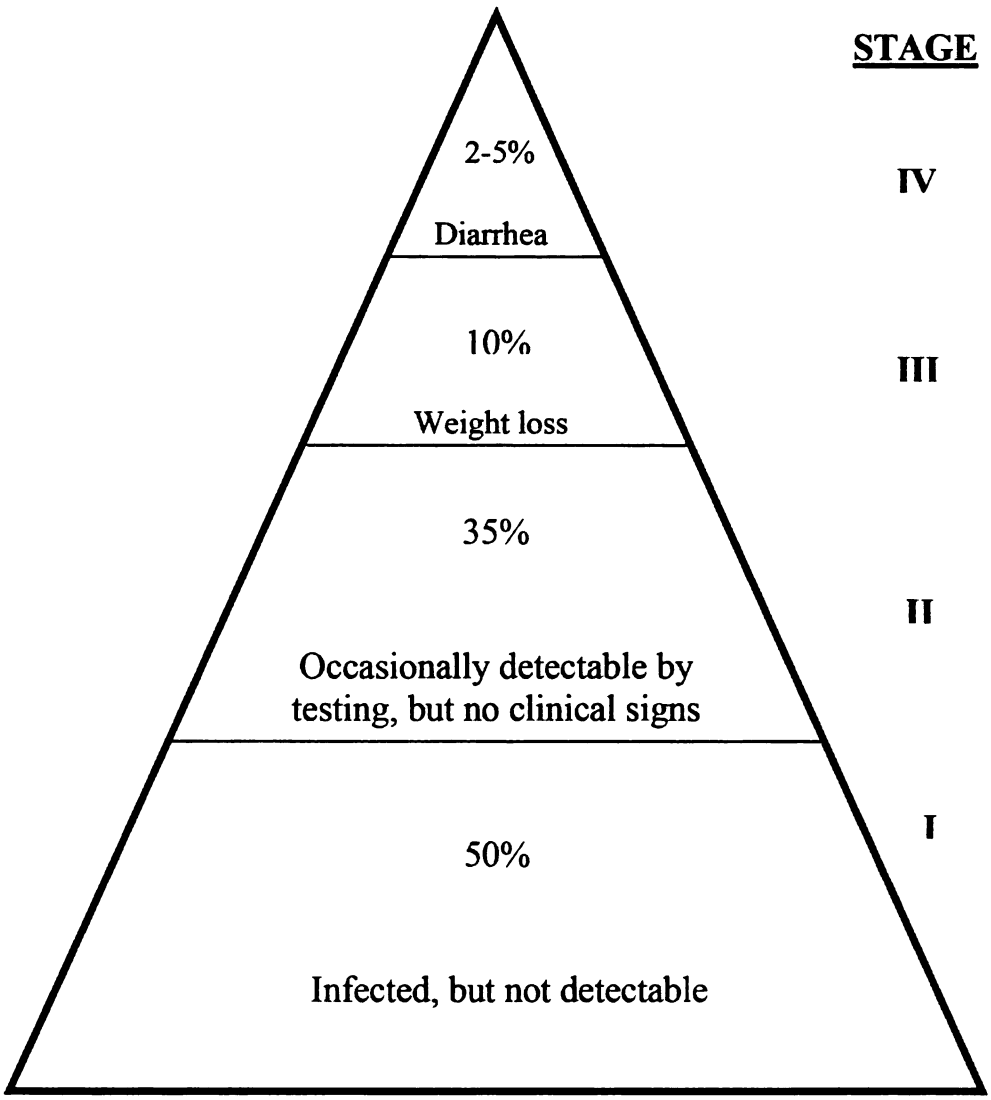
Clinical manifestations of Johne's disease

Infection with MAP has been divided into four stages depending on the severity of the clinical signs, the potential for shedding MAP into the environment, and the ease in diagnosing the disease (Whitlock, 1992). At any given time, the number of MAP infected animals in a population decreases in each subsequent stage of disease, resulting in the so-called "iceberg" effect of JD (Figure 1.1). In MAP infected herds, for every animal in the advanced clinical stage of JD, it is likely there are as many as 25 more animals infected; and only 15-20% of these infected animals will ever be detected, even with the most sensitive testing techniques (Whitlock and Buergelt, 1996).

A. Stage I: "silent" infection

This is the earliest stage of the disease. It is called "silent" because there is no way to distinguish MAP infected animals in this stage from uninfected herdmates. They have no clinical signs of infection. There are no measurable subclinical effects in terms of retarded growth or weight gain; and there are no cost effective diagnostic tests to detect the infection (Whitlock and Buergelt, 1996; Tiwari, et al., 2006). The only way to detect animals in this stage of JD is through the demonstration of MAP in the tissues, either through culture or histologic examination of the affected intestine and/or associated

Figure 1.1: "Iceberg" effect: Relative proportion of cattle infected with *Mycobacterium avium paratuberculosis* distributed through the four stages of the disease



(Adapted from Whitlock, 1992)

lymph nodes. However, these animals may shed MAP into the environment intermittently, and at extremely low levels, below the detection threshold (Whitlock and Buergelt, 1996). This stage tends to contain the largest number (>50%) of MAP infected animals in a population, and lasts the longest, often months to years. The animals in this group often include calves, replacement heifers, as well as adult cows (Whitlock, 1992).

B. Stage II: subclinical infection

Stage II of JD consists of animals, generally adults, in the subclinical stage of infection. Only a small proportion (15-25%) of animals at this stage of the disease is detectable by currently available diagnostic tests. These animals do not have overt signs of JD, such as weight loss or diarrhea, but inflammation resulting from the cell-mediated immune response starts to affect intestinal tract function. Nutrient absorption is less than optimal, resulting in a lower nutritional plane, impairing performance and production. As a result, many of these animals are culled from the herd for reasons other than JD, never being identified as infected (Whitlock and Buergelt, 1996; Tiwari, et al., 2006). Frequently, these animals will be shedding MAP into the environment, potentially infecting other susceptible animals. Many MAP infected cows remain in the subclinical stage for years before progressing into the third, or clinical, stage of JD; and some may mount a successful immune response, such that they never progress to the clinical stage (Whitlock, 1992). It is generally believed the transition from a predominately cell-mediated immune response to a humoral response, with the production of antibodies against MAP, occurs at the end of stage II, and precedes the onset of clinical signs (Chiodini, 1996; Tiwari, et al., 2006).

C. Stage III: clinical infection

The onset of clinical signs of JD follows an extended incubation period of 2-10 years (Whitlock and Buergelt, 1996; Collins, 2003). The first sign is generally weight loss in spite of a normal or sometimes increased appetite. This sign is often missed because the onset of clinical signs is often precipitated by parturition (Harris and Barletta, 2001; Whittington and Sergeant, 2001; Greig, 2005). Cows normally lose weight during early lactation, and that weight loss is unlikely to draw attention if a healthy appetite is maintained. The weight loss is a consequence of the progressive impairment of the functioning of the intestinal mucosa due to inflammation, hindering the absorption of nutrients. A further consequence soon follows in the form of diarrhea that is malabsorptive in nature. The diarrhea may be intermittent initially, with periods of normal manure consistency, but eventually becomes persistent. Thirst may be increased in these cows, but otherwise all other vital signs (appetite, temperature, heart and respiratory rate) remain normal (Whitlock and Buergelt, 1996).

Only 10-15% of cows survive to the clinical stage (Olsen, et al., 2002; Tiwari, et al., 2006). Cows rarely remain in stage III longer than 3-4 months before progressing to stage IV, or, more likely, being culled (Tiwari, et al., 2006). Most cows in this stage of the disease will test positive on fecal culture and have detectable antibodies. On gross pathology, the small intestines of these cows will have the characteristic corrugated cardboard appearance, and the associated mesenteric lymph nodes will be enlarged (Whitlock and Buergelt, 1996; Olsen, et al., 2003).

D. Stage IV: advanced clinical infection

Most cows are culled prior to reaching this stage. These cows are emaciated, weak, lethargic, and have the "pipe-stream" or "water-hose" diarrhea characteristic of JD. At this point, the damage to the intestinal tract has become so extensive that it has essentially ceased to function and absorb nutrients. This necessitates the utilization of body stores of fat and protein for survival; leading to cachexia, and the development of hypoproteinemia, resulting in submandibular edema, or bottle jaw. The condition of cows at this stage deteriorates rapidly, generally within a period of days; and often they cannot be salvaged and die as a result of dehydration and cachexia (Whitlock and Buergelt, 1996). For all intents and purposes, these cows starve to death.

Cows with advanced clinical JD may not test positive for antibodies to JD, as they may have reached immune anergy (Chiodini, 1996; Stabel, 2000). Without the immune system to hold the MAP in check, it rapidly disseminates throughout the entire body, and is readily detectable on culture and histopath (Whitlock and Buergelt, 1996).

1.5. Control and prevention of Johne's disease

Due to the insidious nature of MAP and its complex pathobiology, control and prevention of JD is extremely challenging (Sweeney, et al., 2006b). Johne's disease control programs are multifaceted, and consist of any combination of the following: (on rare occasions) treatment, vaccination, diagnostic testing to identify MAP infected cattle, and (perhaps most importantly) the implementation of farm management practices aimed at preventing infection. Each will be discussed in turn, with the greatest emphasis placed on management including: the role of the environmental burden of MAP in sustaining JD

within a herd; risk factors associated with the spread of JD within a herd; and the proposed practices to prevent the transmission of MAP to susceptible cattle.

Therapeutic treatment of MAP infections

Treatment of MAP infection in production livestock is generally unrewarding and not practical due to the cost of the drugs, the hassle of continued daily administration, and protracted drug-residue withholding times for both milk and meat (St. Jean, 1996). Occasionally, there have been instances where treatment of MAP has been attempted using various antimicrobial agents that have demonstrated effectiveness in treating other mycobacterial diseases (tuberculosis and leprosy) such as rifampin, clofazimine, rifabutin isoniazid, pyrazinamide, and streptomycin, alone or in combination. However, in all cases, the drug protocol was unsuccessful in eliminating the infection. It only succeeded in temporarily alleviating clinical signs, and did not prevent shedding of MAP into the environment (St. Jean, 1996; Stabel, 1998; Belloli, et al. 2001). Treatment of MAP is, therefore, generally reserved for companion animals with strong sentimental value, or animals with high genetic value, in an effort to alleviate symptoms long enough to harvest embryos (St. Jean, 1996).

More recently, monensin, a common feed additive in ruminant diets, has been associated with reducing the severity of lesions caused by MAP (Brumbaugh, et al., 2000), decreasing the odds of testing positive for MAP (Hendrick, et al., 2006a), and marginally reducing shedding of MAP from infected cows (Whitlock, et al., 2005; Hendrick, et al., 2006b). Monensin is an ionophore antibiotic that modifies biological cell membrane permeability (Merck, 1991; Prescott, et al., 2000). It also alters the

proportion of volatile fatty acids produced in the rumen to favor proprionic acid production, which, in turn, improves feed efficiency and hence production (Merck, 1991). It is unknown if the mechanism for the positive effect of monensin on JD is due to increasing the permeability of the bacterial cell wall allowing for easier bacterial cell destruction (Whitlock, et al., 2005; Hendrick, et al., 2006b); or if improved feed efficiency puts the cow on a better nutritional plane, thereby allowing the maintenance of an active immune response for a longer period of time; or some combination of both. In reality, the suppressive effect of monensin on the progression of JD is a beneficial side-effect for most dairy producers. It has long been used as a coccidiostat in replacement heifers (Merck, 1991); and with its approval for use in lactating cows in November 2004 (FDA, 2004), it is now widely included in rations to enhance milk production.

For all practical purposes, JD disease is untreatable in production livestock (Wells and Wagner, 2000). If and when treatment is attempted, clinical improvement should not be confused with cure of the disease (Belloli, et al., 2001).

Vaccination for Johne's disease

Vaccination for the control of JD is controversial (Collins, 1994; Stabel, 1998). Multiple experimental and field studies have demonstrated that vaccination reduces fecal shedding, the number of clinically affected animals, and the severity of pathologic lesions (Cramwell, 1993; Kormendy, 1992; Juste, et al., 1994; Kormendy, 1994; Wentink, et al, 1994; van Schaik, et al., 1996; Gwozdz, et al., 2000; Rast and Whittington, 2005; Reddacliff, et al., 2006). It does not, however, completely prevent infection or the spread of disease to susceptible animals (Kormendy, 1994; Wentink, et al., 1994; van Schaik, et

al., 1996; Reddacliff, et al., 2006). Use of vaccine is regulated in the US due to the potential for cross-reactivity leading to false positive tests for bovine tuberculosis (*M. bovis*) in vaccinated animals (Stabel, 1998; Harris and Barletta, 2001). Vaccinating for JD precludes the use of serological tests for diagnostic purposes, and granulomatous lesions can result at the injection site in cattle (Spangler, et al., 1991), as well as in people in the event of accidental self-injection (Patterson, et al., 1988). Vaccination may have a beneficial role in herds heavily infected with JD by alleviating symptoms of the disease and reducing economic losses, but it must always be used in conjunction with improved management practices to control further transmission of the infection (Harris and Barletta, 2001).

Diagnostic testing to identify MAP infected animals

Multiple diagnostic tests have been developed to diagnose JD. These tests fall into one of two categories; those that detect the actual bacterium, or those that detect the immune system's response to it (Collins, 1996; Tiwari, et al., 2006). Given the pathobiology of a MAP infection, the efficacy of a test to correctly identify a MAP infected animal is dependent upon the stage of the disease process (Whittington and Sergeant, 2001). Almost without exception, the tests do very well confirming MAP infection in animals in the more advanced, or clinical, stages of the disease. They do not, however, do a particularly good job identifying animals in the early, or subclinical, stages of the disease (Collins, 1996; Step, et al., 2000; Olsen, et al., 2002; Dieguez, et al., 2008). The most commonly used diagnostic tests currently being used for JD will be briefly discussed and summarized.

A. Tests that detect MAP

Bacterial culture

Culturing MAP from infected tissues is the most definitive method for diagnosing JD (Collins, 1996; Stabel, 1998; Whittington and Sergeant, 2001). However, collecting tissue samples for an antemortem diagnosis can be problematic; and instead fecal culture is performed more commonly and is often used as the "gold standard" for confirming a diagnosis of JD (Collins, et al., 1991; Sockett, et al., 1992; Collins, et al., 1994; Sweeney, et al, 1995; Whitlock, et al., 2000; Dargatz, et al., 2001; Stabel, et al., 2002; van Schaik, et al., 2003a; Collins, et al. 2005; van Schaik, et al., 2005; Nielsen and Toft, 2006; Tiwari, et al., 2006). Aside from necropsy and tissue biopsy, fecal culture is the most sensitive of the diagnostic tests currently available for JD (Whittington and Sergeant, 2001; Collins, et al., 2006). This is because shedding of MAP often occurs before the production of measurable antibodies (Whitlock and Buergelt; 1996; Whittington and Sergeant, 2001; Sweeney, et al., 2006a). The disadvantages of fecal culture arise from the slow-growing and fastidious nature of MAP. It takes 8-16 weeks to grow MAP *in-vitro* and requires special, mycobactin enriched media (Collins, 1996). Also, because contamination is often a problem when culturing feces, an aggressive decontamination procedure is necessary to prevent overgrowth of other fungal and bacterial microorganisms. This procedure is labor intensive and inadvertently decreases the number of viable bacteria in the sample, adding to the time it takes for detection (Stable, 1998; Readdacliff, et al., 2003). Additionally, bacteria grown on culture need to be verified as MAP by acid-fast staining procedures and/or polymerase chain reaction

(PCR), generally for the IS900 gene sequence (Collins, 1996; Tiwari, et al., 2006). Because of the time, special media, and experience required to culture MAP, fecal culture is relatively expensive as compared to other tests (Collins, 1996; Kalis, et al., 1999; Stabel, 1998; Tiwari, et al., 2006). The recent development of automated liquid culture systems has reduced the amount of time required to detect MAP to about half that required using Herrold's egg yolk solid culture (from 16 weeks to 6-8 weeks), and improved sensitivity from 50% to 60-65% (Kim, et al., 2004; Motiwala, et al., 2005; Collins, et al., 2006; Rajeev, et al., 2006). However, it has not changed the decontamination procedure and requires additional specialized equipment; therefore, it has not reduced the cost ($15-23/sample; Michigan USDA certified Johne's laboratories, 2008).

Despite its better sensitivity, due to the cost, individual animal fecal culture is not recommended for routine screening of herds (Nielsen, et al., 2002a; Wells, et al., 2002b; Collins, et al., 2006). Instead, for herd screening purposes, culturing pooled fecal samples (mixing fecal samples from 5-10 cows together) or environmental samples from high-traffic adult cows area, have proven to be valid and cost-effective methods to identify infected herds and get a rough estimate of within herd JD prevalence (Wells, et al., 2002a; van Schaik, et al., 2003b; Wells, et al., 2003; Kalis, et al., 2004; Raizman, et al., 2004; Tavornpanich, et al., 2004; Berghaus, et al., 2006; Lombard, et al., 2006; van Schaik, et al, 2007). In short, culturing for MAP remains a mainstay for diagnosing JD in infected animals and is used as an aid in herd control programs.

Genetic probe

The evolution of PCR technology has made it possible to identify MAP antigens in samples using genetic probes. The genetic element most commonly used for the diagnosis of JD is a highly conserved insertion element of MAP, IS900; with multiple copies often present within each bacterium (Vary, et al., 1990; Collins, 1996; Harris and Barletta, 2001). While PCR is most frequently used to confirm the identification of MAP in cultured samples (Collins, 1996; Tiwari, et al., 2006), it can also be used on samples obtained directly from the animal (Stabel, 1998). Similar to culture, PCR has a specificity of >99%, but a much lower sensitivity (~30%) due to its inability to detect MAP antigens in animals shedding low numbers of bacteria (Stabel, 1998; Harris and Barletta, 2001; Collins, et al., 2006). In a study comparing direct PCR to fecal culture, PCR only identified 60% of cattle positive on fecal culture (Whipple, et al., 1992). Studies investigating different genetic probes to improve sensitivity while maintaining specificity are ongoing, but are not yet commercially available (Stabel, 1998). The advantage of PCR over culture is its speed; requiring only three days for test completion. However, the required skills and equipment necessary to conduct the PCR test makes it as, or more, expensive as culture; hence, prohibiting it use for routine herd screening purposes (Collins, 1996).

B. Tests that detect the immune response to MAP

Serological or antibody tests

Three techniques have been developed to detect antibodies to MAP: complement fixation (CF), agar-gel immunodiffusion (AGID), and enzyme-linked immunosorbent assay (ELISA) (Collins, 1996). There are different types of commercially available ELISA tests, and all are superior in sensitivity to either the AGID or CF. The ELISA is, therefore, currently the most commonly used assay to detect MAP antibodies (Olsen et al., 2002; Tiwari, et al., 2006). The advantages of ELISA tests include: ease of sample collection (serum or milk), availability of results within days, and relatively low cost ($6/sample; Michigan USDA certified Johne's laboratories, 2008) (Collins, 1996, Tiwari, et al., 2006). The main disadvantage of the ELISA test is its overall lack of sensitivity (30%) (Collins, et al., 2006).

The humoral immune response to MAP, with the production of antibodies, generally does not occur until well after infected animals start shedding MAP (Chiodini, 1996; Whitlock and Buergelt, 1996; Whittington and Sergeant, 2001); making the ELISA less effective in detecting subclinically infected animals than individual fecal culture (Dargatz, et al., 2001; Tiwari, et al., 2006; van Schaik, 2007). So typically one would expect that ELISA positive animals would be fecal culture positive, but that is not always the case. In one study 30 out of 33 cows (91%) positive on serum ELISA were negative on concurrent fecal culture (Pinedo, et al., 2008). In studies where cows with positive serum ELISA's were followed up with fecal culture, 6% and 20% respectively, were fecal culture negative (Stabel, et al., 2002; Muskens, et al., 2003bb). These contradictory JD test results may be partially explained by the intermittent shedding that is not uncommon with MAP infections during the subclinical stages of the disease (Whitlock

and Buergelt, 1996). Another possibility is the potential for false positive ELISA test results. While the ELISA is generally assumed to have excellent specificity (Collins, et al., 2006), it is not perfect, and false positive tests do occur (Hendrick, et al., 2005b). There are documented cases with a disproportionate number of false positive ELISA tests thought to be the result of exposure to other environmental mycobacteria (Grooms, et al., 2006; Roussel, et al., 2007). Also documented, is substantial variation in the level of antibodies upon serial testing, possibly due to stage of lactation and status of the immune system (Hirst, et al., 2002; Nielsen, et al., 2002a; Barrington, et al., 2003; van Schaik, et al., 2003a). Moreover, it has been reported there is very little to only moderate agreement between concurrent milk and serum ELISA results (Hardin, et al., 1996; Hendrick, et al., 2005a); suggesting MAP antibody levels can vary between different tissues within the same cow on the same day. Because of all these things, it is advocated that ELISA test results be interpreted quantitatively, rather than as simply positive or negative; taking into consideration the origin of the sample (milk vs. serum), the clinical presentation of the individual animal, and the JD history of the herd, (Adaska, et al., 2002; Collins, et al., 2005).

The bottom line is the low cost and quick turn around time for results has made the ELISA test, the JD test of choice for many producers and veterinarians, despite its many drawbacks. As with culture and PCR, the accuracy of the ELISA tests improves as the disease progresses (Collins, 1996; Whitlock, et al., 2000; Stabel, et al., 2002; van Schaik, et al.,2003a). It is probably best used as a cost-effective method for screening purposes to identify infected herds, monitor disease burden over time, and aid in the identification and removal of the most infectious animals in a herd; although confirming

the diagnosis with follow-up fecal culture is recommended before making decisions regarding individual animals (Dargatz, et al., 2001; Wells, et al., 2002b; van Schaik, et al., 2003a; van Schaik, et al., 2007).

Tests to detect the cell-mediated immune response

Key to any disease control program is the accurate, early identification and removal of infected animals before they have a chance to transmit the disease to others. The consistent problem with the diagnostic tests for JD discussed so far is their inability to detect cows in the early and subclinical stages of the disease; they only detect animals after they have become infectious. The earliest stage of MAP infection is characterized by a cell-mediated immune response (Chiodini, 1996; Stabel, 2000; Storset, 2003; Coussens, 2004). It is believed this response occurs prior to bacterial shedding, and its waning contributes to shedding and the progression of the disease (Chiodini, 1996; Stabel, 2000). Being able to accurately identify the cell-mediated immune response to MAP would identify animals prior to them becoming infectious, and would go a long ways toward controlling JD (Stabel and Whitlock, 2001).

Cell-mediated immune function can be assessed by the following two methods: antigen-specific delayed-type IV hypersensitivity reactions, and *in-vitro* T lymphocyte proliferation and cytokine stimulation assays (Stabel and Whitlock, 2001). The most commonly used test for the delayed-type IV hypersensitivity reaction is the skin test, where pathogen specific antigens are injected intradermally. If the animal is infected, swelling will occur at the injection site over a period of three days. Skin testing has been

the cornerstone for diagnosing tuberculosis in both people and cattle. Skin testing for JD has not been successful, most likely due to cross-reactivity with other ubiquitous mycobacteria in the environment (Collins, 1996; Olsen, et al., 2002).

The primary cytokine responsible for modulating the cell-mediated immune response is IFNγ (Stabel, 2000; Storset, 2003). IFNγ assays have been successfully developed and used to diagnose and control bovine tuberculosis (Wood, et al, 1990). Likewise, IFNγ assays have been developed for diagnosing MAP (Collins, 1996). Unfortunately, MAP shares many antigens with other mycobacteria commonly found in the environment resulting in cross-reactivity and unsatisfactory test sensitivity and specificity. Studies optimizing the antigen formulations used for the JD IFNγ assay to improve test sensitivity and specificity are ongoing (Stabel and Whitlock, 2001; Jungersen, et al., 2002; Kalis, et al., 2003). Aside from diagnosing MAP infected cattle prior to the onset of shedding, an accurate IFNγ assay for JD would be valuable for routine monitoring of young heifers as an aid in evaluating the effectiveness of control programs (Jungersen, et al., 2002).

C. Summary of testing

In general, diagnostic tests used for identifying MAP infected animals have excellent specificity, but only marginal sensitivity when used for screening populations (Collins, et al., 2006). Across the board, test sensitivity improves dramatically when used to confirm a diagnosis in an animal in the clinical stages of JD (Collins, 1996; Whittington and Sergeant, 2001; Tiwari, et al., 2006). The reason for the less than desirable sensitivity is due more to the pathobiology of MAP, than any innate fault of the

respective tests. MAP has an extremely long incubation period and subclinical stage during which infection cannot be detected. The majority of infected cows in a herd are in the silent or subclinical stage of the disease, while only 10-15% of cows reach the clinical stage (Whitlock, 1992). In some ways JD is similar to cancer; the more advanced the disease, the easier it is to diagnose, but the worse the prognosis for the patient.

It is not uncommon for the results of different JD tests, run concurrently, to disagree (Pinedo, et al., 2008). This is a function of both the tests and the pathobiology of the bacteria. Take, for example, fecal culture and the ELISA test. Fecal culture detects the actual bacteria, while the ELISA test detects antibodies, or the immune system's response to the bacteria. The onset of MAP shedding does not necessarily coincide with the production of antibodies. Test agreement will only occur when these two events overlap. It is important to keep this in mind when choosing which tests to use, and interpreting the results (Rossiter and Burhans, 1996).

Testing for JD is expensive and often represents the biggest cash cost of a control program (Rossiter and Burhans, 1996). Multiple testing strategies have been proposed for diagnosing JD, including the pooling of samples to reduce cost, or running different tests in parallel or sequence to improve overall sensitivity (Collins, 1996; Rossiter and Bruhans, 1996; Wells, et al., 2002a; Kalis, et al., 2004; Tavornpanich, et al., 2004; Tavornpanich, et al., 2008; van Schaik, 2007). Each strategy has its own merit, and there is no one best strategy to fit all. Choosing which test(s) and testing strategy to use needs to be made on a case-by-case basis (Rossiter and Burhans, 1996); taking into consideration: the purpose of testing (confirming a diagnosis vs. screening for control and management), costs, and the goals and capabilities of the operation (Collins, et al.,

2006). Testing can play an important role in a JD control program, but only if the test results are utilized. If JD test status does not guide action to prevent the spread of the disease, testing as part of a control program is useless and a waste of money (Rossiter and Burhans, 1996).

Implementation of farm management practices to control Johne's disease

Regarding the control and prevention of JD, treatment using therapeutic agents is not practical or efficacious (St. Jean, 1996). Vaccination is controversial, only partially protective, and is generally considered a band-aid at best for JD control (Stabel, 1998; Collins, 1994). Diagnostic testing and culling of test positive animals facilitates the removal of the most infectious animals from the herd, and reduces disease burden (Holmes, et al., 2004; Jubb and Galvin, 2004); but is not very effective in eliminating the disease (Groenendaal, et al., 2002; Collins, 2003; Dorshorst, et al., 2006; McKenna, et al., 2006; Kudahl, et al., 2007). Instead, control of JD must focus on implementing farm management practices that minimize the transmission of MAP to susceptible animals (Thoen and Moore, 1989; Collins, 2003; Hoe and Ruegg, 2006; McKenna, et al., 2006).

Before farm management changes for the control of JD can be recommended, a full understanding of the disease, its reservoirs, and the factors associated with increasing or decreasing the risk of infection is necessary. The pathogen and the disease have already been discussed. Attention will now focus on the environmental reservoir and risk factors for JD specific to dairy herds, along with a brief discussion of recommended control practices.

A. Environmental reservoir of MAP on dairy herds

It is generally accepted that the primary route of MAP infection is through the ingestion of bacteria from a contaminated environment (Sweeney, 1996; Step, et al., 2000; Whittington and Sergeant, 2001; Olsen, et al., 2002; Greig, 2005). Thus, the environment is a major reservoir for infection. Understanding how long MAP can survive, under what conditions, and the areas of the farm that are commonly contaminated is critical for developing strategies to minimize or eliminate exposure of susceptible animals to the bacteria.

As previously discussed, MAP is an obligate intracellular pathogen and does not replicate outside the animal host (Whittington and Sergeant, 2001). The thick bacterial cell wall of MAP enables it to withstand exposure to environmental elements for extended periods of time. Substrate (feces, urine, water, milk), temperature, and pH are all factors that influence the length of time MAP will survive in the environment. (McKenna, et al., 2006). Documented survival times in farm environments include: river water – 163 days; pond water – 270 days; feces incorporated with black soil – 11 months; urine – 7 days; low ambient temperatures (<14 C) - >1 year (Chiodini, et al., 1984). While MAP has been cultured on pasture for more than a year following the removal of all livestock, the capacity for infectivity declines significantly after six months, provided MAP is not continuing to be excreted into the environment (Whittington, et al., 2003). While MAP may be hardier than many other pathogens, it is still susceptible to long-term desiccation, large fluctuations in temperature; repeated freeze-thaw cycles, exposure to sunlight, and soils with alkaline pH or low iron content (Richards and Thoen, 1977; Johnson-Ifearulundu and Kaneene, 1997; Johnson-

Ifearulundu and Kaneene, 1999; Whittington, et al., 2003; Ward, et al., 2004; Grewal, et al., 2006; McKenna, et al., 2006).

On infected dairy farms, MAP has been isolated from many different areas including: return alleys from parlor; holding pens; high-traffic alleyways, sick cow pens; maternity pens; post-weaned calf pens; manure storage areas; and manure handling equipment (Raizman, et al., 2004; Berghaus, et al., 2006; Lombard, et al., 2006). The areas most commonly contaminated with MAP were manure storage areas, holding pens, and high-traffic cows areas where manure accumulated from adult cows on a daily basis (Raizman, et al., 2004; Lombard, et al., 2006). Also, there was a positive association between the distribution of MAP contamination in the environment and within herd JD prevalence (Raizman, et al., 2004; Fyock, et al., 2005; Berghaus, et al., 2006).

To summarize, MAP is capable of surviving for extended periods of time in the environment of infected dairy herds, serving as a reservoir of infection for susceptible cattle. It is widely distributed in the environment of dairy farms. MAP is often found in areas where adult cows, the animals most likely to be shedding the bacteria, are housed. It is not uncommon for it to also be found in areas to which young calves, the animals most susceptible to infection, have access, such as the maternity and weaned heifer pens. Finally, the greater the environmental reservoir of MAP, the greater the infectious burden in the herd.

B. Risk factors for Johne's disease on dairy herds

The identification of factors or practices associated with increasing or decreasing the risk of MAP infection is vital information when assessing farm operations and

designing JD control programs. Once identified, they need to be carefully evaluated in an attempt to explain the association; determine whether they are biologically plausible; and, most importantly from a disease control standpoint, decide if something can be manipulated to mitigate further transmission of the infection. Multiple studies have investigated the risk factors for JD. Factors and/or practices associated with an increasing risk for JD are listed in Table 1.1, and those associated with a decreasing risk of JD are summarized in Table 1.2.

Increasing age or parity as a risk factor for MAP infection is consistent with the pathobiology of the bacterium and available diagnostic capabilities. The further the infection progresses, the more likely it is to be detected, and the older the animal.

Several studies have associated an increased risk of JD with large herds. One explanation for this association is that the higher cattle density of larger herds contributes to a higher bacterial load in the environment, increasing the infection pressure of susceptible calves and promoting infection (Daniels, et al., 2002; Muskens, et al., 2003bb). Large herd size has also been associated with the purchase of cattle (USDA, 2005). The addition of purchased cattle is considered the primary method that JD is transmitted between herds (Sweeney, 1996). Thus, the association of herd size with JD may also be a reflection of the introduction of disease through purchased cattle, with the infection being subsequently sustained within the herd (Hirst, et al., 2004).

Since the primary means of JD transmission between herds is through the purchase and addition of subclinically infected cattle, a possible way to mitigate this risk would be to screen purchased cows for JD prior to purchase. Due to the lack of diagnostic sensitivity for detecting subclinically infected cows, this has been proven to be

Table 1.1: Factors associated with an increased risk of Johne's disease in dairy herds

Factor	Reference
Increasing age/parity	Nielsen, et al., 2002a; Berghaus, et al., 2006; Nielsen and Toft, 2006
Large herd size	Collins, et al., 1994; Wells and Wagner, 2000; Daniels, et al., 2002; Muskens, et al., 2003; Hirst, et al., 2004; Crossley, et al., 2005
Purchased cattle	Goodger, et al. 1996; Cetinkaya, et al., 1997; Obasanjo, et al., 1997; Wells and Wagner, 2000; Chi, et al., 2002b; Carpenter, et al., 2004; Hirst, et al., 2004; Korvich, et al., 2006
Jersey and Guernsey breeds	Cetinkaya, et al., 1997
MAP infected dam	Aly and Thurmond, 2005; Nielsen, et al., 2002b; Antognoli, et al, 2007
Exposure to wildlife (including birds) &/or wildlife access to feed stores	Cetinkaya, et al., 1997; Daniels, et al., 2002; Fredricksen, et al., 2004
Exposure to sheep	Daniels, et al., 2002
Commercial operation (vs. purebred registered herds)	Obasanjo, et al., 1997

Table 1.1 (continued): Factors associated with an increased risk of Johne's disease in dairy herds	
Factor	**Reference**
Previous clinical signs or cases of JD	Obasanjo, et al., 1997; Muskens, et al., 2003; Hirst, et al., 2004; Kobayashi, et al., 2007
Co-mingling of pre-weaned calves	Wells and Wagner, 2000
Group maternity pen	Cetinkaya, et al., 1997; Wells and Wagner, 2000
Exposure of calves to feces from adult cows (directly or through contaminated equipment)	Obasanjo, et al., 1997; Nielsen and Toft, 2007
Access to unrestricted housing (exercise lots, pasture, manure pack, etc.)	Johnson-Ifearulundu and Kaneene, 1998; Fredricksen, et al., 2004; Kobayashi, et al, 2007; Nielsen and Toft, 2007
Application of stored manure on pasture	Obasanjo, et al., 1997; Daniels, et al., 2002
High young stock density	Nielsen and Toft, 2007
Feeding waste milk	Ridge, et al., 2005

Table 1.2: Factors associated with a decreased risk of Johne's disease in dairy herds	
Factor	**Reference**
Frequent cleaning of maternity pens	Obasanjo, et al., 1997; Johnson-Ifearulundu and Kaneene, 1998
Frequent cleaning of adult cow barn	Obasanjo, et al., 1997
Feeding milk replacer	Muskens, et al., 2003
Routine culling of cows with clinical signs of JD	Muskens, et al., 2003
Applying lime to pasture	Johnson-Ifearulundu and Kaneene, 1998

of minimal benefit (Carpenter, et al., 2004). Instead it is advocated, rather than testing individual cows that are to be purchased, performing some type of herd screening test on the herd of origin (environmental culturing; ELISA test on subset of adult cows), or buying cows only from herds with a known low prevalence (Carpenter, et al., 2004; Kovich, et al., 2006).

The increased risk of MAP infection associated with the observation of clinical signs, or confirmed cases of JD (unless in purchased cows), is indicative of an established MAP infection within the herd. Cows with clinical JD are only the "tip of the iceberg"; they have likely been infectious for several months prior to the onset of clinical signs, potentially infecting several of their herdmates (Whitlock, 1992). Subsequently, routinely culling cows with clinical signs of JD has been associated with a decreased risk of JD (Muskens, et al., 2003b). The age of onset of clinical signs can be used as a predictor of the infection rate within a herd. Although relative, the observation of clinical signs of JD at an earlier age suggests a high infection rate in the herd where the cow was born; translating to high infection pressure for young calves (Collins, 2003).

The main route of MAP transmission is fecal-oral (Sweeney, 1996). Therefore, any practice promoting the exposure of susceptible animals (young calves) to MAP contaminated feces (most likely from adult cows) will be associated with an increased risk of JD. This could be directly, particularly in group maternity pens (Cetinkaya, et al., 1997; Wells and Wagner, 2000), or indirectly through contaminated feed or equipment (Obasanjo, et al., 1997; Nielsen and Toft, 2007). The application of stored manure to pasture has also been associated with an increased risk of JD, and again is consistent with fecal-oral transmission (Obasanjo, et al., 1997; Daniels, et al., 2002). Potential for

exposure to MAP contaminated feces also likely plays a role in the increased risk of JD with access to unrestricted group housing when compared to free-stall or tie-stall housing (Johnson-Ifearulundu and Kaneene, 1998; Fredricksen, et al., 2004; Kobayashi, et al, 2007; Nielsen and Toft, 2007). In stall housing, the location of defecation is predetermined, there is less opportunity for fecal contamination of feed, and the stalls are cleaned more often than unrestricted exercise lots or manure packs (Obasanjo, et al., 1997; Johnson-Ifearulundu and Kaneene, 2000). To underscore the importance of fecal-oral transmission, frequent cleaning of pens is associated with a decreased risk of JD (Obasanjo, et al., 1997; Johnson-Ifearulundu and Kaneene, 1998).

The commingling of preweaned calves as an increased risk for JD is not easy to explain. Again, it may be a consequence of some unknown characteristic or management practice on MAP infected farms (Collins, et al., 1994). More likely it is due to calf-to-calf shedding following initial transmission from an infected cow (Wells and Wagner, 2000). Recent studies have documented the shedding of MAP in young calves, even prior to weaning (Bolton, et al., 2005; vanRoermund, et al., 2005). These calves could easily spread the infection to other susceptible calves if housed together.

Calves born to MAP seropositive dams were 6.6 times more likely to test seropositive in their lifetime (Aly and Thurmond, 2005). Another study determined that the dam's JD test status was responsible for significant variation in the antibody level of their offspring (Nielsen, et al., 2002b). Whether this association is due to vertical or horizontal transmission is unknown, and academic in terms of control. MAP can be transmitted *in-utero* (Whittington and Windsor, 2007), and is shed in the colostrum (Streeter, et al., 1995), milk (Taylor, et al., 1981; Sweeney, et al., 1992b) and feces

(Sweeney, 1996) of infected cows; all of which potentially exposes their offspring to infection.

The protective effect of feeding milk replacer in decreasing JD is most probably due to the potential for MAP to be transmitted in milk (Taylor, et al., 1981; Sweeney, et al., 1992b). It has long been a common practice for farms that feed whole milk to calves, to feed waste milk. This is milk that cannot be sold for human consumption and would include transitional milk from fresh cows, mastitic milk, or milk from cows that were sick and contains antibiotic residues. Shedding of MAP into milk occurs more frequently just following parturition (Harris and Barletta, 2001) or when otherwise stressed (McKenna, et al., 2006). Hence, the practice of feeding whole milk to calves often results in feeding the most infectious milk to the animals most susceptible to becoming infected (Ridge, et al., 2005). Pastuerization of waste milk can be cost-effective (Godden, et al., 2005) and has been shown to eliminate (Stabel, et al., 2004) or at least significantly reduce (McDonald, et al., 2005) the infectious load; making it one option for JD control. The other, commonly used option to minimize the infectious dose of MAP in calves' diets is to feed milk replacer.

Certain breeds of cows, Jerseys and Guernseys in particular, have been associated with an increased risk for JD (Cetinkaya, et al, 1997). The reason for this is unknown, although several hypotheses have been proposed. One possible explanation is there is inherent variation in the susceptibility of cattle to MAP (Koets, et al., 2000), and these breeds are somehow genetically predisposed to infection. Another explanation suggests it has nothing to do with the genetic susceptibility of the breed, but rather with the management practices of herds with these breeds (McKenna, et al., 2006). Jersey and

Guernsey herds tend to be smaller and have a lower culling rate resulting in a higher average herd age, a factor also associated with JD (Cetinkaya, et al., 1997).

Differences in herd management practices may also explain the increased risk of JD in commercial herds as compared to registered herds. Commercial herds tend to be larger than registered herds, and are more likely to purchase cattle on a routine basis (Obasanjo, et al., 1997); both risk factors for JD already discussed.

Other domestic ruminants, such as sheep and goats, can readily become infected with MAP, as can wildlife. Interspecies transmission has been documented (Williams, et al, 1983; Daniels, et al., 2001). So if infected with MAP, these animals could play the same role as subclinically infected cattle in JD transmission when cows are exposed to these animals. Exposure of cattle, or their feed stores, to wildlife to the extent of promoting interspecies JD transmission could also be an indirect reflection of other substandard herd management practices for minimizing the spread of MAP infection.

Finally, multiple anecdotal reports have associated the application of lime to pastures and other cattle housing areas with decreasing the number of clinical cases of JD (Jansen, 1948; Kopecky, 1977; Richards, 1989). The mechanism for lime decreasing the incidence of JD is unknown, but is believed to be connected to an increase in the environmental pH (Johnson-Ifearulundu and Kaneene, 1998). Studies have shown that MAP survives better in acidic conditions (Johnson-Ifearulundu and Kaneene, 1997; Ward and Perez, 2004). The theory is, as environmental pH increases, the bioavailabilty of iron is decreased. Iron is essential for MAP survival, so limiting iron exacerbates MAP destruction (Johnson-Ifearulundu and Kaneene, 1998).

C. Farm practices to control Johne's disease

The list of farm management practices recommended for controlling JD is extensive (Rossiter and Burhans, 1996; Benedictus and Kalis, 2003), and it can be overwhelming to producers (Ridge, et al., 2005). In the end, the goal of each of those practices is the same; prevent, or minimize, the exposure of susceptible animals to MAP. Obviously, there are many ways to go about achieving that goal; and what works for one operation may not work for another. This means the management practices implemented as part of a JD control program need to be designed specifically for each operation, taking into consideration the JD burden in the herd, the risk for MAP transmission, the goals of the operation, and the resources available both in terms of money and manpower (McKenna, et al., 2006). The reason most JD control programs fail is because they were not designed to meet the unique needs and capabilities of the operation (Rossiter and Burhans, 1996; Collins, 2003).

Designing a JD control program consists of three steps. The first step is to have an open and frank discussion with the producer, and determine what the operation's goals are in regards to JD (Collins, 1994). Implementing a JD control program is long term commitment, and the producer must understand and be willing to make that commitment (Collins, 1994; Jubb and Galvin, 2004) The second step to is assess the risk of JD transmission on the operation (Collins, 1994). Over the years, different risk assessment tools have been developed for JD and assessed using a logical, scientific, systematic approach similar to that used in the successful beef and dairy milk and meat quality assurance programs (Pence, et al., 2004; Berghaus, et al., 2005; Raizman, et al., 2006). Recently, in the US, the National Johne's Disease Working Group put together a

consensus risk assessment for JD, which has been approved by the USDA for use in the National Voluntary Johne's Disease Control Program (Appendix A). The third, and final, step is to recommend farm management practices that are most likely to minimize the spread of MAP on that specific farm (Collins, 1994).

Almost all the recommended farm practices for controlling JD are based on what is currently known about the pathogenesis of MAP, how it is transmitted, and factors associated with increasing or decreasing risk of infection. Validation of control practices in the field is limited, due to the chronic nature of the disease and the diagnostic difficulty in identifying infected animals (Ridge, et al., 2005). Instead, farm practices to control JD have been simulated, using existing theory for control, expert opinion, and the limited field data available (Collins and Morgan, 1992; Groenendaal, et al., 2002; Dorshorst, et al., 2006; Kudahl, et al., 2007).

The general consensus of all (expert research opinion, observational field studies, and simulated studies) is that improved calf hygiene is a critical component of any JD control program (Thoen and Moore, 1989; Collins and Morgan, 1992; Collins, 1994; Goodger, et al., 1996; Groenendaal, et al., 2002; Jubb and Galvin, 2004; Pence, et al., 2004; Ridge, et al., 2005; Dorshorst, et al., 2006; Kudahl, et al., 2007). Johne's disease control starts with breaking the chain of infection (Kudahl, et al., 2007). Given that young cattle are more susceptible to infection with MAP than older cows, management should focus on roughly the first six months of life (Collins, 1994). This means eliminating, or minimizing contact of neonatal and young calves with colostrum, milk and/or feces from infected adult cows (Pence, et al., 2004; McKenna, et al., 2006). How this is done will likely vary by farm (Ridge, et al., 2005). Some of the more common and

easily implemented changes are: prompt removal of calf from dam after birth, cleaning maternity pen after each use, housing calves in separate pens well away from contact with adult cattle, only feeding colostrum from JD test negative cows; and feeding calves milk replacer or pasteurized whole milk (Collins, 1994; McKenna, et al., 2006).

Aside from calf management, other commonly recommended practices to control JD are to cull all cows with clinical signs of weight loss and diarrhea and improve overall farm cleanliness; thereby removing the most infectious animals and reducing the environmental reservoir of MAP (Collins, 1994; Goodger, et al., 1996; McKenna, et al., 2006).

In simulation models, improving calf hygiene was more cost-effective than testing (Groenendaal and Galligan, 2003; Dorshorst, et al., 2006); although improving calf hygiene and the use of a test-and-cull strategy provided the quickest means of control (Collins, 1992). Diagnostic testing for JD control purposes is not always necessary and should not be recommended in all herds (Dorshorst, et al., 2006). To support these simulations, a field evaluation of the Victorian (Australia) Johne's Disease Test and Control Program (TCP), which consisted of testing and culling MAP positive animals along with improving calf management, found that within herd JD prevalence and the incidence of clinical cases did not decline significantly until the herds consisted mainly of cows born after the TCP was started (Jubb and Galvin, 2004).

In short, farm management practices to control JD must focus on eliminating or minimizing exposure of calves to MAP (Collins, et al., 1994; Pence, et al., 2004; McKenna, et al., 2006). It is unlikely the success of a JD control program will depend on any single management change. Instead success will depend on a series of changes, each

with different degrees of importance, and that will be different from farm to farm (Ridge, et al., 2005). Thus, JD control programs must be designed specifically for each herd, only after understanding the goals and capabilities of the operation and an assessment of the areas at greatest risk for JD transmission on the farm is performed (Collins, 1994; Rossiter and Burhans, 1996; Ridge, et al., 2005). Finally, a JD control program is a long term commitment, and it may take years before the program has noticeable impact on within herd JD prevalence and/or incidence (Collins, 1994; Judd and Galvin, 2004).

1.6. Economics of Johne's disease

The economic costs of JD to producers can be divided into two broad categories: (1) the economic costs due to the disease as a result of impaired productivity and performance, and (2) the economic costs associated with diagnosing and controlling the disease. The decision to invest in a JD control program will often hinge on the magnitude of the difference between these two categories. One can look at the economic costs due to JD as an estimate of the potential benefits of controlling the disease (Groenendaal and Wolf, 2008 in press). In other words, if JD was eradicated, the producer could potentially realize an increase in revenue equal to the estimated losses caused by the disease. If the cost associated with diagnosing and controlling JD is greater than the potential benefits of reducing or eradicating it, investing in a control program may not be a sound economic decision. In short, if controlling JD costs more than what the disease is costing the producer in terms of lost production and performance, it will be difficult to convince him to implement a control program.

There have been numerous studies attempting to define and quantify the economic costs due to lost production and performance as a result of JD (see Table 1.3). Few, have attempted to quantify costs of diagnosing and controlling the disease, and those that have are based on expert opinion and assumption; not on real farm data (Benedictus, et al., 1987).

Production and performance losses due to Johne's disease

Few studies in the literature have attempted to quantify, in monetary terms, the production losses caused by JD because such economic indices are so unstable from year to year and across different regions (Hasonova and Pavlik, 2006). Instead, most have addressed production losses qualitatively, and then tried to estimate the magnitude of each qualitative loss. The reported impact of JD on common dairy production and performance indices varies greatly from study to study, and sometimes even within the same study (Spangler, et al., 1992; Hendrick, et al., 2005c). The most likely reason for the discrepancy of reported results is again, due to the chronic nature of JD and the associated difficulty of identifying MAP infected animals. The reported outcomes were dependent on: study design, the population being studied (cull cows vs. cows retained in the herd; subclinical cows vs. cows with clinical signs), prevalence of JD in the population, and the method used to identify infected cows; none of which were uniform or standardized. Therefore, it would be a mistake to try to make direct comparisons between studies. Instead the literature has been evaluated qualitatively and the results summarized in Table 1.3.

Table 1.3: Qualitative summary on the impact of Johne's disease on dairy production and performance parameters. (References are sorted according to findings)

Parameter	Production/Performance		
	Worse in infected cows/herds	**No difference between infected and uninfected cows/herds**	**Better in infected cows/herds**
Milk production	Buergelt and Duncan, 1978; Benedictus, et al., 1987; Kormendy, et al., 1989; Spangler, et al., 1992;[1] Wilson, et al., 1993; Nordlund, et al., 1996; Ott, et al., 1999; Hendrick, et al., 2005c;[1,3] Lombard, et al., 2005; Beaudeau, et al., 2007; Gonda, et al., 2007; Raizman, et al., 2007	McNab, et al., 1991; Spangler, et al., 1992;[2] Johnson, et al., 2001; Hendrick, et al., 2005c[2]	
Fat and protein production	Gonda, et al., 2007	Nordlund, et al., 1996; Johnson, et al., 2001; Hendrick, et al., 2005c; Lombard, et al., 2005	

[1] Fecal culture positive cows
[2] Serum ELISA positive cows
[3] Milk ELISA positive cows

Table 1.3 (continued): Qualitative summary on the impact of Johne's disease on dairy production and performance parameters. (References are sorted according to findings)

Parameter	Production/Performance		
	Worse in infected cows/herds	**No difference between infected and uninfected cows/herds**	**Better in infected cows/herds**
SCC/Mastitis	Merkal, et al., 1975 Buergelt and Duncan, 1978 McNab, et al., 1991	Nordlund, et al., 1996 Hendrick, et al., 2005c Lombard, et al., 2005 Gonda, et al., 2007	Wilson, et al., 1993
Reproduction	Merkal, et al., 1975 Buergelt and Duncan, 1978 Johnson-Ifearulundu, et al., 2000 Raizman, et al., 2007	McNab, et al., 1991	Gonda, et al., 2007 Marce, et al., 2007
Productive lifetime	Buergelt and Duncan, 1978 Kormendy, et al., 1989 Wilson, et al., 1993 Hendrick, et al., 2005c Gonda, et al., 2007		
Cull value	Benedictus, et al., 1987 Johnson-Ifearulundu, et al., 1999 Ott, et al., 1999		
Mortality	Johnson-Ifearulundu, et al., 1999 Ott, et al., 1999		

By far the most consistent finding in the literature was decreased milk production by MAP infected cows. In the four studies in which no production difference was noted between infected and uninfected cows (McNab, et al., 1991; Spangler, et al., 1992; Johnson, et al., 2001; Hendrick, et al., 2005c), all were based on comparing subclinically infected cows, as determined by serum ELISA, to test negative cows. In one study (Johnson, et al, 2001), the mean average parity of the study herds was <2, and it was hypothesized, that in "young" herds, subclinical MAP infection may have "little impact on milk production." In two studies, when infection status was determined by fecal culture (Spangler, et al., 1992; Hendrick, et al., 2005c) or milk ELISA (Hendrick, et al., 2005c), milk production was significantly lower in test positive cows compared to test negative cows; yet, in the same studies, there was no production difference between infected and uninfected cows when infection status was determined by serum ELISA. This underscores the difference in sensitivity between different diagnostic tests, potentially leading to a lack of agreement when they are run concurrently.

Only a few studies reported the magnitude of reduced milk production in MAP infected cows compared to uninfected cows. The milk production loss in cows with subclinical JD ranged from 2-6% (Nordlund, et al., 1996; Hendrick, et al., 2005c). For cows culled with clinical JD, the reported loss in milk production was 14% (Raizman, et al., 2007). One study compared milk production in cull cows for the current lactation and the two previous lactations respectively. Cows with subclinical JD produced 6% less milk in the lactation they were culled as compared to the next previous lactation, and 16% less milk than the second previous lactation. Cows with clinical JD produced 5% less milk in the lactation during which they were culled compared to the next previous

lactation, and 19.5% less than the second previous lactation (Benedictus, et al., 1987). In one study, the comparison was made based on fecal culture test status without reporting clinical status. Milk production loss in this study ranged from 6.6-14% (Wilson, et at., 1993). These results suggest that the drop in milk production gets worse as the severity of the disease progresses, and is consistent with an increasingly negative energy balance.

Also consistent across studies, was a decrease in the productive lifetime of cows infected with JD (Buergelt and Duncan, 1978; Kormendy, et al., 1989; Wilson, et al., 1993; Hendrick, et al., 2005c; Gonda, et al., 2007). This was generally reported as an increased risk of culling for infected cows. No attempt was made to quantify this loss in any of the studies, but was theorized to be significant due to suboptimal culling resulting in the lost future production of the cow culled and the cost of replacing her.

Three studies reported a lower cull value for cows with JD. This was due to weight loss resulting in lower slaughter weight (Johnson-Ifearulundu, et al., 1999), or poorer body condition (Ott, et al., 1999). One study reported a 30% reduction in the cull value of cows with clinical JD (Benedictus, et al., 1987). In another, a 10% increase in serum ELISA JD test prevalence corresponded with a 33.4 kg (73.5 lb) decrease in the mean cull cow weight for the herd (Johnson-Ifearulundu, et al., 1999).

Also uncontested across the studies evaluated, was the finding of an increased mortality rate in JD infected dairy herds. In one study the mortality rate was 3% higher in JD infected herd as compared to uninfected herds. In the 1996 US National Animal Health Monitoring Service (NAHMS) dairy study, the mortality rate in herds with a "low-clinical" rate of JD was 15% greater than in uninfected herds; while in herds with a

"high-clinical" rate of JD, the mortality rate was 45% greater than in uninfected herds (Ott, et al., 1999).

The impact of JD on the other parameters assessed in the studies was not so clear cut. Most controversial was the impact of MAP infection on udder health. Two of the three studies that reported an increase in the incidence of mastitis in infected cows were comparing fecal culture positive clinical cows to culture negative cows (Merkal, et al., 1975; Buergelt and Duncan, et al., 1978). This would suggest that the cows were in the more advanced stages of the disease. For the four studies in which MAP infection was found to have no significant effect on udder health or the incidence of mastitis, two of them were comparing subclinically infected cows to JD test negative cows (Nordlund, et al.., 1996; Hendrick, et al., 2005c); one did not report the clinical status of the infected group, but did report the body condition score of >85% of the infected cows was "normal" (Lombard, et al., 2005); while in the remaining study, the clinical status of the test positive cows was not reported (Gonda., et al., 2007). Thus, it would seem that the disease process in the infected cows in these studies was not as advanced. Perhaps the reason for the conflicting findings regarding udder health across these studies is due to the immune status of the infected cows. The more advanced the JD process, the more compromised the immune system becomes, making the infected cow more susceptible to other infections, such as mastitis. However, the reason for MAP infected cows having a reduced incidence of mastitis in one study remains unexplained (Wilson, et al., 1993); but the study consisted of only one herd, so it may be a phenomenon specific to that herd and should be interpreted accordingly.

The most controversial findings in the literature concerned the impact of MAP infection on reproductive performance. In many of the studies, reproductive performance was poorly defined as simply "infertility" (Merkal, et al., 1975; Buergelt and Duncan, 1978) or "poorer reproductive performance" (Raizman, et al., 2007) so it is difficult to contrast the studies and draw any conclusions. It seems reasonable as JD progresses, and the cow enters into an increasingly negative energy balance, that reproductive performance would be adversely affected, resulting in potential economic losses.

To summarize, the economic impact of MAP infection on the production and performance of dairy cattle is due primarily to reduced milk production and cull value of infected cows, resulting in increased replacement costs (Ott, et al., 1999; Wells and Wagner, 2000). Other losses due to JD associated with concurrent disease and reproductive performance are possible, but the literature is less clear, and sometimes contradictory, regarding the direction of the infection's impact. Thus, these economic losses are probably minor compared to those resulting from reduced milk production and cull value. Finally, the magnitude of the economic costs caused by JD, are positively correlated with the stage of the disease; the more advanced the infection, the greater the economic costs (Ott, et al., 1999).

Economic costs associated with production losses caused by Johne's disease

The estimated monetary costs of the production and performance losses caused by JD have been estimated at both the industry and herd levels. The estimates vary depending on study design and what was or was not included in the calculation.

Based on data collected in the 1996 NAHMS dairy study, it was estimated that JD costs the US dairy industry $200 – 250 million annually (Ott, et al., 1999). The estimated national prevalence of MAP infected dairy herds at the time was 21.6% (USDA, 1997). In the most recent NAHMS dairy study in 2007, the estimated prevalence of MAP infected dairy herds was 68.1% (USDA, 2008); a roughly three-fold increase. The associated estimated economic costs of JD have yet to be released, but it is probable that with more infected herds, the costs will have gone up as well. If a linear association is assumed between costs and prevalence, JD could currently be costing the US dairy industry $600 – 750 million per year.

As with the assessment of JD control programs, simulated models have been developed to assess the economic costs associated with JD at the herd level. In a Canadian based model, the estimated cost of JD was (US equivalent) $33 per cow (Chi, et al., 2002a). A second simulation model estimated the cost of JD to an average midsize US dairy to be $30 per cow in inventory the first year following introduction of the disease into the herd, and increasing to $70 per cow per year by year 20 after infection in the absence of a control program (Groenendaal and Galligan, 2003). These data were in close agreement to those reported in field studies. Data from the 1996 NAHMS study was also used to calculate the cost of JD at the herd level (Ott, et al., 1999). Herds infected with JD lost $97 per cow in inventory per year as compared to uninfected herds. The economic costs increased to $245 per cow in inventory for JD infected herds with a high prevalence (>10% of cull cows having clinical signs). When aggregated across all cows in the US, the economic cost of JD was estimated at $22 – 27 per cow. The

economic losses due to JD reported in other studies ranged from $20 – 26 per cow after standardizing the milk price and cull value used in the calculation (Ott, et al., 1999).

Economic costs of Johne's disease diagnostic and control programs

Diagnostic testing often represents the largest cash cost of a JD control program, and should only be done if the test results are going to be used to guide management decisions (Rossiter and Burhans, 1996). Testing is not a necessary component in all JD control programs. Although, it will aid in reducing the disease burden in the herd by identifying the most infectious cows so they can be culled (Collins, 1992; Rossiter and Burhans, et al., 1996). Cost is almost always the deciding factor on whether to undertake a JD testing strategy. For many herds, low costs tests are more useful than more sensitive, but more expensive tests (Dorshorst, et al., 2006). The current costs (2008) of the most commonly used Johne's diagnostic tests offered by the USDA certified Johne's testing laboratories in Michigan are summarized in Table 1.4.

Published, real farm data on the cost of implementing management practices to control JD, and their impact on the JD burden within dairy herds is lacking. The production losses caused by JD are substantial, and would seem to warrant disease control efforts. However, further research is needed on the costs of changing herd management to control JD, in terms of capital, supplies, and labor; before the cost effectiveness of control programs can be determined.

Table 1.4: Costs of commonly used Johne's disease diagnostic tests offered by the USDA certified Johne's testing laboratories in Michigan

(prices current as of January 2008)

Lab	ELISA		Fecal Culture		PCR
	Serum	Milk	Standard	Liquid	
Geagley Lab, Michigan Department of Agriculture	$6	NA	$16	NA	NA
Diagnostic Center for Population and Animal Health, Michigan State University	$6	NA	NA	$23	NA
Antel Bio	$6	$6	$35	NA	$30 – $100*

NA: Not available

* $30 for results in 2 weeks, $100 for results in 3 days

1.7. Zoonotic potential of MAP

Johne's disease is classified as a reportable, but non-actionable, disease in many states in the US (Step, et al., 2000). It is classified by the Office of International des Epizooties (OIE) as a list B disease, meaning it has the potential for substantial socioeconomic or public health consequences (Wells, et al., 1998). Part of the reason these reporting classifications were made is the ongoing concern that MAP is a zoonotic pathogen. Other mycobacteria (*M. tuberculosis, M. bovis, M. leprae, M. avium, M. africanum*) are zoonotic (Hugh-Jones, et al.,1995), so it is not inconceivable that MAP is as well. More importantly, over the years, there has been a growing body of evidence

linking MAP to Crohn's disease, a chronic granulomatous ileocolitis, in people (Chiodini and Rossiter, 1996). In a recent, comprehensive, meta-analysis, a specific and positive association was found between MAP and Crohn's disease, but the causal role remained undetermined. MAP could be a causative agent of Crohn's disease, a secondary pathogen exacerbating the disease, or an incidental colonist (Feller, et al., 2007).

1.8. Conclusion

With most infectious diseases, eradication is the ultimate goal. However, many experts question if JD eradication is practical or possible (Collins, et al., 2006). Whether eradication is possible or not, the first step needs to be JD control. The increasing number of herds infected with JD suggests that the US cattle industry is a long ways from controlling JD. Thus, further research on how best to manage and control JD in a realistic and cost-effective manner is warranted.

CHAPTER 2

Pillars, R., Grooms, D.L., Kaneene, J.B., in press. Longitudinal study of the distribution of *Mycobacterium avium paratuberculosis* in the environment of dairy herds participating in the Michigan Johne's Disease Control Demonstration Herd Project. Can Vet J.

CHAPTER 2

LONGITUDINAL STUDY OF THE DISTRIBUTION OF *MYCOBACTERIUM AVIUM PARATUBERCULOSIS* IN THE ENVIRONMENT OF DAIRY HERDS PARTICIPATING IN THE MICHIGAN JOHNE'S DISEASE CONTROL DEMONSTRATION HERD PROJECT

2.1 Abstract

The objective of this study was to describe the distribution of *Mycobacterium avium paratuberculosis* (MAP) in the environment of infected dairy farms over time. Johne's Disease (JD) prevalence was monitored annually in seven Michigan dairy herds. Environmental samples were collected bi-annually and cultured for MAP. A total of 731 environmental samples were cultured, of which 81 (11%) were positive. The lactating cow floor and manure storage were the areas most commonly contaminated, representing 30% and 33% of positive samples respectively. When herd prevalence was >2%, MAP was cultured from the lactating cow floor and/or manure storage 75% of the time. When herd prevalence was ≤2%, MAP was never cultured from samples collected. For every one unit increase in number of positive environmental samples, within herd JD prevalence increased 1.62%. Environmental contamination with MAP is consistent over time on infected dairy farms, and management practices to reduce environmental contamination are warranted.

2.2 Introduction

Mycobacterium avium paratuberculosis (MAP), the causative agent of Johne's disease (JD), is prevalent worldwide. The National Animal Health Monitoring and Surveillance (NAHMS) Dairy 2007 study, estimated 68.1% of the dairy herds in the US were infected with MAP (USDA, 2008). This is up from 21.6% reported in the NAHMS Dairy 1996 study (USDA, 1997). Based on data from the 1996 NAHMS study, annual economic losses for the dairy industry due to JD were estimated to range from $200-250 million (Ott, et al., 1999). With increasing prevalence, economic loses are likely to

increase. Cattle generally become infected with MAP as young calves, but do not exhibit signs of the disease until years later (Sweeney, 1996). Due to the chronic nature of the disease, and its long incubation period, testing and culling infected animals as a method for controlling JD has been relatively ineffective by itself (Collins, et al., 2003; Dorshorst, et al., 2006; Kudahl, et al., 2007). Instead, strategies for controlling JD have focused on minimizing the exposure of calves, the animals most susceptible to becoming infected, to MAP, thereby preventing new infections.

While calves can become infected with MAP *in utero* (Seitz, et al., 1989; Sweeney, et al., 1992), or through ingestion of colostrum or milk from infected cows, this generally only occurs when the dam is in the latter stages of the disease (Sweeney, et al., 1992; Streeter, et al., 1995). It is believed most post-natal infections occur through the ingestion of the bacterium from a contaminated environment (Sweeney, 1996; Harris and Barletta, 2001). Thus, factors playing a role in transmission include the amount of MAP being shed into the environment, the location contaminated, and the length of time the bacteria survives in that environment.

As an obligate intracellular pathogen, MAP does not replicate outside the host (Harris and Barletta, 2001), but it can survive for months to over a year in the environment (Whittington, et al., 2004). Wildlife, birds (Beard, et al., 2001; Corn, et al., 2005; Raizman, et al., 2005), even invertebrates such as flies and worms (Fischer, et al., 2001; Pavlik, et al., 2002; Fischer, et al., 2005) commonly found around dairy farms can become infected with MAP, and occasionally shed the bacterium into the environment. While the amount of MAP shed by these nontraditional hosts is negligible compared to that shed by cattle (Tiwari, et al., 2006), it does represent a way by which the bacterium

can persist and multiply outside of the primary host. Recently, the discovery of "dormancy-related genes" in the MAP genome suggests that, in the absence of essential nutrients, MAP may enter a state of dormancy and then return to a viable, infectious state when conditions again become favorable (Whittington, et al., 2004). Under field conditions in Australia, using the sheep strain of MAP, the bacterium was cultured from pasture twelve months after removing livestock from the property (Whittington, et al., 2003).

Studies have been conducted to determine the extent of MAP contamination on infected dairy farms (Raizman, et al., 2004; Berghaus, et al., 2006; Lombard, et al., 2006). The bacterium has been found in numerous locations on dairy farms including calving pens and post-weaned calf pens (Berghaus, et al., 2006), both of which are high-risk areas for transmitting the disease to the next generation of herd replacements. The areas most commonly culture positive for MAP are those where manure accumulates from adult cattle, the animals most likely to be shedding the bacterium. These include manure storage areas (lagoons, manure spreaders) and high-traffic, common cow areas (feed alleys, holding pens, return alleys, etc.) (Raizman, et al., 2004; Berghaus, et al., 2006; Lombard, et al., 2006). As a result, targeted culturing of these areas can be used to identify MAP infected herds. In the most recent revision of the USDA's Johne's Program Standards (USDA, 2005), targeted environmental culturing was approved as an entry-level screening test for dairy herds desiring to participate in the Voluntary Johne's Disease Control Program. Evidence also suggests the number of positive environmental cultures, and the amount of MAP in those samples, is positively correlated with the

within herd prevalence (Raizman, et al., 2004; Fyock, et al., 2005; Berghaus, et al., 2006).

To date, studies investigating MAP contamination on dairy farms have been cross-sectional in nature, with the environment being sampled at only one point in time (Raizman, et al., 2004; Fyock, et al., 2005; Berghaus, et al., 2006). The temporal relationship between MAP environmental contamination and within herd JD prevalence remains undefined. There is limited information on how MAP contamination in the environment changes as within herd JD prevalence changes. Therefore, the objective of this study was to characterize the distribution MAP in the environment of infected dairy farms, and describe if, or how, that distribution changes as within herd prevalence changes. The intention being to identify areas on infected farms that consistently culture positive for MAP. By understanding what areas on infected farms are consistently contaminated with MAP, even in the face of changing herd prevalence, more focused and economical herd screening programs can be developed.

2.3 Materials and Methods

Farms

This study was part of the larger Michigan Johne's Disease Control Demonstration Project. A total of seven Michigan dairy herds participated in this study. Herds were selected based on the following criteria: 1) herds were known to be infected with JD upon enrollment; 2) the producer was willing to participate in a longitudinal study for at least 5 years; and 3) the herd was representative of a typical Michigan dairy

farm in terms of herd size and housing management. Upon enrollment, and annually thereafter, a JD risk assessment was performed for each herd. Based on the risk assessment and the individual herd's goals and management capabilities, a JD control program was implemented on each herd and updated as necessary throughout the study. Study herd size ranged from 94-513 adult cows. Only one herd expanded significantly (231 to 445 cows) during the course of this study. Herd size for the other six herds remained fairly consistent throughout the study period. Housing management practices consisted of total confinement (4 herds), combination of confinement and grazing (2 herds), and one rotational grazing herd which was confined during the winter months. Confinement housing consisted of free stalls (6 farms) or a combination of tie stalls and free stalls (1 farm).

Determination of Herd Prevalence

Fecal culture was performed on all adult cows in each herd annually. Prevalence was calculated as the number of cows with positive fecal culture results, divided by the total number of cows tested that year.

Environmental Sampling

Every six months environmental samples were collected from each farm. At each visit, one sample was collected from the feeding area, primary water source and floor from each of the following areas: pre-weaned calf, weaned heifer, maternity, and lactating cow. A sample from the primary manure storage area (generally a lagoon or manure spreader) was also collected. Thus, a total of 13 environmental samples were

collected at each herd visit. In addition, samples of pasture, pasture water sources, deer feces, and recycled sand bedding were collected and cultured when appropriate.

An attempt was made to get as representative a sample from each designated area as possible. For feed and flooring samples respectively, a clean, gloved hand was used to collect 10 random "grab" samples from various locations in the designated area. The samples were mixed together thoroughly and placed in 720 ml sterile Whirl-Pak bags. A composite sample from all sources (buckets, water tanks, automatic waterers, ponds, etc.) providing drinking water to cattle in a given area was collected in a sterile, 1L bottle. The water sample was thoroughly agitated before filling a 120 ml plastic specimen cup and submitting for culture. For manure lagoons, samples were collected 15 cm below the surface from 4-6 different locations and pooled to fill a 120 ml specimen cup. For manure spreaders, a 120 ml sample was collected from the beaters (box spreaders) or dispensing area (liquid spreaders). For recycled sand bedding and pastures respectively, five random "grab" samples were collected from the surface and five underlying the surface at depths varying from 6-24 cm. All samples from each respective area were mixed together in a clean bucket and a pooled sample placed in a 720 ml sterile Whirl-Pak bag for culture submission. During each farm visit, the farmstead, particularly around feed storage areas, pastures, fields and any adjacent woods where deer sightings were reported, were walked and samples of deer feces collected when found. Environmental samples were collected from January 2003 through November 2006.

Bacterial Culture

All fecal and environmental samples were submitted for MAP culture to the Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing, MI. Prior to June 2004, all samples were cultured on standard solid culture using Herrold's Egg Yolk (HEY) media. Thereafter, samples were cultured using the ESP® culture system II (ESP II, TREK Diagnostics Systems, Inc., Cleveland, OH). Processing and decontamination of samples prior to inoculation of culture media was the same throughout the study, and consisted of a modification of the Cornell method described previously (Stabel, 1997). Briefly, 2 grams of each sample was added to 35 ml sterile distilled water. The sample was vigorously shaken for 15 seconds, and then allowed to set at room temperature for 30 minutes. Five ml from the center of the supernatant was pipetted into a centrifuge tube containing 25 ml ½XBHI-HPC (half strength brain heart infusion broth with 0.9% 1-Hexadecylpyridinium) and gently mixed. Tubes were incubated at 35-37° C overnight. Samples were then centrifuged at 3000 G for 20 minutes at >22° C. The supernatant was decanted. One ml of antibiotic mixture (50 µg amphotericin B, 100 µg vancomycin, and 100 µg naladixic acid in ½ XBHI) was added to the sample and vortexed to resuspend the pellet for final decontamination. Samples were incubated at 35-37° C overnight before inoculating onto culture media.

Culture positive samples were confirmed as MAP using Kinyoun's acid-fast stain and real-time PCR for the IS900 insertion sequence. Real-time PCR was performed after 42 days on all signal negative ESP II samples. Samples were only reported as negative if they were signal negative on ESP II and negative on PCR.

Descriptive Data Analysis

Culture results were recorded in, and descriptive statistics generated, using a commercial computer spreadsheet (Microsoft Office Excel™, Microsoft Corporation, Redmond, WA).

Statistical Data Analysis

The number of environmental samples collected at each collection date varied across and within herds depending on the housing management (pasture vs. confinement), season (pastures were not sampled during winter months when cows were confined and/or access was restricted due to snow cover), and availability (deer feces were not consistently found on all farms). The association between the within herd JD prevalence and the number of culture positive environmental samples over time was therefore restricted to only those samples that were consistently collected on all farms (feed, flooring, and water from the pre-weaned calf, weaned heifer, lactating cow, and maternity areas, and manure storage area). Environmental samples were collected every six months, while herd prevalence was only calculated once every 12 months. Thus, for every year, two samples were collected from each area on the farm. For ease of analysis, environmental culture results were aggregated by calendar year and animal location (pre-weaned calf, weaned heifer, lactating cow, maternity, and manure storage areas). Using within herd JD prevalence as the outcome of interest, its association with time (study year) and the number of positive environmental samples was assessed using linear regression, controlling for repeated measures within herds using generalized estimating equations (GEE) using an exchangeable correlation structure. The regression model was

built starting with univariable analysis for study year and the total number of positive environmental samples each year. To determine if within herd prevalence was associated with MAP contamination in specific areas on the farm, similar univariable linear regression models were assessed using the number of positive environmental samples in the pre-weaned calf, weaned heifer, lactating cow, maternity, and manure storage areas as the independent variables, respectively. Area-specific variables with a p-value of >0.15 on univariable analysis were then considered in a multivariable linear regression model using step-wise backward selection. The final multivariable model consisted of only those variables with a p-value of <0.05.

Model fit for all respective regression models was assessed using an extension of cumulative residuals as discussed in Lin, et al. (2002). Briefly, the cumulative sums of the residuals for each independent variable in the respective regression models were plotted, along with the residuals of 10,000 simulated realizations from a zero-mean Gaussian distribution. The Kolmogorov-type supremum test was calculated along with its associated p-value. This process was repeated with alternative functional forms of the variable based on the initial pattern of the cumulative sums of residuals in an attempt to improve model fit when warranted. The greater the Kolmogorov-type test statisitic and its p-value, the better the model fits the data, and p-values <0.05 were considered indicative of poor, or insufficient, model fit.

All statistical analysis was performed using commercially available software (Proc Genmod, SAS 9.1, SAS Institute, Inc., Cary, NC, USA).

2.4. Results

Herd Prevalence

Initial apparent JD prevalence based on whole herd fecal culture in the study herds ranged from 2-11%. Over the four-year course of this study, apparent JD prevalence within these herds ranged from 0-42%. In one herd, the prevalence increased dramatically, from 7% to 42% in the second year of the study then gradually declined to 12% by year four. This occurred despite the herd having been closed for over 20 years and herd size remaining constant. Apparent prevalence within the herd that purchased cattle to double herd size increased slightly (9-11%) over the study period. Johne's disease prevalence in the other five herds tended to decrease or plateau between years three and four of this study.

Environmental Culturing

A total of 731 environmental samples were collected with 81 (11%) culturing positive for MAP. Culture results by location are summarized in Table 2.1. Over the four-year course of the study, positive environmental samples were identified on six of the seven farms. The one farm with no positive environmental samples had extremely low fecal culture prevalence, ranging from 0-2%.

The areas most commonly contaminated were the lactating cow floor and the manure storage area, representing 30% and 33% of the positive samples respectively. One or both of these areas was positive on 75% of the environmental collection dates.

Table 2.1: Distribution of *Mycobacterium avium paratuberculosis* (MAP) in the environment of seven Michigan Dairy Farms

Location	No. Samples	No. Positive	Location %	Total %
Calf Feed	51	2	3.9	2.5
Calf Floor	57	4	7.0	4.9
Calf Water	49	0	0.0	0.0
Heifer Feed	50	0	0.0	0.0
Heifer Floor	53	3	5.7	3.7
Heifer Water	52	0	0.0	0.0
Maternity Feed	52	0	0.0	0.0
Maternity Floor	56	8	14.3	10.0
Maternity Water	54	5	9.3	6.2
Lactating Cow Feed	52	2	3.8	2.5
Lactating Cow Floor	54	24	44.4	30.0
Lactating Cow Water	53	2	3.8	2.5
Lagoon/Manure Spreader	53	27	50.9	33.3
Recycled Sand	5	4	80.0	4.9
Other	40	0	0.0	0
Total	731	81	11.1	100.0

Both of these areas were positive in the six herds with positive environmental samples at least once, and often multiple times, on different sampling dates.

Ten percent of the positive environmental samples came from the maternity floor and 6% from maternity water samples. The maternity area was positive for MAP at least once in four of the six herds. Fecal culture prevalence in those herds at the time the maternity area was positive ranged from 5.4-42%.

The pre-weaned calf area was found contaminated in three of the six herds. Apparent prevalence of MAP shedding in those herds at the time ranged from 8.6-17%. On one of the farms, the calves were housed in a group pen across an alley from a contaminated maternity pen, with the potential for cross contamination. On the other two farms, the calves were housed in separate barns, well away from any possible contamination or run-off from adult cattle.

Recycled sand bedding represented 5% of the positive environmental samples; however, these samples came from only one farm with fecal culture prevalence ranging from 12-42% at the time the samples were collected.

The majority of environmental samples contaminated with MAP originated from flooring or manure storage (n=70) as compared to feed (n=4) or water (n=7). Two of the positive feed samples came from the calf area adjacent to a contaminated maternity pen on a farm when within herd JD prevalence was 14%. The other two positive feed samples came from fence-line feed alleys in free stall barns housing lactating cows. All of the MAP positive water samples originated from adult cow areas, with five occurring in the maternity area and two in the lactating cow area.

When compiled, the number of positive environmental samples decreased as herd prevalence decreased (Figure 2.1). Once herd prevalence fell to below 2%, MAP was never cultured in the environment of any area sampled. When herd prevalence was >2%, MAP was cultured from the lactating cow floor and/or manure storage areas 75% of the time. All the positive samples in the 2-5% herd prevalence category originated from either the lactating cow floor or manure storage areas. When herd prevalence exceeded 5%, MAP began to be isolated from areas in addition to the lactating cow floor or manure storage areas, with the most common area being the maternity floor. Within individual herds, the trend for decreasing MAP environmental contamination (based on the percent of culture positive environmental samples) with decreasing within herd JD prevalence was not always as obvious (Table 2.2).

Over the course of this study, the JD prevalence within each herd changed, and the herds moved up and down across the prevalence categories outlined in Figure 2.1. For example, the <2% category represents data from three different herds; the 2-5% category, five herds; the 6-15% category, six herds; and the >15% category, three herds.

Figure 2.1: Percentage *Mycobacterium avium paratuberculosis* (MAP) positive environmental samples by within herd Johne's Disease prevalence

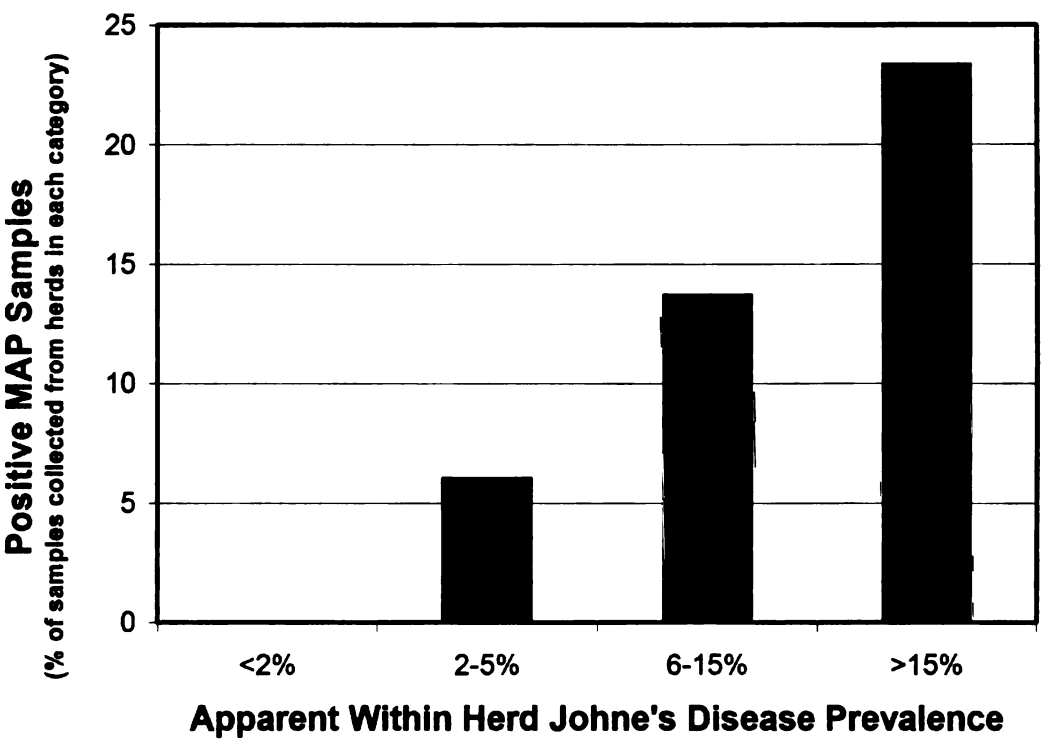


Table 2.2: Percent of MAP culture positive cows and environmental samples by herd over time

Herd	2003[a]		2004		2005		2006	
	Cows (%)	Environment (%)	Cows (%)	Environment (%)	Cows (%)	Environment (%)	Cows (%)	Environment (%)
1	10.3	0	14[a]	24.1[a]	20.3	18.4	4.4	6.5
2	10.2	6.7	4.1	3.6	2.9	7.4	1.9	11.5
3	8.6	14.3	5.4	17.9	10.6	21.4	11	11.5
4	10.6	0	6.4[a]	14.8[a]	2	0	4	7.1
5	NT	NT	5.3	19.2	5	7.7	6.3	19.2
6	7	6.7	42.1	17.2	16.9	28.6	12.1	23.8
7	1.8	0	0.6	0	0.6	0	2	0

[a] Indicates samples cultured on Herrold's Egg Yolk (HEY)

NT Herd not tested

Statistical data analysis

The results of univariable linear regression models to assess the association between herd prevalence over time and the number of positive environmental samples overall and in each respective area are summarized in Table 2.3. The results of the final multivariable linear regression model assessing the association of within herd JD prevalence with MAP environmental contamination in specific areas of the farm are shown in Table 2.4.

Table 2.3: Univariable linear regression analysis using within herd Johne's disease prevalence as outcome

Model	Estimate	95% Confidence Limit		p-value	Kolmogorov-type supremum test p-value
		lower	Upper		
Study year	-0.54	-1.43	0.35	0.2374	0.38
Number positive environmental samples - total	1.62	0.82	2.42	<0.0001	0.53
Number positive environmental samples – pre-weaned calf area	3.01	1.41	4.60	0.0002	0.30
Number positive environmental samples – weaned heifer area	6.01	1.16	10.85	0.0152	0.32
Number positive environmental samples – lactating cow area	1.03	-0.82	2.89	0.2737	0.11
Number positive environmental samples – maternity area	6.24	0.77	11.72	0.0254	0.29
Number positive environmental samples – manure storage area	1.41	-0.0012	2.83	0.0502	0.21

Table 2.4: Final multivariable linear regression model using within herd Johne's disease prevalence as the outcome

Variable	Estimate	95% Confidence Limits		p-value	Kolmogorov-type supremum test
		Lower	Upper		p-value
Number positive environmental samples – pre-weaned calf area	5.12	3.58	6.65	<0.0001	0.48
Number positive environmental samples – weaned heifer area	6.19	2.52	9.87	0.0010	0.42
Number positive environmental samples – maternity area	5.68	2.10	9.25	0.0018	0.20

The regression estimate for the association between within herd JD prevalence and study year was negative, suggesting that the prevalence in these herds declined over time, even though that decline was not statistically significant. Regardless, there was a significant association between decreasing JD herd prevalence and number of positive environmental samples. For every one unit increase in the number of annual positive environmental samples, the within herd JD prevalence increased 1.62%. ($p = <0.0001$). When contamination within specific areas of the farm were assessed, for every one unit increase in the number of positive environmental samples in the pre-weaned calf, weaned calf, and maternity areas, within herd JD prevalence increased by 5.12%, 6.19%, and 5.68% respectively. Environmental contamination in the lactating cow and manure storage areas were not statistically associated with increasing JD prevalence because these were the areas that were consistently contaminated on the farms, even when within herd prevalence was very low.

2.5. Discussion

The strength of this study is its longitudinal nature, such that changes in the distribution of environmental MAP contamination could be monitored as within herd JD prevalence changed on infected dairy farms following the implementation of on-farm JD control programs. MAP was cultured consistently (75% of the time) over time in the manure storage area and/or the lactating cow floor when within herd culture prevalence was >2%; indicating a consistent reservoir of MAP contamination, even when relatively few cows in the herd are actively shedding. However, once the number of cows shedding the bacterium in the herd fell to <2%, MAP was not cultured from any location sampled.

Logically, the fewer cows shedding MAP, the less contamination there is in the environment; and the less likely it is for an environmental sample to be collected containing MAP at a level detectable by currently available culture methods. It is also possible manure management and sanitation practices implemented by the herds for JD control purposes resulted in a less manure accumulation, thereby decreasing the potential for MAP environmental contamination. The fact MAP was never cultured from environmental samples of one herd that consistently had low within herd JD prevalence (<2%) does not mean the environment on this herd was not contaminated with MAP. More likely the level of MAP contamination was minimal and below the detection threshold of the sampling protocol used in this study.

Herd prevalence had to increase only slightly to 5% before MAP was cultured in areas in addition to the lactating cow floor and manure storage areas, with the most common area being the maternity floor. This is not surprising, as this is an area populated with adult cows. From a JD control standpoint, it is concerning because calves, the animals most susceptible to becoming infected with MAP, are being born in those areas. It emphasizes the importance of maternity pen management in any JD control program.

A surprising finding in this study was the positive environmental samples in the pre-weaned calf area on three different farms. While it was possible to explain cross contamination from a contaminated maternity pen across an alley in the same barn on one farm; the other two farms with positive calf areas had separate calf barns, located well away from adult cattle. It is possible these areas became contaminated through farm personnel or feeding/cleaning equipment traveling between cow and calf barns. The

other possibility is some of the calves on these farms were shedding MAP. While it has been traditionally thought newly infected cattle do not start shedding MAP for several months, or until adulthood; recent reports suggest calves may indeed shed MAP, albeit transiently, and typically, at low levels (Bolton, et al., 2005; vanRoermund and deJong, 2005). Regardless, the finding of MAP in the pre-weaned calf area should be considered a risk for infection to the calves housed there, and appropriate precautions taken.

Isolating MAP in four of five (80%) samples of recycled sand bedding (although originating from only one farm) was also an interesting finding, and raises the issue of where that bedding should be used. If the traditional JD paradigm that cattle become less susceptible to infection with age is accepted, using this sand to bed the adult herd likely represents minimal risk for spreading the infection. However, care should be taken to ensure it is not used in calf, young heifer, or maternity pens.

Our findings were similar to those reported in previous studies (Raizman, et al., 2004; Berghaus, et al., 2006; Lombard, et al., 2006), in that the areas most commonly contaminated with MAP on infected dairy farms were those where there was the greatest concentration of manure (lactating cow floor and manure storage) from adult cows, the animals at greatest risk of shedding the bacterium. Also, as in previous studies (Raizman, et al., 2004; Fyock, et al., 2005; Berghaus, et al., 2006), there was an overall tendency for the amount of MAP in the environment to increase as within herd JD prevalence increased (Tables 2.3 and 2.4, Figure 2.1). The difference between this study and those referenced, was this study was longitudinal in nature while the others were cross-sectional. The significance being that these findings were consistent over time in the face of increasing and decreasing JD prevalence within the same herds. Thus, adding strength

to the importance of environmental contamination as a source of MAP transmission to susceptible cattle.

At the individual herd level, there was not an obvious consistent downward trend in MAP environmental contamination as within herd JD prevalence decreased in all herds (Table 2.2). Factors to consider are: the potential for one or two "super-shedders" in the herd, the relatively long time MAP survives in the environment and the diligence each herd gave to sanitation. The "super-shedder" phenomenon in regards to MAP infection is a recently introduced theory in which one infected cow sheds billions of bacterium into the environment each day (Whitlock, et al., 2006). Thus, one or two "super-shedders" in a herd could disproportionately contaminate the environment, resulting in a high environmental load of MAP when compared to the absolute number of shedders in the herd. The prolonged survivability of MAP in the environment may result in a lag period following the removal of cows actively shedding MAP during which the environmental load of the bacteria in the herd's environment is maintained. That environmental load does not decrease to undetectable levels until the MAP finally dies, or it is physically removed. As in the general population, some of the herds in this study did a better job at cleaning and manure removal than others. Subjectively, the herds that did not follow the expected pattern of decreasing environmental MAP contamination in conjunction with decreasing within herd JD prevalence, were the ones that were less diligent in cleaning. Regardless, as long as MAP is detectable in the environment, susceptible cattle in the herd are at risk of becoming infected.

In contrast, linear regression analysis did demonstrate a statistically significant association between within herd JD prevalence and the number of positive environmental

samples. As the number of contaminated environmental samples increased, so did within herd prevalence. Also, the culturing of MAP from areas other than the lactating cow and manure storage areas was likewise associated with increasing herd prevalence. The reason a similar association was not found in the lactating cow and manure storage areas is likely due to the fact that these were the areas most consistently contaminated over time, even when prevalence was relatively low.

Potential factors influencing the results of this study were sample collection and culture system used. An attempt was made to collect representative environmental samples from the same areas on each farm throughout the course of the study. All the study herds were infected with MAP. It is certainly possible any given area may have been contaminated with MAP, but the sample collected either did not contain the bacteria, or did not contain enough of it to be detectable by the culture methods used. Therefore, due to sampling error, the MAP contamination on these dairy herds is likely to be more extensive than reported.

During the course of this study, the lab switched from solid culture on HEY media to the ESP II liquid culture system. Subsequently, the number of positive environmental cultures increased, while JD prevalence on most of the study herds was on a downward trend. The most likely explanation for this is the ESP II culture system is more sensitive than HEY and able to detect MAP at lower levels (Rajeev, et al., 2006).

Quantitative analysis of the level of MAP contamination in environmental samples over time, in conjunction with changing within herd JD prevalence would have strengthened this study. A high volume of contamination in the environment is associated with increased infection pressure, and subsequently higher within herd

prevalence (Collins, 2003). In a cross-sectional study, Raizman, et al. (2004), reported a positive correlation between the volume of MAP isolated from environmental samples and herd prevalence. Unfortunately, the laboratory reporting of quantitative culture results was inconsistent over the course of this study, precluding such analysis. The lack of quantitative analysis of MAP environmental contamination on these herds over time is a limitation of this study, and something that remains to be pursued in the future.

In this study, when herd fecal culture prevalence was >2%, MAP was isolated from the lactating cow floor and/or manure storage area 75% of the time. If samples cultured using only the ESP II culture system are considered, the lactating cow floor and/or the manure storage area was positive 81% of the time. Thus, culturing these two areas was a sensitive method for determining the presence of, and, to a lesser degree, the extent of MAP in a dairy herd. This protocol could be adapted to monitor the progress of JD control programs at the individual herd or regional level. Periodically culturing these areas on an individual farm could provide some indication whether or not the herd's JD control program is working. As within herd JD prevalence declines, eventually the lactating cow area and manure storage should consistently culture negative for MAP. At the state, regional, or national level, culturing the lactating cow and manure storage areas could provide an economical and efficient method for determining the number of dairy herds infected with JD, which in turn, could help direct the allocation of JD control resources.

In conclusion, MAP was widely distributed in the environment of the Michigan dairy farms participating in this study. The lactating cow floor and manure storage area (areas with the greatest concentration of manure from the greatest number of adult cows),

were the locations on the farms that most commonly cultured positive for MAP. Periodic targeted sampling of these areas may provide an efficient and economic tool for monitoring the progress of JD control programs at the individual herd and regional levels. An increasing number of MAP positive environmental cultures was associated with increasing within herd JD prevalence. It was not uncommon for MAP to be cultured from the maternity and pre-weaned calf areas, areas where there is a high risk for transmitting JD to the next generation of herd replacements. This underscores the need to emphasize cleanliness in these areas when recommending JD control programs. Finally, as long as MAP is present and detectable in the herd environment, susceptible cattle are at risk of becoming infected with JD. Thus, when using testing as part of a JD control program, targeted testing of the environment may be as important as testing individual cattle.

CHAPTER 3

LONGITUDINAL STUDY TO EVALUATE THE EFFECTIVENESS OF MANAGEMENT PRACTICES IMPLEMENTED TO CONTROL JOHNE'S DISEASE ON INFECTED DAIRY FARMS IN MICHIGAN

3.1. Abstract

A five-year longitudinal study was conducted on seven Michigan dairy herds infected with Johne's disease (JD) to evaluate the effectiveness of management practices implemented to control the disease. The JD incidence and prevalence was monitored in each herd annually by serum ELISA and/or fecal culture of all adult cows. A JD control program was designed specifically for each herd based on the results of an initial risk assessment. The risk assessment was repeated annually and the control program updated as needed. Herd risk assessment scores were used as a measure of which control practices were implemented and to what extent. The risk assessment scores were extrapolated to each cow consistent with when she was present in each assessed area as determined by her birth date. To assess the overall effectiveness of the control programs in preventing new infections, the JD incidence rate by lactation was compared between cows born after implementation of the JD control program to cows born prior to the control program. Over the first three lactations, the incidence of JD was consistently lower in cows exposed to the JD control program as calves than in cows not exposed to the control program as calves; providing evidence that, overall, the control programs implemented on these herds were successful in preventing new infections. The effectiveness of specific management practices in preventing JD infection was evaluated using logistic regression; controlling for clustering of cows within herds with generalized estimating equations (GEE). Univariable and multivariable models were built. The final multivariable model consisted of the following two variables: exposure to adult cows other than dam at birth (OR = 1.09, 95% CI: 1.06 – 1.13), and feeding colostrum from one cow to multiple calves (OR = 1.10, 95% CI: 1.09 – 1.12). Thus, for every one point

increase in the risk assessment scores in each of these areas, the odds of a cow testing positive for JD was increased by 9% and 10% respectively. Conversely, lowering the risk assessment score will decrease the odds of a cow testing positive for JD. When designing JD control programs, implementing management practices that minimize the exposure of newborn calves to *Mycobacterium avium paratuberculosis* being shed by infected adult cows should take priority.

3.2. Introduction

Johne's disease (JD) is a chronic disease of cattle and other ruminants caused by *Mycobacterium avium paratuberculosis* (MAP). It is prevalent worldwide and is becoming increasingly so in the US dairy industry. In the most recent National Animal Health Monitoring Survey (NAHMS) in 2007, it was estimated that 68.1% of US dairy herds were infected with MAP (USDA, 2008). This is up from 21.6% reported in the NAHMS Dairy 1996 survey (USDA, 1997). The economic costs are likewise substantial. Based on data from the 1996 NAHMS study, it was estimated that JD cost the US dairy industry, on average, $22-27 per cow, or $200-250 million annually (Ott, et al 1999); due primarily to reduced milk production, premature culling and reduced cull value (Wells and Wagner, 2000). While cost estimates from the 2007 study have yet to be released, with an increase in the number of herds infected with MAP, it is likely the economic losses due to JD will also have increased. Due to its significant impact on herd productivity, along with the potential public health consequences should MAP be linked to Crohn's disease in humans, understanding JD and how best to manage and control its

spread within and between herds is a priority for the US livestock industry (Linnabary, et al, 2001).

Infection with MAP results in a slowly progressive granulamatous enteritis, causing the walls of the intestine to become thickened; which in turn, impairs the absorption of nutrients from the gastro-intestinal tract. Clinically, what is observed as the disease progresses is decreased production; weight loss despite a good appetite; intermittent, progressing to chronic diarrhea; and ultimately death if the cow is not culled prior (Whitlock and Buergelt, 1996). There is no approved or practical treatment for JD in production animals (St. Jean, 1996; Wells and Wagner, 2000). Cows generally become infected with MAP as young calves, through ingestion of bacteria from a contaminated environment or from contaminated colostrum or milk. The susceptibility of becoming infected seems to decrease as the animal ages. Typically it takes 2-5 years before infected cows develop clinical signs of the disease. However, as the disease progresses, the risk of shedding MAP, thereby becoming infectious (even in the absence of clinical signs), increases (Sweeney, 1996). Because of the prolonged pre-patent period, testing and culling of MAP infected animals is not very effective in eliminating the disease (Groenendaal, et al., 2002; Collins, 2003; Dorshorst, et al., 2006; McKenna, et al., 2006; Kudahl, et al., 2007). Instead, control of JD focuses on implementing management practices that minimize the transmission of MAP to susceptible animals (Thoen and Moore, 1989; Collins, 2003; Hoe and Ruegg, 2006; McKenna, et al., 2006).

While implementing management changes to minimize JD transmission may sound simple, its actual practice is more problematic. The list of recommended practices to prevent MAP infection is lengthy and complex (Rossiter and Burhans, 1996;

Benedictus and Kalis, 2003). When presented in generic form, JD control recommendations often fail because they are not designed to meet the unique needs and capabilities of each individual farm (Rossiter and Burhans, 1996; Collins, 2003). Moreover, they are often presented to producers in their entirety, and not prioritized in any way to allow for selective or progressive adoption (Ridge, et al 2005). This can overwhelm producers to the point they believe they cannot successfully implement all the recommendations to control JD, so they decide not to implement any.

Part of the reason there is no ranking, or prioritizing, of management practices to control JD is because the current recommendations are all based on hypotheses of what is known about MAP infection and pathogenesis. Research to confirm recommended management practices actually work, or to identify which practices are more effective than others, has been limited. This is due mainly to the prolonged course of the disease, and the difficulty in identifying infected cattle in the early stages of the disease, which makes such studies costly and time consuming (Groenendaal and Galligan 2003). For example, to evaluate the effectiveness of a management practice to prevent MAP infection in newborn calves, the study would have to last a minimum of 3-5 years to allow adequate time for the calf to mature; and, if infected, give the disease time to progress to the point it can be detected. Also necessary, would be extensive, and repeated, individual animal testing to determine if the JD incidence decreased following the implementation of such a control practice. Yet, being able to present producers with a concise and specific plan for JD control, that is not overwhelming in its breadth, is necessary for the widespread adoption of JD control programs on farms.

To date, management practices for the control of JD on dairy farms have been mainly evaluated using computer simulation models. Although the scenarios and assumptions varied across the studies, each concluded that improving and maintaining strict calf hygiene, thereby breaking the primary MAP infection route, was the most critical and cost effective component of a JD control program (Groenendaal, et al., 2002; Groenendaal and Galligan, 2003; Dorshorst, et al., 2006; Kudahl, et al., 2007).

Simulation studies are commonly performed, particularly in the case of a chronic disease such as JD, because they are less expensive and require less time than field studies. The major disadvantage of a simulation study is that it can be difficult to validate. The input data on which the simulation is run is often based on field data supplemented with expert opinion. Therefore, a simulation study cannot be isolated from the field, and, in fact, can only truly be validated with observations made under real farm conditions with a field study.

The primary objective of this five-year longitudinal study was to evaluate the effectiveness of specific management practices implemented to control JD on seven MAP infected dairy herds in Michigan. Specifically, the study was designed to determine which JD control practices were most effective in reducing the incidence of JD. This information could then be used by veterinarians and producers in prioritizing or selecting necessary practices to implement when designing JD control programs for their own operations.

Secondary objectives of the study included: comparing the level of agreement between the serum ELISA and fecal cultures (HEY and ESP II) used to monitor within

herd JD prevalence, and assessing the effect of the dam's JD test status on her offspring's JD test status.

Critical to any disease control program is the early detection of infected animals so they can either be removed from the herd, or managed in a way to mitigate disease transmission to susceptible herdmates. Due to the prolonged incubation period of JD, identifying infected cattle in the early stages of disease is difficult with currently available diagnostic tests (Collins, 1996; Stabel, et al., 2002; Motiwala, et al., 2005; Dieguez, et al., 2008). Culturing MAP from fecal or tissue samples remains the "gold standard" for definitively diagnosing JD (Collins, 1996; Stabel, et al., 2002). . However, ELISA tests to detect antibodies to MAP has become widely accepted for use in routine JD testing because it is inexpensive ($6/sample for ELISA compared to $23/sample for culture, Diagnostic Center for Population and Animal Health, Michigan State University, 2008), and results are generally available within days as opposed to weeks for culture. Multiple studies have assessed the performance of fecal culture and the various commercially available serum ELISA tests for MAP. Findings vary depending on study population, diagnostic test used, and laboratory performing the test, but the general consensus of JD researchers and experts is as follows. Fecal culture detects infected cows earlier in the course of the disease (Sweeney, et al., 2006). Newer, broth-based culture systems are more sensitive and likely detect cows in earlier stages of disease than standard culture on Herrold's egg yolk (HEY) solid media (Kim, et al., 2004; Motiwala, et al., 2005). The ELISA reliably identifies cattle that are shedding large numbers of MAP into the environment (Whitlock, et al, 2000; Stabel, et al., 2002; Sweeney, et al., 2006). However, subclinically infected cows cannot be differentiated from clinically

infected cows based solely on the magnitude of the ELISA test result (Spangler, et al., 1992). There can be substantial variation between serial ELISA tests on the same cattle (Hirst, et al., 2002; Barrington, et al., 2003; van Schaik, et al., 2003; Sweeney, et al., 2006). The sensitivity of the ELISA test decreases with repeated herd testing as the number of heavy shedders in the more advanced stages of the disease are culled (Whitlock, et al., 2000; Stabel, et al., 2002; Sweeney, et al., 2006). Recently, only slight agreement (kappa = 0.14 ± 0.07) was reported between the results of fecal culture on HEY and serum ELISA (Pinedo, et al., 2008). Given this background, it seemed prudent to compare the different JD diagnostic tests used in this study.

While external, or environmental factors, are often the primary focus of JD control programs, vertical transmission should also be considered. Multiple studies have demonstrated that *in-utero* transmission of MAP does occur (Doyle, 1958; Lawrence, 1956; Seitz, et al., 1989; Sweeney, et al., 1992; Manning, et al., 2003). Also important in the transmission of JD is the fact that cows are more likely to shed MAP in their feces, colostrum, and milk around parturition (Harris and Barletta, 2001), the time when there is the closest, and to some extent, unavoidable, contact between calves (the animals most susceptible to infection), and infected cows. On one farm, cows with dams that were MAP ELISA positive were 6.6 times more likely to be ELISA positive themselves (Aly and Thurmond, 2005). Whether it is due to *in-utero* transmission, or occurs through direct contact between infected dams and their offspring, understanding the risk of calves with MAP infected dams becoming infected is important for deciding how to manage infected cows, as well as their calves, if they are to remain in the herd.

3.3. Materials and methods

Herds

Seven Michigan dairy herds were selectively enrolled in this study January 2003 through May 2004, and followed through December 2007. To qualify for this study, each herd had to be infected with JD, be representative of the Michigan dairy industry in terms of herd size and housing management, and the herd owner willing participate in the study for at least five years and implement a JD control program.

Within herd JD prevalence and incidence

Data to calculate and monitor the within herd JD prevalence and lactational incidence rate was obtained by annual whole herd testing of all adult cows. Blood samples were collected, and serum analyzed for MAP antibodies using a commercially available ELISA test kit (Parachek®, Prionics AG, Schlieren-Zurich, Switzerland). Fecal samples were also collected and cultured for MAP. The laboratory switched MAP culture systems midway through the study. Through June 2004, cultures were performed on standard solid culture using Herrold's Egg Yolk (HEY) media. After June 2004, all cultures were performed using an automated liquid culture system (ESP II, ESP® Culture System II, TREK Diagnostic Systems, Cleveland, OH).

Herd risk assessment

A herd risk assessment (RA) was performed annually on each herd. The risk assessment tool used was that approved by the USDA for use in the National Voluntary

Johne's Disease Control Program. It consists of a subjective score given to each of 32 "risk factors" divided into six different areas of the farm: maternity, pre-weaned calf, weaned calf, bred heifer, lactating cow, and additions/replacements. For the purpose of the RA, it is accepted that some risk factors are more important in the transmission of JD than others. For example, exposure to manure as a newborn calf is considered to be more important than exposure to manure as an adult cow. To account for this, the maximum score possible for factors in the maternity area is higher than that in the lactating cow area. In other words, a score of four in the maternity area is not the same as a score of four in the lactating cow area.

Individual cow data

Individual cow data was collected for every cow tested on each annual whole herd test and included, when possible: identification, date of birth, lactation number, dam identification, JD test status, and whether or not she was purchased or raised on the farm.

Implementation and monitoring of JD Control Program

The initial visit to each herd consisted of whole herd testing (as described above) to determine baseline prevalence. Also during this visit, a JD risk assessment was performed; and a JD control program developed based on the risk assessment, farm goals, and capabilities. This RA was repeated annually, and the control program updated as needed. Variation of scores within and between herds was minimized by having the same person (DLG) perform the RA throughout the study.

Although the RA is subjective, it was the best tool we had available to monitor what JD control practices each herd put in place, and the extent to which they were following those practices over time. While the RA scores were assigned at the herd level, the scores were extrapolated to the individual cow level. Each cow was assigned the RA score for each area consistent with when she would have been present in that area based on her birthdate. The initial RA was used as baseline. For analysis purposes, the JD control program was considered to have been implemented on the date of the initial herd visit. Cows were classified as having been "exposed" to the control program if they were born after the initial visit, with the exception of purchased cattle. All other cattle were classified as "unexposed." The vast majority of purchased cattle were introduced into these herds as springing heifers or adults, and information on the conditions under which they were raised was unavailable. As a result they were not assigned any RA scores for those areas and were classified as being "unexposed" to the JD control program. They were dropped from all analyses, except for that comparing JD in purchased cows vs. cows raised on the farm.

Statistical analysis

A. Comparing JD serum ELISA to fecal culture

Agreement between the serum ELISA test and fecal culture was compared using the Kappa statistic. Fecal culture has commonly been used as the "gold standard" for diagnosing JD (Milner, et al., 1990; Collins, et al., 1991; Cox, et al., 1991; Socket, et al., 1992; Collins, et al., 1993; Sweeney, et al., 1995; Dargatz, et al., 2001). The Kappa was

calculated comparing the ELISA to both HEY and ESP II culture systems as well as culture status regardless of system. Kappas were also calculated for each herd and across all herds.

B. Calculating JD prevalence.

The annual JD prevalence within each herd was calculated several ways. ELISA prevalence was calculated as the number of cows testing "positive" (defined as an adjusted OD reading ≥ 0.1 per manufacturer's recommendation) that year divided by the total number of cows tested. The apparent fecal culture prevalence was calculated as the number of cows culturing positive divided by the total number of cows tested. Because the lab switched culture systems midway through the study, and the ESP II system is reported (and appears) to be more sensitive than the HEY system, an adjustment for these differing and imperfect test sensitivities and specificities was made by calculating the "true" fecal culture prevalence using the following equation (Smith, 1995, pp.82):

$$TRUE\ PREVALENCE = \frac{Apparent\ prevalence + Sp - 100\%}{Se + Sp - 100\%}$$

The Sensitivity (Se) and Specificity (Sp) used for each culture system is as follows:

Culture System	Se	Sp	Reference
HEY	50%	99%	Sockett, et al., 1992
ESP II	65%	99%	Kim, et al., 2004

Prevalence was also calculated based on JD test status. Cows testing positive, (either ELISA positive and/or FC positive) were divided by the total tested. This was performed because the agreement between the two tests ranged from poor to moderate depending on herd and culture system. Because JD is a chronic disease with a long and varied incubation period averaging 2-5 years, the prevalence at the herd level may not be an accurate reflection of the effectiveness of the JD control program. The first lactation cows may provide the best indicators of whether or not the control program is working. Thus the ELISA, fecal culture (FC), and JD test prevalence for first lactation cows was calculated by taking the number of first lactation cows testing positive by each respective test and dividing by the total number of first lactation cows tested that year.

The Cochran-Armitage test for trend was calculated for each binary outcome (ELISA, FC, and JD test status) across the years of the study at both the herd level and for first lactation cows only. A test statistic greater than zero suggested prevalence increased over the study period, while a test statistic less than zero suggested decreasing prevalence over time. A p-value of <0.05 was considered statistically significant.

C. Effectiveness of JD control program

Before specific management changes for preventing JD could be evaluated, it first had to be determined if the JD control programs implemented on these farms were effective in reducing the JD burden in the respective herds. The effectiveness of the overall JD control program for each herd was evaluated in two ways: by calculating the relative risk (RR) of cows "exposed" to the control program to those "unexposed," and

by calculating the JD incidence rate by lactation for cows "exposed to the control program as compared to those "unexposed."

Due to the longitudinal nature of the study, many cows were tested for JD multiple times. Johne's disease is a chronic, slowly progressive disease; and while the diagnostic tests used in this study may not have the best sensitivity (30-60%) when applied to random cows within the general population, they do have excellent specificity (>99%) (Collins, et al., 2006). Thus, it was assumed that once a cow tested positive (regardless of test), she was infected with MAP, and remained so for life. As a result, only a single observation to define JD status was kept for each cow. The observation retained was either the first time the cow tested positive for JD; or, for cows always testing negative, the last test performed.

To compare the prevalence of MAP infected cows born prior to the JD control program being implemented to that of cows born and raised with the control program in place, a 2 x 2 table was constructed and the RR and Pearson chi-square calculated for each herd.

Another way to consider the effectiveness of the JD control program is to compare the age at which cows "unexposed" to the control program test positive for JD to that of cows "exposed" to the control program. To do this, the incidence of JD for each group (unexposed and exposed) by lactation was calculated. For lactation 1, the incidence was calculated as the proportion of cows testing positive in their first lactation divided by the total number of first lactation cows tested. The incidence for subsequent lactations was calculated as the number of cows in that lactation testing positive (provided they had not tested positive in a previous lactation) divided by the total number

of cows in that lactation at risk of testing positive for the first time. The fisher's exact test was used to test for statistically significant differences in the incidence between cows exposed and unexposed to the JD control program. For both the RR and incidence rate calculations, a p-value of <0.05 was considered statistically significant.

D. Determining which management practices are effective in the JD control program

Individual cow data, which included the assigned risk scores, was used to determine which management practices significantly affected JD status. The risk of acquiring JD decreases with age, becoming minimal by adulthood. Testing to detect JD did not begin until adulthood. Thus, for practical purposes, the risk of acquiring JD remained unchanged for each cow throughout the observation period. A single observation to define JD status was retained for each cow as described previously. Due to the nature of the study, correlation resulting from clustering of cows within herds was controlled using generalized estimating equations (GEE) with an exchangeable correlation structure. The outcome of interest was JD test status (positive/negative). As JD test status was binary in nature, logistic regression was used to model the probability that the outcome was positive.

The distribution of risk scores for each factor evaluated on the RA was analyzed. Factors with a distribution range of ≤ 3 points were dropped from further analysis. This was deemed appropriate because the scores were subjective in nature, making it difficult to argue that a discrepancy of one point in either direction is biologically significant. Furthermore, there must be sufficient variation in the data to justify incorporating a

variable into a statistical model. To maintain the weighting inherent in the RA for risk factors during multivariable analysis, each risk score was multiplied by the maximum possible score allowed in the RA. For example, the maximum possible score for manure build up in the calving area is ten (Appendix A). So a cow born into a herd when the manure build-up in the maternity pen was rated for would be assigned a risk score of 40 (4 x 10). Accordingly, the maximum possible score for manure contamination in the lactating cow area is four. So an adult cow present in a herd when the manure contamination in the lactating cow area was four, would be assigned a risk score of 16 (4 x 4). This demonstrates that a score of four in the maternity area is not the same as a score of four in the lactating cow area.

Formal interaction terms were not considered in the analysis. However, upon considering the list of risk factors assessed, it seemed likely some factors were linked. For instance, manure build up in the maternity pen was likely associated with manure-soiled udders and legs. In those instances the scores were summed together to form another "risk factor." In the maternity area, the factors fell into three categories, exposure to manure from adult cattle, direct exposure to adult cattle (other than dam), and time spent with dam. In the calf area, the combined categories consisted of colostrum management (potential for colostrum from one cow being fed to multiple calves), feeding of unpasteurized pooled milk, and adult manure contamination of feed or water supplies. Combined terms in the other areas of the farm (weaned calf, bred heifer, and lactating cow) were not necessary because multiple factors in these areas were dropped from the analysis due to lack of variation across scores. To avoid collinearity problems during multivariable modeling, the combined term was used in place of the component risk

factors only when each respective factor was found to be statistically significant on univariable analysis.

Potential confounding factors included lactation number and age. The reason for considering both age and lactation number, which could be used as a proxy for age, will be explained in detail later. Other variables evaluated included culture system (HEY vs. ESP II), exposure to JD control program (yes/no), and source of cows (raised vs. purchased).

Univariable and multivariable models were built using JD test status as the outcome. Variables with a p-value ≤ 0.10 on univariable analysis were considered in the multivariable model. Stepwise backward elimination was used to build the final model. The final multivariable model included only those variables with a p-value of ≤ 0.05.

Model fit was assessed in two ways: a modification of the Hosmer-Lemeshow goodness of fit analysis as described in Horton, et al (1999), and an extension of model checking using cumulative residuals for marginal regression models as discussed in Lin, et al. (2002). Briefly, for the Hosmer-Lemeshow analysis, the data set was divided into groups of approximately equal size based on ordinal ordering of the expected probabilities of observations testing positive for JD. Dummy variables were assigned to each observation, defining into which group it belonged. The regression equation was rerun including the dummy variables. The null hypothesis for the model being, if the model fits the data well, the regression coefficients for all dummy variables will equal zero (Wald χ^2 p-value >0.05). For the residual analysis, the cumulative sums of the residuals for the each respective covariate in the marginal regression model were plotted along with the residuals of 10,000 simulated realizations from a zero-mean Gaussian

distribution. The Kolmogorov-type supremum test was calculated along with its associated p-value. This process was repeated with alternative functional forms of the covariate based on the initial pattern of the cumulative sums of residuals in an attempt to improve model fit when warranted. The greater the Kolmogorov-type test statisitic and its p-value, the better the model fits the data, and p-values <0.05 were considered indicative of poor, or insufficient, model fit. Both the Hosmer-Lemeshow analysis and the cumulative residuals were assessed for the final multivariable regession model as well as for the respective univariable regression models for each covariate included in the final multivariable regression model.

E. Effect of dam's JD test status on offspring's JD status

Another potential risk factor for a calf becoming infected with JD is its dam's JD status. The dam's identity for each cow tested was recorded when available. Over the five-year course of study, dam test information was available on a little over one third of the cows. It also meant there was no dam JD test information on 2/3 of the cows. Thus controlling for dam's JD status in the above described regression analysis would have resulted in only a small proportion of the data being used. Therefore, the association between dam's JD status and that of her daughter was analyzed separately using a 2 x 2 table and calculating the RR and Pearson Chi-square. All cows (dams and daughters) were classified as positive if they were tested at least once during the course of the study, and had a positive ELISA &/or positive FC.

3.4. Results

Descriptive data analysis

A. Herds

In six of the seven herds, both serum ELISA and fecal culture was performed annually. In the remaining herd only serum ELISA testing was performed. Six of the seven herds consisted of Holsteins, or predominately Holstein cows and one herd consisted of Jersey cows. Type of management varied from total confinement (N=3), combination of confinement and grazing (N=3), and rotational grazing with winter confinement (N=1). Two herds (herds 3 and 5) were actively expanding during the study period and were routinely purchasing cattle. Another herd (herd 1) was expanding internally, although occasionally cattle were purchased to improve genetics. The herd size of the other four herds was relatively consistent throughout the study, although three of the four had purchased cows within the five years prior to the start of the study. One herd (herd 6) had been closed for almost 30 years prior to the start of the study. These descriptive statistics are summarized in Table 3.1.

Table 3.1: Herd size, breed and housing management of study herds

Herd	Ave. herd size	Herd size		Breed	Housing
		Start	End		
1	191	170	215	Holstein	Confinement/grazing
2	125	103	137	Holstein	Total confinement
3	378	218	458	Holstein	Confinement/grazing
4	73	75	68	Jersey	Rotational grazing (organic)
5	531	484	641	Holstein	Total confinement
6	155	145	167	Holstein	Total confinement
7	184	209	168	Holstein	Confinement/grazing

B. Cows

Over the course of this study, a total of 8,660 observations were made on 4,123 cows. There were 6,447 observations with concurrent ELISA and fecal culture (FC) results of which 227 (4%) were positive on both tests, 493 (8%) ELISA positive, and 586 (9%) FC positive. There were a total of 6,530 observations with FC results, of which 586 (9%) were positive; and 8,578 total observations with ELISA results, of which 493 (6%) were positive. A total of 787 (9%) observations originated from purchased cows, of which 178 (23%) had concurrent ELISA and FC results while 785 had ELISA results and 180 FC results. Forty-eight (6%) of those observations were positive on ELISA and 46 (26%) had positive FC. Of the 7,873 (91%) observations originating from cows born and raised on their respective farms, 445 (6%) were positive on ELISA and 540 (7%) FC positive. Eighty-two percent (7,137) of the observations from raised cattle came from cows that were born prior to the implementation of the JD control program and 18% (1,523) from cows born and raised with the control program in place. Of the observations made from cattle born prior to the JD control program 421 out of 6,272 observations were ELISA positive and 472 out of 5,073 observations were FC positive. Of the observations from cattle born and raised after the JD control program was implemented, 24 out of 1,520 observations were ELISA positive and 68 out of 1,277 observations were FC positive.

Of the 4,123 cows tested during the course of this study, 416 were purchased and 3,707 were born and raised on their respective farms. Fecal cultures were performed on a total of 2,999 cows of which 460 (16%) were FC positive at least once. Seventy-seven (17%) cows were FC positive two or more times. Serum ELISA tests were performed on

a total of 4,086 cows of which 359 (9%) were positive at least once. Sixty-eight (19%) cows were ELISA positive multiple times. Of the 4,123 cows tested 679 (16%) were ELISA and/or FC positive.

Of the 416 purchased cattle, ELISA tests were performed on 414, with 40 (10%) having at least one positive ELISA result. Fecal cultures were performed on 96 of the purchased cows with 36 (38%) being positive. Overall 63 (15%) of the purchased cows were ELISA and/or FC positive.

Seventy percent (2,610) of the 3,707 cows raised on the farm were born prior to the implementation of the JD control program, while the remaining 30% (1,097) were born and raised with the control program in place. Three hundred fifty-four out of 2,032 (17%) cows were FC positive and raised prior to the JD control program, while 68 cows out of 871 (8%) were FC positive and raised with the control program in place. Two hundred ninety-five cows out of 2,578 (11%) were ELISA positive and born prior to the implementation of the JD control program; while 24 out of 1,094 (2%) cows were ELISA positive and born with the control program in place. Overall, 535 out of 2,610 (20%) cows born prior to the JD control program were ELISA and/or FC positive, while 81 out of 1,097 (7%) cows born after the implementation of the control program were ELISA and/or FC positive.

Statistical data analysis

A. Comparing serum ELISA test with fecal culture.

The kappa statistics comparing the level of agreement beyond chance between the JD serum ELISA test and FC for each herd and across all herds are summarized in Table 3.2. Note kappa is only calculated for six herds because one herd (herd 5) was monitored by annual whole herd serum ELISA only.

Table 3.2: Kappa statistic for comparing agreement between Johne's disease serum ELISA test and fecal culture.

Herd	Kappa for HEY	Kappa for ESP II	Overall Kappa
1	0.11	0.46	0.33
2	0.43	0.28	0.37
3	0.58	0.38	0.43
4	0.58	0.24	0.44
5	N/A	N/A	N/A
6	0.16	0.46	0.41
7	0.06	0.15	0.07
All herds	0.33	0.39	0.37

Kappa level of agreement: Slight=0-0.2, Fair=0.2-0.4, Moderate=0.4-0.6 (Smith, 1995, pp.149)

N/A: Annual herd testing in herd 5 consisted of serum ELISA only

B. Within herd JD prevalence over study period

The within herd prevalence using fecal culture, serum ELISA, and JD test status as the outcomes of interest respectively are shown in the Figures 3.1-3.6 along with the accompanying Cochran-Armitage test for trend (Tables 3.3-3.8).

Figure 3.1: Trend for "true" fecal culture within herd prevalence - all cows

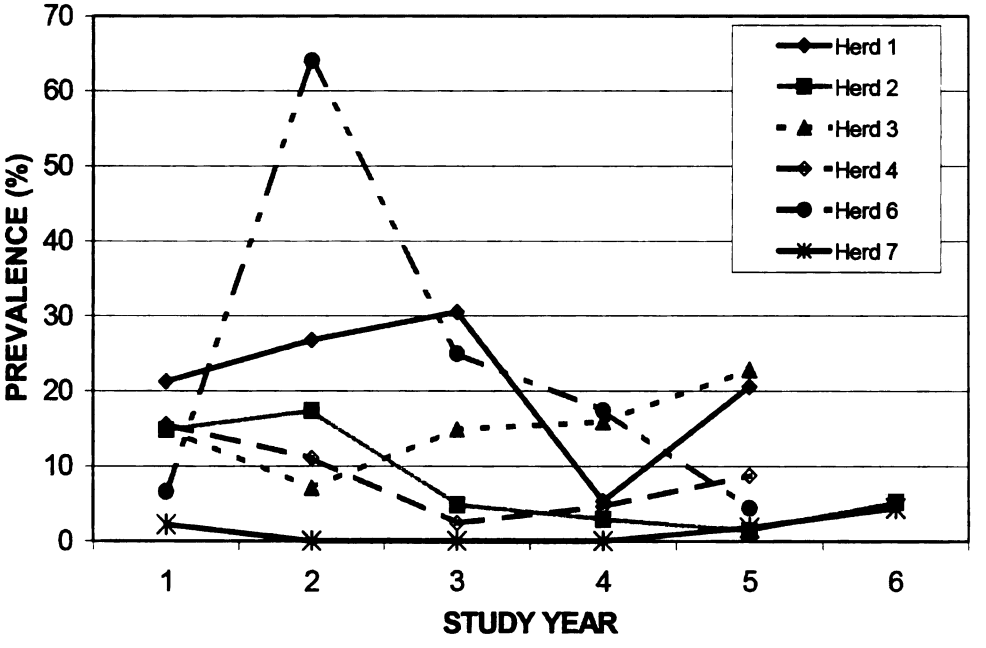


Table 3.3: Cochran-Armitage test for trend for "true" fecal culture prevalence – all cows		
Herd	**Statistic**	**p-value**
1	-0.5422	0.2912
2	-3.4548	0.0003
3	4.4486	<0.0001
4	-0.7848	0.2163
5	N/A	N/A
6	-3.3703	0.0004
7	1.5504	0.0605

N/A Not applicable

Whole herd fecal culture not performed on Herd 5

Figure 3.2: Trend for "true" fecal culture within herd prevalence - first lactation cows only

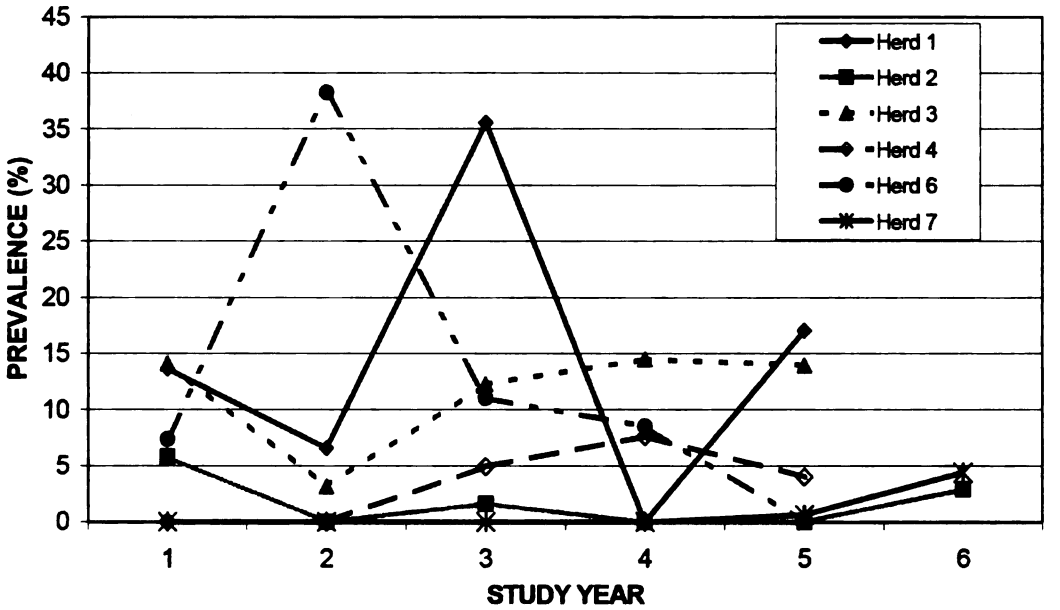


Table 3.4: Cochran-Armitage test for trend for "true" fecal culture prevalence – first lactation cows only		
Herd	**Statistic**	**p-value**
1	0.4751	0.3174
2	-3.6514	0.0001
3	1.9171	0.0276
4	0.0252	0.4899
5	N/A	N/A
6	-2.2172	0.0133
7	2.3979	0.0082
N/A Not applicable Whole herd fecal culture not performed on Herd 5		

Figure 3.3: Trend for ELISA prevalence - all cows

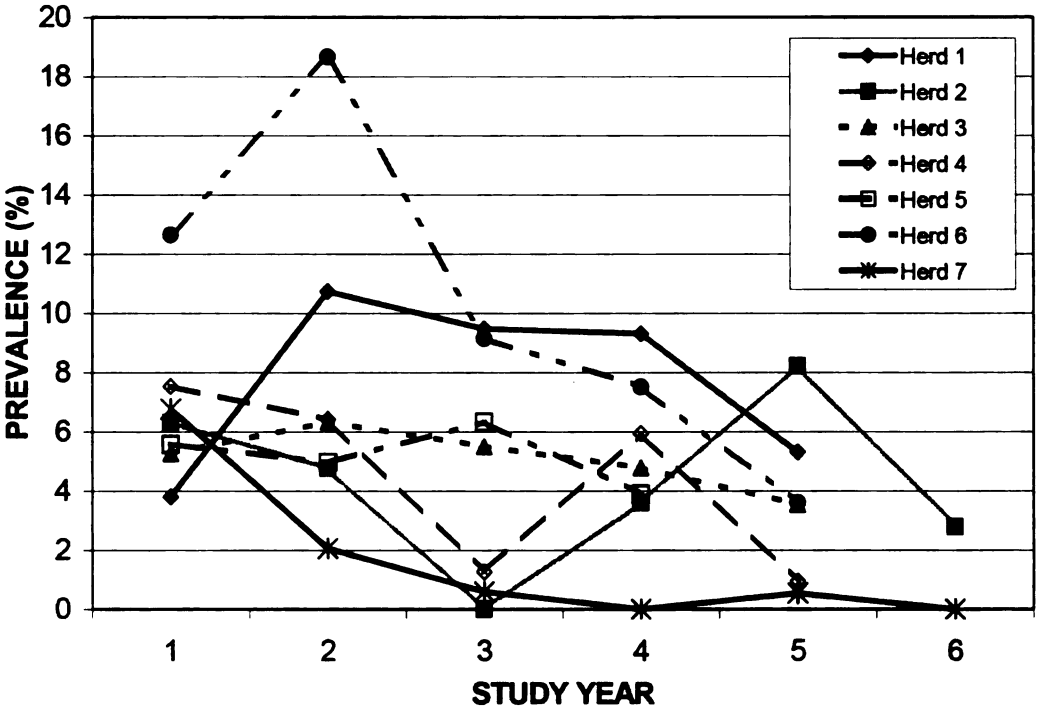


Table 3.5: Cochran-Armitage test for trend for ELISA prevalence – all cows		
Herd	**Statistic**	**p-value**
1	0.2012	0.4203
2	-0.4743	0.3176
3	-1.6445	0.0500
4	-2.3214	0.0101
5	-1.0306	0.1514
6	-3.8153	<0.0001
7	-4.7067	<0.0001

Figure 3.4: Trend for ELISA prevalence – first lactation cows only

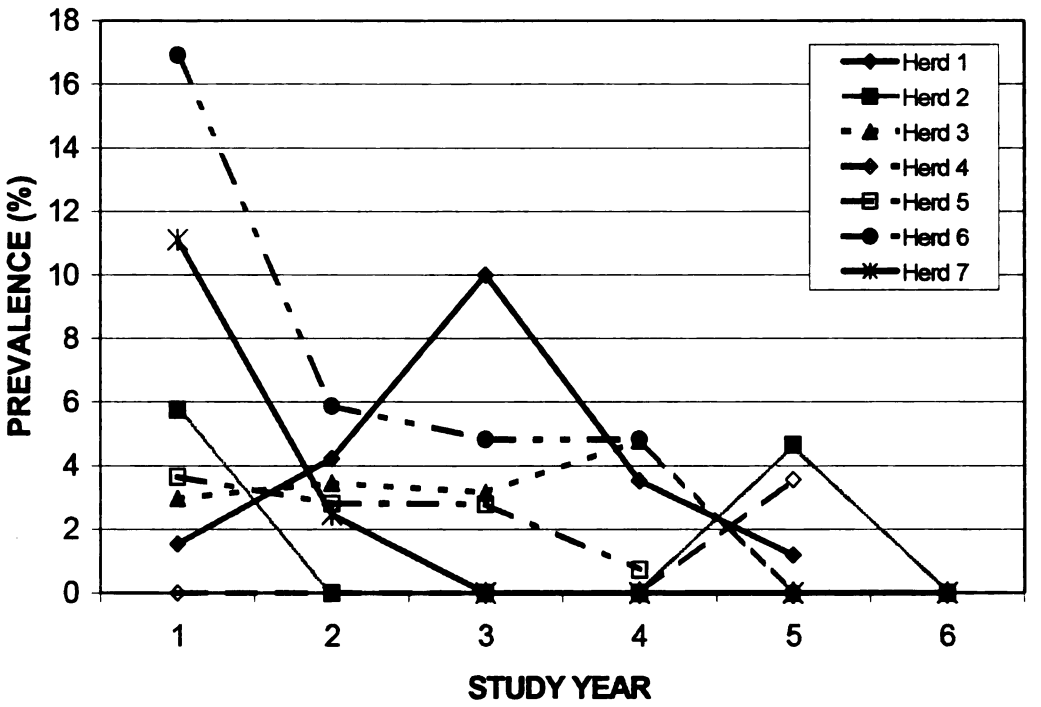


Table 3.6: Cochran-Armitage test for trend for ELISA prevalence – first lactation cows only		
Herd	**Statistic**	**p-value**
1	-0.3967	0.3458
2	-2.3082	0.0105
3	-1.3991	0.0809
4	1.0933	0.1371
5	-1.3068	0.0956
6	-3.2688	0.0005
7	-3.9409	<0.0001

Figure 3.5: Trend for prevalence based on Johne's disease test status = positive all cows

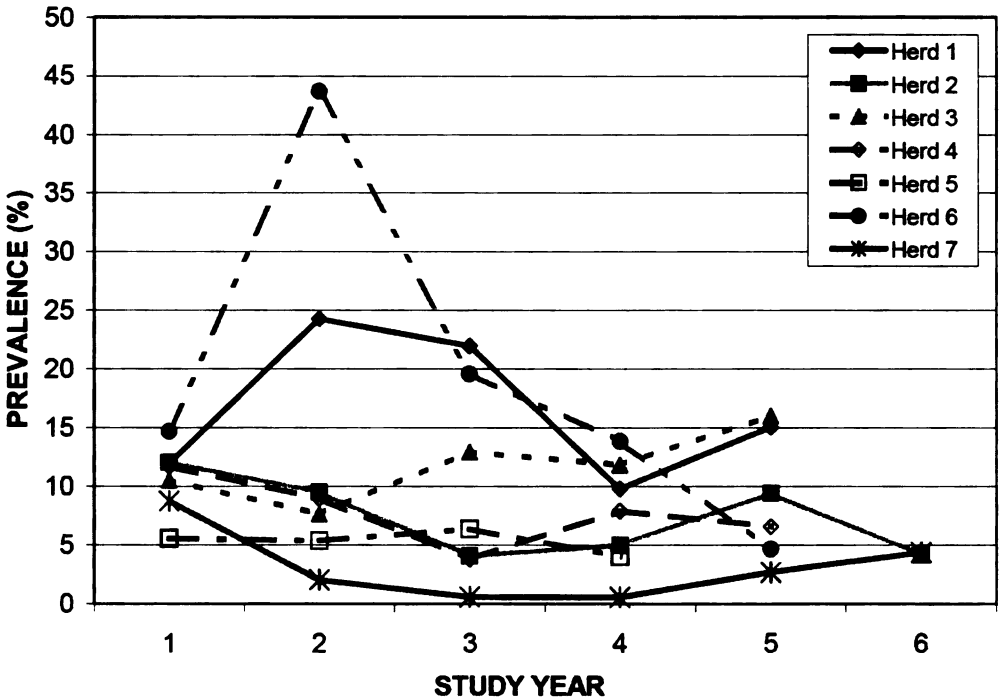


Table 3.7: Cochran-Armitage test for trend for prevalence based on Johne's disease test status = positive – all cows

Herd	Statistic	p-value
1	-1.0365	0.1500
2	-2.5123	0.0060
3	2.9725	0.0015
4	-1.2732	0.1015
5	-1.0306	0.1514
6	-4.8501	<0.0001
7	-2.9047	0.0018

Figure 3.6: Trend for prevalence base on Johne's disease test status = positive first lactation cows only

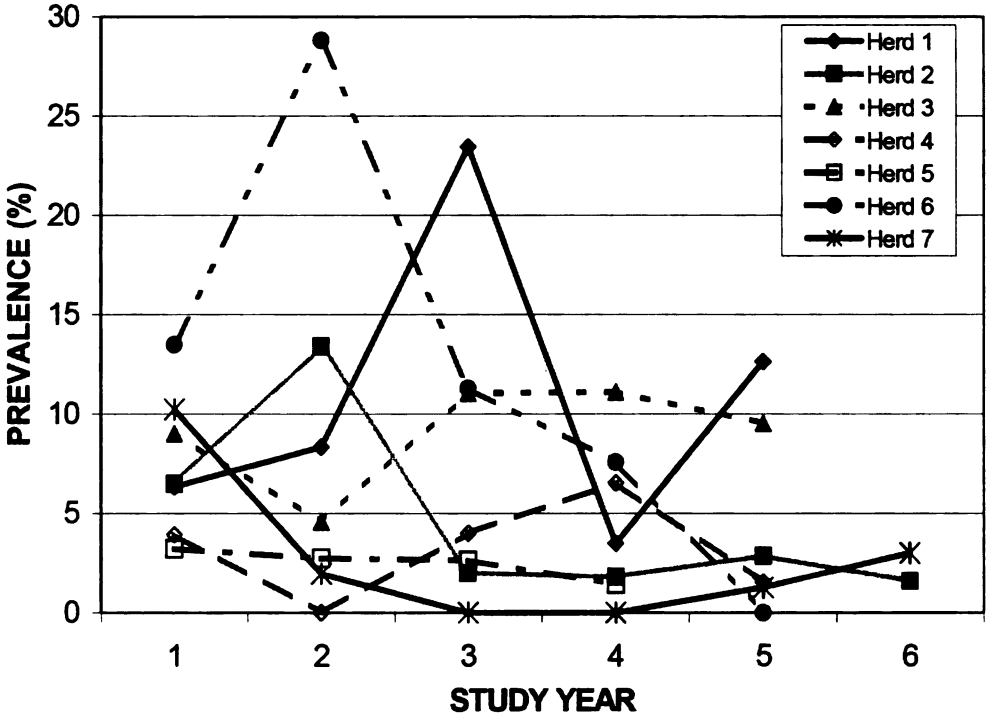


Table 3.8: Cochran-Armitage test for trend for prevalence based on Johne's disease test status = positive - first lactation cows only

Herd	Statistic	p-value
1	0.5612	0.2873
2	-3.6809	0.0001
3	1.3797	0.0838
4	-0.1961	0.4223
5	-1.3068	0.0956
6	-3.8195	<0.0001
7	-2.2360	0.0127

C. Effectiveness of JD control program

Relative risk of cows exposed to JD control program testing positive compared to cows not exposed to JD control program

The relative risk for the following three outcomes: fecal culture, ELISA, and JD test status (fecal culture and/or ELISA positive) were calculated for each herd, and across all herds, along with the 95% confidence limits, and corresponding p-value for the Pearson chi-square and are summarized in Tables 3.9-3.11. All herds had a RR < 1, and all but one (Herd 7 – fecal culture as outcome, p= 0.16) was statistically significant ($p < 0.05$).

Table 3.9: Relative risk of exposure to Johne's disease control program—Fecal culture as outcome

Herd	RR	95% Confidence Interval		Pearson χ^2 p-value
		Lower	Upper	
1	0.4144	0.2420	0.7098	0.0005
2	0.0637	0.0089	0.4580	0.0001
3	0.7274	0.5307	0.9970	0.0440
4	0.2047	0.0486	0.8625	0.0137
5	Whole herd fecal culture not performed			
6	0.0881	0.0284	0.2731	<0.0001
7	0.5279	0.2106	1.3233	0.1610
All herds	0.7505	0.6720	0.8382	<0.0001

Table 3.10: Relative risk of exposure to Johne's disease control program—ELISA status as outcome				
Herd	RR	95% Confidence Interval		Pearson χ^2 p-value
		Lower	Upper	
1	0.1816	0.0666	0.4948	<0.0001
2	0.3227	0.1165	0.8938	0.0187
3	0.2905	0.1403	0.6014	0.0003
4	0.2900	0.0667	1.2621	0.0741
5	0.1485	0.0468	0.4705	0.0001
6	0.1561	0.0495	0.4923	0.0001
7	0 ELISA positive cows exposed to control program			
All herds	0.1936	0..1285	0.2917	<0.0001

Table 3.11: Relative risk of exposure to Johne's disease control program—JD test status = positive as outcome				
Herd	RR	95% Confidence Interval		Pearson χ^2 p-value
		Lower	Upper	
1	0.3591	0.2196	0.5872	<0.0001
2	0.2161	0.0890	0.5245	<0.0001
3	0.6355	0.4685	0.8619	0.0026
4	0.2373	0.0734	0.7671	0.0067
5	0.1430	0.0452	0.4526	<0.0001
6	0.0889	0.0336	0.2356	<0.0001
7	0.2761	0.1135	0.6719	0.0016
All herds	0.3602	0.2883	0.4501	<0.0001

Incidence of JD

Tables 3.12-3.14 summarize the JD incidence rate by lactation over the first three lactations using FC, ELISA, and JD test status as outcomes respectively. The incidence rate was not calculated beyond lactation 3, because at the end of the study, there were no

cows "exposed" to the JD control program exceeding their third lactation. Regarding calculations for FC incidence, all samples from cows in the "exposed" group were cultured using the ESP II system. In the "not exposed" group, lactation 1, 52% (n=42) of positive samples were cultured using HEY and 48% (n=39) by the ESP II system; lactation 2, 11% (n=15) of positive samples were cultured using HEY and 89% (n=104) cultured using the ESP II system; and in lactation 3, all positive samples were cultured using the ESP II system.

Table 3.12: Johne's disease incidence rate over first three lactations for cows not exposed to the control program compared to cows exposed to the control program – using fecal culture as outcome

Cows	Lactation					
	1		2		3	
	Incidence (%)	N	Incidence (%)	N	Incidence (%)	N
Not Exposed to JD control program	5.5	1485	9.8	1084	6.0	726
Exposed to JD control program	4.9	860	12.5	345	0	42
Fisher's exact test p-value	0.57		0.16		0.16	

Table 3.13: Johne's disease incidence rate over first three lactations for cows not exposed to the control program compared to cows exposed to the control program – using ELISA as outcome

Cows	Lactation					
	1		2		3	
	Incidence (%)	N	Incidence (%)	N	Incidence (%)	N
Not Exposed to JD control program	3.7	1822	3.9	1378	9.0	863
Exposed to JD control program	1.3	1038	1.9	366	0	42
Fisher's Exact Test p-value	0.0008		0.08		0.04	

Table 3.14: Johne's disease incidence rate over first three lactations for cows not exposed to the control program compared to cows exposed to the control program – using Johne's disease test status = positive as outcome

Cows	Lactation					
	1		2		3	
	Incidence (%)	N	Incidence (%)	N	Incidence (%)	N
Not Exposed to JD control program	6.6	1822	10.6	1412	8.7	832
Exposed to JD control program	4.7	1041	7.7	351	0	42
Fisher's Exact Test p-value	0.05		0.11		0.04	

D. Determining which management practices were most effective in JD control program

The results of the univariable regression analyses to determine the effect of various risk factors on JD status are provided in Table 3.15. The risk factors analyzed were those assessed in the RA. Due to lack of variation in scores (<3 points) throughout the study period the following factors were dropped from the analysis:

- All adult cow risk factors
- Bred heifer risk factors
    - Direct cow contact or pen contamination with cows' manure
    - Possible manure contamination of feed: refused cow ration, stored feed, equipment, cows, traffic splatter, people or runoff
    - Share pasture with cows
    - Manure spread on forage grazed/harvested same season
- Post-weaned heifer risk factors
    - Share pasture with cows
    - Manure spread on forage grazed/harvested same season
- Pre-weaned calf risk factors
    - Possible manure contamination of feed or water by cows, traffic splatter, equipment or people
    - Direct cow contact or potential manure contamination of pen: by cows, traffic splatter, equipment or people
- Sources of additions & replacements

Table 3.15: Univariable logistic regression analysis of risk factors associated with cows testing positive for Johne's disease

Variable	OR	95% CI		p-value
		Lower	Upper	
Maternity Area Risk Factors				
1. Multiple animal use (Single pen.....dense crowded pen)	1.27	1.12	1.43	0.0001
2. Manure build up risk for calf ingestion (clean, dry.....dirty, wet)	1.17	0.98	1.40	0.0839
3. Area also used for sick cow (never.....always)	1.09	1.01	1.18	0.0288
4. Presence of JD clinicals/suspects	1.03	0.84	1.27	0.7591
5. Manure soiled udders/legs	1.22	1.11	1.34	< 0.0001
6. Calves born in other cow areas	1.27	1.24	1.31	< 0.0001
7. Time calves stay with dam (<30 min.....>24 hrs)	1.27	1.14	1.41	< 0.0001
8. Calves nurse dam	1.21	1.09	1.35	0.0004
Exposure to manure (combine 2 & 5)	1.11	1.04	1.20	0.0039
Exposure to adult cows (combine 1,3,6)	1.13	1.05	1.21	0.001
Time spent with dam (combine 7,8)	1.12	1.06	1.17	< 0.0001
Maternity area total	1.04	1.02	1.06	0.0009

Table 3.15 (continued): Univariable logistic regression analysis of risk factors associated with cows testing positive for Johne's disease

Variable	OR	95% CI		p-value
		Lower	Upper	
Pre-Weaned Heifer Risk Factors				
1. Fed pooled colostrum	1.32	1.26	1.38	< 0.0001
2. Fed colostrum from individual cow to several calves	1.28	1.17	1.39	< 0.0001
3. Fed unpasteurized pooled milk	1.19	1.09	1.30	< 0.0001
4. Possible manure contamination of colostrum or milk: at harvest, utensils, traffic or people	1.02	0.85	1.23	0.8346
Colostrum from one cow going to several calves (combine 1,2)	1.14	1.12	1.16	< 0.0001
Pre-Weaned Heifer area total	1.08	1.04	1.11	< 0.0001
Post-Weaned Heifer Risk Factors				
1. Direct cow contact or pen contamination with cow's manure	1.01	0.77	1.33	0.9470
2. Possible manure contamination of feed: refused cow ration, stored feed, equipment, cows, traffic splatter, people or runoff	1.28	0.96	1.71	0.0877
3. Potential for contamination of supplied or natural water: shared with or by cows, traffic splatter, runoff or people	0.92	0.65	1.30	0.6232
Post-weaned heifer area total	1.04	0.91	1.19	0.5808

Table 3.15 (continued): Univariable logistic regression analysis of risk factors associated with cows testing positive for Johne's disease

Variable		OR	95% CI		p-value
			Lower	Upper	
Bred Heifer Risk Factors					
1. Potential for contamination of supplied or natural water: shared with or by cows, traffic splatter, runoff or people		1.62	0.94	2.79	0.0795
Bred heifer area total		1.09	0.90	1.31	0.3889
Potential Confounders	**N**				
Lactation number	3707	1.00	0.92	1.09	0.9724
Age	3474	1.02	0.94	1.10	0.6307
Exposed to JD control program	3707	0.29	0.16	0.52	<0.0001
Culture system = ESP II	2904	0.49	0.34	0.70	0.0001
Source = purchased	4123	1.57	0.95	2.60	0.0779

Because all the adult cow risk factors were dropped due to lack of score variation, the risk profile for cows being tested did not change throughout the observation period, and only one observation was retained for each cow tested. Several cows tested positive on FC but were negative on the concurrent ELISA, and vice versa. Also, some cows would test positive on one or both tests one year, and then test negative on the same test(s) in subsequent years. Once a cow tested positive (regardless of test), she was assumed to be infected with MAP and remained so for life.

Univariable regression analysis results of all confounders and non-risk factor variables are also shown in Table 3.15. Potential confounders included in the analysis were lactation number and age. Due to the chronic nature of JD, infected cows are more likely to test positive as they age and the disease progresses. Originally, the plan was to use lactation number as a proxy for age because we did not have accurate birthdates on all cows. However, upon closer examination of the data, it was discovered that a proportion of the cows had the same lactation number for two or more test dates. This suggested these cows had prolonged lactations for some reason, and meant their lactation number would not accurately reflect their age. As a result, it was decided to evaluate age as well, dropping cows with unknown birthdates (N=233) from the analysis. Univariable analysis of lactation number and age revealed both to be not statistically significant ($p=0.97$ and $p=0.63$ respectively). Modeling risk factors with and without the cows with missing birthdates resulted in slightly different regression coefficients, but both univariable and multivariable model interpretations were the same. The multivariable regression results for herd management risk factors shown in Table 3.16 are for the full dataset including 3,707 cows.

Table 3.16: Multivariable logistic analysis of risk factors associated with cows testing positive for Johne's disease				
Variable	OR	95% CI		p-value
		Lower	Upper	
Exposure to adult cows other than dam at birth	1.09	1.06	1.13	< 0.0001
Feeding colostrum from one cow to multiple calves	1.10	1.09	1.12	< 0.0001

Other variables evaluated were culture system (HEY vs. ESP II) used, exposure to the JD control program (yes/no), and source of cows (raised vs. purchased). Using ESP II as the referent, analysis of culture system resulted in an OR = 0.49 and a statistically significant p-value (0.0001) on univariable analysis. Likewise, using exposure to the JD control program as the referent on univariable analysis, resulted in an OR = 0.29 and p-value of <0.0001. Regarding the source of the cows, purchased cattle were generally bought as springing heifers or adult cows, and the JD status of the herd of origin was unknown, so risk scores for the areas evaluated could not be assigned and they were dropped from the above described analysis. A separate, but similar regression model to that of the risk factor analysis using GEE, was used to determine the effect of source of cattle (raised versus purchased, N = 4123 cows) on JD test status in the absence of all management risk factors. Purchased cows were 1.6 times (95% CI: 0.95 – 2.60; p = 0.08) more likely to test positive.

E. Regression model fit analysis

Summary statistics for the model fit analysis are presented in Table 3.17. The p-values for all tests performed were >0.05, suggesting adequate fit of the respective regression models to the data.

F. Effect of dam's JD test status on offspring's JD status

Dam JD test information was available for 1,486 cows. Cows born to JD test positive cows (ELISA and/or FC positive) were 1.4 times (95% CI: 1.09 - 1.86, p=0.01) more likely to test positive for JD (ELISA and/or FC positive) themselves than cows born to test negative dams.

3.5. Discussion

Comparing the serum ELISA test with fecal culture

The Kappa statistic was used to compare the amount of agreement beyond chance between the Johne's serum ELISA test and FC. The Kappa does not specify which test is correct, only the level of agreement between the two tests. Because two different culture systems were used during the course of the study, the Kappa was calculated for each culture system separately as well as for all tests combined.

While both the JD serum ELISA and FC are used to identify cows with JD, each test detects something different. The JD serum ELISA detects antibodies, or the immune system's response to MAP, and possibly other closely related organisms. Fecal culture detects live MAP, the actual causative agent. As culture is the "gold standard", it is

Table 3.17: Model fit analysis for univariable and multivariable logistic regression

Variable	Cumulative sums of residuals analysis		Hosmer-Lemeshow Analysis					
	Univariable Model	Multivariable Model	Univariable Model			Multivariable Model		
	Kolmogorov-type supremum p-value	Kolmogorov-type supremum p-value	No. Groups	Size of groups (range)	Wald χ^2 p-value (range)	No. Groups	Size of groups (range)	Wald χ^2 p-value (range)
(A) Exposure to adult cows other than dam at birth	0.55	0.21	6	444-781	0.29-0.43	5	691-888	0.09-0.52
(B) Feeding colostrum from one cow to multiple calves	0.14	0.18	3	1043-1449	0.26-0.29			

assumed that FC is a more reliable test for definitively identifying MAP infected cows (Collins, 1996; Stabel, et al., 2002). However, due to the length of time it takes (6-8 weeks) to complete MAP culture and the higher cost, the use of the serum ELISA test can often be justified. Both the JD ELISA and FC suffer from a lack of sensitivity (ELISA, 30 $\pm$ 5%; FC, 60 $\pm$ 5%), but are generally considered to have specificities above 99% (Collins, et al., 2006). But, because each test detects something different (antibodies to MAP versus the actual MAP), it is likely each test will detect a different subpopulation of the diseased population. The amount of overlap in these two populations will vary depending on several factors including: route of infection, size of infecting dose, stage of infection, strain of MAP, and exposure to other *Mycobacteria.* Obviously variation in the amount of overlap in the populations identified by each test will impact the Kappa.

The Kappa for comparing the serum ELISA test to FC using solid culture on HEY media ranged from 0.06 (slight agreement) to 0.58 (moderate agreement) across the individual herds. The Kappa for comparing the serum ELISA test to FC using the ESP II liquid culture system ranged from 0.15 (slight agreement) to 0.46 (moderate agreement). When all observations with concurrent ELISA and FC results were combined, the overall Kappa ranged from 0.07 (slight agreement) to 0.44 (moderate agreement) across individual herds, with the overall Kappa for all herds combined being 0.37 (fair agreement). There was no consistency in the direction of change in the value of Kappa between the two culture methods (Table 3.2), suggesting that something other than culture method was affecting Kappa. In all instances the overall Kappa fell between the Kappa values for HEY and ESP II.

Upon closer examination (Table 3.2, Figures 3.1 & 3.3), there appeared to be a trend between the Kappa for HEY and that for ESP II based on prevalence. If both ELISA and FC prevalence increased, the Kappa increased (herds 1 and 6). Conversely, if both ELISA and FC prevalence decreased, then Kappa decreased (herds 2 and 4). Also, if FC prevalence increased while ELISA prevalence decreased, the Kappa increased (herd 7, all herds). A possible explanation for this is, assuming that FC is a more reliable indicator of the true disease state, an increasing FC prevalence indicates a higher proportion of JD infected cows in the test population. This should theoretically increase the number of MAP infected cows available to be detected by ELISA. If there is any overlap at all in the populations being detected by the two tests, increasing the number of infected animals should increase the number of positive cows identified by each test, which in turn increases the probability that the tests will agree. Thus, when the true prevalence in a population increases, then the kappa between two diagnostic tests should increase if they are related at all and vice versa. In one herd (herd 3), the FC prevalence decreased at the time of the culture switch while ELISA prevalence increased, resulting in a decrease in the Kappa. Following the above conjecture, a decrease in prevalence would be associated with a decrease in Kappa. However it does not explain the concurrent increase in ELISA prevalence. This leaves one to wonder if there was something particular about this herd, or the strain of MAP infecting the herd, resulting in a higher ELISA prevalence (perhaps proportionately more false positives, i.e. decreased specificity). This herd doubled in size over the course of the study, and did so through the purchase of a large number of cattle with little regard to JD status of individual cows or the herds of origin prior to purchase.

Within herd JD prevalence

Within herd JD prevalence was calculated using three different outcomes: fecal culture prevalence, ELISA prevalence, and JD test status (ELISA &/or FC positive) prevalence. Prevalence was calculated at the herd level as well as for first lactation cows only. The reason for looking at prevalence in first lactation cows was due to the nature of the disease and the control programs implemented. Most of the management practices recommended to control JD started at birth. Moreover, it takes 2-5 years for JD to manifest itself. Thus, monitoring JD prevalence in first lactation animals may provide the earliest indication that the control program is working.

The prevalence trends for each outcome are shown in Figures 3.1-3.6. The Cochran-Armitage test for trend (Tables 3.3-3.8) was conducted to determine if there was a significant change in prevalence over time and the direction of that change. A significant change was defined as a p-value < 0.05. Positive test statistics indicate an increasing trend in prevalence, while negative test statistics indicate a decreasing trend in prevalence. The direction of the trend and the level of statistical significance varied between and within herds depending on the outcome. The Cochran-Armitage test was calculated based on apparent prevalence. The change to the more sensitive ESP II culture system (Kim, et al., 2004) midway through the study may partially explain the unexpected increases in JD prevalence as well as some of the insignificant changes in prevalence trends. Being more sensitive, the ESP II culture likely identified JD infected cows earlier. Therefore, any prevalence dependent on FC culture (FC and JD test status) calculated based on results using the ESP II system were likely inflated as compared to those obtained with the HEY culture system. The ESP II system was put into use in

between the second and third annual herd tests. This corresponded to when cows born and raised with the JD control program in place began to be tested, and when prevalence was expected to decline due to improved management practices. However, regardless of the outcome, a large proportion of the herds had negative Cochran-Armitage test statistics. Thus, overall, there appears to be a general trend for decreasing JD prevalence despite a lack of statistical significance, suggesting that the JD control programs put in place on these herds are working.

Effectiveness of JD control program

Before specific management practices could be evaluated, it first had to be determined if the prevalence and/or incidence had changed in response to implementing the JD control program by each herd. If there was no change in the JD prevalence or incidence, the analysis could go no further. The Cochran-Armitage tests for trend calculated for JD prevalence across each respective herd as well as for prevalence in first lactation cows only provided preliminary support that the JD control programs implemented were working, but more definitive evidence was desired.

One of the easiest ways to evaluate the impact of the respective JD control programs was to calculate the RR of JD prevalence in cows exposed to the control program to that in cows not exposed to the program (Tables 3.9-3.11). For all herds, the RR comparing the JD prevalence of exposed cows to that of unexposed cows was < 1 and statistically significant ($p < 0.05$), or approaching statistical significance. A RR < 1 suggests that the JD control programs implemented on these farms did indeed have a protective effect.

Care must be taken when interpreting these RR's, as they may overestimate the true effect. There is only 4-5 years worth of data on each of these herds. Cows "exposed" to the JD control program were only 2-4 years of age at the end of the study. Potentially, a proportion of these cows were indeed infected, but the disease had not progressed to the point where it could be detected by the tests being used. Meaning, the numerator could, in reality, be higher. Meanwhile, the denominator of the RR would not change much, as the youngest "unexposed" cows were at least four years of age at the end of the study, and it is unlikely a large number of those cows would test positive had the study been continued. However, as the RR's were all rather small, (ranging from 0.09 – 0.64) it seems improbable that the "protective effect" of the JD control program would have been reversed had these cattle been followed further.

The diagnosis of JD at an early age is indicative of high infection rate and high infection pressure on young cattle in the herd of origin (Collins, 2003). It follows then, that if control practices are successful in preventing infection, the infection pressure on young cattle will decrease; which will, in turn, decrease the overall new infection, or incidence rate, and increase the average age at which infected cattle are diagnosed. When the ELISA or JD test status was the outcome, there was a lower incidence of JD in the cows exposed to the control program across all lactations analyzed (tables 3.13-3.14). The fact that this did not hold true when FC was the outcome of interest may be real or may be due to the change in culture systems used. Because it is more sensitive, the ESP II system may have been detecting infected cows earlier than HEY. As all the "exposed" cows were cultured using the ESP II system, it is possible a proportion of those cows culturing positive would have cultured negative with HEY, and not detected until some

later test date. This would explain the increase in the incidence of FC positive cows in lactation 1 in the exposed group as compared to the unexposed group, when it was expected that the opposite would occur if the JD control programs were effective. Furthermore, as JD test status consisted of both FC and ELISA test results, the magnitude of the decrease in the incidence rate between cows exposed and not exposed to the JD control program, may have been obscured by the culture system used to classify the JD test status of the cows. However, as already noted, when JD test status was the outcome of interest, the incidence rate was consistently lower in cows exposed to the control program, and that decrease in incidence was statistically significant in all but the second lactation, and was approaching statistical significance ($p = 0.1$) in lactation 2.

In conclusion, after evaluating the RR and incidence rates of JD by lactation for cows exposed to the control program compared to cows not exposed to the control program, it was determined that the JD control programs implemented were effective in reducing the JD burden in these herds.

Determining which management practices are effective in JD control programs

Given the evidence that the JD control programs implemented on the study herds were successful in reducing the prevalence and incidence of JD in these herds, the next step was to determine which management practices (as measured by RA scores of risk factors) were most effective. Due to the way the outcome was modeled (probability that JD test status was positive), it was expected that all the OR would be >1. After all, the scoring system used was based on biologically proven risks of JD transmission—the higher the scores, the greater the risk of cows becoming infected with JD. The p-values

could then be used to sort out the importance of the respective risk factors to JD control. Also, the direction of the OR is more important than its actual magnitude. This is because the risk scores were subjective and specific to the herds in this study. It would be inadvisable to extrapolate the magnitude of the OR to herds outside of this study. However, the trends established in this study are valid and should extend to the general dairy herd population.

Potential confounders, age and lactation number, were both statistically insignificant on univariable analysis and were not included in the multivariable analysis. Because of the characteristic slow progression of JD, it was expected age, or lactation number as a proxy for age, would be an important risk factor for testing positive for JD. This may be an artifact of the herds in this study. The herds with the highest JD prevalence in this study tended to be "younger" on average than herds with lower prevalence, particularly at the beginning of the study. The question then becomes whether this is a function of the JD process over time. Perhaps high levels of MAP contamination on these herds resulted in the cows being exposed repeatedly to high infectious doses, which accelerated the disease process allowing it to be detected at an earlier age and is consistent with previous observations (Collins, 2003).

In spite of being statistically significant on univariable analysis, neither exposure to the JD control program or culture system was included in the multivariable analysis due to collinearity issues with the risk factor analysis. As expected, cows exposed to the JD control program had lower risk scores than cows that were not exposed to the program. Also, the ESP II system was introduced two years into the study. This concurred with cows exposed to the JD control program entering the test population,

while all the cows tested with the HEY system were not exposed to the JD control program and had higher risk scores.

Being exposed to the JD control program did decrease the probability of a cow testing positive for JD (Table 3.15). This was expected as it was the intent of the study to implement a control program that would decrease the incidence of JD on these farms. It also provides further evidence that the control programs are working. The magnitude of the estimate (OR=0.29) may be overestimated here. Cows exposed to the JD control program in this analysis were still relatively young, 2-4 years old at the conclusion of this study, and a proportion of them may have been infected but tested negative.

In this analysis, cows cultured with the ESP II system were less likely to test positive for JD (OR=0.49) than those cultured with HEY. On the surface this would seem to contradict that the ESP II system is more sensitive than HEY and capable of detecting cows earlier in the course of the disease (Kim, et al., 2004). However, both culture systems were not being run concurrently during the study. It is also important to remember the ESP II system was not put into use until halfway through the study. This means the herds had at least one, and sometimes two years of testing using the HEY system. By the time the ESP II system was put into service, most of the JD infected cows in the more advanced stages of the disease had been culled, and cows exposed to the JD control program were entering the herd. Thus, the overall prevalence in the population of cows cultured using ESP II was lower than that in the population of cows cultured using HEY.

As expected, on univariable analysis, the majority of the risk factors did have OR's >1, and many were statistically significant. However, on multivariable analysis,

only two factors remained in the model: exposure to adult cows other than dam at birth and feeding colostrum (pooled or not) from one cow to multiple calves (Table 3.16). Both seem biologically plausible and are similar to findings in previous studies (Thoen and Moore, 1989; Obasanjo, et al., 1997; Johnson-Ifearulundu and Kaneene, 1998; Wells and Wagner, 2000; Muskens, et al., 2003; Ridge, et al., 2005; Nielsen and Toft, 2007). Adult cows are the animals most likely to be shedding significant amounts of bacteria, so the more cows a calf on an infected farm has contact with, the greater the probability that one of those cows is infected and shedding. Likewise, if a cow is infected with JD and shedding MAP into her colostrum, feeding that colostrum to multiple calves increases the likelihood of infecting all the calves. It was interesting that manure build up in the maternity pen and the cleanliness of the dam fell out of the multivariable model. Manure build up was borderline significant on univariable analysis ($p=0.08$), but manure soiled legs and udders was highly significant ($p<0.0001$). It was assumed that manure build up would result in more manure soiled legs and udders, and so the two were combined in the multivariable analysis, but fell out in the second round. It was expected that a calf being born into a pile of manure to a dam coated in manure would have a high probability of testing positive for JD as an adult. The fact it fell out of the model in this study should not condone the neglect of maternity pen and cow cleanliness. It is more likely the result that, in general, the cows and maternity pens on the farms in this study were fairly clean. The fact that all factors relating to areas other than the maternity or pre-weaned calf areas either were not significant on univariable analysis or fell out of the final multivariable model, underscores the importance of disease transmission at or in the weeks

immediately following birth. It is also consistent with decreasing susceptibility to becoming infected with MAP with age (Larsen, et al., 1975).

Unexpectedly, the results of the univariable risk factor analysis (Table 3.15) revealed one variable with an OR < 1 (contamination of post-weaned heifer water with manure from adult cows), however it was not statistically significant. Upon further investigation, the risk scores for this particular variable were higher in herds with the lowest JD prevalence than in herds with the highest prevalence in this study. As a result, there were proportionately more test positive cows with low scores in these areas than test positive cows with high scores. Thus, while statistically (irrespective of level of significance) this factor may appear "protective," it was simply a function of the herds in this study, and is not biologically plausible given current knowledge of JD.

Model fit analysis for univariable and multivariable logistic regression

Regarding analysis of regression model fit, there were no statistically significant p-values on either the Hosmer-Lemeshow analysis or the cumulative sums of residuals analysis (Table 3.17) that would support a conclusion that the respective univariable and multivariable regression models do not fit the data. In order to perform the Hosmer-Lemeshow analysis the data set needed to be divided into groups of approximately the same size in an ordinal manner. For this analysis, the data set was sorted by the expected probability that the observation would test positive for JD in the respective regression model. The way the risk scores were assigned to each observation in this study resulted in cohorts of cows having the same risk score profile, and thus the same expected probability of testing positive for JD. Unfortunately, the number of observations in

adjacent cohorts varied greatly across the data set. The grouping for the Hosmer-Lemeshow analysis was dictated by the size of these cohorts. The data set was divided into as many groups as possible while trying to retain roughly the same number of observations in each group. As demonstrated in Table 3.17, the data for variable A (exposure to adult cows other than dam at birth) was more equally distributed than that for variable B (feeding colostrum from one cow to multiple calves), which allowed the data set to be divided into more groups when performing the Hosmer-Lemeshow analysis of the univariable model for variable A. Across the respective models there was no data to suggest the models did not fit the data, but the Hosmer-Lemeshow test has limited power to detect departures from the assumed model. Therefore, non-significant p-values may not mean too much, and a cumulative residual analysis was performed as a more sensitive method for assessing model fit.

Given the objective of this study, the regression analysis was set up as a marginal model rather than a subject specific model. Thus, traditional methods of residual analysis and assessing model fit do not apply, and the cumulative sums of residuals analysis has been proposed as a more appropriate method for assessing regression models with aggregated residuals resulting from clustered data (Lin, et al., 2002). From a purely statistical standpoint, the univariable model for variable A appears to fit the data the best (cumulative residual p-value =0.55), as compared to the models including variable B. The univariable model for variable B (p=0.14) does not appear to fit the data particularly well, and contributes to the marginal fit of the multivariable model. Squaring variable B appeared to improve model fit statistically, but it still did not fit the data as well as the model with variable A alone. Reviewing the raw data, it was observed that the

distribution of variable B was highly skewed to the left. Furthermore, only 39% of the observations with the highest risk scores for variable B originated from the herd with the highest within herd JD prevalence. The other 61% of the observations with high variable B scores originated from the three herds with extremely low JD within herd prevalence. Meaning, while statistically there was some evidence to support that high scores for variable B were associated with cows testing positive for JD (as evidenced in the final multivariable regression model); there was also a substantial number of cows with high scores for variable B that tested negative JD. Conversely, in considering the raw data for variable A, there was an obvious trend for herds with the highest within herd JD prevalence to have the highest scores for variable A, leading to a higher proportion of JD test positive cows having high variable A scores than cows testing negative for JD. This is specific for this particular data set, and care should be taken in its interpretation. It does help explain why the models including variable B do not fit the data as well as the univariable model for variable A. From a practical standpoint, it simply suggests that when JD prevalence is low, or absent, a farm can get away with risky practices for JD transmission, such as feeding colostrum from one cow to multiple calves, at least for a time, because there is a lower probability the colostrum came from an infected cow and is contaminated with MAP on these farms.

There are other issues affecting the results of this analysis. First, the majority (70%) of the cows analyzed in this study were born and raised prior to the implementation of JD control programs on these herds. Second, in order to evaluate something statistically you need variability. We could not evaluate all the risk factors assessed on the herd RA because there was little or no variation in the scores for the

herds across the years. This lack of variation, along with the rarity of positive test results, limited the power of the study. The lack of power in this study was evidenced by relatively wide confidence intervals. A formal power analysis was not performed for two reasons. The first being it would be a post-hoc analysis, which is generally frowned upon in most statistical and epidemiological circles. Second, calculating power for longitudinal studies of this design (with cows clustered within herds of unequal size) is still being debated with no apparent consensus.

Effect of dam's JD test status on JD test status of offspring

Cattle become infected with MAP through the ingestion of the bacteria from a contaminated environment, colostrum or milk. Calves can also become infected *in-utero,* but this occurs in small proportion of animals and generally only when the dam is in the more advanced stages of the disease (Sweeney, 1996).

The RR of JD test positive cows having a JD test positive dam was 1.4 and statistically significant (p=0.01). This would suggest that cows with JD positive dams are 40% more likely to test positive themselves. Again, care must be taken in interpreting the magnitude of this effect. Dam JD test information was available for only about one third of all cows tested. It is possible that some cows were born to a JD infected dam, but the dam was culled before she tested positive. Also possible, cows from infected dams were not followed long enough in this study for them to become test positive themselves. In both instances, the calculated RR would be underestimated. Another possibility is that this association, or some part of it, may be due to factors that are confounded with or interacting with dam test status. There was insufficient data to include dam status in the

regression analysis. The positive association between the JD test status of the dam and the JD test status of her offspring could be due to direct contact with the dam, through *in-utero* transmission of MAP, or ingestion of MAP from contaminated colostrum or environment. It could also be reasoned that, because the dam was infected, the environment was so contaminated, or the farm management such, that MAP infection of the calf was likely regardless of dam's test status.

3.6. Conclusion

In summary, while the incidence of JD did decrease following the implementation of JD control programs on these farms, it is difficult to draw any conclusions regarding the ranking of specific risk factors on the transmission of JD. What is apparent is that risk factors associated with the maternity pen and pre-weaned calf areas are critical areas to focus control efforts. This is supported by the fact that the variables that were statistically significant in the multivariable model fell within those areas. Also, the scores for the risk factors in these areas had the greatest range of distribution. The JD risk assessment scores across the herds for the weaned heifer, bred heifer, and cow areas did not vary much, which precluded many of them from this analysis. This suggests that these herds, at least in terms of risk of JD transmission, were managed similarly in these areas. Yet the JD prevalence in these herds varied greatly. The number of purchased cows was small in all but two herds. In fact, the herd with the highest JD prevalence in this study had been completely closed for over 30 years. Thus, the difference in the JD prevalence in these herds must be due to different management practices, and the maternity and pre-weaned calf areas were the areas on these farms where management

varied the most. Finally, focusing JD control from birth to weaning is logical if one accepts that the susceptibility of calves becoming infected with MAP decreases as they mature.

CHAPTER 4

ECONOMIC EVALUATION OF JOHNE'S DISEASE CONTROL PROGRAMS IMPLEMENTED ON SIX MICHIGAN DAIRY FARMS

4.1. Abstract

Johne's disease (JD) is an untreatable, chronic infectious disease that is becoming increasingly prevalent in dairy herds throughout the US and the world; resulting in substantial economic losses. However, information on the costs of controlling the disease is limited, yet necessary, if producers are to make sound decisions regarding JD management. The purpose of this paper is to describe a method for evaluating the cost-effectiveness of implementing management changes to control Johne's disease on infected dairy farms. A five-year longitudinal study of six dairy herds infected with JD was performed. Each herd implemented a JD control program upon study enrollment. Prevalence of JD within each herd was monitored with annual testing of all adult cows using fecal culture and/or serum ELISA. Individual cow production and culling information was collected to estimate the annual economic losses caused by JD. A questionnaire to collect economic data was developed and administered to each herd annually to estimate costs directly attributable to the JD control program. Based on the costs of the control program, and using the losses to estimate the potential benefits, the net present value (NPV) of the control program was calculated for each herd during the study and projected into the future for a total of 20 years. The NPV was calculated for four different scenarios: (1) assuming a linear decline in losses following year five and disease eradication by year 20 with the control program; (2) assuming losses and JD prevalence remain constant at year five levels while continuing the control program; (3) assuming linear increase in losses at rate equal to that in scenario 1 with no control program; and (4) assuming losses remain constant at the same level as the beginning of the study with no control plan implemented. The NPV varied greatly across the herds.

For scenario 1, only three herds had a positive NPV; and only two herds had a positive NPV under scenario 2. In the absence of a control program, the NPV's were always negative. When calculated across all cows in the herd, the costs of the JD control programs implemented on these herds averaged $30/cow/year with a median of $24/cow/year. The annual losses due to JD averaged $79/cow/year with a median of $66/cow/year. Investing in a JD control program can be cost effective, and doing something to control JD was always a better economical decision than doing nothing.

4.2. Introduction

Johne's Disease (JD), an infectious disease of cattle and other ruminants caused by the bacterium *Mycobacterium avium paratuberculosis* (MAP), is becoming increasingly prevalent, especially in US dairy herds. In 1996, the National Animal Health Monitoring Service (NAHMS) Dairy study estimated the prevalence of dairy herds infected with JD in the US to be 21.6% (USDA, 1997). In the 2007 NAHMS Dairy study, that estimate increased to 68.1% (USDA, 2008). Other estimates range from 21-93%, depending on region and testing method used to classify infected herds (Collins, et al., 1994; Obasanjo, et al., 1997; Thorne and Hardin, 1997; Johnson-Ifearulundu and Kaneene 1998; Johnson-Ifearulundu, et al., 1999; Adaska and Anderson, 2003; Hirst, et al., 2004).

Several estimates of the economic impact of JD have been made. These estimates vary depending on study design, JD prevalence, and herd performance. Based on the NAHMS 1996 study, JD cost the US dairy industry an estimated US$ 200-250 million annually (Ott, et al., 1999), due primarily to reduced production and cull value of infected cows and increased replacement costs (Wells and Wagner, 2000). When spread across

all cows in the herd, estimates of losses due to JD range from US$ 20-33/cow (Meyer and Hall, 1994; Ott, et al., 1999; Chi, et al., 2002). In a simulated study, the mean loss due to JD on a typical midsize US dairy herd started at $35/cow in the first year following JD introduction, and increased to > $72/cow by year 20 in the absence of a control program (Groenendaal and Galligan, 2003). As JD prevalence increases so too do the economic losses incurred by the disease.

While quantifying the losses caused by JD is important, it represents just one of the necessary components needed for making on-farm decisions regarding JD management and control. Also needed are estimates of future losses that can be prevented by implementing control practices, and how much those control practices will cost. From a producer perspective, it is difficult to justify investing in a disease control program if the cost of controlling the disease is greater than the costs being incurred it. Only when all three components (estimated losses with and without control and costs of the control program) are known, can return on investment be estimated, allowing producers to make informed economic decisions.

Multiple computer simulation studies have attempted to estimate the benefits and costs of various JD control practices (Groenendaal, et al., 2002; Dorshorst, et al., 2006; Kudahl, et al., 2007). While simulation studies are commonly used and have the advantage of costing less and requiring less time as compared to field studies, they can be difficult to validate. The input data required for these simulations is often based on field data supplemented by expert opinion. Thus, a simulation study cannot be isolated from the field; and, in fact, can only truly be validated with observations made under real farm conditions once a field study is performed.

The chronic and insidious nature of JD is the most likely reason studies attempting to quantify the costs and benefits of JD control have been limited to simulations to date. A field study would have to be of sufficient length that changes in herd performance resulting from a JD control program are observed. As most management practices to control JD focus on preventing young calves from becoming infected with MAP (Collins, 1994; Rossiter and Burhans, 1996), and infection generally does not become detectable until infected calves become adults (Sweeney, 1996), a field study would have to last a minimum of three years to see results from the control program.

The study reported here, to our knowledge, is the first longitudinal observational field study investigating the costs of implementing management practices to control JD on infected dairy farms. The objective of this study was to estimate the net present value (NPV) of the JD control programs implemented on each of six Michigan dairy farms to aid producers in making more informed decisions regarding JD control and management. This chapter consists of a series of case reports. The economic analysis performed is described for each herd separately, and the results summarized across all herds.

4.3. Materials and Methods

Study design

This was a five-year longitudinal observational study of six Michigan dairy herds infected with MAP.

Farms

This study was part of the larger Michigan Johne's Disease Control Demonstration Project. A total of six Michigan dairy herds participated in this study. (Herd 7 discussed in previous chapters was not included in this part of the project. It was a university-owned research herd; and, as such, the economic decisions made on this farm were not necessarily consistent with those of other commercial dairies.) The herds were chosen based on the producer's willingness to participate in a longitudinal study for at least five years, and because they were representative of typical Michigan dairy farms in terms of herd size and housing management. Types of housing management targeted included total confinement, combination of confinement and grazing, and rotational grazing. All herds were infected with JD and initiated a control program upon enrollment into the project. The JD control programs implemented were not standardized or static. Instead, the programs were designed specifically for each herd based on the operation's goals and capabilities, and modified as necessary. The within herd prevalence of JD on these farms was determined annually by fecal culture and/or serum ELISA of all adult cows, and was used for monitoring the effectiveness of the respective control programs. Herds were enrolled in the study beginning in January 2003 with the last herd enrolled in May 2004.

Questionnaire used for economic data collection

A questionnaire was developed to collect data regarding costs directly attributable to the JD control program (Appendix A). This questionnaire was broken down into four sections specific to each farm's management: supplies, management, labor, and capital

investments. The questionnaire was administered to the herd owner/manager beginning the first year following implementation of the JD control program and annually thereafter through calendar year 2007. Additionally, questions regarding the manager's perception of, and satisfaction with, the effectiveness of the JD control program were asked the last time the questionnaire was administered in 2007.

Other data collected

Individual cow production and cull information was collected when available. Information was obtained from the Dairy Health Improvement Association (DHIA) for four herds. One herd had computerized daily milk weights, and the remaining herd had hand-written records only with no individual cow production data.

Data analysis

A. JD prevalence

Within herd JD prevalence was calculated for each herd based on annual whole herd fecal culture and/or serum ELISA. Johne's disease prevalence was calculated as the number of cattle testing positive (regardless of test) divided by the total number tested.

B. Cost of JD control program

The cost of the control program was calculated based on information obtained from the questionnaire.

The supplies category consisted of things such as milk replacer, additional ear tags or other identification for JD test positive animals, change in volume of bedding, sanitation supplies, etc., that were a direct result of implementing the JD control program. For herds that switched from whole milk to milk replacer, an adjustment was made for the sale of milk that would have previously been fed to the calves. It was assumed that one 50 pound (23 kg) bag of milk replacer was needed to wean one calf, and was equivalent to 400 pounds (181 L) of whole milk (Groenendaal and Galligan, 1999). It was further assumed that all milk previously fed to calves was marketable. The number of calves weaned per year was calculated assuming non-seasonal calving, a 14-month calving interval with 50% heifer calves born. All farms sold bull calves within 1-2 days of birth, and thus deacon calves were not included in the calculation. All herds belonged to the same milk marketing cooperative, although one farm became certified organic during the course of the study. The adjustment for additional milk sold was valued as the average base farm price paid by the cooperative for each respective year. For the herd that became certified organic, the cooperative base farm price was used for the adjustment up until certification, with the average yearly price received by the farm used thereafter.

The intent of the management category was to account for the time (hours) herd managers spent coordinating the JD control program. This would include things such as testing, decision-making, and employee education. For herds that had a full-time herd manager (N=2) earning an annual salary, the approximate hourly wage was calculated as the total value of compensation (salary + benefits) divided by the average number of hours worked. In the other four herds where the owner was the herd manager, the value

of management time was based on the owner's estimated value ($/hr) of their time given their education and experience (what they could be making doing something else), or what they estimated they would have to pay to hire a manager. Management costs were then calculated as the hours committed to the JD control program multiplied by the estimated hourly value of management.

The labor category included the additional hours employees spent implementing the JD control program such as improved cow and calf hygiene, increased time spent caring for calves, etc. All farms had hired part-time labor paid hourly wages. Labor costs were calculated as additional hours spent performing tasks required by the JD control program multiplied by the hourly wage paid.

Capital investments included things such as a pasteurizer, calf hutches, fencing, skid-steer, and/or loader buckets purchased as a direct result of the JD control program. Capital investments were converted to annuities based on the useful life of the purchase (as determined by the producer), and those values used annually for the evaluation period. In other words, once the useful life of the investment expired, it would be replaced as necessary. The annual capital cost was calculated as the purchase price multiplied by the annuity factor. The annuity factor was calculated as $i/[1-(1+i)^{-n}]$ (Olson, 2004, pp. 407); where i is the interest rate, assumed to be 7%, and n is the useful life of the investment. Also included in this category was any interest paid on capital purchases that were financed.

The yearly costs by category were summed together to calculate total costs. The annual total costs were then divided by the number of adult cows tested in the herd to

estimate the cost/cow. The annual costs/cow were averaged over the observed study period to estimate the overall cost/cow/year.

C. Losses due to JD

The economic losses for the following three aspects were calculated for JD infected cows identified by annual testing: decreased milk production, loss of future income due to premature or suboptimal culling, and reduced cull value.

Decreased milk production

The mature equivalent 305 day (ME305) milk and butterfat production were obtained for all cows when available. These were used to calculate the 3.5% fact corrected milk (FCM) for each cow using the following equation: 3.5% FCM = (0.4323 x pounds of milk) + (16.216 x pounds of fat) (Hutjens, 2005). The average annual 3.5% FCM production was calculated for each of the following groups of cows annually in each respective herd, controlling for lactation number and days in milk (Proc GLM, SAS 9.1, SAS Institute, Inc., Cary, NC): cows culled due to clinical JD, cows testing positive for JD and culled for reasons other than JD, cows testing negative for JD and culled, cows testing positive for JD and remaining in the milking herd, and cows remaining in the milking herd testing negative. For all cows testing positive for JD, milk production lost due to the disease was estimated by subtracting each individual cow's production from the average milk production of test negative cows remaining in the herd, and then summing the differences over each study year. For test positive cows culled when they

were too fresh for a valid ME305 to be calculated, the average for her respective group was used as a proxy.

For the herd that did not have individual cow production data, the annual pounds of milk and butter fat sold was obtained, the 3.5% FCM calculated and divided by the average number of cows in the herd that year to estimate the yearly rolling herd average. Lost milk production for cows testing positive for JD was imputed based on milk production losses averaged across the other five herds in the study. Cows testing positive for JD but not exhibiting clinical signs were assumed to produce 12% less milk than test negative cows, while cows with clinical JD were assumed to produce 23% less milk than their test negative herdmates. The annual pounds of milk lost due to JD was calculated as the number of cows with subclinical and clinical JD each year multiplied by the product of the rolling herd average times 12% and 23% respectively, then summed together.

The total economic losses resulting from reduced milk production due to JD was calculated by multiplying the estimated number of pounds lost by the average price/cwt received by the producer for each respective year. The cost of milk production lost per cow in the herd was calculated by dividing the total economic value of milk lost each year by the number of adult cows tested in the herd that year.

Loss of future income due to premature culling

When cows are culled due to JD the producer often sustains losses from two aspects. First her net income stream is lost, provided she has not reached optimal culling age. Second, JD infected cows generally weigh less, especially cows exhibiting clinical signs, which lowers their cull value. Future productivity will be addressed first.

A recently developed computer spreadsheet model (OptiCow^{TM}, Model v1.4, Center of Animal Health and Productivity, University of Pennsylvania, Kennett Square, PA) allows the retention pay-off (RPO) value of individual dairy cows to be calculated. The RPO-value of a cow is defined as the total additional expected profit if the cow is kept until her optimal age as compared to her immediate replacement. It is an economic index that can be used to rank cows by their future profitability; the higher the RPO, the more valuable the cow. A negative RPO means replacement is the preferred action (Groenendaal and Galligan, 1999). For herds with individual cow production data (N=5), the RPO values were calculated for cows culled due to clinical JD and/or test positive for JD throughout the study, and summed together to obtain the total RPO-value lost due to JD per year. The loss of future productivity per cow for the herd was estimated by dividing the total RPO-value of all test positive cows culled by the number of adult cows in the herd for each respective year. The loss of future productivity for the herd without individual cow data was not calculated. (The farm input data for the OptiCow^{TM} Model are summarized in Appendix C).

While the RPO-value estimates future production potential, it does not include slaughter value. Due to the pathogenesis of JD, infected cows lose weight and may exhibit diarrhea, depending on the stage of infection. Thus, they tend to weigh less than uninfected cows when culled, resulting in lower slaughter value. The economic loss due to reduced cull income was estimated. Previous studies report losses in slaughter value ranging from 10-37.5% (Benedictus, et al., 1987; Ott, et al., 1999), with higher losses occurring with the more advanced stages of the disease.

Cull cow data was available for all herds, although in some cases it was incomplete. When possible the reduction in cull cow income due to JD was calculated using the following guidelines. Test negative Holstein cull cows were assumed to weigh an average of 1400 pounds (636 kg) and Jerseys 800 pounds (364 kg). Cows that were culled due to clinical JD were assumed to weigh 30% less (420 pounds Holstein, 240 pounds Jersey) than test negative cows during the first year of the study, and 15% less (210 pounds Holstein, 120 pounds Jersey) thereafter. The loss in body weight due to JD was changed because, after enrolling in the JD demonstration project and the start of annual whole herd testing, the working definition of "culled due to clinical JD" changed. Producers were more cognizant of the disease and quicker to cull a cow as soon as she started to lose weight or developed diarrhea, especially if she happened to test positive for JD. Cows that tested positive, but were culled due so some reason other than JD, were assumed to weigh 10% less (140 pounds Holstein, 80 pounds Jersey) than test negative cows throughout the study. In some instances, records were complete enough to identify cows that were sold for slaughter and those that died. Obviously if a cow died, the producer did not realize any cull income. The loss in cull value due to JD was then calculated as the total weight lost due to cows culled with JD (either clinical or test positive) multiplied by the respective slaughter value for each year. In the event a test positive cow died, the loss was calculated as the entire value of the cow, equaling the average weight minus 10%, multiplied by the respective slaughter value for that year. The total losses for each year were then divided by the number of adult cows in the herd that year to estimate the lost cull value per cow in the herd.

Benefits of JD control program

The economic benefits, or the reduction of losses, due to the JD control program were calculated as the difference between the annual economic losses due to JD fore each year following the implementation of the control program and a baseline measure of the losses caused by the disease prior to the control program. The baseline was estimated as the average of the losses due to JD over the first two complete years of the study. It was believed that the completeness of the data for the first year of the study depended on when, in the course of that year, a herd was enrolled in the program (herds enrolled in the spring were more likely to have more complete data than herds enrolled in the fall). Furthermore, as the majority of the management changes implemented to control JD were intended to prevent new infections in young calves, no benefits resulting from the control program were expected until the third year of the study at the earliest. Averaging losses over the first full two years would, therefore, provide a more accurate baseline measure of losses caused by JD in the absence of a control program. Thereafter, the annual benefits of the JD control program were estimated as the losses due to JD at baseline minus the losses due to JD in the subsequent study years.

Both costs and benefits were used to calculate the net present value (NPV) of each farm's JD control program over the course of the study. Simply put, the NPV is the value of the expected future returns of an investment minus the value of expected future costs, discounted to current dollars. It is commonly used in economics as a method for appraising long-term projects. All cash flows used to calculate the NPV were discounted back to the first year of the study to account for the time-value of money and the risk of the investment. Additionally, the NPV was projected over a total of 20 years assuming

four different scenarios: (1) the economic losses beyond the observed study period follow a linear decrease with eradication of JD from the herd 20 years after the start of the control program; (2) the economic losses stay constant at a rate equal to that of the last observed year of the study while continuing to invest in the control program; (3) the economic losses increase from baseline equal to the rate of decrease in scenario 1, in the absence of a JD control program; and (4) the economic losses remain constant at the baseline level in the absence of a control program. The reason scenarios 3 and 4 were calculated was to demonstrate potential economic losses should the farm elect not to implement a JD control strategy. The NPV was calculated as follows:

$$NPV = \sum_{t=1}^{n} \frac{C_t}{(1+r)^t} + \frac{C_f}{r(1+r)^{n+1}}$$

Where:

t = the index of time (year),

n = the total number of periods (years) during which cash flows were estimated,

r = the discount rate,

C_t = the net cash flow for period t, and

C_f = the constant net cash flow expected in years beyond n.

To estimate the NPV beyond 2007 (t = 4), or the observed study period, some assumptions were made. For scenarios 1 and 2, the projected ongoing costs of investing in the JD control program were assumed to equal the average annual cost of the control program during the observed study period. In scenario 1, it was assumed the losses due

to JD would follow a linear decline until disease eradication in year 20, when the losses would equal zero. The observed loss in 2007 (t = 4) was divided by 16, to estimate the necessary annual decrease in JD losses resulting in disease eradication in t = 20. For years t = 5 to 20, this result was subtracted from the previous year's loss to calculate the loss for each respective year t. In scenario 2, the observed loss in 2007 (t = 4) was assumed to remain constant for years t = 5 to 20 respectively. For scenario 3, an amount equal to the annual decrease in JD losses for scenario 1 was added to the baseline beginning in year t = 1, and increased by the same increment in all subsequent years until t = 20. In scenario 4, the baseline loss was held constant throughout the 20-year projection. The opportunity cost of capital is represented by the discount rate, r. The opportunity cost for capital used in agriculture is generally lower than that in other economic sectors. The discount rate was assumed to equal 8%, but was varied later in a sensitivity analysis. The constant net cash flow for years beyond n, C_f, was assumed to be equal to C_t when t = n. Dividing C_f by r resulted in a terminal value, or perpetuity, calculation to reflect future benefits from investing in the JD control program. As with the net cash flows for each period, t, the terminal value, was discounted back to the start of the program.

Sensitivity analysis

A sensitivity analysis was performed on the NPV calculations to determine which inputs had the greatest influence on the final calculation. The discount rate, r, was varied from 5% to 10%. All other input factors were varied by $\pm$ 10% including: overall cost of

JD control program, individual components of the JD control program (supplies, management, labor, and capital investments), milk price, cull price, and RPO-value.

The break-even cost for the JD control program was calculated for the two scenarios (1 and 2) that included investing in a control program.

Testing for JD was also included in the sensitivity analysis. Testing was provided free of charge to the herds during the observed study period and involved annual whole herd fecal culture and/or serum ELISA. It is unlikely the producers would have invested in such an intensive testing program had they incurred the testing costs, yet they used the test results to make management and culling decisions. Moreover, it is probable the producers will continue to do some JD testing after the study, so it was important to estimate the effects testing would have on the estimated NPV. Testing costs used in the sensitivity analysis were set equal to the laboratory costs being charged during the study period: fecal culture, $23/sample; and serum ELISA, $6/sample. The sensitivity analysis included the scenario for what actually happened, the costs of both tests run in parallel; as well as for serum ELISA testing only.

4.4. Results

Individual herd reports

A. Herd 1

Farm background

This farm has been owned and operated by the same family for over 100 years. At the beginning of the program, the herd was milking approximately 140 Holstein cows (167 total adult cows) with a rolling herd average (RHA) of 31,516 pounds (3.5% FCM). Bred heifers and heifer calves were occasionally purchased, mainly in an attempt to improve herd genetics, not because they were needed to maintain herd size. Considerable thought went into the purchase of these animals and they were purchased directly from herds which were at low risk for disease (including JD).

Johne's disease was first diagnosed in the herd in the mid-1980's. In 2003, 5.5% of the cows culled were due to clinical signs of JD. The youngest animal to develop clinical JD was an 18 month-old home-raised heifer in January of 2002. She was the wake up call the farm needed to realize they needed to take steps to control JD. Aside from JD, this herd had very few other health problems. The producer reported that the annual incidence of all periparturient diseases combined, was less than 5%. There were occasional summer flare-ups of environmental mastitis, and the bulk tank SCC averaged 300,000. The long term goals for this farm at the beginning of the study were:

1. Expand (internally) to milk 200 cows
2. 30,000 lb RHA
3. Market dairy replacements

JD risk assessment

Prior to the implementation of a JD control program, the area at greatest risk for disease transmission on this farm was the calving area. The farm had individual maternity pens, but they were not always cleaned between each calving. No consideration was given to a cow's JD status when placed in the pens. About 5% of calves were born in free stalls. Most of the calves (80%) were removed from the dams within two hours of birth. However, pooled colostrum was used for feeding. The other issue with the calving area was its proximity to weaned calves. It was an old basement barn. Weaned calves were kept in group pens just across a six foot alley from the maternity pens. Occasionally manure slurry ran from the maternity pens, across the alley and into the calf pens.

Other risks for JD transmission prior to the control program included feeding left-over feed from the lactating herd to replacement heifers; and, in the summer, bred heifers had fence-line contact and shared a waterer with dry cows on pasture.

JD control plan

Upon enrolling in the Michigan Johne's Disease Control Demonstration Project, annual testing of all adult cows for JD with serum ELISA and fecal culture began. All cows testing positive on either test were visually identified with a distinct ear tag and flagged in the computer for management. Cows positive on fecal culture and exhibiting clinical signs were culled immediately. Fecal culture positive cows not showing clinical signs were not bred back and were culled when they either began to exhibit clinical signs, or their milk production fell to below a break-even point defined by the farm.

A maternity pen was designated for calving all JD test positive cows. A greater effort was placed on cleaning all maternity pens after each calving. Pooled colostrum was no longer used. Colostrum from JD test positive cows was not fed to heifer calves. Only colostrum from JD test negative cows was frozen to be used as needed. Weaned calves were still housed across the alley from the maternity pens; but with more frequent cleaning, the amount of manure contamination from the maternity pens to the calf pens was reduced. A super hutch and fencing was purchased in the summer of 2005 to house calves that were just weaned, and relieve some of the crowding in the pens across from the maternity area.

Feeding waste feed to replacement heifers was discontinued. Although not done specifically for the JD control program, an existing barn was renovated for dry cows so they no longer had contact with bred heifers.

Descriptive statistics

Descriptive statistics for herd 1 are summarized in Table 4.1.

Table 4.1: Descriptive Statistics for Herd 1

Year	Herd size (adult cows)	RHA (lbs 3.5% FCM)	Cull Rate (%)	Culled due to clinical JD (%)	Mortality Rate (%)
2003	170	31,516	25.3	4.7	4.1
2004	176	29,666	38.1	11.9	5.1
2005	190	33,090	26.8	15.7	2.1
2006	204	33,744	27.5	12.5	2.5
2007	215	32,522	27.9	5.0	1.9

Herd size increased steadily over the study period in accordance with the farm's stated goals. The overall cull rate, with the exception of 2004, was fairly constant over the five-year study period, and was lower than the average state cull rate of 37.7% (Hadley, et al., 2006). This is likely due to the herd trying to expand internally to bring existing facilities to full capacity of 200 cows milking. As the herd reaches this goal, it is likely the overall cull rate will increase. Mortality rate decreased over the course of the study. The number of cows culled for clinical JD increased initially and then decreased back to approximately the same level as when the study began. However, this may have been a larger decrease than suggested numerically. Following study enrollment, the producer was much more cognizant of cows with clinical signs. The farm also had access to the annual JD test results, which figured heavily into the culling decision process. Cows were culled as soon as they started exhibiting signs of weight loss and/or diarrhea. Cows testing positive for JD had one strike against them. As soon as they started exhibiting clinical signs, or developed another problem, they were culled.

JD prevalence

The within herd JD prevalence trend is outlined in Table 4.2. Johne's disease prevalence increased steadily over the first three years of the study, before declining dramatically in 2006. This pattern fit with what the producer reported seeing clinically. The number of cows exhibiting weight loss and diarrhea increased in 2004 and continued through the spring of 2006. Beginning in 2006, the producer began noticing a decline in clinical cases. The increase in prevalence in 2007 was unexpected and remained unexplained at the conclusion of this study.

Table 4.2: Johne's disease prevalence trends 2003-2007 for Herd 1	
Year	Apparent JD prevalence (ELISA &/or FC positive)
2003	12.0%
2004	24.3%
2005	22.0%
2006	9.8%
2007	15.0%

Cost of the JD control program 2003-2007

The costs of the JD control program observed over the five years of this study are summarized in Table 4.3. As part of the Michigan Johne's Disease Control Demonstration Project, the herd did not have to pay for any JD testing beyond the cost of labor to collect samples on the day of the annual test. It is unlikely the herd would have done the extensive testing that was performed if it had to pay for the testing, yet management decisions were made based on those test results, so for the sake of completeness, testing costs are included in the last column of the table.

Table 4.3: Cost of Johne's disease control program 2003-2007 for Herd 1 ($/cow)

Year	No. Cows	Supplies	Management	Labor	Capital Investments	Total	Total (+ testing)
2003	170	$9.72	$1.17	$5.35	$0.00	$16.24	$45.24
2004	176	$9.27	$1.16	$5.85	$0.00	$16.29	$45.29
2005	190	$2.47	$0.47	$7.16	$0.77	$10.88	$39.88
2006	204	$2.21	$0.46	$6.67	$0.72	$10.05	$39.05
2007	215	$2.19	$0.45	$5.95	$0.68	$9.27	$38.27
Ave.	191	$5.17	$0.74	$6.20	$0.43	$12.54	$41.54

The supplies category included the purchase of pink ear tags to identify JD test positive cows, and colostrum replacer over the first two years of the program until the herd had enough colostrum from test negative cows banked to meet its needs. Also included was a charge for increased bedding used as a result of more frequent cleaning of the maternity and calf areas. Management costs were mainly due to record management to keep track of test positive cattle. Labor costs were largely due to the increased time spent cleaning the maternity barn; although time spent assisting in the collecting of samples for JD testing was also included in this category. The capital purchase made by the farm was a super hutch and fencing in 2005 to keep just-weaned calves out of the maternity barn.

Economic losses due to JD 2003-2007

The annual estimated economic losses due to JD, along with the calculated, or assumed, benefits of the control program for Herd 1 are summarized in Table 4.4.

Table 4.4: Economic losses due to Johne's disease and assumed benefits of Johne's disease control program for Herd 1 – 2003-2007

($/cow)

Year	No. Cows	Milk Value	RPO	Cull Value	Total	Assumed Benefits
2003	170	$87.03	$0.31	$11.61	$98.95	N/A
2004	176	$114.93	$0.00	$23.61	$138.53	-$19.79
2005	190	$45.84	$21.80	$12.33	$79.97	$38.77
2006	204	$49.41	$22.55	$11.60	$83.57	$35.17
2007	215	$50.09	$4.11	$6.02	$60.23	$58.51
Ave.	191	$69.46	$9.75	$13.04	$92.25	N/A

N/A Not applicable

NPV calculation

The NPV was calculated for four different scenarios: (1) assuming a linear decline in losses following year five and disease eradication by year 20 with the control program; (2) assuming losses and JD prevalence remain constant at year five levels while continuing the control program; (3) assuming linear increase in losses at rate equal to that in scenario 1 with no control program; and (4) assuming losses remain constant at same level as the beginning of the study with no control plan implemented. The results are shown in Table 4.5. Due to the relatively small about of money invested in the JD control program as compared to the estimated potential benefits, the NPV for scenarios 1 and 2 became positive by the third year of the study. The NPV's for scenarios 3 and 4 remain negative over the entire 20-year projected study period because, in the absence of a control program, there was nothing to offset the losses caused by JD.

Results of sensitivity analysis

The break-even cost for the JD control program is an estimate of the amount of money a farm can invest in the control program and still "break-even", or have a NPV equal to zero. The break-even cost calculated for scenario 1 was $75.64/cow/year, and $49.74/cow/year, for scenario 2.

The NPV was most sensitive to the discount rate (r) used. After the discount rate, milk price, followed by cost of the JD control program had the greatest effect on the NPV. When the individual categories within the JD control program were evaluated, the results were ranked depending on which category represented the highest proportion of the

Table 4.5: NPV of four scenarios for Johne's disease (JD) control on Herd 1

Year of Program	Scenario*			
	1	2	3	4
5	$80.49	$77.93	-$516.88	-$474.10
10	$244.76	$202.85	-$919.80	-$796.76
20	$778.53	$459.88	-$1,944.65	-$1,460.67
Payback Year	3	3	N/A	N/A

N/A Not applicable

Payback Year: Year of control program when NPV became positive

* Scenario 1: Assuming linear decline in losses caused by JD after year 4 due to declining prevalence and eventual eradication after implementation of JD control program

Scenario 2: Assuming losses caused by JD remain constant after year 4 while still investing in JD control program

Scenario 3: Assuming losses caused by JD increase at a rate equal to the decline in Scenario 1 in the absence of JD control program

Scenario 4: Assuming losses caused by JD remain constant at baseline level in absence of JD control program

control program. For this herd, changing the input costs of labor had the greatest impact on the NPV, followed by supplies, management, and capital investments respectfully.

Including testing in the calculations increased the input cost of the JD control program, and hence decreases the NPV. For scenario 1, the NPV decreased from $779 to $705 when ELISA testing only was included, and to $434 when both ELISA and fecal culture was included. For scenario 2, the NPV decreased from $460 to $386 and $115 respectfully, for including ELISA testing only or ELISA and fecal culture together.

Producer perception of the JD control program

Overall, the producer was very pleased with the results of the JD control program, and planned to continue investing in it after the end of the study. Subjectively, herd health improved over the course of the study, which in turn led to improved production and increased revenues. An additional value resulting from the JD control program on this farm was seen in the marketing of herd replacements. Other producers were willing to pay more for cattle raised on this farm because of the JD control program the herd had implemented and the amount of diagnostic testing that was performed.

B. Herd 2

Farm background

This is a second generation dairy farm that entered the Michigan Johne's Disease Control Demonstration Project as a partnership in December 2002. At that time, the herd was milking around 85 Holstein cows (105 total adult cows) with a RHA of 26,569 pounds (3.5% FCM). Purchased cattle were last added to the herd in 2000 when 10 yearling heifers were bought from a single source with a low risk of JD (vouched for by the herd's veterinarian). Prior to that, in 1999, six bred heifers were purchased from a single source with unknown JD status. Johne's disease was first diagnosed in the herd in 1992, within five years of purchasing six yearling heifers. The youngest clinical case of JD occurred in an 18 month-old bred heifer raised on the farm in 2002. In 2002, approximately 7% (N=7) of cows were culled due to clinical signs of JD. Aside from JD,

the herd historically has had problems getting cows bred back, but few overt health problems. The bulk tank SCC averages 210,000. The stated long term goals for the farm at the beginning of the study were (in order):

1. Maintain quality personal time for owners and employees
2. 60% of the adult herd pregnant at all times
3. 30,000 lb. RHA
4. Maintain or expand herd size

In 2004, the partnership dissolved, and one partner was forced to buy out the other. This, along with the low milk prices at the time, put the farm in a tenuous financial position. As part of the buyout, all cows testing positive for JD were culled, regardless of production or reproductive status. This temporarily decreased the size of the milking herd, and cows that would have been culled previous to the partnership break-up were retained in order to meet cash flow needs. Labor was also an issue. Retaining quality, dependable employees was a challenge; and the farm had a high employee turnover rate, including the herd manager. As a result of these distractions, improving herd health and facilities were not top priorities, as it was a struggle to maintain the status quo. Beginning in 2007, things began to stabilize. Time and improved milk prices put the farm on better financial footing, the labor issues seemed to have been resolved, and focus was again being placed on the cows.

JD risk assessment

Prior to the implementation of a JD control program, the areas at greatest risk for JD transmission were the calving and pre-weaned calf areas; although there were also some risks in the weaned and bred heifer areas. The farm had one maternity pen bedded with straw. It was cleaned infrequently (about once every 10 calvings), although fresh bedding was occasionally added between calvings. No attempt was made to segregate JD suspect cows and JD test negative cows in the maternity pen or the adjacent close-up dry cow area. Calves were generally removed from the cow within two hours of birth, unless they were born during the night, then they might remain with the cow for 6-8 hours. If multiple cows were calving, colostrum was pooled and fed to the calves. All calves were fed pooled, unpasteurized, whole milk. Weaned calves were fed hay in an alley adjacent to the lactating cow area, where feed could be contaminated by manure from adults. Bred heifers and dry cows were housed together in the same pen. Breeding age heifers were housed in a pen adjacent to the bred heifers/dry cows and shared the same water source. Breeding age heifers were also fed leftover feed from the lactating cows when available.

JD Control Plan

Upon enrolling in the project, annual testing of all adult cows for JD with serum ELISA and fecal culture began. All cows testing positive on either test were not bred back, and were culled when they developed clinical signs, or milk production decreased below some break-even point determined by the farm. Test positive cows were visually identified with a notch in their ear tag and calves born to test positive cows identified

with a blue ear tag with a "J". An effort was made to clean the maternity pen more often, although not always after each calving. The maternity area was finally remodeled in October of 2007, with multiple calving pens, which theoretically will be cleaned after each calving. Colostrum from JD test positive cows was no longer used, and colostrum was no longer pooled. Extra colostrum from individual JD test negative cows was frozen for use as needed. In the absence of colostrum, a colostrum supplement (Colostrix™) was used. All heifer calves were fed milk replacer. Bull calves being raised as steers continued to receive pooled waste milk as available. Bottles used for feeding milk were sanitized after each feeding. An off-the-floor hay feeder was constructed in the weaned calf area. Feeding of leftover feed from the cows to breeding age heifers was discontinued.

Descriptive statistics

Descriptive statistics for herd 2 are summarized in Table 4.6. Herd size increased over the study period, and by 2007, the farm was milking approximately 120 cows, which was the desired herd size, with existing facilities at capacity. The overall cull rate fluctuated over the study period. It started off at 36%, which was close to the average state cull rate of 37.7% (Hadley, et al 2006). It increased dramatically in 2004 as a result of the dissolution of the partnership. This was followed by a substantial decrease in cull rate in 2005, likely due to the herd trying to recover from the buyout, and increase herd size to increase cash flow. The increase in the number of cows culled due to clinical JD in 2004 may be due, in part, to misclassification. Recall that all JD test positive cows were culled as part of the partnership settlement. So some of the cows in 2004 may

Table 4.6: Descriptive Statistics for Herd 2					
Year	Herd size (adult cows)	RHA (lbs 3.5% FCM)	Cull Rate (%)	Culled due to clinical JD (%)	Mortality Rate (%)
2003	103	26,569	35.9	6.8	2.9
2004	121	25,081	47.9	10.7	2.5
2005	134	23,854	21.6	0.7	3.0
2006	132	22,022	27.3	5.3	3.0
2007	137	25,231	25.5	1.4	6.6

have been culled due to "JD" but were not actually exhibiting clinical signs. Following the "JD cleansing" of the herd in 2004, it was not surprising the number of cows culled due to clinical JD dropped to less than 1% in 2005. The JD test positive cows, the cows in the most advanced stages of the disease, and therefore most likely to develop clinical signs, had been culled the previous year. In 2005, the herd was relatively young (as compared to previous years), with a small proportion of test positive cows, so there were fewer cows culled due to clinical signs. The number of cows culled due to clinical JD crept up again in 2006. This is likely a return to what it would have been in the absence of the buyout. Since the cleansing in 2004, all the remaining cows, and the majority of heifers entering the herd had not been exposed to the JD control program. Two years later, in 2006, cows with JD had matured and were more likely to exhibit clinical signs. The decline in 2007 was likely due to a couple of different factors. First management had improved, with more attention being paid to the cows, so JD test positive cows were being removed before they had a chance to develop clinical signs. Second, the heifers entering the herd had been born and raised with the JD control program in place. Thus, it

was simply a reflection of the decreasing prevalence of JD in the herd. The mortality rate was fairly consistent throughout the study period with the exception of 2007 when it doubled. The reason for that increase was not reported.

JD Prevalence

The within herd JD prevalence trend is outlined in Table 4.7. The culling of all JD test positive cows occurred a couple of months prior to the 2004 test, which explains the decline in JD prevalence from 2003 to 2004. The subsequent rebound in prevalence is most likely the result of infected heifers not exposed to the JD control program maturing, entering the milking herd, and testing positive over the course of their first and second lactations.

Table 4.7: Johne's disease prevalence trends 2003-2007 for Herd 2

Year	Apparent JD prevalence (ELISA &/or FC positive)
2002	12.1%
2003	9.5%
2004	4.1%
2005	5.0%
2006	9.4%
2007	4.2%

Cost of the JD control program 2003-2007

The costs of the JD control program observed over the five-year period of 2003-2007 are summarized in Table 4.8. As part of the Michigan Johne's Disease Control

Table 4.8: Cost of Johne's disease control program 2003-2007 for Herd 2 ($/cow)

Year	No. Cows	Supplies	Management	Labor	Capital Investments	Total	Total (+ testing)
2003	103	$9.68	$4.12	$7.96	$1.80	$23.57	$52.57
2004	121	$1.49	$4.30	$7.44	$1.53	$14.77	$43.77
2005	134	$3.28	$3.98	$6.72	$1.39	$15.37	$44.37
2006	132	$5.55	$3.33	$6.82	$1.41	$17.10	$46.10
2007	137	-$1.82	$2.31	$5.84	$1.35	$7.68	$36.68
Ave.	125	$3.64	$3.61	$6.95	$1.50	$15.70	$44.70

Demonstration Project, the herd did not have to pay for any JD testing beyond the cost of labor to collect samples on the day of the annual test. It is unlikely the herd would have done the extensive testing that was performed if it had to pay for the testing; yet management decisions were made based on those test results, so for the sake of completeness, testing costs are included in the last column of the table.

The supplies category included the purchase of blue ear tags to identify calves from JD test positive cows, milk replacer to feed all heifer calves, and colostrum supplement. It also included increased cost of straw due to more frequent cleaning and bedding of the maternity pen. The cost of supplies was adjusted to reflect the sale of milk that was previously fed to calves, and this adjustment explains the "negative" supply cost in 2007. Management costs reflect the time spent on record keeping, making management decisions regarding JD test positive cows, and employee education. Labor costs reflect the increased time spent cleaning the maternity pen, mixing milk replacer, fresh cow and calf care, as well as time spent assisting in the collection of samples during JD testing. The capital investment made by this farm was a second skid-steer bucket

purchased in January 2003 so one bucket could be dedicated to feed and one to manure handling.

Economic losses due to JD 2003-2007

The annual estimated economic losses due to JD, along with the calculated, or assumed, benefits of the control program for Herd 2 are summarized in Table 4.9. The mass culling of JD test positive cows occurred in 2004, which likely explains the increase in losses seen in that year, and the subsequent decrease in losses in 2005. The increase in total losses beginning in 2006 reflects MAP infected cows maturing (and the disease progressing), but needing to be retained in the milking string to help meet cash flow needs. With the farm management and finances finally stabilizing in 2007, the JD losses once again began to decline.

Table 4.9: Economic losses due to Johne's disease and assumed benefits of Johne's disease control program for Herd 2 – 2003-2007

($/cow)

Year	No. Cows	Milk Value	RPO	Cull Value	Total	Assumed Benefits
2003	103	$0.00	$0.00	$15.85	$15.85	N/A
2004	121	$0.00	$20.49	$28.19	$48.67	-$16.41
2005	134	$13.33	$2.90	$1.82	$18.05	$14.21
2006	132	$53.60	$15.80	$12.73	$82.13	-$49.87
2007	137	$42.07	$2.12	$21.97	$66.17	-$33.91
Ave.	125	$21.80	$8.26	$16.11	$46.17	N/A

N/A Not applicable

NPV calculation

The NPV was calculated for four different scenarios: (1) assuming a linear decline in losses following year five and disease eradication by year 20 with the control program; (2) assuming losses and JD prevalence remain constant at year five levels while continuing the control program; (3) assuming linear increase in losses at a rate equal to that in scenario 1 with no control program; and (4) assuming losses remain constant at same level as the beginning of the study with no control plan implemented. The results are shown in Table 4.10. The reason the NPV's for both scenarios 1 and 2 were negative was because the baseline value for the losses due to JD was lower than losses in the subsequent years of the study. Thus, mathematically, it appeared the control program had "negative" benefits that never exceeded zero when summed over the projected study period. The NPV's for scenarios 3 and 4 remain negative over the entire 20-year projected study period because, in the absence of a control program, there was nothing to offset the losses caused by JD.

Results of sensitivity analysis

The break-even cost for the JD control program is an estimate of the amount of money a farm can invest in the control program and still "break-even", or have a NPV equal to zero. The break-even costs were -$1.81 and -$30.27 for scenarios 1 and 2 respectively. Thus, based on the assumptions used to calculate the NPV's in this study, the JD control program on this farm would not break-even, no matter how little money was invested in it.

Table 4.10: NPV of four scenarios for Johne's disease (JD) control on Herd 2

Year of Program	Scenario*			
	1	2	3	4
5	-$144.54	-$147.35	-$175.81	-$128.81
10	-$236.11	-$282.15	-$351.65	-$216.47
20	-$209.42	-$559.51	-$928.57	-$396.85
Payback Year	N/A	N/A	N/A	N/A

N/A Not applicable

Payback Year: Year of control program when NPV became positive

* Scenario 1: Assuming linear decline in losses caused by JD after year 4 due to declining prevalence and eventual eradication after implementation of JD control program

Scenario 2: Assuming losses caused by JD remain constant after year 4 while still investing in JD control program

Scenario 3: Assuming losses caused by JD increase at a rate equal to the decline in Scenario 1 in the absence of JD control program

Scenario 4: Assuming losses caused by JD remain constant at baseline level in absence of JD control program

The NPV was most sensitive to the discount rate (r) used. After the discount rate, milk price, followed by the cost of the JD control program had the greatest effect on the NPV. When the individual categories within the JD control program were evaluated, the results were ranked depending on which category represented the highest proportion of the control program. For this herd, changing the input costs of labor had the greatest impact on NPV, followed by supplies, management, and capital investments respectfully.

Including testing in the calculations increased the input cost of the JD control program, and hence decreased the NPV. For scenario 1, the NPV decreased from -$209

to -$283 when ELISA testing only was included, and to -$554 when both ELISA and fecal culture was included. For scenario 2, the NPV decreased from -$550 to -$633 and -$904 respectfully, for including ELISA testing only or ELISA and fecal culture together.

Producer perception of the JD control program

At the conclusion of this study, the producer stated he was satisfied with the JD control program and planned to continue investing in it. He noted he had not necessarily seen any increased revenue as a result of the control program, but hoped that would change as herd health and production improved.

C. Herd 3

Farm background

This is a third generation farm in the very early stages of transitioning to the next generation. At the beginning of the study, the herd was milking approximately 190 cows (218 total adult cows) with a RHA of 21,865 pounds (3.5% FCM). The long term goals for this farm were:

1. Expand herd size to 450 cows milking (~500 cows total)
2. Transition ownership and management to next generation

In order to meet these goals, the farm built a new free stall barn and remodeled existing facilities in 2003. The herd was open. In preparation for the herd expansion, three smaller herds, including all young stock (approximately 300 cattle total), were purchased and consolidated in 2004. Additionally, six to eight bulls are purchased each year for breeding purposes, and are not screened for JD. Johne's disease was first diagnosed in the herd in 2001, in a 3.5 year old cow raised on the farm. In 2002, approximately 12 cows (5%) were culled exhibiting clinical signs of JD. Aside from JD, the herd was experiencing other "expansion pains" including periparturient metabolic problems and hairy heal warts. Labor management was also a concern as the herd transitioned from primarily family labor to hired Hispanic labor.

JD risk assessment

Prior to the implementation of the JD control program, the areas at greatest risk for disease transmission on this farm were the maternity and pre-weaned calf areas. The close-up dry cows were housed on a manure pack that also served as the maternity pen. Cows suspected of having JD were not segregated in any way. Manure build up in the maternity area was occasionally an issue, and cows in the area were moderately dirty. Calves were generally removed from dams within 4-6 hours of birth. Calves were fed pooled colostrum, then unpasteurized whole milk until weaned. Weaned calves, until five months of age, were housed adjacent to the adult cow area and fed feed refusal from those cows.

JD control plan

Upon enrolling in the Michigan Johne's Disease Control Demonstration Project, annual testing of all adult cows for JD with serum ELISA and fecal culture began. All cows testing positive on either test are visually identified with red cable ties placed through their ear tags, as were as any calves born to these cows. Cows testing positive for JD ware kept in the herd until they developed clinical signs or their production fell below some breakeven point set by the farm. As all breeding was done by natural service, test positive cows were often bred back.

An effort was made to improve the sanitation of the maternity pen by more frequent cleaning and/or bedding. Calves were removed as soon as possible after birth, generally within one hour. Feeding pooled colostrum was discontinued. Calves were fed colostrum only from test negative cows. The farm also switched from unpasteurized whole milk to milk replacer to feed calves.

As a result of the herd expansion, a heifer grower was contracted to raise heifers from the age of six months until they are returned to the farm as springing heifers to freshen. The heifer grower only raised heifers for this farm. A new barn was built for pre-weaned calves, and an existing barn remodeled for calves from the time of weaning until they were sent to the heifer grower. This removed calves from direct contact with adult cows, but they were still occasionally fed feed refusal from the adult herd.

Descriptive statistics

Descriptive statistics for herd 3 are summarized in Table 4.11.

Table 4.11: Descriptive Statistics for Herd 3				
Year	Herd size (adult cows)	RHA (lbs 3.5% FCM)	Cull Rate (%)	Culled due to clinical JD (%)
2003	218	21,865	21.6	3.2
2004	369	25,643	24.1	3.0
2005	412	26,028	27.7	1.2
2006	432	26,329	32.9	3.7
2007	458	26,210	29.7	2.6

Herd size more than doubled over the study period due to the purchase of cattle as already discussed. No additional replacement cattle have been purchased since 2004. As of 2007, the plan was for the herd to expand internally to reach the desired herd size of 500 adult cows. As a result of trying to increase herd size, the overall cull rate was relatively low throughout the study period. To cash flow the new free stall barn, cows were needed in every stall. Cows that would have been culled previous to the expansion were kept to fill stalls. Once the facility reaches capacity, it is likely the cull rate will go up as space becomes a limiting factor, and cows will need to be culled to make room for more productive heifers. The number of cows culled due to clinical JD has been fairly consistent throughout the study period. As of the conclusion of the study, there had not been much culling pressure on JD test positive cows. Test positive cows were managed only in so far as to prevent disease transmission to calves through colostrum or milk. Otherwise, they were managed as any other cow in the herd. The mortality rates are not reported due to insufficient data.

JD prevalence

The within herd JD prevalence is outlined in Table 4.12. Johne's disease prevalence remained relatively unchanged over the course of this study. This might lead one to believe that the JD control program implemented on this farm was ineffective in preventing JD transmission. However, it must be remembered that this herd doubled in size, mainly through the purchase of cattle, including young stock, from herds of unknown JD status. As late as 2006, heifers were still entering the herd that had not been exposed to the JD control program implemented by this herd. In 2007, it was estimated over half the milking herd consisted of purchased cattle. Unfortunately, insufficient records prevented differentiating the JD prevalence in purchased cows vs. cows raised on the farm after implementation of the JD control program.

Table 4.12: Johne's disease prevalence trends 2003-2007 for Herd 3

Year	Apparent JD prevalence (ELISA &/or FC positive)
2003	10.5%
2004	7.7%
2005	12.9%
2006	11.8%
2007	16.0%

Cost of JD control program 2003-2007

The costs of the JD control program observed over the five years of this study are summarized in Table 4.13. As part of the Michigan Johne's Disease Control Demonstration Project, the herd did not have to pay for any JD testing beyond the cost of

labor to collect samples on the day of the annual test. It is unlikely the herd would have done the extensive testing that was performed if it had to pay for the testing, yet management decisions were made based on those test results, so for the sake of completeness, testing costs are included in the last column of the table.

Table 4.13: Cost of Johne's disease control program 2003-2007 for Herd 3 ($/cow)

Year	No. Cows	Supplies	Management	Labor	Capital Investments	Total	Total (+ testing)
2003	218	$1.76	$21.83	$34.02	$0.62	$58.22	$87.22
2004	369	-$5.39	$16.34	$30.15	$0.36	$41.46	$70.46
2005	412	-$2.66	$15.00	$27.01	$0.33	$39.68	$68.68
2006	432	-$0.60	$14.88	$27.19	$0.31	$41.77	$70.77
2007	458	-$8.79	$14.41	$25.64	$0.29	$31.55	$60.55
Ave.	378	-$3.14	$16.49	$28.80	$0.38	$42.54	$71.54

The supplies category included the purchase of milk replacer to feed calves and colostrum supplement, as well as cable ties to identify JD test positive cows. It also included increased cost for straw due to more frequent cleaning and bedding of the maternity pen. The cost of supplies was also adjusted to reflect the sale of milk that would have previously been fed to calves. This adjustment explains the negative supply costs for 2004-2007. Over the course of this study, the farm was in the process of transitioning from family labor to hired labor. The herd manager was still doing many things that labor would do on other farms. Management costs for this herd reflect time spent on JD testing, record keeping, making management decisions regarding JD test positive cows, buying and selling cows, and capital purchases. It also includes time spent

handling cattle and colostrum, making sure that only colostrum from test negative cows is fed to calves. Labor costs are due primarily to increased time spent cleaning the maternity pen, mixing milk replacer, and fresh cow and calf care. The capital investment made by this farm was a mixing vat to mix large amounts of milk replacer purchased in the summer of 2003.

Economic losses due to JD 2003-2007

The annual estimated economic losses due to JD, along with the calculated, or assumed, benefits of the control program for Herd 3 are summarized in Table 4.14.

Table 4.14: Economic losses due to Johne's disease and assumed benefits of Johne's disease control program for Herd 3 – 2003-2007

($/cow)

Year	No. Cows	Milk Value	RPO	Cull Value	Total	Assumed Benefits
2003	218	$10.06	$0.87	$6.94	$17.86	N/A
2004	369	$16.82	$0.00	$3.72	$20.54	-$1.34
2005	412	$44.59	$8.50	$2.66	$55.75	-$36.55
2006	432	$97.41	$15.80	$5.08	$118.29	-$99.09
2007	458	$53.13	$8.67	$4.74	$66.54	-$47.34
Ave.	378	$44.40	$6.77	$4.63	$55.80	N/A

N/A Not applicable

NPV calculation

The NPV was calculated for four different scenarios: (1) assuming a linear decline in losses following year five and disease eradication by year 20 with the control program; (2) assuming losses and JD prevalence remain constant at year five levels while continuing the control program; (3) assuming linear increase in losses at rate equal to that in scenario 1 with no control program; and (4) assuming losses remain constant at same level as the beginning of the study with no control plan implemented. The results are shown in Table 4.15. The reason the NPV's for both scenarios 1 and 2 were negative was because the baseline value for the losses due to JD was lower than losses in the subsequent years of the study. Thus, mathematically, it appeared the control program had "negative" benefits that never exceeded zero when summed over the projected study period. The NPV's for scenarios 3 and 4 remain negative over the entire 20-year projected study period because, in the absence of a control program, there was nothing to offset the losses caused by JD.

Results of sensitivity analysis

The break-even cost for the JD control program is an estimate of the amount of Money a farm can invest in the control program and still "break-even", or have a NPV equal to zero. The break-even costs were -$17.85 and -$46.46 for scenarios 1 and 2 respectively. Thus, based on the assumptions used to calculate the NPV's in this study, the JD control program on this farm would not break-even, no matter how little money was invested in it.

Table 4.15: NPV of four scenarios for Johne's disease (JD) control on Herd 3

Year of Program	Scenario*			
	1	2	3	4
5	-$333.12	-$335.95	-$123.92	-$76.66
10	-$533.87	-$580.17	-$264.77	-$128.83
20	-$730.64	-$1,082.68	-$770.86	-$236.19
Payback Year	N/A	N/A	N/A	N/A

N/A Not applicable

Payback Year: Year of control program when NPV became positive

* Scenario 1: Assuming linear decline in losses caused by JD after year 4 due to declining prevalence and eventual eradication after implementation of JD control program

Scenario 2: Assuming losses caused by JD remain constant after year 4 while still investing in JD control program

Scenario 3: Assuming losses caused by JD increase at a rate equal to the decline in Scenario 1 in the absence of JD control program

Scenario 4: Assuming losses caused by JD remain constant at baseline level in absence of JD control program

The NPV was most sensitive to the discount rate (r) used. After the discount rate, the cost of the JD control program, followed by milk price had the greatest effect on the NPV. When the individual categories within the JD control program were evaluated, the results were ranked depending on which category represented the highest proportion of the control program. For this herd, changing the input costs of labor had the greatest impact on NPV, followed by management, capital investments, and supplies respectfully.

Including testing in the calculations increased the input costs of the JD control program, and hence decreased the NPV. For scenario 1, the NPV decreased from -$731 to -$864 when ELISA testing only was included, and to -$1075 when both ELISA and fecal culture was included. For scenario 2, the NPV decreased from -$1083 to -$1156

and -$1427 respectfully, for including ELISA testing only or ELISA and fecal culture together.

Producer perception of the JD control program

At the conclusion of this study, the producer was satisfied with the JD control program and planned to continue investing in it. Subjectively, herd health had improved over the course of this study as had production which led to increased revenues.

D. Herd 4

Farm background

This is a rotational grazing Jersey herd, although the cows are confined to free stalls during the winter months. The owners sold the herd and got out of the dairy business in 1994. In 1995, they changed their minds and reassembled a herd with the purchase of 17 cows from multiple sources. The last outside cattle added to the herd occurred in 1997 with the purchase of ten cows and two springing heifers from a single herd in North Carolina. In 2003, they were milking approximately 70 cows (75 total adult cows) with a RHA of 12,446 pounds, and were in the final stages of becoming a certified organic dairy farm. The herd's goals were as follows:

1. Become a certified organic dairy farm
2. Decrease herd size to milk ~50 cows
3. Maintain a low input operation (rotational grazing)
4. Sell dairy replacements

The herd gained organic certification in the spring of 2005, at which time they contracted the price they receive for milk at $34/cwt; more than double the price the other herds in this study were receiving.

Johne's disease was first diagnosed in the herd in the winter of 2002, in a three-year old cow that was raised on the farm, but whose dam was purchased. A total of four cows were culled in 2002, two of which were believed to have JD based on clinical signs.

JD risk assessment

Prior to the implementation of a JD control program, this herd was at high risk for disease transmission in almost every area on the farm. The maternity pen doubled as the sick cow pen. It was a manure pack that was cleaned infrequently. Calves were often left to nurse the dam, or surrogate dam, for one week, up to one month. Otherwise calves, were fed unpastuerized whole milk, and housed in a pen adjacent to the maternity pen with direct contact with adult cows. After weaning, heifers were housed in a pen adjacent to the barnyard where they had nose-to-nose contact with adult cows. Bred heifers were housed with the adult herd two months prior to calving, and grazed with the lactating herd during the summer months. Finally, the same loader bucket was used for feed and manure handling.

JD control plan

Upon enrolling in the Michigan Johne's Disease Control Demonstration Project, annual testing of all adult cows for JD with serum ELISA and fecal culture began. Cows positive on fecal culture and exhibiting clinical signs were culled immediately. Cows testing positive on fecal culture and/or ELISA and not showing clinical signs were not rebred, and were culled as soon as they began showing clinical signs, or their milk production fell below a break-even point defined by the farm.

The most immediate management changes focused on the maternity and calf areas. The maternity area was cleaned weekly with lime put down under fresh straw bedding. Calves were removed as soon as possible from the dams, generally within two hours. An existing barn was renovated for pre-weaned calves to remove them from contact with adult cows. Colostrum was not pooled, and only colostrum from JD test negative cows was fed to calves and frozen to be used as needed. Instead of whole milk, calves were fed milk replacer until weaned.

Weaned heifers were still housed next to the barn yard, but the feeding area was moved to minimize the potential for feed contamination by manure from the lactating herd. Bred heifers were still grazed with lactating cows during the summer, and springing heifers were housed with the lactating herd two months prior to calving.

In 2005, a front-end loading tractor was purchased, mainly for the purpose of the JD control program. This, in addition to their old tractor, allowed one tractor to be used exclusively for handling feed and the other for handling manure.

Descriptive statistics

Descriptive statistics for Herd 4 are summarized in Table 4.16. Aside from becoming a certified organic dairy farm in 2005, this herd is unique from the other herds in this study for several reasons; but the main one is the personal attachment the owner has with the cows. The owner recognizes each cow by name, without the aid of any other identification such as ear tags or neck chains. Culling decisions are difficult for the owner, and sentiment plays a much larger role than on other farms. This is evident in the relatively low cull rates up until 2006. In 2006, a concentrated effort was made to decrease herd size, and the majority of cows culled were sold to other dairy farms rather than to slaughter. Irregardless of sentiment, cows with a positive JD test (ELISA and/or fecal culture) were culled as soon as possible. As within herd JD prevalence declined, so did the number of cows being culled for JD. The mortality rate remained low throughout the study. The increase in 2006 was due to a total of three cows dying for various reasons, as compared to only one cow in each of the other study years.

Table 4.16: Descriptive Statistics for Herd 4

Year	Herd size (adult cows)	RHA (lbs 3.5% FCM)	Cull Rate (%)	Culled due to clinical JD (%)	Mortality Rate (%)
2003	75	12,446	28.0	9.3	0.0
2004	74	12,149	28.4	12.2	1.4
2005	77	12,307	24.7	1.3	1.3
2006	72	12,578	50.0	2.8	4.2
2007	68	13,429	44.0	2.9	1.5

JD prevalence

The within herd JD prevalence trend is outlined in Table 4.17. Prevalence decreased steadily until 2005, and then rebounded in 2006 and 2007. This has been somewhat disconcerting for the owner, as the number of cows exhibiting clinical signs has declined. In fact, in 2007, there were no cows reported with clinical signs.

Table 4.17: Johne's disease prevalence trends 2003-2007 for Herd 4

Year	Apparent JD prevalence (ELISA &/or FC positive)
2003	11.7%
2004	9.0%
2005	3.8%
2006	7.9%
2007	6.6%

Cost of the JD control program 2003-2007

The costs of the JD control program observed over the five years of this study are summarized in Table 4.18. As part of the Michigan Johne's Disease Control Demonstration Project, the herd did not have to pay for any JD testing beyond the cost of labor to collect samples on the day of the annual test. It is unlikely the herd would have done the extensive testing that was performed if it had to pay for the testing, yet management decisions were made based on those test results, so for the sake of completeness, testing costs are included in the last column of the table.

The supplies category included costs associated with switching to milk replacer to feed calves and the purchase of colostrum replacer to be used when real colostrum from

JD test negative cows was unavailable. The reason for the relatively large "negative" supply costs 2005-2007 was because of the adjustment made for the sale of milk that was previously fed to calves. As a certified organic dairy farm, the cost of the milk replacer the farm was allowed to use tended to be higher than that used by the other herds in this study. However, upon becoming certified, the price the farm was receiving for their milk more than compensated for the switch to milk replacer.

Table 4.18: Cost of Johne's disease control program 2003-2007 for Herd 4 ($/cow)

Year	No. Cows	Supplies	Management	Labor	Capital Investments	Total	Total (+ testing)
2003	75	$7.40	$18.00	$17.78	$0.00	$43.18	$72.18
2004	74	$2.18	$30.01	$25.14	$0.00	$57.33	$86.33
2005	77	-$41.44	$29.57	$24.16	$55.70	$67.99	$96.99
2006	72	-$39.85	$32.99	$25.83	$56.59	$75.56	$104.56
2007	68	-$38.00	$35.87	$26.65	$56.55	$81.07	$110.07
Ave.	73	-$21.94	$29.29	$23.91	$33.77	$65.02	$94.02

The management costs for this herd seemed extremely high when compared to the other, although larger, herds in the study. Initially, the producer reported spending a half hour each day managing JD and valued this time at $50 per hour. Even if this time was limited to weekdays, that equates to $85 per cow per year. As that seemed like an unrealistic number, further conference with the producer resulted in the above estimate, which was adjusted to reflect ten minutes per weekday spent managing the JD control program. While this still seems very high in comparison to the other herds, only the

producer knows what is happening on the farm. This demonstrates one of the issues with the method employed to determine the costs of the JD control programs in this study. The questionnaire used to collect information, particularly in regards to the management and labor sections, was dependent upon the producer recalling their daily routine and allotting time to a particular enterprise.

The labor category reflects costs associated with annual herd testing and additional fresh cow and calf care. Again, it is subject to the producer's recall and allotment of time to the JD control program.

The capital investment made by this farm was the purchase of a new loader tractor in January 2005. This purchase was financed, and the costs reflect the annuity value for the tractor as well as the annual interest paid. While the producer stated the tractor was purchased as a direct result of the JD control program; it should be noted that, from a JD control standpoint, the farm could have achieved the same outcome by purchasing a second bucket for their existing tractor at a much lower cost.

Economic losses due to JD 2003-2007

The annual estimated economic losses due to JD, along with the calculated, or assumed, benefits of the control program for Herd 4 are summarized in Table 4.19. The loss due to suboptimal culling (RPO) could not be calculated for this herd because individual cow production data was not available. Thus, the total losses due to JD are underestimated for this herd.

Table 4.19: Economic losses due to Johne's disease and assumed benefits of Johne's disease control program for Herd 4 – 2003-2007

($/cow)

Year	No. Cows	Milk Value	RPO	Cull Value	Total	Assumed Benefits
2003	75	$58.94	$0.00	$11.03	$69.97	N/A
2004	74	$68.87	$0.00	$8.31	$77.18	-$3.60
2005	77	$32.06	$0.00	$0.84	$32.90	$40.67
2006	72	$70.09	$0.00	$7.62	$77.71	-$4.14
2007	68	$56.40	$0.00	$3.53	$59.93	$13.64
Ave.	73	$57.27	$0.00	$6.27	$63.54	N/A

N/A Not applicable

NPV calculation

The NPV was calculated for four different scenarios: (1) assuming a linear decline in losses following year five and disease eradication by year 20 with the control program; (2) assuming losses and JD prevalence remain constant at year five levels while continuing the control program; (3) assuming linear increase in losses at rate equal to that in scenario 1 with no control program; and (4) assuming losses remain constant at same level as the beginning of the study with no control plan implemented. The results are shown in Table 4.20. The reason the NPV's for both scenarios 1 and 2 were negative was because, on average, the costs of the control program exceeded the potential benefits. Thus, mathematically, it appeared the control program had "negative" benefits that never exceeded zero when summed over the projected study period. However, when interpreting these figures, it should be remembered that the economic losses, and hence,

Table 4.20: NPV of four scenarios for Johne's disease (JD) control on Herd 4

Year of Program	Scenario*			
	1	2	3	4
5	-$225.08	-$227.63	-409.90	-$367.33
10	-$325.55	-$367.25	-$689.69	-$567.26
20	-$337.46	-$654.53	-$1,460.20	-$978.62
Payback Year	N/A	N/A	N/A	N/A

N/A Not applicable

Payback Year: Year of control program when NPV became positive

* Scenario 1: Assuming linear decline in losses caused by JD after year 4 due to declining prevalence and eventual eradication after implementation of JD control program

Scenario 2: Assuming losses caused by JD remain constant after year 4 while still investing in JD control program

Scenario 3: Assuming losses caused by JD increase at a rate equal to the decline in Scenario 1 in the absence of JD control program

Scenario 4: Assuming losses caused by JD remain constant at baseline level in absence of JD control program

the potential benefits, were underestimated because the RPO could not be calculated. Also, the JD control program was footing the entire cost of the new loading tractor. The tractor was likely being used for other enterprises on the farm, even though the producer stated the tractor would not have been purchased if not for the JD control program. As a result, the costs of the JD control program for this herd were robust. A more conservative estimate of costs could have been achieved by assigning the cost of the bucket for the tractor to the JD control program, and the remaining cost of the tractor to some other farm enterprise. The net result of these issues on the benefits and costs was to lower the NPV. The NPV's for scenarios 3 and 4 remain negative over the entire 20-year

projected study period because, in the absence of a control program, there was nothing to offset the losses caused by JD.

Results of sensitivity analysis

The break-even cost for the JD control program is an estimate of the amount of Money a farm can invest in the control program and still "break-even", or have a NPV equal to zero. The break-even costs were $38.86 for scenario 1 and $13.08 for scenario 2. Thus, had this herd been able to cut the cost of the JD control program by 40% and 80% respectively for scenarios 1 and 2, the NPV would have equaled zero; and cutting costs even more would have resulted in a positive NPV.

The NPV was most sensitive to the discount rate (r) used. After the discount rate, the cost of the JD control program, followed by milk price had the greatest effect on the NPV. When the individual categories within the JD control program were evaluated, the results were ranked depending on which category represented the highest proportion of the control program. For this herd, changing the input costs of capital investment had the greatest impact on NPV, followed by management, labor, and supplies respectfully.

Including testing in the calculations increased the input costs of the JD control program, and hence decreased the NPV. For scenario 1, the NPV decreased from -$337 to -$411 when ELISA testing only was included, and to -$682 when both ELISA and fecal culture was included. For scenario 2, the NPV decreased from -$655 to -$728 and -$999 respectfully, for including ELISA testing only or ELISA and fecal culture together.

Producer perception of the JD control program

Overall, the producer was pleased with the results of the JD control program, and planned to continue investing in it after the end of the study. Subjectively, herd health improved over the course of the study which resulted in increased production and increased revenues. An additional value resulting from the JD control program on this herd was seen in the marketing of herd replacements. Other producers were willing to pay more for cattle raised on this farm because of the JD control program the herd had implemented and the amount of diagnostic testing that was performed.

E. Herd 5

Farm background

This farm has been in existence at the present location for approximately 50 years. In 2004, when the herd enrolled in the Michigan Johne's Disease Control Demonstration Project, it was milking approximately 440 Holstein cows (484 total adult cows) with a RHA of 26,839 pounds. The farm was expanding with the following goals:

1. Milk 600 cows by 2006
2. Milk 1200 cows within 10 years
3. Build new facilities, including a new dry cow and maternity barn

As part of the expansion, the herd was purchasing cattle on a routine basis. Often entire herds were purchased, although they also contracted with a cattle broker to purchase cattle in their stead. As of 2004, approximately 25% of the adult herd had been purchased. The JD status of the cows or herds that were purchased was not considered. The first case of clinical JD was diagnosed in this herd in a two year-old purchased cow in 1999. Since that time, the number of cows diagnosed and being culled for JD had increased; and by 2004, JD had become a concern for the producer.

Aside from JD, the herd was experiencing the normal "expansion" woes, but no one particular problem seemed to stand out. Bulk tank SCC averaged around 200,000, and most of the mastitis problems were environmental in nature. When the herd expansion began around 2000, calves were moved off-site to a heifer grower's facility, where they are raised and bred. This grower raises heifers only for this farm. The heifers are returned to the home farm when they were 6-7 months pregnant.

JD risk assessment

Prior to the implementation of a JD control program, the area at greatest risk for disease transmission on this farm was the calving area. The farm had a group maternity pen that housed approximately 10-20 cows at all times. It was cleaned every 2-3 months, but was bedded "as needed," and the cows were not always clean. As it was a bedded pack near the parlor, sick cows with poor mobility were often kept in this area. There was no attempt to segregate cows suspected of having JD, and all cows calved in this pen. Calves were often left with the dam in the group pen for 12-24 hours, and allowed to nurse, before being moved to a remote calf barn with individual pens. The calves were

fed pooled colostrum and unpasteurized waste or whole milk. In January 2004, a pasteurizer was purchased, and calves were then fed pasteurized milk. Upon weaning, all calves were moved off-site to the heifer grower's facility, and returned to the home farm when they were 6-7 months pregnant. The springing heifers were housed in a barn separate from the lactating herd, but fed feed refusal from the adult cows.

JD control program

Upon enrolling in the Michigan Johne's Disease Control Demonstration Project, annual testing of all adult cows for JD with serum ELISA began. All cows testing positive were flagged in the computer for management. Cows with a positive ELISA had "one strike" against them in terms of culling. However, these cows were often bred back, and kept in the herd until they developed clinical signs of JD, some other problem warranting culling, or their production fell below some break-even point determined by the farm. The use of pooled colostrum was discontinued. Only colostrum from test negative cows was fed to heifer calves and banked for use as needed. A corner in the maternity pen was gated off to serve as a holding area for newborn calves until they could be moved to the calf barn. In 2007, a new maternity barn was built with individual calving pens. Cows were kept in the pens for the minimum time necessary, and one pen is still reserved as a holding area for calves. Calves were moved to this holding area as soon as possible after birth. This was achieved by offering employees a monetary incentive for moving the calves. This worked well, as the maternity area was located adjacent to the milking parlor, with employees passing by several times a day as they

moved cows for milking. Calves were still fed whole milk until weaning, but beginning in January 2004, all milk fed to calves was pasteurized.

Descriptive statistics

Descriptive statistics for Herd 5 are summarized in Table 4.21. Herd size increased by approximately one-third over the course of the study. This was accomplished mainly through the purchase of cattle. Herd size was fairly stable for the first three years of the study and milk production gradually increased. In 2007, a bunch of purchased cattle were added to the herd, and the result was a 5% production decrease. The cull data was incomplete for this herd, and the overall cull rate and mortality rate was not reported. The number of cows culled due to clinical signs of JD was reported, and decreased over the course of the study; although it remained consistently low. As long as cattle continue to be purchased and added to the herd with no regard to their JD status, it is likely this number will remain fairly consistent, or even increase in the future.

Table 4.21: Descriptive Statistics for Herd 5

Year	Herd size (adult cows)	RHA (lbs 3.5% FCM)	Culled due to clinical JD (%)
2004	484	26,839	2.5%
2005	500	28,470	2.6%
2006	497	29,668	0.6%
2007	641	28,232	0.5%

JD prevalence

The within herd JD prevalence trend, as determined by serum ELISA, is outlined in Table 4.22. For all practical purposes, the JD ELISA prevalence remained unchanged in this herd over the course of the study. This was not unexpected, given that a significant proportion of the herd was purchased from multiple sources with unknown JD status. In fact, the proportion of test positive cows that were purchased ranged from 26-50% per year. As long as cattle from herds with unknown JD status continue to be added to the herd, the best this herd can hope for is to maintain the JD prevalence at the current level.

Table 4.22: Johne's disease prevalence trends 2003-2007 for Herd 5

Year	Apparent JD prevalence (serum ELISA)
2003	NT
2004	5.6%
2005	5.4%
2006	6.4%
2007	4.0%

NT – Not Tested

Cost of the JD control program 2004-2007

The costs of the JD control program observed over the five years of this study are summarized in Table 4.23. As part of the Michigan Johne's Disease Control Demonstration Project, the herd did not have to pay for any JD testing beyond the cost of labor to collect samples on the day of the annual test. It is unlikely the herd would have

Table 4.23: Cost of Johne's disease control program 2004-2007 for Herd 5 ($/cow)							
Year	No. Cows	Supplies	Management	Labor	Capital Investments	Total	Total (+ testing)
2004	484	$6.11	$1.36	$4.73	$2.65	$14.85	$43.85
2005	500	$5.91	$0.74	$6.00	$2.56	$15.22	$44.22
2006	497	$5.95	$0.39	$6.04	$2.58	$14.95	$43.95
2007	641	$5.79	$0.31	$4.78	$2.00	$12.87	$41.87
Ave.	531	$5.94	$0.70	$5.39	$2.45	$14.47	$43.47

done the extensive testing that was performed if it had to pay for the testing, yet management decisions were made based on those test results, so for the sake of completeness, testing costs are included in the last column of the table.

The supplies category included the increased costs of operating the pasteurizer, such as electricity and sanitation supplies. Management included time the herd manager spent aiding in annual JD testing, inputting test results into the computer, and making buying and selling decisions. Labor included time spent aiding in JD testing, removing calves from dams as soon as possible after birth, pasteurizing milk, and sanitizing the pasteurizer. The capital investments made by this farm included the pasteurizer and a bulk tank to hold the milk after pasteurization.

Economic losses due to JD 2004-2007

The annual estimated economic losses due to JD, along with the calculated, or assumed, benefits of the control program for Herd 5 are summarized in Table 4.24.

Table 4.24: Economic losses due to Johne's disease and assumed benefits of Johne's disease control program for Herd 5 – 2004-2007

($/cow)

Year	No. Cows	Milk Value	RPO	Cull Value	Total	Assumed Benefits
2004	484	$23.61	$1.98	$3.99	$29.58	N/A
2005	500	$41.54	$2.82	$3.55	$47.91	-$9.16
2006	497	$27.48	$6.07	$2.00	$35.55	$3.20
2007	641	$17.51	$1.02	$0.71	$19.24	$19.51
Ave.	531	$27.54	$2.97	$2.56	$33.07	N/A

N/A Not applicable

NPV calculation

The NPV was calculated for four different scenarios: (1) assuming a linear decline in losses following year five and disease eradication by year 20 with the control program; (2) assuming losses and JD prevalence remain constant at year five levels while continuing the control program; (3) assuming linear increase in losses at rate equal to that in scenario 1 with no control program; and (4) assuming losses remain constant at same level as the beginning of the study with no control plan implemented. The results are shown in Table 4.25. The NPV for scenario 1 became positive in year nine, and that for scenario 2 became positive in year 14. The NPV's for scenarios 3 and 4 remain negative over the entire 20-year projected study period because, in the absence of a control program, there was nothing to offset the losses caused by JD.

Table 4.25: NPV of four scenarios for Johne's disease (JD) control on Herd 5				
Year of Program	Scenario*			
	1	2	3	4
5	-$17.89	-$20.26	-$167.57	-$154.71
10	$10.70	-$6.57	-$296.98	-$259.99
20	$128.39	$21.59	-$622.13	-$476.64
Payback Year	9	14	N/A	N/A

N/A Not applicable

Payback Year: Year of control program when NPV became positive

* Scenario 1: Assuming linear decline in losses caused by JD after year 4 due to declining prevalence and eventual eradication after implementation of JD control program

Scenario 2: Assuming losses caused by JD remain constant after year 4 while still investing in JD control program

Scenario 3: Assuming losses caused by JD increase at a rate equal to the decline in Scenario 1 in the absence of JD control program

Scenario 4: Assuming losses caused by JD remain constant at baseline level in absence of JD control program

Results of sensitivity analysis

The break-even cost for the JD control program is an estimate of the amount of money a farm can invest in the control program and still "break-even", or have a NPV equal to zero. The break-even cost calculated for scenario 1 was $24.90/cow/year, and for scenario 2, $16.21/cow/year.

The NPV was most sensitive to the discount rate (r) used. After the discount rate, milk price, followed by cost of the JD control program had the greatest effect on the NPV. When the individual categories within the JD control program were evaluated, the results were ranked depending on which category represented the highest proportion of

the control program. For this herd, changing the input costs of supplies had the greatest impact on the NPV, followed by labor, capital investments, and management respectfully.

Including testing in the calculations increased the input costs of the JD control program, and hence decreased the NPV. For scenario 1, the NPV decreased from $128 to $55 when ELISA testing only was included, and to -$216 when both ELISA and fecal culture was included. For scenario 2, the NPV decreased from $22 to -$52 and -$323 respectfully, for including ELISA testing only or ELISA and fecal culture together.

Producer perception of the JD control program

At the conclusion of this study, the producer was satisfied with the JD control program and planned to continue investing in it. Subjectively, herd health had improved over the course of this study, as had production, which led to increased revenues.

F. Herd 6

Farm background

This herd has been in existence at the current location since the early 1970's. In 2003, the herd was milking around 130 adult Holstein cows (145 total cows) with a RHA of 26,875 pounds (3.5% FCM). The herd had been completely closed for 30 years. The goals for this farm were:

1. Continue dairy farming
2. Transfer farm to next generation
3. Maintain a 28,000 pound RHA
4. Minimize JD in the herd
5. Expand

An ongoing threat to this farm was bovine tuberculosis (TB). This farm was located in the middle of the bovine TB zone in the northeastern lower peninsula of Michigan. Bovine TB had been diagnosed on an adjacent farm, and that herd had been depopulated twice since 1995. On this farm, a son had come of age, and wanted to continue dairying, but major improvements to, or replacement of, the existing facilities was necessary. While the farm was doing everything it could to protect the herd from becoming infected with TB; knowing it was nearby, and the uncertainty over the state's long term plan for eradicating or controlling TB (depopulation vs. test and cull), made the producer reluctant to invest a large amount of money into new facilities. So, over the course of this study, expanding was put on hold, and the existing facilities were repaired and remodeled to meet the herd's needs.

The first case of clinical JD was diagnosed in the herd in 2002, in a second lactation cow. In the year following, a total of six (4%) cows were culled due to clinical signs of JD. Given that both bovine TB and JD are caused by *Mycobacterium*, and there is potential for cross-reactivity between screening tests for the two diseases, controlling JD became a high priority for this herd.

Aside from JD, this herd reported occasional problems with metabolic diseases such as displaced abomasums, ketosis, fatty livers, and acidosis. Mastitis was generally not a problem, and the bulk tank SCC ranged between 200,000-300,000.

JD risk assessment

In terms of controlling JD, this herd was doing everything wrong. Cows were calved on a bedded pack in a group maternity pen. Manure often built up before fresh bedding was added, resulting in soiled udders and legs. All cows calved in this pen, regardless of their JD status, or suspected status. Also, sick and/or treated cows were housed in this pen, and milked in adjacent stanchions with a bucket milker until their milk was good to go into the bulk tank. Then they were moved back to the main herd. Once a cow calved she was moved with her calf to an individual pen where they would stay for 3-5 days until the cow's milk was okay to go into the bulk tank. During this time the calf was allowed to nurse the cow. Colostrum and waste milk was pooled and fed to all calves in the maternity barn. Once the dam was moved to the main herd, the calf remained in the maternity barn as long as there was waste milk available to feed it. Once waste milk was no longer available, the calf was moved to an individual pen in a calf barn and switched to milk replacer.

Once calves were weaned they were housed in a super hutch or group pen for approximately 1-2 months before being moved to a heifer barn that housed all replacements and the far-off dry cow group. Regardless of group, a common skid-steer bucket was used for feed and manure handling.

JD control program

Upon enrolling in the Michigan Johne's Disease Control Demonstration Project, annual testing of all adult cows for JD with serum ELISA and fecal culture began. All cows testing positive were visually identified with colored ear tags and/or neck chains. Cows positive on fecal culture and exhibiting clinical signs were culled immediately. Fecal culture positive cows not showing clinical signs were not bred back, and were culled when they either began to exhibit clinical signs, or their milk production fell to below a break-even point defined by the farm. Cows positive on serum ELISA had one strike against them, and were evaluated on a case by case basis.

In the winter of 2003, the maternity barn was remodeled. Sand-bedded free stalls took the place of the bedded pack to house the close-up dry cow group. Adjacent to the free-stalls were individual calving pens. Cows were moved to individual pens when they began to calve. An attempt was made to calve all JD test positive cows in a pen separate from where JD test negative cows calved. Pooled colostrum was no longer fed. Only colostrum from JD test negative cows was fed to heifer calves and frozen for use as needed. Heifer calves were removed from JD test positive dams as soon as possible. Otherwise, bull calves or calves born to JD test negative cows were allowed to stay with the dam until her milk was saleable and she was moved to the main parlor. Despite recommendations against this practice, it was the producer's belief that fresh cows transitioned better if they were allowed to stay with their calf for a couple of days.

In 2004, a second skid-steer was purchased, allowing one to be dedicated to feed handling and one to manure handling.

Descriptive statistics

Descriptive statistics for Herd 6 are summarized in Table 4.26. Herd size increased by approximately 20 cows, however it remained within the capacity of existing facilities. The overall cull rate was consistently lower than the state average of 37.7% (Hadley, et al 2006), and varied by less than 6 % throughout the study. The number of cows culled due to clinical JD increased and then decreased. This was consistent with the pattern set by JD prevalence. The mortality rate increased over the course of this study. The reason for this is unknown, as the data simply reported which cows died; it did not detail why they died.

Table 4.26: Descriptive Statistics for Herd 6

Year	Herd size (adult cows)	RHA (lbs 3.5% FCM)	Cull Rate (%)	Culled due to clinical JD (%)	Mortality Rate (%)
2003	145	26,875	ID	ID	ID
2004	143	27,987	30.1	2.8	5.6
2005	153	27,326	29.4	10.5	5.9
2006	169	26,593	32.5	5.3	7.1
2007	167	26,899	26.9	4.2	7.8

ID Insufficient data

JD prevalence

The within herd JD prevalence trend is outlined in Table 4.27. There was a dramatic increase in JD prevalence between 2003 and 2004. The reason for this increase was unknown, and samples were retested to rule out laboratory error. Test results

confirmed estimated prevalence, and coincided with the producer observing an increase in the number of cows exhibiting signs of weight loss and diarrhea. This explains the sharp increase in the number of cows culled due to JD, with over one third of the cows culled in 2005 being culled due to clinical signs of JD. Subsequently, the declining prevalence of JD was associated with a decreasing incidence of cows developing clinical signs of the disease.

Table 4.27: Johne's disease prevalence trends 2003-2007 for Herd 6

Year	Apparent JD prevalence (ELISA &/or FC positive)
2003	14.7%
2004	43.7%
2005	19.6%
2006	13.9%
2007	4.7%

Cost of the JD control program 2003-2007

The costs of the JD control program observed over the five years of this study are summarized in Table 4.28. As part of the Michigan Johne's Disease Control Demonstration Project, the herd did not have to pay for any JD testing beyond the cost of labor to collect samples on the day of the annual test. It is unlikely the herd would have done the extensive testing that was performed if it had to pay for the testing, yet management decisions were made based on those test results, so for the sake of completeness, testing costs are included in the last column of the table.

Table 4.28: Cost of Johne's disease control program 2003-2007 for Herd 6 ($/cow)							
Year	No. Cows	Supplies	Management	Labor	Capital Investments	Total	Total (+ testing)
2003	145	$0.34	$4.83	$0.62	$0.00	$5.79	$34.79
2004	143	$0.00	$7.58	$1.86	$27.88	$37.33	$66.33
2005	153	$0.00	$7.27	$1.74	$34.30	$43.30	$72.30
2006	169	$0.00	$6.86	$1.64	$29.70	$38.20	$67.20
2007	167	$0.00	$3.74	$1.66	$28.62	$34.02	$63.02
Ave.	155	$0.07	$6.06	$1.50	$24.10	$31.73	$60.73

The costs for the supplies category were minor, and only consisted of the purchase of colored neck strings to identify JD test positive cows. Management included extra time spent aiding annual JD testing, record keeping, and making capital investment decisions. Labor included extra time spent on fresh cow and calf care. The capital purchase made by this farm was second skid-steer bought in the summer of 2004. It was financed, so interest paid is also included in the costs for capital investments. Remodeling the maternity barn was necessary and planned prior to the JD control program, so none of its costs are included in the cost of the control program.

Similar to the purchase of a new tractor in Herd 4, assigning the entire cost of the new skid steer to the JD control program resulted in a robust estimate for the costs to this herd as compared to the other study herds. From a JD control standpoint, the objective of separate equipment to handle feed and manure could have been achieved with the purchase of a second bucket for the existing skid-steer, at less cost; even after adjusting labor costs to reflect the extra time required for changing the buckets between feeding

and cleaning pens. However, this study sought to estimate the actual costs of the JD control program as reported by the producer, and it was the producer's choice to buy the new skid-steer and assign that cost to the JD control program.

Economic losses due to JD 2003-2007

The annual estimated economic losses due to JD, along with the calculated, or assumed, benefits of the control program for Herd 6 are summarized in Table 4.29. Economic losses due to JD were calculated based on the calendar year. This herd was enrolled in the study in the fall of 2003, and annual testing occurred every fall thereafter. As a result, JD fecal culture results were generally not available until the following year, which is when management decisions based on those results were made. Thus, there often was a lag period between when JD prevalence was estimated and when the losses associated with that prevalence occurred. In other words, the estimated losses due to JD in 2004 were more reflective of the JD prevalence in 2003, and so on throughout the rest of the study. Also, availability of the herd data needed for this study was sketchy prior to study enrollment, which is why the estimated losses due to JD were so low in 2003.

Table 4.29: Economic losses due to Johne's disease and assumed benefits of Johne's disease control program for Herd 6 – 2003-2007

($/cow)

Year	No. Cows	Milk Value	RPO	Cull Value	Total	Assumed Benefits
2003	145	$0.00	$13.39	$4.99	$18.38	N/A
2004	143	$112.07	$43.03	$16.09	$171.19	-$27.51
2005	153	$182.96	$0.00	$43.24	$226.20	-$44.79
2006	169	$212.81	$15.85	$14.82	$243.48	-$20.85
2007	167	$194.71	$3.24	$21.59	$219.54	-$7.13
Ave.	155	$140.51	$15.10	$20.14	$175.76	N/A

N/A Not applicable

NPV calculation

The NPV was calculated for four different scenarios: (1) assuming a linear decline in losses following year five and disease eradication by year 20 with the control program; (2) assuming losses and JD prevalence remain constant at year five levels while continuing the control program; (3) assuming linear increase in losses at rate equal to that in scenario 1 with no control program; and (4) assuming losses remain constant at same level as the beginning of the study with no control plan implemented. The results are shown in Table 4.30. The estimated losses due to JD dictated the rate of increasing benefits projected over the remaining 20 years. The high estimated losses in 2007 for this herd resulted in rapidly increasing benefits in scenario 1, and led to a positive NPV by year 18. However, in scenario 2, the estimated losses beyond the observed study period were held constant at the rate equal to the losses in 2007. As the 2007 losses for this herd

exceeded its estimated baseline JD losses, the control program appeared to have "negative" benefits projected across the remaining 16 years of the calculation, resulting in a negative NPV. The NPV's for scenarios 3 and 4 remain negative over the entire 20-year projected study period because, in the absence of a control program, there was nothing to offset the losses caused by JD.

Table 4.30: NPV of four scenarios for Johne's disease (JD) control on Herd 6

Year of Program	Scenario*			
	1	2	3	4
5	-$202.46	-$211.79	-$949.26	-$793.32
10	-$201.91	-$354.67	-$1,781.75	-$1,333.24
20	$512.91	-$648.64	-$4,208.35	-$2,444.18
Payback Year	18	N/A	N/A	N/A

N/A Not applicable

Payback Year: Year of control program when NPV became positive

* Scenario 1: Assuming linear decline in losses caused by JD after year 4 due to declining prevalence and eventual eradication after implementation of JD control program

Scenario 2: Assuming losses caused by JD remain constant after year 4 while still investing in JD control program

Scenario 3: Assuming losses caused by JD increase at a rate equal to the decline in Scenario 1 in the absence of JD control program

Scenario 4: Assuming losses caused by JD remain constant at baseline level in absence of JD control program

Results of sensitivity analysis

The break-even cost for the JD control program is an estimate of the amount of money a farm can invest in the control program and still "break-even", or have a NPV

equal to zero. The break-even cost calculated for scenario 1 was $74.20/cow/year, and for scenario 2, -$19.22/cow/year.

The NPV was most sensitive to the discount rate (r) used. After the discount rate, milk price, followed by cost of the JD control program had the greatest effect on the NPV. When the individual categories within the JD control program were evaluated, the results were ranked depending on which category represented the highest proportion of the control program. For this herd, changing the input costs of capital investments had the greatest impact on the NPV, followed by management, labor, and supplies respectfully.

Including testing in the calculations increased the input costs of the JD control program, and hence decreased the NPV. For scenario 1, the NPV decreased from $513 to $439 when ELISA testing only was included, and to $168 when both ELISA and fecal culture was included. For scenario 2, the NPV decreased from -$649 to -$722 and -$993 respectfully, for including ELISA testing only or ELISA and fecal culture together.

Producer perception of the JD control program

At the conclusion of this study, the producer was satisfied with the JD control program and planned to continue investing in it. Subjectively, herd health had improved over the course of this study, as had production, which led to increased revenues.

Summary results

A. Farms and JD prevalence

Table 4.31 outlines each study herd in terms of herd size, breed, and housing management, while the within herd JD prevalence is shown in Table 4.32. Over the five-year course of this study, several changes occurred in the management of these farms, beyond the simple implementation of a JD control program that may have had some impact on the JD status of the herd. For example, in 2004, herd 2, went through the dissolution of a partnership, placing the farm in a tenuous financial position accompanied by management instability and labor issues as it transitioned to a sole proprietorship. More importantly, from a JD prevalence standpoint, all cows that had ever tested positive for JD were culled from the herd as part of the partnership buyout. Herds 3 and 5 underwent significant expansion during the course of this study. Both expansions occurred through the purchase of a substantial number of cattle, and neither farm gave any consideration to the JD status of the individual cows purchased, or their herds of origin. Herd 3 increased herd size by purchasing and consolidating three small herds, including all young stock, in a period of about four months in 2004; and then did not buy any more cows, although they did continue to purchase breeding-age bulls. Herd 5 also purchased a couple of smaller herds, but continued to buy cows through a cattle broker throughout the study. As a result of their respective expansions, both herds decided to contract their replacements to heifer growers. Herd 3 keeps heifers through weaning to four months of age before sending them to the grower, while Herd 5 sends heifers to the grower at weaning. Both growers only raise heifers for the respective farms. Herd 4 became certified organic in the spring of 2005, contracting their milk at more than double the price on the commercial market. Herd 6 had been an entirely closed herd for 30 years

prior to the start of the study and remained so throughout the study. However, there was an unexplained increase in JD prevalence from 15% to 44% between 2003 and 2004. Samples were retested to rule out lab error and verify the estimate of prevalence.

Table 4.31: Herd size, breed and housing management of study herds

Herd	Ave. herd size	Herd size		Breed	Housing
		Start	End		
1	191	170	215	Holstein	Confinement/grazing
2	125	103	137	Holstein	Total confinement
3	378	218	458	Holstein	Confinement/grazing
4	73	75	68	Jersey	Rotational grazing (organic)
5	531	484	641	Holstein	Total confinement
6	155	145	167	Holstein	Total confinement

Table 4.32: Johne's Disease test prevalence (fecal culture and/or ELISA positive) for herds during observed study period 2003-2007

Year	Herd					
	1	2	3	4	5	6
2003	12%	10%	11%	12%	NT	15%
2004	24%	4%	8%	9%	6%	44%
2005	22%	5%	13%	4%	5%	20%
2006	10%	9%	12%	8%	6%	14%
2007	15%	4%	16%	7%	4%	5%

NT: Not tested

B. Cost of the JD control program

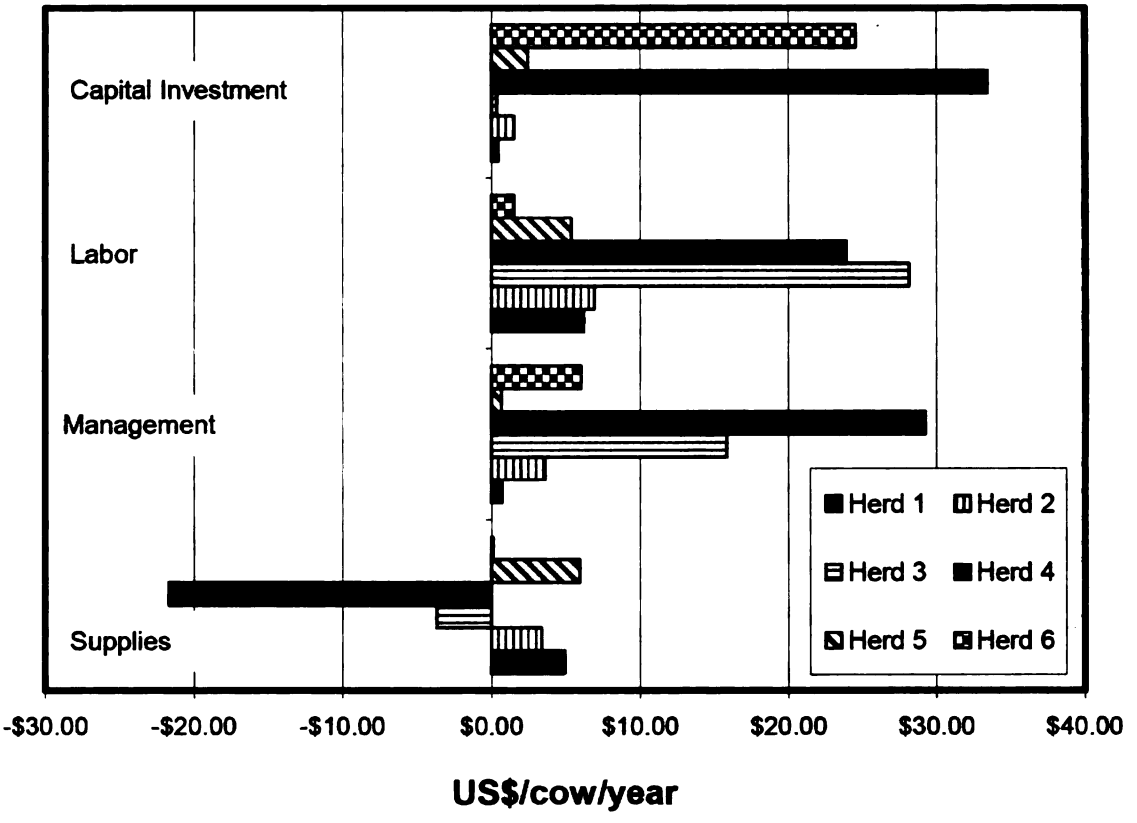
The annual costs of the JD control program for each herd are summarized in Table 4.33. The range was $5.79 - $81.07/cow/year with an average cost of $30.33/cow/year and median cost of $23.71/cow/year. Figure 4.1 shows the average annual costs of the JD control program broken down by category. While how much each herd spent varied greatly by category, many of the herds spent the greatest proportion of their money on labor and management. The negative supply cost for herds 3 and 4 is due to the adjustment to costs for additional milk sold by switching from whole milk to milk replacer to feed calves. In other words, the cost of milk replacer was less than the market value of an equivalent amount of whole milk. Of course, herd 4 was the organic Jersey farm, so they were receiving, on average, over twice as much for their milk as compared to the other herds.

Table 4.33: Annual Cost of Johne's disease control programs implemented by study herds 2003-2007. All costs represent $/cow in herd.

	Herd					
Year	1	2	3	4	5	6
2003	$16.24	$23.57	$58.22	$43.18	NC	$5.79
2004	$16.29	$14.77	$41.46	$57.33	$14.85	$37.33
2005	$10.88	$15.37	$39.68	$67.99	$15.22	$43.30
2006	$10.05	$17.10	$41.77	$75.56	$14.95	$38.20
2007	$9.27	$7.68	$31.55	$81.07	$12.87	$34.02
Average	$12.54	$15.70	$42.54	$65.02	$14.47	$31.73

NC: Not calculated

Figure 4.1: Average costs of Johne's disease control program 2003-2007 broken down by category. Values are shown in $/cow/year.



C. Economic losses due to JD

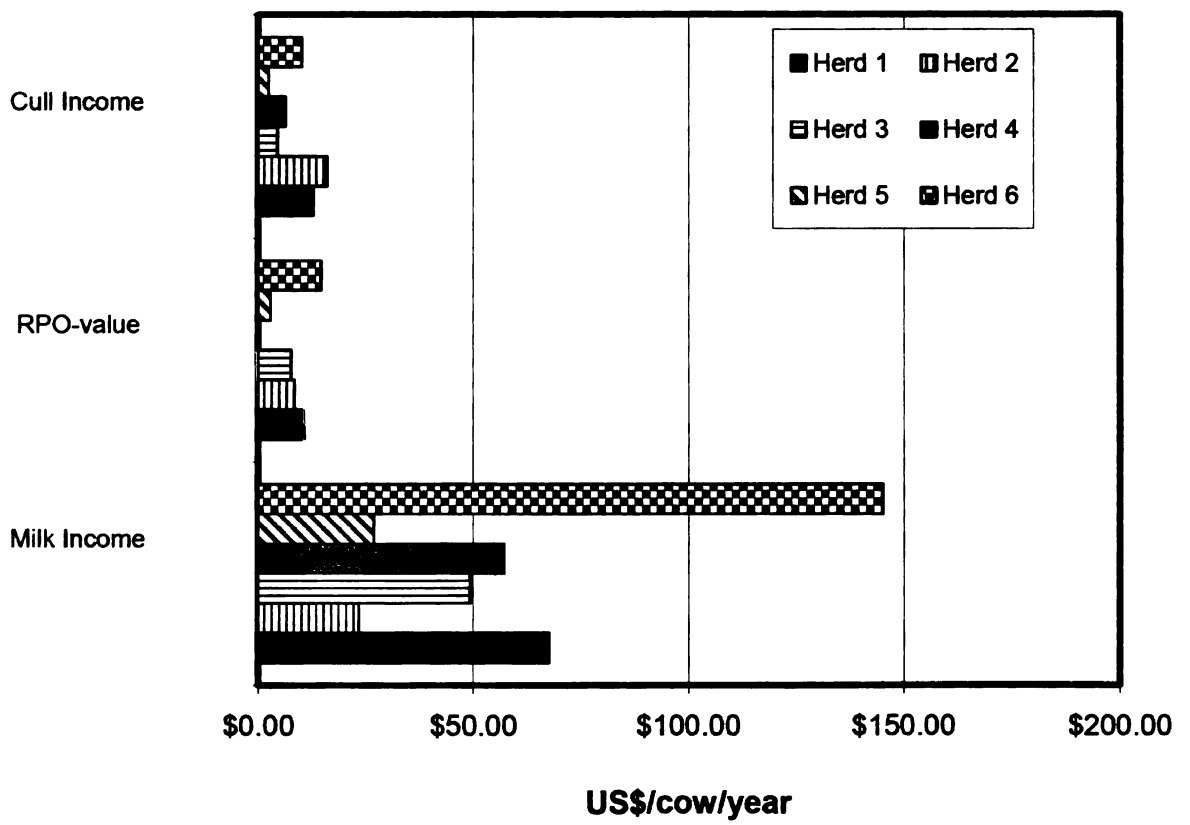
The annual losses due to JD for each herd are summarized in Table 4.34. The range of the losses was $15.85 - $243.48/cow/year with an average loss of $79.31/cow/year and a median loss of $66.17. Figure 4.2 shows the average annual losses due to JD broken down by category. As with the costs of the JD control program, the losses due to JD also varied greatly across herds. The one consistent thing in all herds was that the highest proportion (49 - 90%) of the JD economic losses was due to lost income from decreased milk production.

Table 4.34: Annual losses due to Johne's disease for study herds 2003-2007. All values represent $/cow in herd.

Year	Herd 1	2	3	4	5	6
2003	$98.95	$15.85	$17.86	$69.97	NC	$18.38
2004	$138.53	$48.67	$20.54	$77.18	$29.58	$171.19
2005	$79.97	$18.05	$55.75	$32.90	$47.91	$226.20
2006	$83.57	$82.13	$118.29	$77.71	$35.55	$243.48
2007	$60.23	$66.17	$66.54	$59.93	$19.24	$219.54
Average	$92.25	$46.17	$55.80	$63.54	$33.07	$169.85

NC: Not calculated

Figure 4.2: Average losses due to Johne's disease 2003-2007 broken down by category. Values are shown in $/cow/year.



D. NPV results

The NPV of the JD control program with a 20-year projection horizon for each of four different scenarios for each herd is summarized in Table 4.35. For scenario 1, in which JD control program was assumed to result in disease eradication after 20 years, only three of the six herds (herds 1, 5, and 6) had a positive NPV. The average NPV for all herds under scenario 1 was $24 per cow with a median of -$41 per cow. For scenario 2, in which it was assumed that, in spite of continued investing in the JD control program, the losses caused by the disease would remain at the level observed in 2007 for the remainder of the 20-year projection period, only 2 herds (herds 1 and 5) had a positive NPV. The average NPV for all herds under scenario 2 was -$411 per cow and a median of -$604 per cow. The NPV's for scenarios 3 and 4 were negative for all herds, as there were no benefits because no control program was implemented. These two scenarios simply estimated the potential economic losses should the farm do nothing for JD control.

E. Results of sensitivity analysis

The break-even cost for the JD control program is an estimate of the amount of money a farm can invest in the control program that will "break-even", or result in a NPV equal to zero. The break-even costs were calculated for scenarios 1 and 2 for each herd and are shown in Table 4.36. All the break-even costs for herds 2 and 3 are negative, suggesting that, given the assumed benefits outlined in each scenario, no amount of money invested into a JD control program will yield a zero NPV. On the other hand, if herd 4 could decrease the cost of its JD control program by 40% and 80% for scenarios 1

Table 4.35: Net present value of Johne's Disease (JD) control program after 20 years for four different scenarios. All values are $/cow in herd.

Scenario*	Herd					
	1	2	3	4	5	6
1	$778.53	-$209.42	-$730.64	-$337.46	$128.39	$512.91
2	$459.88	-$559.51	-$1,082.68	-$654.53	$21.59	-$648.64
3	-$2,050.21	-$947.91	-$777.28	-$1,447.79	-$656.87	-$4,362.72
4	-$1,577.53	-$428.60	-$255.08	-$1,019.31	-$514.77	-$2,639.71

* Scenario 1: Assuming linear decline in losses caused by JD after year 4 due to declining prevalence and eventual eradication after implementation of JD control program

Scenario 2: Assuming losses caused by JD remain constant after year 4 while still investing in JD control program

Scenario 3: Assuming losses caused by JD increase at rate equal to that in Scenario 1 in absence of JD control program

Scenario 4: Assuming losses caused by JD remain constant at baseline level in absence of JD control program

Table 4.36: Break-even point for cost of Johne's disease control program for scenarios 1 and 2. All values are in $/cow/year.

Scenario*	Herd					
	1	2	3	4	5	6
1	$75.64	-$1.81	-$17.85	$38.86	$24.90	$75.20
2	$49.74	-$30.27	-$46.46	$13.08	$16.21	-$19.22

* Scenario 1: Assuming linear decline in losses caused by JD after year 4 due to declining prevalence and eventual eradication after implementation of JD control program

Scenario 2: Assuming losses caused by JD remain constant after year 4 while still investing in JD control program

and 2 respectively, the expected NPV would equal zero; and cutting costs even more would result in a positive NPV.

Across all herds, the NPV was most sensitive to the discount rate (r) used. After the discount rate, milk price, followed by cost of the JD control program had the greatest effect on the NPV for all herds except herds 3 and 4. For those two herds the reverse was true, aside from the discount rate, the cost of the JD control program had the greatest effect on the NPV with milk price following. When the individual categories within the JD control program were evaluated, the results varied between herds depending on which category represented the highest proportion of the control program (Figure 4.1).

The NPV calculations including JD testing are shown in Table 4.37 and can be compared to the NPV's calculated without testing in Table 4.35. Including testing in the calculation increased the cost of the JD control program and hence decreased the NPV. Although not shown, the NPV value for running fecal culture alone would fall in between that of running the ELISA alone and running the two tests in parallel.

F. Producer perceptions of JD control program

At the end of the five-year study period producers were asked for their assessment of the JD control program implemented on their farms. All six producers stated that they were satisfied with the program and planned to continue it after the conclusion of the study. In support of this decision was the number of cows with clinical JD had decreased, and there was a feeling that overall herd and calf health had improved. Five of the six herds reported that because of improved herd health, production had improved resulting in increased income. Herd 2 reported not seeing any increase in revenue as a

Table 4.37: Net present value of Johne's Disease (JD) control program after 20 years for four different scenarios including testing. All values are $/cow in herd.

Scenario*	Herd											
	1		2		3		4		5		6	
	FC & ELISA	ELISA only	FC & ELISA	ELISA only	FC & ELISA	ELISA only	FC & ELISA	ELISA only	FC & ELISA	ELISA only	FC & ELISA	ELISA only
1	$434	$705	-$554	-$283	-$1075	-$804	-$682	-$411	-$216	$55	$168	$439
2	$115	$386	-$904	-$633	-$1427	-$1156	-$999	-$728	-$323	-$52	-$993	-$722

* Scenario 1: Assuming linear decline in losses caused by JD after year 4 due to declining prevalence and eventual eradication after implementation of JD control program

Scenario 2: Assuming losses caused by JD remain constant after year 4 while still investing in JD control program

FC: Fecal culture

result of the control program, but was optimistic that would change as herd health improved. Two of the herds (herds 1 and 4), claimed additional value from the control program in the marketing of herd replacements. Other producers were willing to pay more for cattle from these herds because of the JD testing and control practices they had in place. Moreover, after five years, the practices put in place to control JD on these farms had become standard operating procedure, making the control program more efficient to the point that the producers give little thought they were putting any extra effort into JD control.

4.5. Discussion

This study is one of the first field studies attempting to quantify the costs and benefits of implementing JD control programs on infected dairy farms. The NPV of the JD control programs implemented on these herds, assuming disease eradication in 20 years (scenario 1, Table 4.35) ranged from -$731 to $779 per cow with an average of $24 per cow and a median of -$41 per cow. So when considered as a whole, it was estimated that the herds in this study would basically break-even after investing in a JD control program for 20 years and assuming JD eradication. However, care must be taken in interpreting these results; as there is a great deal of difference between a NPV of $779 and a NPV of -$731. These differences should be examined because, regardless of what the NPV's predict, all the producers in this study were satisfied with the results of the JD control program they had implemented after five years, and planned to continue investing in it. Thus, suggesting there were some collateral benefits of the JD control program (such as improved calf health) that were not accounted for in the NPV calculation; or

producer perception is sometimes as, or more, important than mathematical calculations, when decisions are made at the farm level.

Arguments can be made for and against the assumptions used to create the four scenarios summarized in Table 4.35. No one can predict exactly what effect implementing management practices to prevent JD transmission will have on disease prevalence or losses on a given herd over an extended period of time. Instead assumptions have to be made. The assumptions for defining the four scenarios in this study were made to demonstrate the best case and worse case scenarios of implementing, and not implementing, a JD control program. Reality will fall somewhere in between the best case and worst case, but at least the exercise provides some guidelines when decisions have to be made. Additionally, following these herds for another 3-5 years would help take some of the uncertainty out of the estimates, and minimize the assumptions that had to be made to calculate the NPV.

In general, if making decisions based only on NPV, the option with the highest NPV is the best option to choose. When comparing the four NPV calculations outlined for each herd in Table 4.35, the NPV for scenario 1 (invest in a JD control program assuming eventual disease eradication) was the highest for all herds except herd 3. For herd 3, scenario 4 (assume the ongoing losses caused by JD remain constant in the absence of a control program) had the highest NPV, although the reality of losses remaining constant in the absence of efforts to prevent JD transmission is subject to argument. A discussion of why herd 3 differed from the other herds, along with why the NPV's for some of the herds were negative, is warranted and follows.

The NPV, as calculated in the four scenarios in this study, was highly dependent on the starting baseline value against which estimated future benefits of the control program were compared, the losses estimated for the final year (2007) of the study, and the average cost of the JD control program. The baseline value was important because it was the starting point for all NPV calculations, and was assumed to be a measure of the herd's losses caused by JD in the absence of a control program (doing nothing). It was further assumed, that if the JD control program was working, the losses in subsequent years would decline as disease prevalence declined. Therefore, the difference between the baseline and the losses in subsequent years would be an estimate of the "benefits" of the control program. If the baseline value was lower than the losses in subsequent years, then mathematically the control program had a negative benefit which, when the annual cost of the control program is subtracted, yields an even greater negative cash flow for that particular year. This in turn will result in a lower NPV than had the baseline been higher such that "negative" benefits were avoided. This is part of the reason the NPV for herds 2 and 3 were negative; their baseline values were lower than the losses due to JD in the subsequent study years.

The losses caused by JD in the final year (2007) of the study were important because the magnitude of this loss dictated the forecasted rate of increase in benefits in scenario 1 and decrease in benefits in scenario 3, as well as the constant benefits in scenario 2. A high ending value for losses in 2007 would result in a rapidly increasing rate of annual benefits in scenario 1 which would have a positive effect on NPV (as observed in herd 6, Table 4.35), and a rapidly decreasing rate of benefits in scenario 3. In scenario 2, as compared to scenario 1, herd 6 went from a positive NPV of $513 to a

negative NPV of $649. The reason for this was because the losses due to JD in 2007 exceeded its baseline level, resulting in a negative effect on the NPV as discussed previously. Mathematically, because losses, and thus benefits, are held constant at 2007 levels, the NPV in scenario 2 will always be less than that of scenario 1. The greater the magnitude of the losses in 2007, the greater the difference in NPV's between the two scenarios.

The annual cost of the JD control program is important to the NPV calculation because it represents the input cost that must be exceeded by its benefits to result in a positive NPV. The herds that invested the most money in their JD control program (herds 3 and 4, Table 4.33) had the lowest NPV's. In fact, in herd 4, the average annual cost of the JD control program exceeded the average annual losses caused by the disease; although the losses for this herd were underestimated because individual cow information was unavailable, which precluded estimating losses due to premature culling (RPO). Herd 1, the herd with the highest NPV, invested the least amount of money (average $12.54/cow/year) into its control program. Herd 6, while having the third highest costing JD control program in the study, still maintained a positive NPV in scenario 1. This was because the losses, and thus the potential benefits, were extremely high due to high prevalence. As with all things, there is a limit to just how much money can be reasonably spent controlling a disease. This limit is determined by marginal costs and benefits. In other words, the additional cost of lowering JD prevalence by 1% should not exceed its benefits. Table 4.36, shows the amount of money each herd could spend on JD control and still break even under the assumptions in scenarios 1 and 2. It shows that had herd 4 cut its JD control costs, the NPV of the control program could have been positive.

While it is important to understand the mathematics behind a negative NPV, it is probably more important to understand what was happening on the farm that resulted in the numbers that led to that value. This goes beyond simply looking at JD prevalence (Table 4.32), although that is a start. For example, in herd 2, JD prevalence decreased in 2004, but the decrease in losses caused by the disease was not seen until 2005, and both increase again in 2006. All of this is a reflection of the partnership buyout. The annual herd test occurred in the fall of 2004, after all previous test positive cows were culled in the spring and summer of that year, hence the low prevalence. However, as all the previous test positive cows were culled in 2004, their losses were included for that year and the decrease in losses resulting from the decreased prevalence were not seen until 2005. This mass culling of all JD test positive cows had another consequence for the herd. It only removed the cows previously detected by testing. Infected cows remained in the herd, and as the disease progressed past the detection threshold, prevalence again increased. However, the buyout put the herd in a tenuous financial position, and more cows were milked to meet cash flow needs. Thus, infected cows that would have been culled previous to the buyout were retained in the herd. Keeping infected cows in the latter years of this study increased the annual JD losses above baseline for this herd, which adversely affected the NPV calculations.

Herd 3 had the lowest NPV calculations for the JD control program; -$731 and -$1083 for scenarios 1 and 2 respectively. The mathematics behind the negative NPV's has already been discussed. On face value, based on NPV as well as a relatively unchanged JD prevalence, it would appear that the control program on this herd failed. However, that is not necessarily the case. Recall that during expansion, this herd

purchased three smaller herds, including all young stock. This occurred in 2004. The JD control program implemented on this farm in the summer of 2003 focused primarily on preventing MAP infection at birth and shortly thereafter. The purchased cattle, including the vast majority of the young stock, were never exposed to this farm's JD control program. In 2007, it was estimated that over half of the adult milking herd consisted of purchased cattle. As late as 2006, purchased heifers were entering the herd that had not been exposed to a JD control program as calves. Therefore, the annual JD losses estimated in this study, and thus the NPV calculations, do not truly reflect this farm's investment in a JD control program, but rather the lack of a JD control program in the purchased herds. Unfortunately, inadequate herd records prevented the separation of purchased cows and cows raised on the farm. To get a more accurate estimate of the costs and benefits of the control program on this herd, the study would have to be continued for a few more years; until the infected purchased cows are culled and replaced by heifers raised with this farm's JD control program in place. The bottom line is, making judgments on the value of implementing a JD control program based solely on a NPV calculation is dangerous unless one understands what is actually happening on the farm influencing the numbers used in that calculation.

The sensitivity analysis showed that the NPV calculation was most sensitive to changes in the discount rate. The discount rate, reflecting the opportunity cost of capital in agriculture is generally lower than that for other industries, and was assumed to be 8% in the base calculations. Increasing the discount rate will increase the denominators in the NPV calculation which will decrease the absolute value of the present value of the cash flows and vice versa. The net effect changing the discount rate has on the NPV will

depend on the signs (positive or negative) of the net cash flows, both present and future, and their summation over time.

Following the discount rate, the NPV was most sensitive to the milk price for herds in which the losses, or inversely, the benefits, were proportionally higher than the costs of the JD control program (herds 1, 2, 5, and 6). As milk price increased, so did the potential economic benefits, which in turn increased NPV. This is consistent with the fact that the majority of economic losses due to JD, and hence benefits, is due to decreased milk production (Figure 4.2). For these herds, following the discount rate, and milk price, the cost of the JD control program was the next most influential factor on the NPV. Increasing the cost of the control program cuts into potential profits, which will lower the NPV, and vice versa. The category of the JD control program that influenced the NPV the most varied across herds, but was consistent with the proportion each category contributed to the total cost of the control program as shown in Figure 4.1. In other words, the greater proportion a particular category contributed to the total cost of the control program, the greater influence it had on the NPV. For herds 3 and 4, where the cost of the control program exceeded or was almost equal to the losses caused by the disease, the cost of the JD control program swapped places with milk price in the sensitivity analysis.

During this study the producers did not have to bear the cost of the diagnostic testing. Yet, they were informed of the test results, and many used this information to make culling and management decisions, which ultimately affected the benefits of the control program estimated here. Including testing in the calculation, increased the input costs, and decreased the NPV's across the board for all herds (Tables 4.35 and 4.37). It is

unlikely any of the herds will continue the intensive whole herd testing with both fecal culture and ELISA now the study is over. It is more likely they will use some form of targeted sampling of high-risk cows using the less expensive ELISA test. It was for the sake of completeness and comparison that testing was included in the sensitivity analysis.

Annual losses due to JD have been reported in several previous studies (Meyer and Hall, 1994; Ott, et al., 1999; Chi, et al., 2002). However, studies differ in their design and methodology, so it is always a good idea to compare your results to those previously published to see if they are comparable, and, if not, determine why. The average annual losses due to JD in this study are summarized in Table 4.34. When standardized to a common milk price and cull price as reported by Ott, et al. (1999), the average annual loss due to JD in these herds was $61.24/cow/year. This was in agreement to the $61/cow/year lost reported by Ott, et al. (1999) given the prevalence of JD in the herds.

In general, as the within herd JD declined so did the losses incurred by the disease (Tables 4.32 and 4.34) and vice versa, although sometimes there was a lag period depending on when the losses actually occurred in relation to testing to determine herd prevalence. Losses were calculated based on a calendar year. Annual JD prevalence was based on a single whole herd test occurring at the same time each year. As previously noted, in 2004, the losses for Herd 2 increased even though the JD prevalence declined to its lowest rate. This was due to the mass culling of all JD test positive cows as part of the partnership buyout that occurred prior to the annual test to determine prevalence. Herd 6 is another example where there appears to be a disassociation between JD prevalence and losses. This herd enrolled in the study the fall of 2003, and annual testing occurred each

fall thereafter. In 2003, the JD prevalence was 15% and losses reported at $18/cow. Enrollment late in the year, combined with incomplete data availability prior to enrollment, explains the low estimate of losses due to JD in 2003. In subsequent years however, fecal culture results, and thus management related to those results, were often not available until January of the following year. Thus the losses reported in 2007 ($220/cow) when prevalence reached its low point of 5%, more accurately reflects the 14% prevalence reported in 2006.

Other factors affecting the magnitude of losses due to JD from one year to the next were the milk and cull prices. Cull prices were fairly consistent ranging from $0.47 - $0.54/pound. Milk price, however, was particularly volatile during this study, ranging from an average of $12.64/cwt in 2003 to $18.39/cwt in 2007. The change in milk price was even greater for Herd 4 once becoming certified organic and contracting its milk for $34/cwt beginning in 2005.

To our knowledge this is the one of the first studies that has attempted to report the costs of controlling JD (Table 4.33). A study using similar methods to evaluate the costs and benefits of vaccinating for JD was performed with favorable results (van Schaik, et al., 1996). In this study, the average annual costs of the JD control programs implemented on these farms ranged from $13 - $65 per cow, with an average of $30 per cow and median of $24 per cow. This can be compared to the annual losses summarized in Table 4.34 that ranged from $33 - $170 per cow, with an average of $74 per cow and median of $52 per cow. Only in herd 4 did the average annual cost of the JD control program exceed the average annual losses due to the disease. Therefore, if the complicated NPV calculations previously discussed are ignored, looking at losses as

potential benefits, and given that costs were, on average, less than the benefits for these herds, it would seem that JD control is economically feasible. However, as the NPV calculations demonstrate, first impressions can be deceiving; although, consideration of what is happening on the farm to generate the numbers is as important as the actual calculation.

Stott, et al. (2005) reported there was little financial incentive for dairy producers to invest in controlling JD. This study would seem to contradict that. While investing in a JD control program might not guarantee much, if any, return on investment over time, doing nothing (scenarios 3 and 4, Table 4.35) was certainly a worse option when predicted NPV's are compared. Even when cows with clinical signs of JD are not observed, and within herd prevalence is relatively low, significant economic losses can still occur due to subclinical JD. For example, Herd 4 had no cows with clinical JD in 2007 with only 7% prevalence (Table 4.32), yet still lost an estimated $60/cow in the herd due to JD (Table 4.34). Most of that loss (87%) was attributed to lost milk production. This was the organic herd which was receiving $34/cwt at the time, as compared to the other herds in the study, which were averaging $18.39/cwt. Adjusting herd 4's loss to reflect the milk income lost on the commercial market still resulted in a loss of over $34/cow in the herd. Johne's disease is an insidious disease that causes losses even when not observed clinically, and will not just go away without some effort.

Finally, there were only six herds in this study, and they were not randomly chosen. Moreover, the results for six herds in Michigan are likely different than those for six herds in California, based on differences in climate, herd size, and herd management; so these results should not be extrapolated to the overall dairy farm population.

Additionally, economic decisions regarding JD control should be made on an individual farm basis. So while the actual results from this study are not broadly applicable to all dairy farms, the principles for the economic evaluation of JD control programs are.

4.6. Conclusion

The primary purpose of this paper was to describe one method for evaluating the cost-effectiveness of implementing management practices to control JD on infected dairy herds. The NPV for the implemented JD control programs varied across the herds, with some results suggesting JD control is very cost-effective (positive NPV in three of six herds) and others suggesting it is not (negative NPV in three of six herds). Regardless of the NPV, all the producers in this study felt the JD control program was a good investment, and planned to continue the program after the end of the study. Making financial investment decisions should be performed on a case-by-case basis. The one consistent thing across all the herds in this study, from a purely economical perspective, was that doing something to control JD was always a better option than doing nothing.

CHAPTER 5

OVERALL SUMMARY

5.1. Introduction

In May 1922, the following statements appeared in the University of Wisconsin Agricultural Experiment Station Bulletin (Bulletin #343):

> "Johne's disease is not at all widespread. .....It does occur, however, and as the years go by it will become more and more common and will place a great tax on the cattle industry."

Unfortunately, this prediction has come true. Johne's disease (JD) is becoming increasingly prevalent in the US with over 68% of its dairy herds infected. Largely ignored by the cattle industry until recent years, JD control has now become a priority due to the economic losses being incurred by the disease and the growing body of evidence linking the causative agent, *Mycobacterium avium paratuberculosis* (MAP), to Crohn's disease in people.

Over the years, extensive research on JD has been performed. Because of the protracted course of the disease, most of this research has been experimental, cross-sectional, or simulated in nature. While such studies are necessary, and have been invaluable in creating the foundation on which current JD control recommendations are made; the next step is to validate if the recommendations for JD control (which are really theories) actually work over time in a natural farm setting.

This research project was a longitudinal, observational study of seven MAP infected dairy herds. The goal this project was to answer the following four questions to better understand JD in a natural farm setting over time, particularly after the implementation of a control program:

5) What is the extent of the MAP infectious burden in the environment of infected farms in relation to the JD burden in the herd, and does it change over time?

6) Do farm management practices designed to limit the transmission of MAP infection actually decrease the JD burden in a herd over time?

7) What specific management practices are the most effective in decreasing the JD burden in a herd?

8) Are management practices to control JD cost effective?

The following summarizes the findings of this research project in an effort to answer these questions.

5.2. What is the extent of the MAP infectious burden in the environment of infected farms in relation to the JD burden in the herd, and does it change over time?

Central to any infectious disease control program is identifying the reservoir for the agent, and, if possible, eliminating it. In the case of JD, there are two primary reservoirs: the environment and infected cattle. It has been well documented that MAP can survive for months to years under various environmental conditions that commonly

occur on dairy farms. Also, the primary route of infection for susceptible cattle is the ingestion of MAP from a contaminated environment. Thus, understanding what areas on infected farms are commonly contaminated with MAP, and how that distribution changes as the number of infected cattle in the herd changes over time, will provide important information on the extent and replenishment of the environmental reservoir. This, in turn, will provide insight into where control efforts should be focused to minimize exposure of susceptible calves to MAP.

In this study the environment of seven MAP infected dairy herds was serially cultured over four years. Samples of feed, water, and flooring were collected every six months from the: pre-weaned calf, post-weaned heifer, maternity, and lactating cow areas, as well as manure storage and pasture when applicable. Eighty-one (11%) of the 731 samples collected were culture positive for MAP. The areas most commonly contaminated with MAP were the lactating cow floor and manure storage area. This was not surprising, as these are areas where manure from adult cows, the animals most likely to be shedding the bacterium, is concentrated. Preventing young calves, the animals most susceptible to becoming infected, from being exposed to these areas is not difficult and is commonly practiced on most dairy farms. More concerning was the fact that 16% of the positive environmental samples originated from maternity area. This is the one area on the farm where calves cannot be completely isolated from all adult cows, certainly not from the calf's own dam. The objective then is to minimize the time the calf spends in this area, and keep it as clean as possible to limit exposure of the calf to MAP.

When all the data was compiled, there was a trend for the number of culture positive samples to increase as herd prevalence increased (Figure 2.1). Once within herd

prevalence fell to below 2%, MAP was never cultured from any area sampled. This does not mean that the environment was not contaminated with MAP. More likely the volume of MAP was below the detection threshold of the sampling procedure and culture methods used. When herd prevalence was >2%, MAP was cultured from the lactating cow floor and/or the manure storage area 75% of the time. Prevalence only had to increase to just over 5% before MAP started being cultured from other areas on the farm, the most common being the maternity area.

In summary, MAP was widely distributed in the environment of infected dairy farms. As the within herd prevalence increased, so did the number and distribution of culture positive samples on the farm. This was expected as the greater the number of MAP infected cows in a herd, the greater the potential for replenishing the environmental reservoir.

5.3. Do farm management practices, designed to limit the transmission of MAP infection, actually decrease the JD burden in a herd over time?

With the environmental reservoir for MAP investigated, the next step was to study the source that replenishes it, MAP infected cattle. Farm management practices recommended for the prevention and control of JD are well documented, but there is little data from real-life farm settings validating their effectiveness. Again, this is due mainly to the slowly progressive and chronic nature of JD that would require such studies be

conducted over a period of many years. This project is one of the few studies that has endeavored to validate the effectiveness of JD control programs implemented on naturally-infected dairy herds over a period of several years.

This project was a series of intervention studies; one for each of the seven herds. A logical way of assessing the effectiveness of the intervention, or JD control program, is to compare some measure of disease before and after the implementation of the control program. As most of the management practices implemented to control JD focused on minimizing transmission of MAP in young calves, the comparison was the prevalence or incidence of JD in cows born after the control program to that in cows born prior to the control program.

Regarding JD prevalence, the relative risk (RR) was calculated for each herd. In all instances, the risk of testing positive for JD was lower for cattle born after the start of the control program than in cattle born before the control program (Table 3.11); providing summary evidence that the JD control programs were effective in preventing JD transmission.

Another way to assess the effectiveness of the control programs implemented was to compare the JD incidence by lactation between cows born before and after the control program. If the JD control program was working, disease burden in the herd would be reduced, as would MAP exposure, which would lower the infectious dose and delay the onset of disease. Thus, not only would the number of infected cattle be lower in cows exposed to the control program, the age at which they are detected will increase. Over the first three lactations, the incidence of JD in cows exposed to the control program as calves was consistently less than in cows not exposed to the control program. This

provided further evidence that the JD control programs in these herds were successful in minimizing disease burden.

5.4. What specific management practices are the most effective in decreasing the JD burden in a herd?

Once it was verified that the control programs put in place on the study herds were successful in preventing or minimizing JD transmission, the next step was to determine which of the multiple management practices implemented were most effective in preventing disease. If one looks at the recommendations to control JD, the list is quite lengthy. This can overwhelm producers if presented in its entirety with no ranking, or prioritizing, of the practices in terms of "getting the most bang for the buck" so to speak. Convincing producers to adopt JD control programs is easier if management practices can be invested in over time, with the changes having the greatest impact on preventing MAP infection being implemented first.

The specific management practices put in place to control JD, and the extent to which they were implemented, were unique to each herd and varied greatly; making it impossible to look at each practice individually. So in lieu of analyzing specific practices, the risk of JD transmission, as assessed by a standardized risk assessment, was used to determine the areas of the farm in which management changes had the greatest effect on reducing disease. Of the risks for JD transmission assessed, exposure to adult cows other than the dam at birth and feeding colostrum from one cow to multiple calves

had the most significant effect on cows testing positive for JD as adults (Table 3.16). In both instances, the probability of exposure of susceptible calves to MAP being shed by infected cows is increased.

These results are consistent with the fact that the susceptibility of cattle becoming infected with MAP decreases with age. Also, the results seemed plausible, considering observations made on these farms. The JD prevalence in these herds varied greatly over the course of this study (0.6% to 43%, Figure 3.5); even ignoring the two herds (herds 3 and 5) that underwent significant expansion through the purchase of a large number of cows and whose JD prevalence remained relatively static. In fact, the herd with highest JD prevalence in this study was closed for over 30 years prior to the start of the program. This suggested that something in the management of these herds, beyond the purchase of cattle, was contributing to the spread of JD within these herds. When the risk assessments were compared, the risk of JD transmission was similar across the herds once calves were weaned. The areas where there was the greatest difference in management in terms of risk for JD transmission were the maternity and pre-weaned calf areas. Thus, it seems reasonable that the bulk of MAP infections occur in these areas. With these areas identified, management practices for JD control can be recommended that are designed specifically for each herd's unique needs and capabilities.

5.5. Are management practices to control JD cost effective?

Proving that JD control programs are effective in minimizing the disease burden in infected dairy herds, is not enough for the widespread adoption of management practices to control and prevent JD disease. The costs and benefits of investing in JD control, as well as the economic losses of JD in the absence of control, need to be quantified. Producers are unlikely to invest in JD control programs if the cost of controlling the disease is greater than what the disease is costing them. To determine the cost-effectiveness of the JD control programs implemented by the herds in this study, the net present value (NPV) of the control program was projected over a 20-year period from the start of the program. The costs and benefits of the respective control programs observed over the five-year course of this study were extrapolated, and the NPV calculated based on different assumptions on the extent of disease burden in the presence, and absence, of a JD control program.

Assuming JD could be eradicated after 20 years, the control program netted a positive return on investment in half the herds in this study; while resulting in a net loss for the other half of the herds. If it was assumed that JD prevalence and the economic losses remained at the same level as those observed at the end of the study for the remaining 20-year projection, the control program yielded a positive return on investment in only two herds. However, across all herds, when the potential ongoing losses due to JD in the absence of a control program were considered, investing in a control program was always a better economic choice than doing nothing (Table 4.35).

Moreover, irregardless of the calculated NPV of the JD control program, all the producers in this study were generally satisfied with their control programs, and planned to continue investing in them into the future. While the assessment was subjective, the producers stated that following the implementation of the control program, they saw an overall improvement in herd health (beyond a reduction in the number of cows with clinical JD) that translated into improved production and increased revenues.

This suggests two things. First, producer perception of, and satisfaction with, the JD control program are just as important in the on-farm decision-making process as complicated calculations projected over an extended period of time and necessarily based on assumptions. Second, the calculations underestimated the NPV of the JD control program. Certainly there was the opportunity for error to enter into the calculations. The questionnaire used to gather data on the economic costs of the JD control program, particularly regarding management and labor, relied on the producers' ability to recall time spent on JD control. Also, the economic losses, from which the benefits of the control program were estimated, were only those directly attributed to JD. It did not take into consideration any ancillary effects the control program might have. Many of the management practices implemented to control JD are also recommended for the control of a multitude of other infectious diseases such as *Salmonella, E. coli, Mycoplasma,* bovine leukosis, etc. Unfortunately, baseline records for cow and calf health aside from JD were not available for the herds in this study. Future studies on the cost-effectiveness of JD control programs should include some measure for collateral improvement in overall herd health. This measure is potentially significant based on the subjective

accounts of the producers in this study. As a result, the estimates of the cost-effectiveness of JD control programs reported here are probably conservative.

5.6. Conclusion

In conclusion, the four questions posed at the beginning of this research project have been answered. Serial culturing of the environment of infected dairy farms found that MAP is widely distributed; with the areas most commonly contaminated being those where manure from adult cows is concentrated. As JD prevalence in the herds declined so too did the number of culture positive environmental samples and the areas from which those samples originated. The JD control programs implemented on these farms were successful in minimizing disease transmission as evidenced by a reduced prevalence and incidence of JD in cattle exposed to the control program as calves compared to those not exposed to the program. Evidence also suggested that the majority of MAP infected cows acquired the infection at, or shortly after, birth. Therefore, management practices to minimize exposure of calves to MAP in the maternity and pre-weaned calf areas should take priority in any JD control program. Finally, JD control programs can be cost-effective. Investing in a JD control program was always a better economic decision than doing nothing. Moreover, the producers in this study were generally satisfied with the JD control programs they had implemented. They reported a decrease in clinical cases, an improvement in overall herd health resulting in better production and increased revenues, and they planned to continue investing in the JD control program into the future.

APPENDICES

Risk Assessment Scores (based on visual observation of each environment and investigation of policy)
Estimate the risk for fecal/oral and colostrum/milk disease spread, or gap in farm's biosecurity, for each management practice. Follow the logical order. *Observe Proper Biosecurity!* Note how current management conditions differ from past. Ideally producer & veterinarian score risks independently then compare & discuss relative importance in plan development of management.

A. Calving Area Risk Factors (Place an X in the box to the right of the management practice that most closely signifies the risk for that item.)	0	1. V. Low	2. Low	3	4	5. Moderate	6	7	8. High	9	10. V. High	**Notes / Current vs. Past**
1. Multiple animal use [Single pen---Dense crowded pen]												
2. Manure build up risk for calf ingestion [clean dry---- dirty wet]												
3. Area also used for sick cows [Never----Always]												
4. Presence of JD clinicals / suspects [Never----Always]												
5. Manure soiled udders / legs [Never----Always]												
6. Calves born in other cow areas [Never----Always]												
7. Time calves stay with dam [<30 minutes ---- >24 hours]												
8. Calves nurse dam [Never----Always]												

Maximum score = 80. Your herd score is _____. Consider the impact of JD prevalence on ability to reduce risks.
Estimate the likely risk for spreading Johne's in the calving area: **Very Low Low Moderate High Very High**
(Mark an X on the line)

B. Pre-Weaned Heifer Risk Factors	0	1. V. Low	2. Low	3	4	5. Moderate	6	7	8. High	9	10. V. High
1. Fed pooled colostrum [Never or JD negative —— High risk cows]											
2. Fed colostrum from individual cow to several calves [Never or JD negative —— High risk cows]											
3. Fed unpasteurized pooled milk [JD negative cows — high risk cows]											
4. Possible manure contamination of colostrum or milk at harvest, utensils, traffic or people [None any source —— Frequent many sources]											
5. Possible manure contamination of feed or water: by cows, traffic splatter, equipment or people [None any source —— Frequent many sources]											
6. Direct cow contact or potential manure contamination of pen: by cows, traffic splatter, equipment or people [None any source —— Frequent many sources]											

Notes / Current vs. Past

Maximum score = 60. Your herd score is _____. Consider the impact of JD prevalence on ability to reduce risks.
Estimate the likely risk for spreading Johne's in pre-weaned heifers: **Very Low Low Moderate High Very High**
(Mark an X on the line)

C. Post-Weaned Heifer Risk Factors	0	1. V. Low	2	3	4. Moderate	5	6	7. V. High
1. Direct cow contact or pen contamination with cows' manure [None ---- Always]								
2. Possible manure contamination of feed: refused cow ration, stored feed, equipment, cows, traffic splatter, people or runoff. [Never ---- Frequently]								
3. Potential for contamination of supplied or natural water: shared with or by cows, traffic splatter, runoff or people. [Never ---- Frequently]								
4. Share pasture with cows. [Never ---- Frequently]								
5. Manure spread on forage grazed / harvested same season [Never ---- Frequently]								

Notes / Current vs. Past

Maximum score = 35. Your herd score is _____. Consider the impact of JD prevalence on ability to reduce risks.
Estimate the likely risk for spreading Johne's in post-weaned heifers: **Very Low Low Moderate High Very High**
(Mark an X on the line)

D. Bred Heifer Risk Factors	0	1. V. Low	2	3. Moderate	4.	5. V. High
1. Direct cow contact or pen contamination with cows' manure [None ---- Always]						
2. Possible manure contamination of feed: refused cow ration, stored feed, equipment, cows, traffic splatter, people or runoff. [Never ---- Frequently]						
3. Potential for contamination of water sources: shared with or by cows, traffic splatter, runoff or people. [Never ---- Frequently]						
4. Share pasture with cows. [Never ---- Frequently]						
5. Manure spread on forage grazed / harvested same season [Never ---- Frequently]						

Notes / Current vs. Past

Maximum score = 25. Your herd score is _____. Consider the impact of JD prevalence on ability to reduce risks.
Estimate the likely risk for spreading Johne's in bred heifers: **Very Low Low Moderate High Very High**
(Mark an X on the line)

E. Cow Risk Factors	0	1. Low	2	3.	4. High
1. Possible cow manure contamination of feed: when fed or stored, by equipment, traffic splatter, runoff or people. [Never ---- Frequently]					
2. Possible manure contamination of water by cows, traffic splatter, runoff or people. [Never ---- Frequently]					
3. Direct access to accumulated or stored manure [Never ---- Frequently]					
4. Manure spread on forage grazed / harvested same season [Never ---- Frequently]					

Notes / Current vs. Past

Maximum score = 16. Your herd score is _____. Consider the impact of JD prevalence on ability to reduce risks.
Estimate the likely risk for spreading Johne's among cows: **Very Low Low Moderate High Very High**
(Mark an X on the line)

Appendix B: Economic Questionnaire

Name_____	Date_____

Cost and revenue changes due to Johne's Disease Control Program

Please read through the following questionnaire that relates to biosecurity and Johne's disease control changes and investments. Check the appropriate box (□) next to any change(s) you have made or plan on making that was directly related to the Johne's control program on your farm. Also provide quantities and dollar values where appropriate. If you have a change that is not listed please fill it in under "other." Use the space provided including the back of pages as necessary.

This survey covers the period from the start of the Johne's disease Control Program (2003) until the current time

A. Operations management changes because of Johne's Control Program

1. ***Has any part of the farm enterprise changed as a <u>direct result of the Johne's Control Program</u> (ie. Outsourced replacements to custom heifer raiser or eliminated steer enterprise)?***

Yes ***No***

If yes, what is the approximate cost of that change in terms of either additional expense or lost revenue?

2. Changes in supplies <u>DUE TO JOHNE'S CONTROL PROGRAM</u>

Supply	**Increase**	**Decrease**	**Change in quantity** (specify unit)	**Cost** ($/unit)	**Once**	**Ongoing**	**<u>Date</u> or <u>Frequency</u> of change**
Milk replacer	☐	☐			☐	☐	
Colostrum replacer/supplement	☐	☐			☐	☐	
Ear tags/Animal Identification	☐	☐			☐	☐	
Sanitation supplies	☐	☐			☐	☐	
Bedding	☐	☐			☐	☐	
Other (specify):	☐	☐			☐	☐	
Other (specify):	☐	☐			☐	☐	
Other (specify):	☐	☐			☐	☐	

Comments:_____

3. Changes in managerial time and responsibilities ***DUE TO JOHNE'S CONTROL PROGRAM***

Enterprise	Increase	Decrease	Change (hours/month)	Describe	Once	Ongoing	Date or Frequency of change
Johne's diagnosis/testing	☐	☐			☐	☐	
Record-keeping	☐	☐			☐	☐	
Buying/Selling decisions	☐	☐			☐	☐	
Animal logistics (where to calve cows, house calves, etc.)	☐	☐			☐	☐	
Capital Investment decision making	☐	☐			☐	☐	
Other (specify):	☐	☐			☐	☐	
Other (specify):	☐	☐			☐	☐	
Other (specify):	☐	☐			☐	☐	

What would it cost the operation to hire equivalent management services (gross or amount/hr)?_____

_____

_____

_____

What could the person making these management decisions earn doing something else (gross or amount/hr)?

_____

_____

_____

_____

4. Changes in labor and custom hired services ***DUE TO JOHNE'S CONTROL PROGRAM***

Labor use	**Increase**	**Decrease**	**Change** (hours/year)	**Describe**	**Once**	**Ongoing**	**Date or Frequency of change**
Johne's diagnosis/testing	❑	❑			❑	❑	
Record-keeping	❑	❑			❑	❑	
Sanitation/cleaning	❑	❑			❑	❑	
Calf care	❑	❑			❑	❑	
Cow care	❑	❑			❑	❑	
Animal handling	❑	❑			❑	❑	
Other (specify):	❑	❑			❑	❑	
Other (specify):	❑	❑			❑	❑	

What is the labor cost for the reporting period? (e.g., $/hour)? Include all costs of hired labor such as benefits.

B. Capital investments (or anticipated investments) DUE TO JOHNE'S CONTROL PROGRAM. Do NOT include capital investments that were made irrespective of Johne's Disease Control

Category	Y	Specific Item	Total Cost	Financing[1]		
				% equity	% debt	% Cost-share
Manure storage facilities	☐					
Feed storage facilities	☐					
Feed handling equipment	☐					
Cattle feeding facilities	☐					
Livestock housing	☐					
Maternity pens	☐					
Manure equipment						
Spreader	☐					
Skid-steer	☐					
Other (specify):	☐					
Pasteurizer	☐					
Other machinery	☐					
Improvements	☐					
Fencing	☐					
Other (specify):	☐					
Other (specify):	☐					
Other (specify):	☐					

[1] Equity includes farm or personal resources such as savings. Debt refers to borrowed money. Cost share refers to money obtained through programs such as EQIP.

C. Producer Perceptions

1. Since beginning the Johne's control program, have you seen any increases in revenues (e.g. higher price replacement heifers)? Explain.

2. Since beginning the Johne's control program, have any other changes happened (even changes not related to Johne's disease at the farm? Explain.

3. Since beginning the Johne's control program, do you *think* the farm is doing better (or worse) financially? Explain.

4. Since beginning the Johne's control program, do you *think* the farm is doing better (or worse) production-wise? Explain.

5. Since beginning the Johne's control program, do you *think* herd-health is better (or worse)? Explain.

6. On average, how much lower is the slaughter value of Johne's disease clinical animals? Also, do Johne's disease test-positive animals have a lower slaughter value? Explain.

7. Are you glad that you joined the Johne's disease demo-herd program? Do you think it was a good financial decision? Explain.

8. Do you plan to continue taking steps to manage and reduce Johne's disease in your herd after the end of the Johne's disease demo-herd program? Explain.

Appendix C: Farm input data for OptiCow™ Model

Farm Input	Source	US Dollars				
		2003	2004	2005	2006	2007
Herd average milk yield/yr (lbs/cow/yr)	OptiCow™ calculation from inputted cow data	Herd specific				
Ave. milk price ($/cwt)	Ave. MMPA base farm price	$14.34	$18.07	$15.92	$14.02	$18.39
Value replacement heifer	ERS-USDA	$1572	$1871	$2021	$1878	$2004
Calf value	ERS-USDA	$108	$130	$145	$140	$154
Weight @ birth	OptiCow™ default	90 lbs				
Mature live weight	OptiCow™ default	1350 lbs				
Price/lb carcass weight	ERS-USDA	$0.53	$0.58	$0.58	$0.50	$0.50
Veterinary costs ($/cow/year)	Michigan Dairy Farm Business Analysis Summary	$100.69	$106.50	$106.43	$114.67	$119.83
Financial losses at disposal ($/case)	OptiCow™ default	$50				
Insemination ($/insemination)	OptiCow™ default	$12				
Heat detection rate	OptiCow™ default	40%				
Conception rate	OptiCow™ default	40%				
VWP	Herd DHIA data	Herd specific				
Age at first calving	Herd DHIA data					

REFERENCES

REFERENCES

Adaska, J.M., Munoz-Zanzi, C.A., Hietala, S.K., 2002. Evaluation of result variability with a commercial Johne's disease enzyme-linked immunosorbent assay kit and repeat testing of samples. J Vet Diagn Invest. 14, 423-426.

Adaska, J.M., Anderson, R.J., 2003. Seroprevalence of Johne's disease infection in dairy cattle in California, USA. Prev Vet Med. 60, 255-261.

Aly, S.S., Thurmond, M.C., 2005. Evaluation of *Mycobacterium avium* subsp *paratuberculosis* infection of dairy cows attributable to infection status of the dam. J Am Vet Med Assoc. 227, 450-454.

Antognoli, M.C., Hirst, H.L., Garry, F.B., Salman, M.D., 2007. Immune response to and faecal shedding of *Mycobacterium avium* ssp *paratuberculosis* in young dairy calves, and the association between test results in the calves and the infection status of their dams. Zoonoses Public Health. 54, 152-159.

Ayele, W.Y., Bartos, M., Svastova, P., Pavlik, I., 2004. Distribution of *Mycobacterium avium* subsp. *paratuberculosis* in organs of naturally infected bull-calves and breeding bulls. Vet Microbiol. 103, 209-217.

Barrington, G.M., Gay, J.M., Eriks, I.S., Davis, W.C., Evermann, J.F., Emerson, C., O'Rourke, J.L., Hamilton, M.J., Bradway, D.S., 2003. Temporal patterns of diagnostic results in serial samples from cattle with advanced paratuberculosis infections. J Vet Diagn Invest. 15, 195-200.

Beard, P.M., Daniels, M.J., Henderson, D., Pirie, A.A., Rudge, K., Buxton, D., Rhind, S., Greig, A., Huthings, M.R., McKendrick, I., Stevenson, K., Sharp, J.M., 2001. Paratuberculosis infection in nonruminant wildlife in Scotland. J Clin Microbiol. 39, 1517-1521.

Beaudeau, F., Belliard, M., Joly, A., Seegers, H., 2007. Reduction in milk yield associated with *Mycobacterium avium* subspecies *paratuberculosis* (Map) infection in dairy cows. Vet Res. 38, 625-634.

Belknap, E.B., Getzy, D.M., Johnson, L.W., Ellis, R.P., Thompson, G.L., Shulaw, W.P., 1994. *Mycobacterium paratuberculosis* infection in two llamas. J Am Vet Med Assoc. 204, 1805-1808.

Belloli, A., Carli, S., Pravettoni, D., Proverbio, D., Giangaspero M., Lauzi, S., Bonizzi, L., 2001. Case study – Attempted treatment of a cow with *Mycobacterium avium* enterocolitis. Bov Prac. 35, 56-60.

Benedictus, G., Dijkhuizen, A.A., Stelwagen, J., 1987. Economic losses due to paratuberculosis in dairy cattle. Vet Rec. 121, 142-146.

Benedictus, G., Kalis, C.J.H., 2003. Paratuberculosis: eradication, control and diagnostic methods. Act Vet Scand. 44, 231-241.

Berghaus, R.D., Lombard, J.E., Gardner, I.A., Farver, T.B., 2005. Factor analysis of a Johne's disease risk assessment questionnaire with evaluation of factor scores and a subset of original questions as predictors of observed clinical paratuberculosis. Prev Vet Med. 72, 291-309.

Berghaus, R.D., Farver, T.B., Anderson, R.J., Jaravata, C.C., Gardner, I.A., 2006. Environmental sampling for detection of Mycobacterium avium ssp. paratuberculosis on large California dairies. J Dairy Sci. 89, 963-970.

Bielanski, A., Algire, J., Randall, G.C.B., Surujballi, O., 2006. Risk of transmission of *Mycobacterium avium* ssp. *paratuberculosis* by embryo transfer in vivo and in vitro fertilized bovine embryos. Theriogenology. 66, 260-266.

Bolton, M.W., Grooms, D.L., Kaneene, J.B., 2005. Fecal shedding of Mycobacterium avium subsp. paratuberculosis in calves: implications for infection control and management. Proc 8th Int Coll Paratuberculosis. 596-600.

Brumbaugh, G.W., Edwards J.F., Roussel, A.J., Thomson, T.D., 2000. Effect of monensin sodium on histological lesions of naturally occurring bovine paratuberculosis. J Comp Path. 123, 22-28.

Buergelt, C.D., Duncan, J.R., 1978. Age and milk production data of cattle culled from a dairy herd with paratuberculosis. J Am Vet Med Assoc. 173, 478-480.

Buergelt, C.D., Ginn, P.E., 2000. The histopathologic diagnosis of subclinical Johne's disease in North American Bison (*Bison bison*). Vet Microbiol. 77, 325-331.

Buza, J.J., Hikono, H., Mori, Y., Nagata, R., Hirayama, S., Bari, A.M., Aodon-geril, Shu, Y., Tsuji, N.M., Momotani, E., 2004. Neutralization of interleukin-10 significantly enhances gamma interferon expression in peripheral blood by stimulation with Johnin purified protein derivative and by infection with *Mycobacterium avium* subsp. *paratuberculosis* in experimentally infected cattle with paratuberculosis. Infect Immun. 72, 2425-2428.

Carpenter, T.E., Gardner, I.A., Collins, M.T., Whitlock, R.H., 2004. Effects of prevalence and testing by enzyme-linked immunosorbent assay and fecal culture on the risk of introduction of *Mycobacterium avium* subsp *paratuberculosis*-infected cows into dairy herds. J Vet Diagn Invest. 16, 31-38.

Cetinkaya, B., Erdogan, H.M., Morgan, K.L., 1997. Relationships between the presence of Johne's disease and farm and management factors in dairy cattle in England. Prev Vet Med. 32, 253-266.

Chi, J., VanLeeuwen, J.A., Weersink, A., Keefe, G.P., 2002a. Direct production losses and treatment costs from bovine viral diarrhoea virus, bovine leukosis virus, *Mycobacterium avium* subspecies *paratuberculosis*, and *Neospora caninum*. Prev Vet Med. 55, 137-153.

Chi, J., VanLeeuwen, J.A., Weersink, A., Keefe, G.P., 2002b. Management factors related to seroprevalences to bovine viral-darrhoea virus, bovine-leukosis virus, *Mycobacterium avium* subspecies *paratuberculosis*, and *Neospora caninum* in dairy herds in the Canadian Maritimes. Prev Vet Med. 55, 57-68.

Chiodini, R.J., Van Kruiningen, H.J., 1983. Eastern white-tailed deer as a reservoir of ruminant paratuberculosis. J Am Vet Med Assoc. 182, 168-169.

Chiodini, R.J., Van Kruiningen, H.J., Merkal, R.S., 1984. Ruminant paratuberculosis (Johne's disease): The current status and future prospects. Cornell Vet. 74, 217-262.

Chiodini, R.J, 1996. Immunology: Resistance to paratuberculosis. Vet Clin North Am Food Anim Pract. 12, 313-343.

Chiodini, R.J., Rossiter, C.A., 1996. Paratuberculosis: a potential zoonosis. Vet Clin North Am Food Anim Pract. 12, 457-467.

Collins, MT, Sockett, D.C., Ridge, S., Cox, J.C., 1991. Evaluation of a commercial enzyme-linked immunoassay for Johne's disease. J Clin Microbiol. 29, 272-276.

Collins, M.T., Morgan, I.R., 1992. Simulation model of paratuberculosis control in a dairy herd. Prev Vet Med. 14, 21-32.

Collins, M.T., 1994. Clinical approach to control bovine paratuberculosis. J Am Vet Med Assoc. 204, 208-210.

Collins, M.T., Sockett, D.C., Goodger, W.J., Conrad, T.A., Thomas, C.B., Carr, D.J., 1994. Herd prevalence and geographic distribution of, and risk factors for, bovine paratuberculosis in Wisconsin, J Am Vet Med Assoc. 204, 636-641.

Collins, M.T., 1996. Diagnosis of paratuberculosis. Vet Clin North Am Food Anim Pract. 12, 357-371.

Collins, M.T., 2003. Paratuberculosis: Review of present knowledge. Acta Vet Scand. 44, 217-221.

Collins, M.T., Wells, S.J., Petrini, K.R., Collins, J.E., Schultz, R.D., Whitlock, R.H., 2005. Evaluation of five antibody detection tests for diagnosis of bovine paratuberculosis. Clin Diagn Lab Immunol. 12, 685-692.

Collins, M.T., Gardner, I.A., Garry, F.B., Roussel, A.J., Wells, S.J., 2006. Consensus recommendations on diagnostic testing for the detection of paratuberculosis in cattle in the United States. J Am Vet Med Assoc. 229, 1912-1919.

Corn, J.L., Manning, E.J.B., Sreevatsan, S., Fischer, J.R., 2005. Isolation of *Mycobacterium avium* subsp. *paratuberculosis* from free-ranging birds and mammals on livestock premises. Appl Env Microbiol 71, 6963-6967.

Coussens, P.M., 2004. Model for immune responses to *Mycobacterium avium* subspecies *paratuberculosis* in cattle. Infect Immun. 72, 3089-3096.

Cramwell, M.P., 1993. Control of Johne's disease in a flock of sheep by vaccination. Vet Rec. 133, 219-220.

Daniels, M.J., Ball, N., Hutchings, M.R., Greig, A., 2001. The grazing response of cattle to pasture contaminated with rabbit faeces and the implications for the transmission of paratuberculosis. Vet J. 161, 306-313.

Daniels, M.J., Hutchings, M.R., Allcroft, D.J., McKendrick, I.J., Greig, A., 2002. Risk factors for Johne's disease in Scotland – the results of a survey of farmers. Vet Rec. 150, 135-139.

Daniels, M.J., Henderson, D., Greig, A., Stevenson, K., Sharp, J.M., Hutchings, M.R., 2003a. The potential role of wild rabbits *Oryctolagus cuniculus* in the epidemiology of paratuberculosis in domestic ruminants. Epidemiol Infect. 130, 553-559.

Daniels, M.J., Hutchings, M.R., Greig, A., 2003b. The risk of disease transmission to livestock posed by contamination of farm stored feed by wildlife excreta. Epidemiol Infect. 130, 561-568.

Dargatz, D.A., Byrum, B.A., Barber, L.K., Sweeney, R.W., Whitlock, R.H., Shulaw, W.P., Jacobson, R.H., Stabel, J.R., 2001. Evaluation of a commercial ELISA for diagnosis of paratuberculosis in cattle. J Am Vet Med Assoc. 218, 1163-1166.

Davidson, W.R., Manning, E.J.B., Nettles, V.F., 2004. Culture and Serologic survey for *Mycobacterium avium* subsp. *paratuberculosis* infection among Southeastern white-tailed deer (*Odocoileus virginianus*). J Wildl Dis. 40, 301-306.

de Lisle, G.W., Yates, G.F., Cavaignac, S.M., Collins, D.M., Paterson, B.M., Montgomery, R.H., 2002. *Mycobacterium paratuberculosis* in wild ferrets – a potential wildlife reservoir of Johne's disease. Proc 7th Int Coll Paratuberculosis. pp. 90.

Dieguez, F.J., Gonzalez, A.M., Menendez, S., Vilar, M.J., Sanjuan, M.L., Yus, E., Arnaiz, I., 2008. Evaluation of four commercial serum ELISAs for detection of *Mycobacterium avium* subsp. *paratuberculosis* infection in dairy cows. Vet J. in press.

Dorshorst, N.C., Collins, M.T., Lombard, J.E., 2006. Decision analysis model for paratuberculosis control in commercial dairy herds. Prev Vet Med. 75, 92-122.

Ellingson, J.L.E., Anderson, J.L., Koziczkowski, J.J., Radcliff, R.P., Sloan, S.J., Allen, S.E., Sullivan, N.M., 2005. Detection of viable *Mycobacterium avium* subsp. *paratuberculosis* in retail pasteurized whole milk by two culture methods and PCR. J Food Prot. 68, 966-972.

Ellner, J.J., Wallis, R.S., 1989. Immunologic aspects of mycobacterial infections. Rev Infect Dis. Suppl 2, S455-S459.

FDA, Center for Veterinary Medicine, 2004. FDA Approves Rumensin® for increased milk production efficiency in dairy cows. Press release. Available from: http://www.fda.gov/cvm/CVM_Updates/monensinup.htm. Last accessed July 27, 2008.

Featherstone, C., 1997. M cells: portals to the mucosal immune system. Lancet. 350, 1230.

Feller, M., Huwiler, K., Stephan, R., Altpeter, E., Ahang, A., Furrer, H., Pfyffer, G.E., Jemmi, T., Baumgartner, A., Egger, M., 2007. *Mycobacterium avium* subspecies *paratuberculosis* and Crohn's disease: a systematic review and meta-analysis. Lancet. 7, 607-613.

Fischer, O.A., Matlova, L., Dvorska, L., Svastova, P., Bartl, J., Melicharek, I., Weston, R.T., Pavlik, I., 2001. Diptera as vectors of mycobacterial infections in cattle and pigs. Med Vet Entomol. 15, 208-211.

Fischer, O.A., Matlova, L., Dvorska, L., Svastova, P., Bartos, M., Weston, R.T., Kopecna, M., Trcka, I., Pavlik, I., 2005. Potential risk of Mycobacterium avium subspecies paratuberculosis spread by syrphid flies in infected cattle farms. Med Vet Entomol. 4, 360-366.

Fredriksen, B., Djonne, B., Sigurdardottir, O., Tharaldsen, J., Nyberg, O., Jarp, J., 2004. Factors affecting the herd level of antibodies against *Mycobacterium avium* subspecies *paratuberculosis* in dairy cattle. Vet Rec. 154, 522-526.

Frehel, C., deChastellier, C., Lang, T., Rastogi., N., 1986. Evidence for inhibition of fusion of lysosomal and prelysosomal compartments with phagosomes in macrophages infected with pathogenic *Mycobacterium avium*. Infect Immun. 52, 252-262.

Frehel, C., Thorel, M.F., Rastogi, N., 1989. Evidence that host-recycling of *Mycobacterium avium* preserves its ability to hinder macrophage killing functions. Acta Leprol. 7 Suppl. 160-163.

Fyock, B.A., Smith, J., Hoving, E., Schukken, Y., Whitlock, R.H., 2005. Whole herd fecal pools to assess herd MAP bioburden compared to composite environmental samples. Proc 8th Int Coll Paratuberculosis. 258.

Gilmour, N.J.L., Nisbet, D.I., Brotherston, J.G., 1965. Experimental oral infection of calves with *Mycobacterium johnei*. J Comp Path. 75, 281-286.

Godden, S.M., Fetrow, J.P., Feirtag, J.M., Green, L.R., Wells, S.J., 2005. Economic analysis of feeding pasteurized nonsaleable milk versus conventional milk replacer to dairy calves. J Am Vet Med Assoc. 226, 1547-1554.

Gollnick, N.S., Mitchell, R.M., Baumgart, M., Janagama, H.K. Sreevatsan, S., Schukken, Y.H., 2007. Survival of *Mycobacterium avium* subsp. *paratuberculosis* in bovine monocyte-derived macrophages is not affected by host infection status but depends on the infecting bacterial genotype. Vet Immunol Immunopathol. 120, 93-105.

Gonda, M.G., Chang, Y.M., Shook, G.E., Collins, M.T., Kirkpatrick, B.W., 2007. Effect of *Mycobacterium paratuberculosis* infection on production, reproduction, and health traits in US Holsteins. Prev Vet Med. 80, 103-119.

Goodger, W.J., Collins, M.T., Nordlund, K.V., Eisele, C., Pelletier, J., Thomas, C.B., Sockett, D.C., 1996. Epidemiologic study of on-farm management practices associated with prevalence of *Mycobacterium paratuberculosis* infections in dairy cattle. J Am Vet Med Assoc. 208, 1877-1881.

Grant, I.R., Ball, H.J., Neill, S.D., Rowe, M.T., 1996. Inactivation of *Mycobacterium paratuberculosis* in cow's milk at pasteurisation temperatures. Appl Environ Microbiol. 62, 631-636.

Grant, I.R., Ball, H.J., Rowe, M.T., 1998. Effect of high temperature, short time pasteurisation on milk containing low numbers of *Mycobacterium paratuberculosis*. Lett Appl Microbiol. 26, 166-170.

Grant, I.R., 2003. *Mycobacterium paratuberculosis* and milk. Act Vet Scand. 44, 261-266.

Greig, A., 2005. *Mycobacterium avium* subsp. *paratuberculosis* – its plans for survival. Proc 8th Int Coll Paratuberculosis. pp. 583-586.

Grewal, S.K., Rajeev, S., Sreevatsan, S., Michel, F.C., 2006. Persistence of *Mycobacterium avium* subsp. *paratuberculosis* and other zoonotic pathogens during simulated composting, manure packing, and liquid storage of dairy manure. Appl Environ Microbiol. 72, 565-574.

Groenendaal, H., Galligan, D.T., 1999. Economic consequences of Johne's disease control programs. Center of Animal Health and Productivity, School of Veterinary Medicine, University of Pennsylvania, Animal Health Economics Staff Paper. Available from: http://cahpwww.vetupenn.edu/field/johne_report.pdf. Last accessed 10/31/2008.

Groenendaal, H., Nielen, M., Jalvingh, A.W., Horst, S.H., Galligan, D.T., Hesselink, J.W., 2002. A simulation of Johne's disease control. Prev Vet Med. 54, 225-245.

Groenendaal, H., Galligan, D.T., 2003. Economic consequences of control programs for paratuberculosis in midsize dairy farms in the United States. J Am Vet Med Assoc. 223, 1757-1763.

Grooms, D.L., Durst, P., Kaneene, J.B., 2006. Low *Mycobacterium avium* subspecies *paratuberculosis* ELISA specificity in a dairy herd. Proc 39th Ann Conv Am Assoc Bov Prac. pp. 323-324.

Gwozdz, J.M., Thompson, K.G., Manktelow, B.W., Murray, A., West, D.M., 2000. Vaccination against paratuberculosis of lambs already infected experimentally with *Mycobacterium avium* subspecies *paratuberculosis*. Aust Vet J. 78, 560-566.

Hadley, G.L., Wolf, C.A., Harsh, S.B., 2006. Dairy cattle culling patterns, explanations, and implications. J Dairy Sci. 89, 2286-2296.

Hagan, W.A., 1938. Age as a factor in susceptibility to Johne's disease. Cornell Vet. 38, 34.

Hansen, D., Rossiter, C.A., 1999. Tools to use against Johne's disease in cattle herds. Bov Prac. 33, 188-191.

Hardin, L.E., Thorne, J.G., 1996. Comparison of milk with serum ELISA for the detection of paratuberculosis in dairy cows. J Am Vet Med Assoc. 209, 120-122.

Harris, N.B., Barletta, R.G., 2001. *Mycobacterium avium* subsp. *paratuberculosis* in veterinary medicine. Clin Microbiol Rev. 4, 489-512.

Hasonova, L., Pavlik, I., 2006. Economic impact of paratuberculosis in dairy cattle herds: a review. Veterinarni Medicina, 51, 193-211.

Hendrick, S.H., Duffield, T., Leslie, K., Lissemore, K., Archambault, M., Kelton, D., 2005a. The prevalence of milk and serum antibodies to *Mycobacterium avium* subspecies *paratuberculosis* in dairy herds in Ontario. Can Vet J. 46, 1126-1129.

Hendrick, S.H., Duffield, T.F., Kelton, D.F., Leslie, K.E., Lissemore, K.D., Archambault, M., 2005b. Evaluation of enzyme-linked immunosorbent assays performed on milk and serum samples for detection of paratuberculosis in lactating dairy cows. J Am Vet Med Assoc. 226, 424-428.

Hendrick, S.H., Kelton, D.F., Leslie, K.E., Lissemore, K.D., Archambault, M., Duffield, T.F., 2005c. Effect of paratuberculosis on culling, milk production, and milk quality in dairy herds. J Am Vet Med Assoc. 227, 1302-1308.

Hendrick, S.H., Duffield, T.F., Leslie, K.E., Lissmore, K.D., Archambault, M., Bagg, R., Dick, P., Kelton, D.F., 2006a. Monensin might protect Ontario, Canada dairy cows from paratuberculosis milk-ELISA positivity. Prev Vet Med. 76, 237-248.

Hendrick, S.H.., Kelton, D.F., Leslie, K.E., Lissemore, K.D., Archambault, M., Bagg, R., Dick, P., Duffield, T.F., 2006b. Efficacy of monensin sodium for the reduction of fecal shedding of *Mycobacterium avium* subsp. *Paratuberculosis* in infected dairy cattle. Prev Vet Med. 75, 206-220.

Hill, B.B., West, M., Brock, K.V., 2003. An estimated prevalence of Johne's disease in a subpopulation of Alabama beef cattle. J Vet Diagn Invest. 15, 21-25.

Hirst, H.L., Garry, F.B., Salman, M.D., 2002. Assessment of test results when using a commercial enzyme-linked immunosorbent assay for diagnosis of paratuberculosis in repeated samples collected from adult dairy cattle. J Am Vet Med Assoc. 220, 1685-1689.

Hirst, H.L., Garry, G.B., Morley, P.S., Salmon, M.D., Dinsmore, R.P., Wagner, B.A., McSweeney, K.D., Goodell, G.M., 2004. Seroprevalence of *Mycobacterium avium* subsp *paratuberculosis* infection among dairy cows in Colorado and herd-level risk factors for seropositivity. J Am Vet Med Assoc. 225, 97-101.

Hoe, F.G.H., Ruegg, P.L., 2006. Opinions and practices of Wisconsin dairy producers about biosecurity and animal well-being. J Dairy Sci. 89, 2297-2308.

Holmes, I.R.L., Jubb, T.F., Callinan, A.P.L., 2004. Infection rates in reactors to an absorbed ELISA used in a test and cull program for bovine Johne's disease. Aust Vet J. 82, 233-235.

Horton, N.J., Bebchuk, J.D., Jones, C.L., Lipsitz, S.R., Catalano, P.J., Zahner, G.E.P., Fitzmaurice, G.M., 1999. Goodness-of-fit for GEE: An example with mental health service utilization. Statist Med. 18, 213-222.

Hugh-Jones, M.E., Hubbert, W.T., Hagstad, H.V. (Eds.), 1995. Zoonoses, Recognition, Control and Prevention. Iowa State University Press, Ames, Iowa.

Hutjens, M.F., 2005. Dairy efficiency and dry matter intake. Proc. 7th Western Dairy Management Conf. pp. 71-76.

Jansen, J., 1948. Paratuberculosis. J Am Vet Med Assoc. 112, 52-54.

Johnson-Ifearulundu,Y., Kaneene, J.B., 1997. Relationship between soil type and *Mycobacterium paratuberculosis*. J Am Vet Med Assoc. 210, 1735-1740.

Johnson-Ifearulundu,Y., Kaneene, J.B., 1998. Management-related risk factors for *M. paratuberculosis* infection in Michigan, USA dairy herds. Prev Vet Med 37, 41-54.

Johnson-Ifearulundu, Y., Kaneene, J.B., Lloyd, J.W., 1999. Herd-level economic analysis of the impact of paratuberculosis on dairy herds. J Am Vet Med Assoc. 214, 822-825.

Johnson-Ifearulundu, Y., Kannene, J.B., Sprecher, D.J., Gardiner, J.C., Lloyd, J.W., 2000. The effect of subclinical *Mycobacterium avium paratuberculosis* infection on days open in Michigan, USA dairy cows. Prev Vet Med. 46, 171-181.

Johnson, Y.J., Kaneene, J.B., Gardiner, J.C., Lloyd, J.W., Sprecher, D.J., Coe, P.H., 2001. The effect of subclinical *Mycobacterium paratuberculosis* infection on milk production in Michigan dairy cows. J Dairy Sci. 84, 2188-2194.

Jubb, T.F., Galvin, J.W., 2004. Effect of a test and control program for bovine Johne's disease in Victorian dairy herds 1992-2002. Aust. Vet J. 82, 228-232.

Judge, J., Kyriazakis, I., Greig, A., Hutchings, M.R., 2005. Quantification of the faecal-oral route of *Mycobacterium avium* subspecies *paratuberculosis* transmission from rabbits to livestock. Proc 8th Int Coll Paratuberculosis. pp. 632.

Jungersen, G., Huda, A., Hansen, J.J., Lind, P., 2002. Interpretation of the Gamma Interferon test for diagnosis of subclinical paratuberculosis in cattle. Clin Diagn Lab Immunol. 9, 453-460.

Juste, R.A., Garcia Marin, J.F., Peris, B., Saez de Ocariz, C., Badiola, J.J., 1994. Experimental infection of vaccinated and non-vaccinated lambs with *Mycobacterium paratuberculosis*. J Comp Pathol. 110, 185-194.

Kalis, C.H.J., Hesselink, J.W., Russchen, E.W., Barkema, H.W., Collins, M.T., Visser, I.J.R., 1999. Factors influencing the isolation of *Mycobacterium avium* subsp. *paratuberculosis* from bovine fecal samples. J Vet Diagn Invest. 11, 345-351.

Kalis, C.H.J., Collins, M.T., Hesselink, J.W., Barkema, H.W., 2003. Specificity of two tests for the early diagnosis of bovine paratuberculosis based on cell-mediated immunity: the Johnin skin test and the gamma interferon assay. Vet Microbiol. 97, 73-86.

Kalis, C.H.J., Collins, M.T., Barkema, H.W., Hesselink, J.W., 2004. Certification of herds as free of *Mycobacterium paratuberculosis* infection: actual pooled faecal results versus certification model predictions. Prev Vet Med. 65, 189-204.

Keller, L.L., Harrell, C.D., Loerzel, S.M., Rae, D.O., 2004. Johne's disease: seroprevalence of *Mycobacterium avium* subspecies *paratuberculosis* in Florida beef and dairy cattle. Bov Prac. 38, 135-141.

Khalifeh, M.S., Stabel, J.R., 2004. Effects of gamma interferon, inteleukin-10, and transforming growth factor-β on the survival of *Mycobacterium avium* subsp. *paratuberculosis* in monocyte-derived macrophages from naturally infected cattle. Infect Immun. 72, 1974-1982.

Kim, S.G., Kim, E.H., Lafferty, C.J., Miller, L.J., Koo, H.J., Stehman, S.M., Shin, S.J., 2004. Use of conventional and real-time polymerase chain reaction for confirmation of *Mycobacterium avium* subsp. *paratuberculosis* in a broth-based culture system ESP II. J Vet Diagn Invest. 16, 448-453.

Klijn, N., Harrewegh, A.A.P.M., deJong, P., 2001. Heat inactivation data for *Mycobacterium avium* subsp. *paratuberculosis*: implications for interpretation. J Appl Microbiol. 91, 697-704.

Kobayashi, S., Tsutsui, T., Yamamoto, T., Nishicuchi, A., 2007. Epidemiologic indicators associated with within-farm spread of Johne's disease in dairy farms in Japan. Bacteriology. 69, 1255-1258.

Koets, A.P., Adugna, G., Janss, L.L.G., van Weering, H.J., Kalis, C.H.J., Wentink, G.H., Rutten, V.P.M.G., Schukken, Y.H., 2000. Genetic variation of susceptibility to *Mycobacterium avium* subsp *paratuberculosis* infection in dairy cattle. J Dairy Sci. 83, 2702-2708.

Kopecky, K.E., 1977. Distribution of bovine paratuberculosis in the US. J Am Vet Med Assoc. 170, 320-324.

Kormendy, B., Kopal, T., Balint, T., Szilagyi, M., Beki, L., 1989. Economic losses caused by paratuberculosis in a dairy herd: case report. Acta Vet Hung. 37, 54-53.

Kormendy, B., 1992. Paratuberculosis vaccine in a large dairy herd. Acta Vet Hung. 40, 171-184.

Kormendy, B., 1994. The effect of vaccination on the prevalence of paraturculosis in large dairy herds. Vet Microbiol. 41, 117-125.

Kovich, D.A., Wells, S.J., Friendshuh, K., 2006. Evaluation of the voluntary Johne's disease herd status program as a source of replacement cattle. J Dairy Sci. 89, 3466-3470.

Kruip, T.A.M., Muskens, J., vanRoermund, H.J.W., Bakker, D., Stockhofe-Zurwieden, N., 2003. Lack of association of *Mycobacterium avium* subsp. *paratuberculosis* with oocytes and embryos from moderate shedders of the pathogen. Theriogenology. 59, 1651-1660.

Kudahl, A.B., Ostergaard, S., Sorensen, J.T., Nielsen, S.S., 2007. A stochastic model simulating paratuberculosis in a dairy herd. Prev Vet Med. 78, 97-117.

Lambrecht, R.S., Carriere, J.F., Collins, M.T., 1988. A model for analyzing growth kinetics of a slowly growing *Mycobacterium* sp. Appl Environ Microbiol. 54, 910-916.

Larsen, A.B., Merkal, R.S., Cutlip, R.C., 1975. Age of cattle as related to resistance to infection with *Mycobacterium paratuberculosis*. Am J Vet Res. 36, 255-257.

Lawrence, W.E., 1956. Congenital infection with *Mycobacterium johnei* in cattle. Vet Rec. 68, 312-312.

Lin, D.Y., Wei, L.J., Ying, Z., 2002. Model-checking techniques based on cumulative residuals. Biometrics. 58, 1-12.

Linnabary, R.D, Meerdink, G.L, Collins, M.T., Stabel, J.R., Sweeney, R.W., Washington, M.K, Wells, S.J., 2001. Johne's disease in cattle. Council for Agricultural Science and Technology. Issue Paper. 17, 1-10.

Lombard, J.E., Garry, F.B., McCluskey, B.J., Wagner, B.A., 2005. Risk of removal and effects on milk production associated with paratuberculosis status in dairy cows. J Am Vet Med Assoc. 227, 1975-1981.

Lombard, J.E., Wagner, B.A., Smith, R.L., McCluskey, B.J., Harris, B.N., Payeur, J.B., Garry, F.B., Salman, M.D., 2006. Evaluation of environmental sampling and culture to determine Mycobacterium avium subspecies paratuberculosis distribution and herd infection status on US dairy operations. J Dairy Sci. 89, 4162-4171.

Manning, E.J.B., Augenstein, M., Collins, M.T., Nelson, K.M., 2003. Case report – Johne's disease: the recipient risk. Bov Prac. 37, 20-22.

Marce, C., Beaudeau, F., Bareille, N., Seegers, H., Fourichon, C., 2007. Effects of infection by *Mycobacterium avium paratuberculosis* on fertility of dairy cows. Proc 7th Int Coll Paratuberculosis. pp. 114.

McDonald, W.L., O'Riley, K.J., Schroen, C.J., Condron, R.J., 2005. Heat inactivation of *Mycobacterium avium* subsp. *paratuberculosis* in milk. Appl Environ Microbiol. 71, 1785-1789.

McKenna, S.L.B., Keefe, G.P., Tiwari, A., VanLeeuwen, J., Barkema, H.W., 2006. Johne's disease in Canada part II: Disease impacts, risk factors, and control programs for dairy producers. Can Vet J. 47, 1089-1099.

McNab, W.B., Meek, A.H., Martin, S.W., Duncan, J.R., 1991. Associations between dairy production indices and lipoarabinomannan enzyme-immunoassay results for partuberculosis. Can J Vet Res. 55, 356-361.

McQueen, D.S., Russel, E.G., 1979. Culture of *Mycobacterium paratuberculosis* from bovine fetuses. Aust Vet J. 55, 203-204.

Merck Veterinary Manual, 7th Ed.. Merck & Co., Inc, New Jersey, 1991. pp. 1474, 1540.

Merkal, R.S., Larsen, A.B., Booth, G.D., 1975. Analysis of the effect of inapparent bovine paratuberculosis. Am J Vet Res. 36, 837-838.

Merkal, R.S., Miller, J.M., Hintz, A.M., Bryner, J.H., 1982. Intrauterine inoculation of *Mycobacterium paratuberculosis* into guinea pigs and cattle. Am J Vet Res. 43, 676-678.

Meyer, A.L., Hall, H.H., 1994. Economic analysis of the impact of paratuberculosis on the Kentucky cattle industry. Department of Agricultural Economics, University of Kentucky. Staff paper #343.

Miltner, E., Daroogheh, K., Mehta, P.K., Cirillo, S.L.G., Cirillo, J.D., Bermudez, L.E., 2005. Identification of *Mycobacterium avium* genes that affect invasion of the intestinal epithelium. Infect Immun. 73, 4214-4221.

Momotani, E., Whipple, D.L, Thiermann, A.B., Cheville, N.F., 1988. Role of M cells and macrophages in the entrance of *Mycobacterium paratuberculosis* into domes of ileal Peyer's patches in calves. Vet Pathol. 25, 131-137.

Motiwala, A.S., Strother, M., Theus, N.E., Stich, R.W., Byrum, B., Shulaw, W.P., Kapur, V., Sreevatsan, S., 2005. Rapid detection and typing of strains of *Mycobacterium avium* subsp. *paratuberculosis* from broth cultures. J Clin Microbiol. 43, 2111-2117.

Muskens, J., Elbers, A.R.W. vanWeering H.J., Noordhuizen, J.P.T.M., 2003a. Herd management practices associated with paratuberculosis seroprevalence in Dutch dairy herds. J. Vet Med. 50, 372-377.

Muskens, J., Mars, M.H., Elbers, A.R.W., van Mannen, K., Bakker, D., 2003b. The results of using faecal culture as confirmation test of paratuberculosis-seropositive dairy cattle. J Vet Med. 50, 231-234.

Nielsen, S.S., Gronbaek, C., Agger, J.F., Houe, H., 2002a. Maximum-likelihood estimation of sensitivity and specificity of ELISAs and faecal culture for diagnosis of paratuberculosis. Prev Vet Med. 53, 191-204.

Nielsen, S.S., Grohn, Y.T., Quaas, R.L., Agger, J.F., 2002b. Paratuberculosis in dairy cattle: variation of the antibody response in offspring attributable to the dam. J Dairy Sci. 85, 406-412.

Nielsen, S.S., Toft, N., 2006. Age-Specific characteristics of ELISA and fecal culture for purpose-specific testing for paratuberculosis. J Dairy Sci. 89, 569-579.

Nielsen, S.S., Toft, N., 2007. Assessment of management-related risk factors for paratuberculosis in Danish dairy herds using Bayesian mixture models. Prev Vet Med. 81, 306-317.

Nordlund, K.V., Goodger, W.J., Pelletier, J., Collins, M.T., 1996. Associations between subclinical paratuberculosis and milk production, milk components, and somatic cell counts in dairy herds. J Am Vet Med Assoc. 208, 1872-1876.

Obasanjo, I.O., Grohn, Y.T., Mohammed, H.O., 1997. Farm factors associated with the presence of *Mycobacterium paratuberculosis* infection in dairy herds on the New York State paratuberculosis control program. Prev Vet Med. 32, 243-251.

Olsen, I., Sigurdardottir, O.G., Djonne, B., 2002. Paratuberculosis with special reference to cattle: a review. Vet Quarterly. 32, 12-28.

Olsen, K.D., 2004. Farm Management Principles and Strategies. Iowa State Press, Ames, Iowa.

Ott, S.L., Wells, S.J., Wagner, B.A., 1999. Herd-level economic losses associated with Johne's disease on US dairy operations. Prev Vet Med. 40, 179-192.

Palmer, M.V., Stoffregen, W.C., Carpenter, J.G., Stabel, J.R., 2005. Isolation of *Mycobacterium avium* subsp *paratuberculosis* (Map) from feral cats on a dairy farm with Map-infected cattle. J Wildlife D. 41, 629-635.

Patterson, C.J., LaVenture, M., Hurley, S.S., Davis, J.P., 1988. Accidental self-inoculation with *Mycobacterium paratuberculosis* bacterin (Johne's bacterin) by veterinarians in Wisconsin. J Am Vet Med Assoc. 192, 1197-1199.

Pavlik, I., Yayo Ayele, W., Fischer O.A., 2002. Role of the external environment, plants, and non-vertebrates for the spread of Mycobacterium avium subsp. paratuberculosis. Proc 7th Int Coll Paratuberculosis. pp. 90.

Pearson, J.K.L., McClelland, T.G., 1955. Uterine infections and congenital Johne's disease in cattle. Vet Rec. 67, 615-617.

Pearson, L., 1908. A note on the occurrence in America of chronic bacterial dysentery of cattle. Am Vet Rev. 32, 602-605.

Pence, M., Baldwin, C., Carter-Black, C., 2003. The seroprevalence of Johne's disease in Georgia beef and dairy cull cattle. J Vet Diagn Invest. 15, 475-477.

Pence, M., Thomson, J.U., Cole, D. 2004. Important elements of the HACCP process to control Johne's disease, BVDV, and BLV. Bov Prac. 38, 126-132.

Perez, V., Tellechea, J., Badiola, J.J., Gutierrez, M., Garcia Marin, J.F., 1997. Relation between serologic response and pathologic findings in sheep with naturally acquired paratuberculosis. Am J Vet Res. 58, 799-803.

Pinedo, P.J., Rae, D.O., Williams, J.E., Donovan, G.A., Melendez, P., Buergelt, C.D., 2008. Association among results of serum ELISA, faecal culture and nested PCR on milk, blood and faeces for the detection of paratuberculosis in dairy cows. Tranbound Emerg Dis. 55, 125-133.

Prescott, J.F., Baggot, J.D., Walder, R.D. (Eds.) 2000. Antimicrobial Therapy in Veterinary Medicine. 3rd ed. Iowa State University Press, Ames, Iowa.

Raizman, E.A., Wells, S.J., Godden, S.M., Bey, R.F., Oakes, M.J., Bentley, D.C., Olsen, K.E., 2004. The distribution of Mycobacterium avium ssp. paratuberculosis in the environment surrounding Minnesota dairy farms. J Dairy Sci. 87, 2959-2966.

Raizman, E.A., Wells, S.J., Jordan, P.A., DelGiudice, G.D., Bey, R.R., 2005. *Mycobacterium avium* subsp. *paratuberculosis* from free-ranging deer and rabbits surrounding Minnesota dairy herds. Can J Vet Res. 69, 32-38.

Raizman, E.A., Wells, S.J., Godden, S.M., Fetrow, J., Friendshuh, K., Oakes, J.M., 2006. Characterization of Minnesota dairy herds participating in a Johne's disease control program and evaluation of the program risk assessment tool. Prev Vet Med. 75, 22-33.

Raizman, E.A., Fetrow, J., Wells, S.J., Godden, S.M., Oakes, M.J., Varquez, G., 2007. The association between *Mycobacterium avium* subsp. *paratuberculosis* fecal shedding or clinical Johne's disease and lactation performance on two Minnesota, USA dairy farms. Prev Vet Med. 78, 179-195.

Rajeev, S., Shulaw, W., Berghaus, R., Zhang, Y., Byrum, B., 2006. A testing scheme for the detection of *Mycobacterium avium* subsp. *paratuberculosis* in bovine feces utilizing the ESP *para*-JEM liquid culture system. J Vet Diagn Invest. 18, 529-535.

Rankin, JD., 1961. The experimental infection of cattle with *Mycobacterium johnei* III. Calves maintained in an infectious environment. J Comp Pathol. 71, 10-15.

Rankin, JD., 1962. The experimental infection of cattle with *Mycobacterium johnei* IV. Adult cattle maintained in an infectious environment. J Comp Pathol. 72, 113-117.

Rast, L., Whittington, R.J., 2005. Longitudinal study of the spread of ovine Johne's disease in a sheep flock in southeastern New South Wales. Aust. Vet J. 83, 227-232.

Rastogi, N., David, H.L., 1988. Mechanisms of pathogenicity in mycobacteria. Biochimie. 70, 1101-1120.

Reddacliff, L.A., Vadali, A., Whittington, R.J., 2003. The effect of decontamination protocols on the numbers of sheep strain *Mycobacterium avium* subsp. *paratuberculosis* isolated from tissues and faeces. Vet Microbiol. 95, 271-282.

Reddacliff, L., Eppleston, J., Windsor, P., Whittington, R., Jones. S., 2006. Efficacy of a killed vaccine for the control of paratuberculosis in Australian sheep flocks. Vet Microbiol. 15, 77-90.

Richards, W.D., Thoen, C.O., 1977. Effect of freezing on the viability of *Mycobacterium paratuberculosis* in bovine feces. J Clin Microbiol. 6, 392-395.

Richards, W.D., 1989. Environmental acidity may be the missing piece in the Johne's disease puzzle. In: Millner, A.R., Wood, P.R. (Eds.), Johne's Disease: Current Trends in Research Diagnosis and Management. CSIRO, Victoria, Australia, pp. 90-103.

Ridge, S.E., Baker, I.M., Hannah, M., 2005. Effect of compliance with recommended calf-rearing practices on control of bovine Johne's disease. Aust Vet J. 83, 85-90.

Rossiter, C.A., Burhans, W.S., 1996. Farm-specific approach to paratuberculosis (Johne's disease) control. Vet Clin North Am Food Anim Pract. 12, 383-415.

Roussel, A.J., Libal, M.C., Whitlock, R.H., Hairgrove, T.B., Barling, K.S., Thompson, J.A., 2005. Prevalence of and risk factors for paratuberculosis in purebred beef cattle. J Am Vet Med Assoc. 226, 773-778.

Roussel, A.J., Fosgate, G.T., Manning, E.J.B., Collins, M.T., 2007. Association of fecal shedding of mycobacteria with high ELISA-determined seroprevalence for paratuberculosis in beef herds. J Am Vet Med Assoc. 230, 890-901.

Rowe, M.T., Grant, I.R., 2006. *Mycobacterium avium* ssp. *paratuberculosis* and its potential survival tactics. Lett Appl Microbiol. 42, 305-311.

Ryter, A., Frehel, C., Rastogi, N., David, H.L., 1984. Macrophage interaction with mycobacteria including M. leprae. Acta Leprol. 2, 211-226.

Seitz, S.E, Heider, L.E., Heuston, W.D., Bech-Nielsen, S., Rings, D.M., Spangler, L., 1989. Bovine fetal infection with Mycobacterium paratuberculosis. J Am Vet Med Assoc. 194, 1423-1426.

Smith, R.D., 1995. Veterinary Clinical Epidemiology: A Problem-Oriented Approach. CRC Press, Inc., Boca Raton, Florida.

Sockett, D.C., Carr, D.J., Collins, M.T., 1992. Evaluation of conventional and radiometric fecal culture and a commercial DNA probe for diagnosis of *Mycobacterium paratuberculosis* infections in cattle. Can J Vet Res. 56, 148-153.

Spangler, E., Heider, L.E., Bech-Nielsen, S., Dorn, C.R., 1991. Serologic enzyme-linked immunosorbent assay responses of calves vaccinated with a killed *Mycobacterium paratuberculosis* vaccine. Am J Vet Res. 52, 1197-1200.

Spangler, E., Bech-Nielsen, S., Heider, L.E., 1992. Diagnostic performance of two serologic tests and fecal culture for subclinical paratuberculosis, and associations with production. Prev Vet Med. 13, 185-195.

St. Jean, G., 1996. Treatment of clinical paratuberculosis in cattle. Vet Clin North Am Food Anim Pract. 12, 417-430.

Stabel, J.R., 1997. An improved method for cultivation of Mycobacterium paratuberculosis from bovine fecal samples and comparison to three other methods. J Vet Diagn Invest. 9, 375-380.

Stabel, J.R., 1998. Johne's disease: A hidden threat. J. Dairy Sci. 81, 283-288.

Stabel, J.R., 2000. Transitions in immune responses to *Mycobacterium paratuberculosis*. Vet Microbiol. 77, 465-473.

Stabel, J.R., Whitlock, R.H., 2001. An evaluation of a modified interferon-γ assay for the detection of paratuberculosis in dairy herds. Vet Immunol Immunopathol. 79, 69-81.

Stabel, J.R., Wells, S.J., Wagner, B.A., 2002. Relationships between fecal culture, ELISA, and bulk tank milk test results for Johne's disease in US dairy herds. J Dairy Sci. 85, 525-531.

Stabel, J.R., Hurd, S., Calvente, L., Rosenbusch, R.F., 2004. Destruction of *Mycobacterium paratuberculosis, Salmonella* spp., and *Mycoplasma* spp. in raw milk by a commercial on-farm high-temperature, short-time pasteurizer. J Dairy Sci. 87, 2177-2183.

Stehman, S.M., 1996. Paratuberculosis in small ruminants, deer, and South American camelids. Vet Clin North Am Food Anim Pract. 12, 441-455.

Step, D.L., Streeter, R.N., Kirkpatrick, J.G., 2000. Johne's disease update. Bov Prac. 34, 6-12.

Streeter, R.N., Hoffsis, G.F., Bech-Nielsen, S., Shulaw, W.P., Rings, D.M., 1995. Isolation of *Mycobacterium paratuberculosis* from colostrum and milk of subclinically infected cows. Am J Vet Res. 56, 1322-1324.

Storset, A.K., Hasvold, H.J., Valheim, M., Brun-Hanse, H., Berntsen, G., Whist, S.K., Djonne, B., Press, C.M., Holstad, G., Larsen, H.J., 2001. Subclinical paratuberculosis in goats following experimental infection. An immunological and microbiological study. Vet Immunol Immunopathol. 80, 271-287.

Storset, A.K., 2003. Immunology of *Mycobacterium avium* subsp. *paratuberculosis* infection – immunodiagnostic methods. Acta Vet Scand. 44, 223-229.

Stott, A.W., Jones, G.M., Humphry, R.W., Gunn, G.J., 2005. Financial incentive to control paratuberculosis (Johne's disease) on dairy farms in the United Kingdom. Vet Rec. 156, 825-831.

Sweeney, R.W., Whitlock, R.H., Rosenber,A.E., 1992a. *Mycobacterium paratuberculosis* isolated from fetuses of infected cows not manifesting signs of the disease. Am J Vet Res. 53, 477-480.

Sweeney, R.W., Whitlock, R.H., Rosenberger, A.E., 1992b. *Mycobacterium paratuberculosis* cultured from milk and supramammary lymph nodes of infected asymptomatic cows. J Clin Microbiol. 30, 166-171.

Sweeney, R.W., Whitlock, R.H., Rosenberger, A.E., 1992c. Isolation of *Mycobacterium paratuberculosis* after oral inoculation of uninfected cattle. Am J Vet Res. 53, 1312-1314.

Sweeney, R.W., Whitlock, R.H., Buckley, C.L., Spencer, P.A., 1995. Evaluation of a commercial enzyme-linked immunosorbent assay for the diagnosis of paratuberculosis in dairy cattle. J Vet Diagn Invest. 7, 488-493.

Sweeney, R.W., 1996. Transmission of paratuberculosis. Vet Clin North Am Food Anim Pract. 12, 305-312.

Sweeney, R.W., Whitlock, R.H., McAdams, S., Fyock, T., 2006a. Longitudinal study of ELISA seroreactivity to *Mycobacterium avium* subsp. *paratuberculosis* in infected cattle and culture-negative herd mates. J Vet Diagn Invest. 18, 2-6.

Sweeney, R.W., Uzonna, J., Whitlock, R.H., Habecker, P.L., Chilton, P., Scott, P., 2006b. Tissue predilection sites and effect of dose on *Mycobacterium avium* subsp. *paratuberculosis* organism recovery in a short-term bovine experimental oral infection model, Res Vet Med. 80, 253-259.

Tavornpanich, S., Gardner, I.A., Anderson, R.J., Shin, S., Whitlock, R.H., Fyock, T., Adaska, J.M., Walker, R.L., Hietala, S.K., 2004. Evaluation of microbial culture of pooled fecal samples for detection of *Mycobacterium avium* subsp. *paratuberculosis* in large dairy herds. Am J Vet Res. 65, 1061-1070.

Tavornpanich, S., Munoz-Zanzi, C.A., Wells, S.J., Raizman, E.A., Carpenter, T.E., Johnson, W.O., Gardner, I.A., 2008. Simulation model for evaluation of testing strategies for detection of paratuberculosis in Midwestern US dairy hreds. Prev Vet Med. 83, 65-82.

Taylor, T.K., Wilks, C.R., McQueen, D.S., 1981. Isolation of *Mycobacterium paratuberculosis* from the milk of a cow with Johne's disease. Vet Rec. 109, 532-533.

Thoen, C.O., Jarnagin, J.L., Richards, W.D., 1975. Isolation and identification of mycobacteria from porcine tissues: a three-year study. Am J Vet Res. 36, 1383-1386.

Thoen, C.O., Moore, L.A., 1989. Control of Johne's disease in four commercial dairy herds in Iowa. J Vet Diagn Invest. 1, 223-226.

Thorne, J.G., Hardin, L.E., 1997. Estimated prevalence of paratuberculosis in Missouri, USA cattle. Prev Vet Med. 31, 51-57.

Tiwari, A., VanLeeuwen, J.A., McKenna, S.L.B., Keefe, G.P., Barkema, H.W., 2006. Johne's disease in Canada part I: Clinical symptoms, pathophysiology, diagnosis, and prevalence in dairy herds. Can Vet J. 47, 874-882.

USDA, 1997. Johne's Disease on U.S. Dairy Operations. NAHMS USDA:APHIS:VS:CEAH, Fort Collins, CO, USA, #N245.1097.

USDA, 2005. Johne's disease on U.S. dairy operations, 2002. USDA:APHIS:VS:CEAH, National Animal Health Monitoring System, Fort Collins, CO #N27.0205. pp. 26-27.

USDA, 2005. Uniform program standards for the voluntary bovine Johne's disease Mycobacterium avium subsp. Paratuberculosis. USDA-APHIS 91-45-016. Available from http://www.aphis.usda.gov/animal_health/animal_diseases/johnes/downloads/johnes-umr.pdf. Last accessed 5/16/2008.

USDA, 2008. Johne's Disease on U.S. Dairies, 1991-2007. NAHMS USDA:APHIS:VS, CEAH, Fort Collins, CO, USA, #N521.0408.

vanRoermund, H.J.W., deJong, M.C.M., 2005. Horizontal transmission experiment of paratuberculosis. Proc 8th Int Coll Paratuberculosis. pp. 672-678.

van Schaik, G., Kalis, C.H.J., Benedictus, G., Dijkhuizen, A.A., Huirne, R.B.M., 1996. Cost-benefit analysis of vaccination against paratuberculosis in dairy cattle. Vet Rec. 139, 624-627.

van Schaik, G., Rossiter, C.R., Stehman, S.M., Shin, S.J., Schukken, Y.H., 2003a. Longitudinal study to investigate variation in results of repeated ELISA and culture of fecal samples for *Mycobacterium avium* subsp. *paratuberculosis* in commercial dairy herds. Am J Vet Res. 64, 479-484.

van Schaik, G., Stehman, S.M., Schukken, Y.H., Rossiter C.R., Shin, S.J., 2003b. Pooled fecal culture sampling for *Mycobacterium avium* subsp. *paratuberculosis* at different herd sizes and prevalenc. J Vet Diagn Invest. 15, 233-241.

van Schaik, G., Stehman, S.M., Jacobson, R.H., Schukken, Y.H., Shin, S.J., Lein, D.H., 2005. Cow-level evaluation of a kinetics ELISA with multiple cutoff values to detect fecal shedding of *Mycobacterium avium* subspecies *paratuberculosis* in New York State dairy cows. Prev Vet Med. 72, 221-236.

van Schaik, G., Pradenas, M.F., Mella, A.N., Kruze, J.V., 2007. Diagnostic validity and costs of pooled feal samples and individual blood or fecal samples to determine the cow- and herd-status for *Mycobacterium avium* subsp. *paratuberculosis.* Prev Vet Med. 82, 159-165.

Vary, P.H., Andersen, P.R., Green, E., Hermon-Taylor, J., McFadden, J.J., 1990. Use of highly specific DNA probes and the polymerase chain reaction to detect *Mycobacterium paratuberculosis* in Johne's disease. J Clin Microbiol. 28, 933-937.

Ward, M.P., Perez, A.M., 2004. Association between soil type and paratuberculosis in cattle herds. Am J Vet Res. 65, 10-14.

Waters, W.R., Miller, J.M., Palmer, M.V., Stabel, J.R., Jones, D.E., Koistinen, K.A., Steadham, E.M., Hamilton, M.J., Davis, W.C., Bannantine, J.P., 2003. Early induction of humoral and cellular immune responses during experimental *Mycobacterium avium* subsp. *paratuberculosis* infection of calves. Infect Immun. 71, 5130-5138.

Wells, S.J., Ott, S.L., Hillberg Seitzinger, A., 1998. Key health issues for dairy cattle – new and old. J. Dairy Sci. 81, 3029-3035.

Wells, S.J., Wagner, B.A., 2000. Herd-level risk factors for infection with *Mycobacterium paratuberculosis* in US dairies and association between familiarity of the herd manger with the disease or prior diagnosis of the disease in that herd and use of preventive measures. J Am Vet Med Assoc. 216, 1450-1457.

Wells, S.J., Whitlock, R.H., Lindeman, C.J., Fyock, T., 2002a. Evaluation of bacteriologic culture of pooled fecal samples for detection of *Mycobacterium paratuberculosis.* Am J Vet Res. 63, 1207-1211.

Wells, S.J., Whitlock, R.H., Wagner, B.A., Collins, J., Garry, F., Hirst, H., Lawrence, J., Saville, W.J.A., Larew-Naugle, A.L., 2002b. Sensitivity of test strategies used in the Voluntary Johne's Disease Herd Status Program for detection of *Mycobacterium paratuberculosis* infection in dairy cattle herds. J Am Vet Med Assoc. 220, 1053-1057.

Wells, S.J., Godden, S.M., Lindeman, C.J., Collins, J.E., 2003. Evaluation of bacteriologic culture of individual and pooled fecal samples for detection of *Mycobacterium paratuberculosis* in dairy cattle herds. J Am Vet Med Assoc. 223, 1022-1025.

Wentink, G.H., Bonger, J.H. Zeeuwen, A.A., Jaartsveld, F.H., 1994. Incidence of paratuberculosis after vaccination against *M. paratuberculosis* in two infected dairy herds. J Vet Med Ser B. 41, 517-522.

Whan, L., Grant, I.R., Ball, H.J., Scott, R., Rowe, M.T., 2001. Bactericidal effect of chlorine of *Mycobacterium paratuberculosis* in drinking water. Lett Appl Microbiol. 33, 227-231.

Whipple, D.L., Kapke, P.A., Andersen, P.R., 1992. Comparison of a commercial DNA probe test and three cultivation procedures for the detection of *Mycobacterium paratuberculosis* in bovine feces. J Vet Diagn Invest. 4, 23-27.

Whitlock, R.H., 1992. Diarrhea in Cattle: Johne's disease. In: Anderson, N.V. (Ed.), Veterinary Gastroenterology. 2nd Ed. Philadelphia, pp. 783-789.

Whitlock, R.H., Buergelt, C., 1996. Preclinical and clinical manifestations of paratuberculosis (including pathology). Vet Clin North Am Food Anim Pract.. 12, 345-356.

Whitlock, R.H., Wells, S.J., Sweeney, R.W., Van Tiem, J., 2000. ELISA and fecal culture for paratuberculosis (Johne's disease): sensitivity and specificity of each method. Vet Microbiol. 77, 387-398.

Whitlock, R.H., Sweeney, R.W., Fyock, T., McAdams, S., Gardner, I.A., McClary, D.G., 2005. Johne's disease: the effect of feeding monensin to reduce the bioburden of *Mycobacterium avium* subspecies *paratuberculosis* in neonatal calves. 2005. Proc 38th Annu Conv Am Assoc Bov Prac. pp. 191- 192.

Whiltock,.R.H., Gardiner, I.A., Mangold, B.L., Smith, J., Sweeney, R.W., Schukken, Y., 2006. Johne's disease: Mycobacterium paratuberculosis super-shedders: detection and contribution to passive shedding (false-positive fecal cultures). Proc 39th Annu Conv Am Assoc Bov Prac. 286-288.

Whittington, R.J., Sergeant, E.S.G., 2001. Progress towards understanding the spread, detection and control of *Mycobacterium avium* subsp *paratuberculosis* in animal populations. Aust Vet J. 79, 267-278.

Whittington, R.J., Marsh, I.B., Taylor, P.J., Marshall, D.J., Taragel, C., Reddacliff, L.A., 2003. Isolation of *Mycobacterium avium* subsp *paratuberculosis* from environmental samples collected from farms before and after destocking sheep with paratuberculosis. Aust Vet J. 81, 559-563.

Whittington, R.J., Marshall, D.J., Nicholls, P.J., Marsh, I.B., Reddacliff, L.A., 2004. Survival and dormancy of Mycobacterium avium subsp. paratuberculosis in the environment. Appl Environ Microbiol. 70, 2989-3004.

Whittington, R.J., Windsor, P.A., 2007. In utero infection of cattle with *Mycobacterium avium* subsp *paratuberculosis*. Proc 9th Int Coll Paratuberculosis. pp. 112.

Williams, E.S., Snyder, S.P., Martin, K.L., 1983. Experimental infection of some North American wild ruminants and domestic sheep with *Mycobacterium paratuberculosis*: clinical and bacteriological findings. J Wildl Dis. 19, 185-191.

Wilson, D.J., Rossiter, C., Han, H.R., Sears, P.M., 1993. Association of *Mycobacterium avium paratuberculosis* infection with reduced mastitis, but with decreased milk production and increased cull rate in clinically normal dairy cows. Am J Vet Res. 54, 1851-1857.

Wood, P.R., Corner, L.A., Plackett, P., 1990. Development of a simple, rapid in vitro cellular assay for bovine tuberculosis based on the production of γ-interferon. Res Vet Sci. 49, 46-49.

Zubrick, B.G., Czuprynski, C.J., 1987. Ingestion and intracellular growth of *Mycobacterium paratuberculosis* within bovine blood monocytes and monocyte-derived macrophages. Infect Immun. 55, 1588-1593.

Zubrick, B.G., Follett, D.M., Czuprynski, C.J., 1988. Cytokine regulation of the intracellular growth of *Mycobacterium paratuberculosis* in bovine monocytes. Infect Immun. 56, 1692-1697.