INVESTIGATION OF THE DISTRIBUTION AND RISK FACTORS ASSOCIATED WITH *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* IN COW-CALF HERDS IN CANADA

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ABSTRACT

This thesis summarizes an investigation of *Mycobacterium avium* subspecies *paratuberculosis* (Map) as a pathogen within the cow-calf industry in Canada. The specific objectives of this project were to describe the distribution of this pathogen in this industry provincially, as well as at the individual farm level in wildlife species, and in the environment. Secondary objectives of this project were to identify on-farm management risk factors that are associated with this disease and to examine potential options for herd level diagnostic capabilities. Nationally, 0.8% (95%CI = 0.4-1.1%) of the cows in the cow-calf industry were seropositive for Map with 11.7% (95%CI=7.0-16.5%) of the herds sampled having a minimum of one positive test result or 4.5% (95%CI=1.4-7.5%) of the herds having a minimum of two positive test results. The true cow prevalence was estimated as 1.8% (95%CI= 0.4 – 3.1). No Map was detected in any of the non-ruminant wildlife species sampled on cow-calf operations suggesting that these species were not of primary concern when dealing with the management of this disease. In a study not focussed on a cow-calf operation, Map was detected in one cluster of trapped coyote samples in a region with cow-calf production. The prevalence of Map infection in this cluster of coyotes was calculated to be 9.1% (CI: 5.7-12.5). The prevalence of infection in coyotes including all sites, ignoring the effect of clustering, was calculated to be 3.7% (CI: 2.3-5.1). The use of a commercial colostrum replacement on farm (Odds Ratio =3.96; 95% CI = 1.10–14.23, p=0.035) and the presence of wild deer interacting with the cattle (Odds Ratio = 14.32; 95% CI = 1.13–181.90, p=0.040) were positively associated with being a herd infected with paratuberculosis. The use of rotational grazing practices was protective (Odds Ratio = 0.20; 95% CI = 0.04–0.93, p=0.039). It was possible to

detect environmental contamination with Map on cow-calf farms using bacterial culture and PCR for confirmation. No water samples were positive to Map; however, 6.2% of the non-water environmental samples were positive. The use of an environmental sampling protocol had a herd sensitivity of 29.6%. This finding led to a simulation modelling study to evaluate how various testing methods would compare in the broader population of cow-calf herds. The final mean risk of selecting a herd infected with Map that was not identified as positive via the herd screen test strategy was 12.9%, 9.8%, 9.6%, and 6.1% for no herd screen test, environmental sampling, ELISA serology, and pooled fecal culture strategies, respectively.

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DEDICATION

I would like to dedicate this thesis to my family, especially…

to Lisa for your love and support

to Peter and Juliana for the joy and motivation that you bring

to Mom and Dad for instilling in me the importance of hard work and education

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LIST OF ABBREVIATIONS

AP	Apparent prevalence
CI	Confidence interval
CrI	Credible interval
df	Degrees of freedom
ELISA	Enzyme-Linked Immunosorbent Assay
HNPV	Herd negative predictive value
Hp	Herd level prevalence
HSens	Herd sensitivity
HSpec	Herd specificity
M.	*Mycobacterium*
M. paratuberculosis	*Mycobacterium avium* ssp. *paratuberculosis*
Map	*Mycobacterium avium* ssp. *paratuberculosis*
NAHMS	National Animal Health Monitoring System
n	number of animals tested
No.	Number
OR	Odds ratio
p	Within herd prevalence
PCR	Polymerase chain reaction
PLDC	Production Limiting Disease Committee
pos.	Positive
RFLP	Restriction fragment length polymorphism

RR	Risk Ratio
Sen	Sensitivity
Spec	Specificity
ssp.	Subspecies
USDA	United States Department of Agriculture
UV	Ultraviolet
WCVM	Western College of Veterinary Medicine
+	Positive
-	Negative

CHAPTER 1

INTRODUCTION

1.1 Background

Johne's disease, or paratuberculosis, is a chronic, granulomatous, bacterial enteritis that leads to diarrhea, cachexia and death in ruminants. It is caused by the bacterium known as *Mycobacterium avium* subspecies *paratuberculosis* (Map). First described in 1826 (Chiodini et al., 1984), paratuberculosis remains a disease of importance for cattle industries, not only due to the losses associated with limiting trade and on-farm production, but also as a potential zoonotic concern (Chiodini et al., 1984; Collins and Manning, 1995; Hermon-Taylor, 2000; Manning, 2001, Bull et al., 2003; Chiodini and Rossiter, 1996). The ability of Map to infect multiple species and survive in the environment adds to the complexity of the ecology of this disease. Understanding the distribution of Map in the farm ecosystem is necessary if appropriate control measures are to be determined.

Due to the complicated pathogenesis of the disease that involves a long latent period of infection and a slow immune response there is currently no perfect gold standard test available for detection of Map infection. The sensitivity and specificity of diagnostic tests for Map have had variable results depending on the age of the animal at the time of infection and of sample collection, as well as the type of test and methodology used (Neilsen and Toft, 2008). Most Map control strategies include recommendations of

purchasing replacement cattle from low risk or disease free herds. Identifying herds as low risk or disease free involves having a herd history of disease as well as some consideration of testing protocols used. Many different testing strategies have been used as herd screening tests in Map control programs including pooled fecal culture, serological antibody detections tests, and more recently culture of strategically collected environmental samples. Improved diagnostic tests and testing methodologies are required if paratuberculosis is to be truly controlled.

1.2 Investigative Approach

Most of the present research and control efforts have targeted the dairy industry and therefore very little is presently known about the epidemiology of Map in beef herds. This research was commenced to provide some initial data on epidemiology of Map in cow-calf herds in western Canada in order to determine on-farm management risk factors, to describe the environmental distribution, and finally to evaluate potential new diagnostic strategies for this population. These investigations were entirely based on field research and surveys of current producers from the four western provinces. The objectives of this research included:

1. to report the seroprevalence for Map infection in cow-calf herds in Canada
2. to identify potential risk factors associated with cow-calf herds infected with Map.
3. to describe the distribution of Map in the envionment of infected cow-calf farms in Western Canada throughout the year

4. to determine the distribution and prevalence of Map in the non-cattle species found on infected cow calf farms as well as assessing the value of using wild species as sentinel species for regional Map infection.
5. to evaluate the potential use of environmental sampling as a alternative to herd serological and pooled fecal culture on cow-calf herds by suggesting a new herd screen test strategy and modelling the risks assosiateed with the use of this strategy.

The literature on Map was reviewed as it pertained to the objectives of this thesis. This set the background for the following projects which aimed to add to the present knowledge of the subject area. In Chapter 3, the seroprevalence of Map in cow-calf herds was described along with a preliminary study of the on-farm management risk factors associated with having an infected herd. An expanded study looking at risk factors associated with on-farm management and herd infection is described in Chapter 4. Chapter 5 examines the temporal and locational distribution of Map on infected farms both in the environment and the species other than cattle. This was a pilot study undertaken to direct and focus further projects. The potential use of a wild species, coyotes, as a sentinel species was assessed in Chapter 6. An expanded study of the environmental distribution was conducted in Chapter 7. The results of this study were used as a model for environmental sampling as a herd screen test that would be evaluated in Chapter 8 using a stochastic modeling approach to risk analysis comparing the use of the various herd screening methods used throughout this thesis. In conclusion, Chapter 9

summarizes the findings of this research along with some of the limitations of this study, while including some suggestions for further required research.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Johne's disease, or paratuberculosis, is a chronic, granulomatous, bacterial enteritis that leads to diarrhea, cachexia and eventually death. First described in 1826, Johne's disease continues to plague the cattle industry today. While the presence of acid-fast bacilli in diseased intestines of a cow was first demonstrated by Johne and Frothingham in 1895, it was Twort and Ingram that identified the causative organism in 1912 and named it *Mycobacterium enteriditis chronicae pseudotuberculosis bovis johne* (Chiodini et al., 1984). This bacterium underwent multiple name changes over time and it is currently being classified as *Mycobacterium avium* subspecies *paratuberculosis* or *M. paratuberculosis,* commonly abbreviated simply as Map. While primarily a disease of domestic ruminants, Map has also been found in many non-domestic ruminant and monogastric species. Over the past 100 years, an immense amount of information has been generated and while it has helped improve current day knowledge of Johne's disease, the disease has not been controlled or eliminated (Kreeger, 1991). This is partly due to the ability of Map to survive long periods of time in harsh conditions, along with a long latency period of infection prior to the onset of clinical disease allowing for effective horizontal and vertical transmission to occur. This enables infections to persist in populations despite a low prevalence of disease. The limitations of the diagnostic tests available for Map make identifying infected animals challenging, especially in the early

stages of the disease further complicating the control measures for Johne's disease (Collins, 1996; Manning and Collins, 2001; Harris and Barletta, 2001). While there are antibiotic combinations that have been suggested as efficacious against Map (Borody et al., 2002; Borody et al., 2007), they are not effective for use in livestock species due to the cost and long term nature of treatment. (Merkal and Larsen, 1973; Merkal and Richards, 1972; St-Jean and Jernigan, 1991).

Johne's disease is important because of the economic losses associated with its ability to limit on-farm production (Ott et al., 1999; McKenna et al., 2006; Tiwari et al., 2008), its effect on world trade (Hasonova and Pavlik, 2006), and also because of its zoonotic potential (Chiodini et al., 1984; Collins and Manning, 1995; Hermon-Taylor, 2000; Manning, 2001). Multiple reviews have been conducted to try to make sense of the variable results researchers have found and while many have concluded that there is a real association between Map infection and Crohn's disease in humans, they have been unable to prove any causal relationship and so the debate over the zoonotic nature of this organism continues (Feller et al., 2007; Behr and Kapur, 2008; Waddell et al., 2008). The importance of Johne's disease has led several countries such as Australia, Netherlands, and United States to develop national control programs for paratuberculosis in their cattle industries (Allworth and Kennedy, 2000; Benedictus et al., 2000; Bulaga, 1998; Groenendaal et al., 2003).

There is a vast amount of literature on Johne's disease and Map spanning both the human and veterinary medicine across the globe. This review is not intended to be

exhaustive review of this research but is focused on enabling readers not already intimately involved in this research area to become aware of some of the complexities of the epidemiology of this organism and the disease it causes. This will hopefully allow the readers to be able to understand the following dissertation and appreciate the need for continuing research in this research area.

2.2 Epidemiology

A thorough understanding of the epidemiology of Johne's disease is important in the development of prevention and control strategies. Factors such as the causative agent, the variety of hosts and the pathogenesis of the disease must be considered if successful management is to be achieved.

2.2.1 Agent

Mycobacterium avium subspecies *paratuberculosis* is a slow growing member of the Mycobacteriaceae. The slow growing members of this bacterial family are known for their association with human and animal disease (Rautiala et al 2004). Map shares >99% DNA homology with *M. avium* subspecies *avium*, which causes tuberculosis in birds and is usually classified as a subspecies of *M. avium*. There remains a segment of the research community that continue to use the name *M. paratuberculosis* due to the significant differences between Map and *M. avium* such as its slower growth rate and its in vitro dependency on mycobactin (Manning, 2001).

There are multiple strains of Map and restriction fragment length polymorphism (RFLP) protocols have been used to distinguish between them. Historically the existence of a cattle (C) and sheep strain (S) was debated (Eamens et al., 2000; Reddacliff et al., 2003; Stehman, 1996; Whipple et al., 1989). The S strain was thought to be found mainly in sheep, and the C strain was more common in cattle (Collins et al., 1993; Eamens et al., 2000; Taylor, 1953). In one study 28 RFLP types were found in 1008 samples from 13 host species and 22 countries (Pavlik et al., 2000). While one type or strain may be more common in a certain species, it is likely that all types of Map can infect all susceptible species of host (Manning, 2001). The complete sequencing of the genome of Map strain K-10 was completed and released in 2004 (Rowe and Grant, 2006).

Map is able to survive harsh conditions for long periods of time and this ability contributes to its epidemiology. Map is the slowest growing of all the cultivatable mycobacteria (Lambrecht et al., 1988). It is surrounded by a complex tripartite lipid-rich cell wall that enables it to persist in the environment and contributes to its resistance to low pH, high temperature and chemical agents (Manning, 2001). The slow growing nature of this organism is also partially due to the waxy cell wall limiting the speed of nutrient uptake (Domingue and Woody, 1997). Research has suggested that chlorination of water may not kill Map, especially when large quantities of the bacteria are present (Whan et al., 2001). When taken up by environmental protozoa such as *Acanthomoeba polyphaga*, Map was even more resistant to the effects of chlorine which would

contribute to this organism's ability to survive in the environment (Whan et al., 2006). The thermotolerance of Map is a significant factor in the potential spread of this pathogen. Not only does the ability of Map to survive thermal stressors increase its environmental survival, but it also increases the risk associated with pasteurized milk as a potential route of exposure for calves or humans. The thermotolerance of Map reduces the effectiveness of pasteurization of contaminated milk (Chiodini and Hermon-Taylor, 1993; Sung and Collins, 1998; Donaghy et al., 2007); however, the risk associated with pasteurized milk remains a topic of ongoing research (Lund et al, 2002; Cerf et al, 2007; Ruzante et al 2008; Stabel 2008). Some of the variability of results can be attributed to the duration of time and heat used for pasteurization, the type of tests used to evaluate the samples (culture versus polymerase chain reaction, PCR), whether or not natural or experimental samples were used, and the presence or absence of clumped colonies of Map within the samples (Lund et al., 2002). For a more extensive summary of this topic several review articles are available (Cerf et al., 2007; Boor, 2001; Lund et al., 2002; Stabel, 2000; Sung and Collins, 1998). Overall, the tolerance of this pathogen to harsh conditions must be considered as an important factor in the epidemiology of this organism and its associated disease.

2.2.2 Host Species

Map is primarily a pathogen of domestic ruminants. Historically Johne's disease has been considered a disease of cattle and sheep; however, over the years many alternate species have been associated with both infection and pathology including both domestic

and wild ruminant and non-ruminant species (Chiodini et al., 1984; Corn et al., 2005; Daniels et al., 2003b). A brief review of the pathogenesis of Johne's disease in cattle will be provided as it has been researched most extensively. Age plays an important role in the susceptibility of cattle to infection with Map (Doyle, 1953; Hagan, 1938; Larsen et al., 1975). Cattle less than six months of age are most susceptible and it has been estimated that approximately one third of calves will develop infections with a single exposure (Chiodini et al., 1984). Older cattle have been shown to require larger doses of Map in order to cause an infection experimentally. This has been attributed to a more developed immune system of the older animal (Manning and Collins, 2001; Rankin, 1961; Rankin, 1962; Sweeney et al., 1992a). The most common route of exposure to Map is via ingestion of contaminated colostrum, milk, or feces (Clarke, 1997; Streeter et al., 1995; Sweeney, 1996b). Map may contaminate milk and colostrum either through direct intramammary shedding or indirectly, via fecal contamination (Taylor et al., 1981; Sweeney et al., 1992; Streeter et al., 1995). There is also evidence of Map being transmitted vertically or transplacentally ((Doyle, 1958; Lawrence, 1956; McQueen and Russell, 1979; Seitz et al., 1989; Sweeney et al., 1992b). It has been suggested that 18%-37% of calves from symptomatic dams and 9% of calves from asymptomatic dams will be infected at birth and that calves infected at birth may become symptomatic themselves more quickly than their counterparts infected after parturition (Sweeney 1996). Map has also been identified in the reproductive tract of infected animals (Ayele et al., 2004; Kopecky et al., 1967; Larsen and Kopecky, 1970) including from the uterine washings from infected cows and in semen of infected bulls (Larsen et al., 1981; Rhode and Shulaw, 1990). The risk of infection from these sources is suspected to be relatively

low, and therefore embryo transfer and artificial insemination are not regarded as a significant risk. (Kruip et al., 2003; Sweeney, 1996b).

2.3 Public Health Significance

In 1913, it was proposed that Map may be a cause of chronic enteritis in humans (Dalziel, 1913). In the last century, much research has been conducted and the public health concern associated with Map is still inconclusive. While it is now generally accepted that there is an association with the presence of Map and Crohn's disease in humans, the issue of causation is still a topic of much debate. Several helpful reviews have recently been published and offer the reader more complete synopsis of this specific topic that is only briefly discussed in this thesis (Grant, 2005; Feller et al., 2007; Uzoigwe et al., 2007; .Abubakar et al, 2008; Behr et al, 2008; Waddell et al., 2008). The public health concern has gained attention since researchers have demonstrated that viable Map organisms can be identified in pasteurized retail milk in multiple countries. In the United States, 2.8% of the retail samples tested identified viable Map organisms. Other potential sources of exposure of public health concern include meat from infected cattle as well as water and non-fluid milk dairy products (Grant, 2005). The production limiting effects of Johne's disease as well as the potential public health risk of Map have made the control of Map a priority for the affected industries. The Office International des Epizooties (OIE) considers Johne's disease a disease of major global importance and has categorized it as a List B transmissible disease (Office International des Epizooties, 2001). Initiatives

have been taken internationally to promote research to gain further understanding of the epidemiology of this disease and to develop regional Johne's disease control and management programs.

2.4 Pathogenesis

Most researchers agree that once ingested the primary site of entry for the Map organism is into the lymphatic system of the small intestine through the M cells, although other sites of entry have been suggested as well (Manning and Collins, 2001). Macrophages then engulf the Map organisms and this is where Map is maintained and multiplies within the host (Obasanjo et al., 1997). These activated macrophages illicit the lymphocytic response. The immune response against Map is similar to the response patterns seen against other pathogenic Mycobacteria and consists of a tuberculoid (Th1) response and a lepromatous (Th2) response. During the initial Th1 response there is primarily a lymphocytic infiltrate surrounding only a few bacteria which may be contained in macrophages and microscopic granulomas. Cytokines are produced including gamma interferon, interleukin 2, and tumour necrosis factor alpha and these are the earliest detectable signs of a Map infection, although they are non specific. Antibodies are not produced at a detectable level during this subclinical phase that can last for years (Manning, 2001). Clinical signs, such as weight loss and diarrhea, develop during the Th2 period of infection. During this period additional cytokines are stimulated which produce an antibody response that does not appear to prevent infection nor pathology. Gross pathologic changes may vary depending on the stage and species

involved. In cattle the most common lesion is a thickened ileum with a classic corrugated appearance along with large edematous mesenteric lymph nodes. The thickened ileum is caused by the dramatic infiltration of inflammatory cells that impedes the ability of the ileum to absorb nutrients (Manning and Collins, 2001). This leads to the malabsorption - protein losing enteropathy that causes the development of the typical fluid diarrhea and bottle jaw often described in the literature. An increase in tumour necrosis factor at this time may lead to tissue catabolism followed by emaciation and systemic shedding of macrophages containing Map beyond the gastrointestinal tract and into various organs throughout the body (Manning, 2001).

It is commonly believed that fecal shedding is initiated through suppression or other changes of the immune system. A complete review of the immunology related to paratuberculosis infection can be found in several sources (Chiodini and Rossiter, 1996; Rideout et al., 2003). It has been suggested that if the host is unable to contain the infection through granuloma formation, then *M. paratuberculosis* continues to proliferate and more mononuclear phagocytes are recruited from peripheral circulation (Chiodini, 1996). The granulomatous lesion will continue to expand until such a point that emigration occurs. It is thought that this migration of macrophages out of the lesion may be a result of lost or diminished macrophage inhibitory factor, or simply the effect of a space-occupying lesion. Macrophages near the epithelial lining emigrate into the intestinal lumen and are passed in the feces making the host "culture positive". The emigration of macrophages is dynamic, and varies as the lesion progresses and regresses.

As each focus of infection expands, *M. paratuberculosis* is periodically shed into the feces through macrophages.

Several longitudinal studies have investigated the temporal patterns of diagnostic results from cattle and other species infected with *M. paratuberculosis* (Barrington et al., 2003; de Lisle et al., 1980; Kurade et al., 2004; Manning et al., 2003; van Schaik et al., 2003a). These studies have all reported considerable variation in the responses of animals to *M. paratuberculosis* for the various tests. This suggests that there are many herd and cow factors that govern these different response patterns. One problem with many of these studies is the small numbers of animals investigated. However, such studies do provide some inferences as to what may be happening both immunologically and in regard to fecal shedding of *M. paratuberculosis*.

For a cow, the periparturient period is commonly regarded as the time of greatest immune suppression. However, studies have failed to consistently show an increase in fecal shedding during periods of stress. One such study demonstrated that force-feeding infected cattle during the peripartum period resulted in improved immunological status, but no difference in fecal shedding during this time (Stabel et al., 2003). This study only had 6 cows in each treatment group with variable stages of infection and it is also possible that the follow-up of these cows was not long enough. Perhaps "stressful" periods provide a "trigger" for shedding, but only when the intestinal lesions are sufficiently advanced.

Corticosteroids have also been used to attempt to stimulate fecal shedding in cattle. Dexamethasone (0.1 mg/kg IM) and prednisolone (0.3 mg/kg IM) were given once daily for 6 days to 10 female cattle (Wentink et al., 1988). These cattle ranged in age from 1 to 3 years and were purchased from 3 infected herds. No clinical signs were present in any of these animals prior to treatment. In two of the cattle, *M. paratuberculosis* was cultured from the ileum and mesenteric lymph nodes, but only one of the two shed *M. paratuberculosis* into its feces after treatment. Overall, corticosteroids were found to alter the immunological reactivity of these cattle, but not to such an extent that clinical disease was developed.

The ability of animals to clear infection has been documented experimentally (Chiodini et al., 1984). Our current understanding of how such phenomena may occur is limited by our knowledge of the immune response to *M. paratuberculosis*. Unfortunately many of the immunological studies completed to date have been focused around diagnosis rather than host-agent interactions (Chiodini, 1996).

Johne's disease is commonly described as having four stages. Stage 1 (or the latent infection stage) consists of the time period after initial infection. During this stage there may be some Map organisms in the feces initially due to the "pass through effect" but the newly infected animal is not yet actively shedding Map. Significant levels of antibodies are not produced at this time and there is a lack of fecal shedding so that, although the animal is truly infected, it is not possible to effectively test for the disease status (de Lisle et al., 1980). The majority of infected cattle under two years of age are

in stage 1 (Whitlock and Buergelt, 1996), although some variability exists due to age of exposure and the dose of Map received (Rankin, 1961; Sockett et al., 1992a). Cattle initially infected at an older age may still remain in this stage for long durations and may not progress to stage 2 prior to being culled for other reasons.

Stage 2 begins when the infected animal begins to shed Map actively in the feces. This is often referred to as the subclinical stage, as there are not yet any clinically apparent symptoms. The amount of Map shed may remain quite low during this stage and only 15%-25% of cattle in this stage can be identified with diagnostic testing. These undetectable cattle continue to shed Map into the environment and can be a significant source of exposure to the remaining herd (Dargatz et al., 2001a; Sweeney et al., 1995). Some stage 2 cattle have been classified as super-shedders and can shed more Map in their feces than 160 heavy shedding cattle or 20,000 low shedding cattle (Whitlock et al., 2005a). Excretion of Map in the feces often occurs between 1 and 2.5 years before the onset of clinical disease (Larsen and Merkal, 1968; Whitlock et al., 1991). Occasionally cattle are capable of shedding Map prior to two years of age including one report of a calf shedding Map at less than 6 months of age (Bolton et al, 2005; Weber et al., 2005). Many of the economic costs due to Map infection begin to occur at this time (Nordlund et al., 1996). Many of the cattle will have developed detectable antibody levels by the end of Stage 2 (van Schaik et al., 2003a). Infected cattle can remain in stages 1 and 2 or progress to the clinical stages after a couple years (Sweeney, 2006).

Due to the long latent and subclinical period of Johne's disease, even though infection usually occurs early in life, clinical signs often begin to appear between 2 and 6 years of age. The development of clinical signs is considered the onset of Stage 3. Manure consistency will start to change. Diarrhea may be periodic at first but will continue to worsen. Weight loss will also become evident (Chiodini et al., 1984). The animal usually remains non-febrile throughout the disease and often maintains a strong appetite during this stage (Sherman, 1985).

At stage 4, or the advanced stage, the diarrhea often progresses dramatically until the classic "pipe stream" diarrhea is present. The chronic weight loss becomes severe wasting and the animal often becomes anorexic. Intermandibular edema, commonly known as bottle jaw, develops and the animal becomes very weak and lethargic (Sweeney, 2006). Although usually culled for welfare reasons, cattle in this final stage will often die within weeks (Manning, 2001). During the clinical stages (3 and 4), the quantity of Map shed in the feces usually increases and the serum antibodies become much more detectable; however, it has been suggested that cattle in the very terminal stage of disease may become so anergic that antibodies are no longer above the threshold level of detection (Bendixen, 1978).

There is no effective treatment for Johne's disease and vaccination is only partially effective (Groenendaal and Galligan, 2003). Current vaccines help to reduce fecal shedding but do not prevent infection or prevent all fecal shedding. They have been shown to reduce the quantity of Map shed and have been used in some jurisdictions

outside of Canada (Benedictus et al., 2000). International collaboration has led to attempts to standardize future vaccination research (Hines II et al., 2007).

2.5 Diagnostic Testing

Diagnostic testing capabilities play a vital role in the effectiveness of any attempts to manage this disease at all levels from the individual animal to international levels. Prompt and accurate testing is part of any successful control strategy. Diagnostic testing for Map is often considered discouraging because of the challenges associated with a long latent period during which it is exceptionally challenging to know how to interpret test results. Diagnostic methods available for Map are considered much more effective at the herd level than at the individual animal level; however, results at both levels are necessary when trying to manage an infected herd. Many diagnostic tests for Map have been suggested and used over the last century and research continues to improve the quality of the tests available. The most common diagnostic methods used today include serological assays, bacterial culture, and polymerase chain reaction (PCR) assays. These may be used individually or in combination, and each has positive and negative attributes to them. There is a vast amount of literature on the topic of specific diagnostic tests for Map and many variations to the methods for each type of test. The details will not be discussed exhaustively here. This review will focus on the characteristics of the tests used in the projects in this thesis and will discuss the ability to use diagnostic tests at a herd level for use in herd level disease control programs.

2.5.1 Culture Methods

The generally accepted "gold standard" for Map diagnosis is bacterial culture. There are many variations in culture method and specimen collection which lead to some challenges when trying to compare results between one studies. It is important to know what tests were used, which animals were sampled, and how the results were interpreted. While culture of tissue samples from the lymph nodes or intestinal tissue is usually considered the definitive test (Whittington et al., 1999), because of cost and technical difficulty, fecal sampling is used much more frequently. In order to grow Map in vitro, an enzyme, mycobactin J, must be added to enable the organism to transfer iron across its membrane. Due to its very slow growth, samples must undergo a significant decontamination process prior to culture to prevent overgrowth of other bacteria and fungi. The most common method for bacterial culture uses solid Herrold's egg yolk medium (HEYM). With this method, slants are incubated for a period of 16 weeks and colony growth is monitored visually. Radiometric broth culture methods, such as the Bactec 12B technique used in this thesis, require only 8 weeks incubation prior to reading the results. This method measures the radioisotopes released during bacterial replication daily and, once a threshold is met, the culture is considered positive. A drawback of the radiometric culture systems is the expense of setting up facilities that meet the safety requirements of working with radioactive media. More recently non-radiometric broth culture methods have been developed that are also capable of results after an 8 week incubation. These systems depend on a repeated pressure or fluorescence measurements

to determine positive bacterial growth. All culture methods generally use a PCR assay on positive cultures to confirm the presence of Map. It has been reported that the Bactec system is the most reliable method when trying to culture strains of Map other than those frequently found in cattle and particularly the S strain commonly found in sheep (Gumber and Whittington, 2007). None of these culture systems exclusively grow Map and so a confirmatory test is required on all positive cultures. The sensitivity of conventional fecal culture and Bactec fecal culture has been reported as 45.1% and 54.5%, respectively (Socket et al., 1992a).

2.5.2 Polymerase Chain Reaction Assays

The most common test used for confirmation of Map is one of the Map specific polymerase chain reaction (PCR) assays. When combined with a confirmatory test, the specificity of bacterial culture is generally accepted to be 100%. There are many variations of PCR assay in use for the detection of Map in all types of specimens. These assays detect Map by lysing the organism to extract the DNA. A particular DNA sequence, unique to Map, is amplified through the PCR process. Most of the PCR assays developed for Map are based upon the insertion sequence 900 (IS900) although it has been suggested that IS1311 may be more specific to Map. The potential sequences that are available specific to Map are quite restricted, as there is over 99% homology between the genome of Map and *M. avium* (McFadden et al., 1987b). The IS900 sequence is repeated approximately 8 to 20 times in the Map genome, making it a good target for amplification (Olsen et al., 2002). The primary advantage of the use of PCR, instead of

culture, is the short turnaround time between sampling and availability of results. Results of a PCR can be received within days as opposed to the weeks to months required for the available culture methods. There are also significant disadvantages to PCR. This test detects sequences of DNA and so there is no way to identify whether the DNA came from viable bacteria or from fragments of dead bacteria. Depending on the purpose for testing this can have important implications. For example, if one is trying to determine the risk of infection of a susceptible animal, the viability of the bacteria is paramount. One other reported disadvantage to PCR is the variability of results. Depending on the method used and experience of the technical staff, the sensitivity of PCR can vary widely. It is generally accepted to have a lower sensitivity than traditional culture techniques.

2.5.3 Enzyme-Linked ImmunoSorbent Assays

Another commonly used diagnostic test for Map is the Enzyme-Linked ImmunoSorbent Assay (ELISA) The first USDA license was granted in 1992 for an Australian developed diagnostic test kit that would be marketed in North America by IDEXX Laboratories Inc. (Westbrook, ME) (Collins and Sockett, 1993). There are several ELISA test kits commercially available today in North America that have reported a range of sensitivities and specificities from 43% to 65% and 98.9% to 99.8%, respectively (Collins et al., 1991; Cox et al., 1991; Milner et al., 1990; Reichel et al., 1999; Ridge et al., 1991; Sockett et al., 1992b). Each of these studies acknowledges the influence of the stage of disease on the outcome of the test; however, the exact magnitude of the effect is not fully discussed. Two studies have evaluated the effectiveness of

commercial ELISAs in multiple groups of cattle at various stages of Johne's disease (Dargatz et al., 2001a; Sweeney et al., 1995). Both studies had similar results, with the sensitivity ranging from 15% in light-shedding, subclinical cattle to 88% in clinical cases of Johne's disease. The overall sensitivity and specificity of the ELISA using the manufacturers recommended cutoff was 45% ± 4.8% and 99% ± 0.9% (Sweeney et al., 1995). Whitlock et al. (2000) estimated that in a typical infected population of cattle, 95% of the detectable individuals will be subclinical (stage 2) and 5% will be clinical cases (stage 3). One study estimated that the sensitivity of a serum ELISA would be 25% in a typical Map infected herd if it consisted of subclinical cattle made up of 70% low fecal shedders, 5% medium fecal shedders, and 20% high fecal shedders (Whitlock et al., 2000). An important consideration is that this estimate excluded cattle that were in stage 1 of the disease, as all cattle were assumed to be shedding Map in the feces. More recently, the sensitivity of two commercial serum ELISAs was determined to be 8.8% and 6.9%, relative to tissue culture, in a slaughterhouse study of culled dairy cattle (McKenna et al., 2005b). Researchers have found a wide range of agreement between the results of different ELISAs reporting Kappa statistics between 0.18 and 0.85 (Collins et al., 2005; McKenna et al., 2006). The lack of sensitivity and agreement between these tests are a significant concern and make interpretation of results a challenging exercise. Some of the reasons for the wide range of reported sensitivities include the stage of disease of cattle tested and also the "gold standard" to which the ELISA results were compared. Bacterial culture techniques have improved over the years, leading to a corresponding reduction in the reported ELISA sensitivities (Whitlock et al., 2000). Tissue culture is more sensitive than fecal culture; however, due to cost and ease of

sampling, fecal culture has frequently been used as the gold standard, which leads to an inflated ELISA sensitivity (McKenna et al., 2005b; Whitlock et al., 2000). It is important to consider these issues when interpreting the results of an individual test. Companies marketing the ELISA tests have recommended that it be used for identifying infected herds and not for individual animal diagnosis. Historically, only serum samples were collected for use with the ELISA; however, recently ELISA tests have been approved for use on milk samples as well. The sensitivity of the ELISA has been reported to be equivalent whether using milk or serum in cattle with low milk production (Lombard et al., 2006). It is believed that high milk production may have a dilution effect and make the antibodies undetectable in certain cattle. The low cost of the ELISA and the quick turnaround time from sampling to receiving results are its primary advantages; however, the low sensitivity especially during the early stages of disease and the difficulty of interpretation at the individual animal level present the user with significant disadvantages.

2.5.4 Herd-Level Screen Tests

Many strategies have been suggested for diagnostic use at the herd level. A consensus report was published in 2006 that outlined the recommendations for various testing scenarios for both the beef and dairy industries depending on the goals of testing as well as the infection status and size of the herd (Collins et al., 2006).

The bacterial culture of pooled fecal samples has been evaluated as an economical option for the detection of Map. Research has indicated that 63% to 81% of age-clustered pools with one or more infected dairy cattle, will test positive on bacterial culture and that the herd sensitivity of these pooled fecal samples ranged from 73% to 94% (Kalis et al., 2000; Wells et al., 2003). In fact, 4% to 7% of the fecal pools consisting of samples from cattle that were negative on individual culture were culture positive for Map. The likelihood of a positive pooled culture result was positively correlated with the quantity of Map in the individual samples (Wells et al., 2003). Pooling fecal samples from 5 cows has a higher sensitivity than pools from 10 cows; however, herd prevalence and size must be considered to optimize the validity and economy of the results (Wells et al., 2002; van Schaik et al., 2003; Tavornpanich et al., 2004; Eamens et al., 2008).

Preliminary results from a single beef herd study, suggest that pooling 4 to 5 fecal samples according to age clusters is more effective than random pooling of samples. The researchers expressed concern that in beef herds with a low level of infection this method may lack the required sensitivity to be recommended for regular use (Jensen et al., 2005). Research in Alberta has shown that when compared to individual fecal culture, the cow level sensitivity of fecal pooling of 5 and 10 cattle in test positive herds was 73% and 77%, respectively, in beef herds and 74% and 63%, respectively, in dairy herds (Scott, 2004). Herd level sensitivity of fecal pools of 5 and 10 cattle was 92% and 83%, respectively, in beef herds and 78% and 78%, respectively, in dairy herds. Dairy cattle fecal samples were twice as likely to develop fungal overgrowth as compared to beef

cattle. It has been suggested that this may be due to the common practice of feeding silage to dairy cattle.

Due to the expense and logistic challenge of sampling individual cattle to determine herd infection status, researchers have attempted to develop alternate methods of herd screening. Some of the original research done on survivability indicated that Map persisted for up to 11 months in feces and 13 months in water under certain conditions (Lovell et al., 1944; Larsen et al., 1956). The organism has been found to survive for up to 55 weeks in a dry, fully shaded environment in Australia (Whittington et al., 2004). In compost, Map has survived for up to 3 weeks (Gobec et al., 2005). UV radiation and especially temperature fluctuations can significantly impact the recovery of viable Map. It has been suggested that dormancy also may play a role in the survival of this organism (Whittington et al., 2004). Several environmental factors such as soil type, aridity, and pH have been associated with the survival of Map (Johnson-Ifearulundu and Kaneene, 1997; Kopecky, 1977). It was reported that both loamy and sandy soils are conducive to the survivability of Map (Ward and Perez, 2004). Higher organic matters in loamy soils and lower pH in leached sandy soils both increased the survival of this pathogen. Soil aridity and pH were identified as significant inhibitors to the environmental survival of Map in Alberta (Scott, 2004). Map has been cultured from water and sediment samples from rivers, lakes, and reservoirs in the United Kingdom. In these cases, positive water samples were significantly associated with recent rainfalls upstream, river height, and flow (Pickup et al., 2005; Pickup et al., 2006). The ability to detect Map in water and sediment from dams has been described (Whittington et al., 2005) and should be

considered a potential route of exposure. Recently it has been described that the ability of Map to survive outside of the host is in part due to its ability to replicate inside of environmental acanthamoebas (Rowe, 2006; Whan et al., 2006; Mura et al., 2006). These studies demonstrate the ability of Map to replicate within the protist, however, it is unlikely that the rate of replication is sufficient to dramatically increase the level of contamination in the environment. While the ability of Map to survive in the environment has been described repeatedly, the area of the farm with the greatest risk for pathogen exposure has had little attention until recent years. Environmental sampling has been demonstrated to be an economical and effective alternative to traditional herd screening techniques when used in the dairy industry (Raizman et al., 2004; Berghaus et al., 2006; Lombard et al., 2006). This method of sampling a herd does not require individual cattle sampling and therefore the need to handle the animals is eliminated. It has been suggested that as few as 3 samples are needed to accurately identify the infection status of the herd (Berghaus et al., 2006). In Minnesota dairy herds with Johne's disease, culture positive environmental samples were found in cow alley-ways (77% of herds), manure storage (68%), calving area (21%), sick cow pen (18%), water runoff (6%), and post-weaned calf areas (3%) (Raizman et al., 2004). In California dairy herds, environmental samples were positive for Map on culture from lagoon water samples (65%), milking parlour exit alleyways (39%) and from the sick/fresh cow pens (36%) (Berghaus et al., 2006). Researchers from the USDA have evaluated six sites for use in environmental sampling of dairy herds and found parlour exits, holding pens, common alleyways, lagoons, manure spreaders, and manure pits to have very similar Map contamination rates between 42-52% of samples being culture positive for Map

(Lombard et al., 2006). These findings are not surprising given that infected mature cattle shed the greatest number of organisms into the environment and sites with mature cattle are more contaminated than sites without mature cattle. Knowing the environmental distribution of Map is useful information when attempting to manage the spread of Map within a herd. The diagnostic value of environmental sampling on dairy herds was analyzed and determined be a cost-effective option as a herd screening test. Raizman et al., (2006), found that it took 30 minutes of sampling time and $100 of laboratory fees to determine the herd disease status with 90% accuracy. Two other studies found that environmental sampling is able to determine herd status with 70-74% accuracy (Berghaus et al., 2006; Lombard et al., 2006). The results of strategically conducted environmental sampling have been shown to be a positively associated with the approximate level of infection within the herd (Berghaus et al., 2006; Lombard et al., 2006). If precise prevalence data or individual cattle infection status is required, further testing would be required. There is a lack of published research focusing on the distribution of Map in the environment of beef herds. One study from the United States was unable to culture Map from environmental samples collected from nine beef cattle ranches in Texas (Norby et al., 2007). It must be noted that although the nine herds sampled in this study were previously seropositive to Map, no fecal or tissue cultures were done to confirm the presence of Map in the cattle at the time of environmental sampling. As with fecal cultures, all positive environmental cultures must be confirmed to be Map using PCR. One disadvantage of environmental testing is that while it can confirm Map on the farm premise it does not specify which animals are infected and does not confirm that the cattle are the source of the Map.

There are many options to consider when developing a testing strategy for Map and all methods have their positive and negative aspects. To determine which strategy is appropriate in a particular situation herd history, the goal of testing, financial resources, and the determination of the owner must be considered. A flowchart of these options was recently developed to help in this process (Collins, 2006).

2.6 Prevalence of Paratuberculosis

2.6.1 Cattle

Many studies have been done around the globe to determine the prevalence of Map in cattle, as well as in other species (Chiodini et al., 1984; Kennedy and Benedictus, 2001). Most of these studies have focused on dairy cattle. The cow level prevalence in the dairy industry varies globally from as low as 0.8% to as high as 18% (Adaska and Anderson, 2003; Chiodini and van Kruiningen, 1986; Dargatz et al., 2001b; Doyle, 1956; Hill et al., 2003; McKenna et al., 2004; McNab et al., 1991a; Merkal et al., 1987; Stephan et al., 2002; VanLeeuwen et al., 2001). In Canada, the herd level prevalence in the dairy industry ranges from 9.8% in Ontario to 58.8% in Alberta when two positive tests are required to classify a herd as positive. The animal level prevalence ranges from 1.3% in Prince Edward Island to 9.1% in Alberta (VanLeeuwen et al., 2001; VanLeeuwen, 2005; Tiwari and VanLeeuwen JA, 2006; VanLeeuwen, 2006; Scott, 2006). The prevalence in

countries varies by region and is affected by the testing strategy used, but the testing provides evidence that Map can be found in cattle herds across the world.

Data related to the prevalence of Map in beef cattle is quite limited. The USDA National Animal Health Monitoring System (NAHMS) 1997 National Beef Survey reported that the US beef industry has a cow level seroprevalence of 0.4%, with 7.9% of beef herds having at least one seropositive animal (Dargatz et al., 2001a). In regional studies, it is evident that prevalence rates in the beef industry vary among regions in North America. Beef herds in Louisiana had a herd seroprevalence of 30% and a cow level seroprevalence of 4.4% (Turnquist et al., 1991). Beef cattle in Florida had a cow level seroprevalence of 8.8% (Braun et al., 1990). When adjusted for test inaccuracies it was estimated that at least 50% of Alabama beef herds were infected with Map, which coincided with a cow level prevalence of approximately 8% (Hill et al., 2003). Roussel et al., (2005) reported that 44% of Texas beef herds had at least one seropositive animal and a cow level seroprevalence of 3%. It was hypothesized that this might be an overestimate of actual Map infection because of false positives caused by non-Map environmental mycobacteria (Roussel et al., 2005). A serological study of beef herds on community pastures in Saskatchewan found a cow level prevalence of 0.8%. If herds were classified as positive on the basis of one positive serological test the herd prevalence was 15.2% as opposed to only 3.0% when 2 positive serological tests were required for a herd to be classified as infected (Waldner et al., 2002). Alberta dairy cattle and herds tend to have a 4-5 times greater prevalence than their beef counterparts (Scott, 2004). The province of Alberta has reported a cow level seroprevalence of 1.5% which was

estimated to equal a true prevalence of 1.2% of the provincial adult beef herd; 28.5% of herds had at least one positive serological test and 7.9% had two or more positive serological tests (Scott, 2004). In Manitoba, 1.7% of beef cattle sampled were seropositive for Map as compared to 4.5% of dairy cattle in the same study. This agrees with findings internationally that show the prevalence within the dairy industry to be consistently higher than that found in the beef industry (Boelaert et al., 2000; Pence et al., 2003; Diθguez et al., 2007). The intensive nature of dairy production as opposed to beef production is assumed to be the explanation of these findings (Chiodini et al., 1984; Dargatz et al., 2001b; Thoen and Baum, 1988).

Viable Map were recovered from the tissues of 15/189 (7.9%) dairy cattle and 1/350 (0.3%) beef cattle during a study of thin market cows at slaughter in the United States (Rossiter et al., 2005). This supports research that shows that the prevalence of Map in beef cattle is relatively low and suggests the potential risk to food safety is also likely quite low. Direct comparisons between various studies are difficult because of the wide variety of sampling strategies and tests used; however, in general the studies do indicate that presently the seroprevalence in beef cattle is relatively low, although certain regions may experience much more significant challenges.

2.6.2 Wildlife

Many other species become infected with Map under natural or experimental conditions (Chiodini et al., 1984; Clarke, 1997; Hines et al., 1995); Map has even been

isolated from flies (Fischer et al., 2001) and an earthworm (Fischer et al., 2003). Further research is required to fully understand the role that these species play in the epidemiology of this disease. Epidemiological studies have evaluated wildlife in the United Kingdom, Norway, and the Czech Republic with regard to paratuberculosis (Beard et al., 2001; Daniels et al., 2003a; Fredriksen et al., 2004; Greig et al., 1999; Machackova et al., 2004). Researchers in Scotland have suggested that various wild species, particularly rabbits (*Oryctolagus cuniculus*), may represent a significant concern to their livestock industries because of the level of Map they are able to shed into the environment and the lack of fecal pellet avoidance behaviour evident in the cattle (Daniels et al., 2003a). In North America, a number of free ranging ruminants as well as various other wild mammals and birds have been identified with Map (Corn et al., 2005; Temple et al., 1979). However, the epidemiologic role that these species play in Johne's disease of livestock has not been described adequately. These species are likely of greatest importance to pasture based management systems, such as beef cow-calf herds, as compared to dairy cattle that are primarily raised in confinement.

2.7 Environmental Factors

The ability of Map to survive in the environment for an extended period of time significantly affects how Johne's disease needs to be managed. The organism has been found to survive for up to 55 weeks in a dry, fully shaded environment (Whittington et al., 2004). Moisture, application of lime, UV radiation, and temperature fluctuations can all significantly impact the recovery of viable Map. It has been suggested that dormancy

may also play a role in the survivability of this organism (Whittington et al., 2004). The ability of Map to survive in feces (desiccated or slurry), urine, various sources of water, silage, and compost have all been investigated (Gobec et al., 2005; Jorgensen, 1977; Larsen et al., 1956; Lovell et al., 1944). The survival times reported in these studies ranged from 21 days in compost to 3 months in cattle slurry and for up to 19 months in tap water.

Several environmental factors have been associated with the survival of Map. Soil type, aridity, and pH are three of these associated factors. Studies in the United Kingdom, the Netherlands, and the United States (Wisconsin and Michigan) have all concluded that Map is self-limiting in alkaline, calcareous soils (Johnson-Ifearulundu and Kaneene, 1997; Kopecky, 1977). More recently it was reported that loamy and sandy soils are conducive to the survival of Map (Ward and Perez, 2004). Higher organic matters in loamy soils, and lower pH in leached sandy soils, improved the survival of this pathogen (Ward and Perez, 2004). Soil aridity and pH were identified as significant inhibitors to Map survival outside of the host in Alberta (Scott, 2004). Map has been cultured from water and sediment samples from rivers, lakes, and reservoirs in the United Kingdom. Positive water samples were significantly associated with recent rainfalls upstream, river height, and flow (Pickup et al., 2005).

The ability of Map to survive in the environment has been well described but the area of the farm with the greatest risk for pathogen exposure has received little attention. In dairy herds with Johne's disease from Minnesota, the bacterium was cultured from

environmental samples collected in cow alley-ways (77% of herds), manure storage (68%), calving area (21%), sick cow pen (18%), water runoff (6%), and post-weaned calf areas (3%) (Raizman et al., 2004). These findings are not too surprising given that infected mature cattle shed the greatest number of organisms into the environment. Knowing the environmental distribution of Map is useful information, especially when combined with the age-related susceptibility to Johne's disease. Further research is required focusing on the environment of beef herds.

2.8 Johne's Disease Management

2.8.1 Risk Factors

The epidemiology of Johne's disease must be well understood when developing effective prevention and control strategies. The 1997 NAHMS Beef Survey found that 92.2% of beef producers were either unaware of Johne's disease or only recognized the disease by name (USDA, 1999). Any successful management of this disease will require a significant focus on producer education. Many of the management recommendations that are currently suggested around the world are based on best management practices that have been developed for the dairy industry. These are often focused on the logical principle of decreasing the exposure of susceptible young stock to common sources of Map such as contaminated feces, milk and colostrum. Many of these recommendations have not been extensively studied to determine their actual effectiveness, or practicality

in the beef cow-calf setting. Given the differences in management between beef and dairy cattle in North America, the need for beef specific research is essential.

Feces, milk, and colostrum are considered the common sources of Map leading to transmission of disease. Feces from a cow actively shedding Map are likely responsible for contributing the largest quantity of bacteria to the environment and would, therefore, represent the most significant risk to calves from uninfected dams. However, when Map is shed in the milk or colostrum, the suckling calf receives an ongoing and direct dose of infectious bacteria as well as being exposed to the environmental contamination due to fecal shedding. When all risk factors are combined these calves likely bear the greatest risk of becoming infected with Map. Aly and Thurmond, (2005) showed that a calf born to an infected dairy dam was 6.6 times more likely to be infected than calves born to uninfected dams. The largest source of infectious bacteria is from the feces of subclinical and clinical shedding cattle and, in particular, from super-shedding cattle. It has been stated that super-shedders represent the greatest risk to the spread of Johne's disease among herd mates. Some super-shedders are able to contaminate the environment with more Map than 160 heavy shedders, more than 2,000 moderate shedders and more than 20,000 low shedders (Whitlock et al., 2005a). However, super-shedders may be detected relatively easily by culture while many subclinically infected cattle remain undetectable.

Several management factors are associated with a reduction of the risk of Johne's disease in dairy herds. Calving management is commonly identified as one of the most critical factors and must be considered in any control strategy. A clean and dry calving

pen, low number of cows sharing a calving area, prompt or immediate removal of a calf from its dam, and collecting colostrum after the udder has been washed, have all been associated with reduced paratuberculosis herd status (Goodger et al., 1996; Johnson-Ifearulundu and Kaneene, 1998; Wells and Wagner, 2000). The housing and feeding of pre-weaned calves is very important (Collins et al., 1994; Goodger et al., 1996; McNab et al., 1992; Obasanjo et al., 1997). Reducing calf exposure to manure from adult cattle is usually the goal of calf rearing recommendations. A benefit of applying these recommendations is that they also reduce the risk of other fecal-oral diseases of cattle such as Salmonella, Campylobacter, Escherichia and Cryptosporidium species (McKenna et al., 2006). The inability of beef producers to remove calves from cows after calving introduces a major challenge when trying to manage this disease.

The application of lime to the environment has been associated with a reduction of risk of having paratuberculosis in the herd (Johnson-Ifearulundu and Kaneene, 1998; Johnson-Ifearulundu and Kaneene, 1999); however, application of lime was not shown to decrease the survivability of Map in the soil (Whittington et al., 2004). Application of manure onto pasture, the use of exercise lots for cows, contact with other cattle, cow nutrition and water sources have been associated with herd Map status in dairy research (Daniels et al., 2002; Goodger et al., 1996; Johnson-Ifearulundu and Kaneene, 1998; McNab et al., 1992). Dairy herds that have had a previous diagnosis of Johne's disease, a herd size of greater than 300 cows, herds that have previously purchased replacement animals, or herds that have overall poor management are at an increased risk of a positive Map status (Daniels et al., 2002; Hirst et al., 2004; Johnson-Ifearulundu and

Kaneene, 1998; McNab et al., 1992; Obasanjo et al., 1997; Wells and Wagner, 2000). Wildlife access to livestock feed and pasture have also been identified as a significant risk factor for dairy farms. Contact with rabbits and deer on dairy farms are of particular concern (Daniels et al., 2002; Raizman et al., 2005).

Cow-calf producers commonly engage in management practices that increase the risk of disease introduction to their cattle such as importing cattle, inconsistently testing for various diseases in imported animals, failing to use quarantine procedures, and the use of communal grazing (Sanderson et al., 2000). Management practices that have been found associated with the Map seropositivity of beef herds include: history of having a dairy-type nurse cow on farm (Odds Ratio=2.1), use of seasonal calving, i.e. spring calving versus any other time of year (OR= 2.2), the use of running streams as a water source (OR=2.2), previous clinical signs of Johne's on farm (OR=2.8), and having *Bos indicus* rather than *Bos taurus* cattle on farm (OR=17.4). When the effects of other risk factors were controlled for, the use of a dairy-type nurse cow and seasonal calving became non significant but cattle species and water source remained significant risk factors (Roussel et al., 2005). Clearly, more research is needed to fully understand the potential factors important in management of Map in beef herds in Western Canada.

The use of vaccination as part of any control strategy for paratuberculosis remains controversial at this time because of variable results of clinical trials. Vaccination may be beneficial in herds with high rates of culling because of clinical disease; trials indicate that while the vaccine does not eliminate disease there is a reduction of the incidence, or

a delay of clinical disease and fecal shedding (Groenendaal and Galligan, 2003, Kalis et al., 2001; Kormendy, 1994; Larsen et al., 1978; Uzonna et al., 2003; van Schaik et al., 1996). Several published studies did not involve commercially available vaccines and did not use natural exposure to Map as the challenge model and this makes interpretation of the results challenging (Chiodini et al., 1984; Kalis et al., 2001; Kormendy, 1994; Larsen et al., 1978). Other concerns with the use of vaccination for Map include potential interference with serological tests for bovine tuberculosis and potential development of inoculation site granulomas (Chiodini et al., 1984; Spangler et al., 1991). Although the limitations of vaccination are evident and can complicate disease eradication efforts, a significant cost benefit, at least in the short term, of using vaccination as part of a control strategy in some circumstances has been shown (Groenendaal and Galligan, 2003). If vaccination is used, it is recommended that it be given within the first 30 days of life (Thoen and Haagsma, 1996).

The use of monensin as a feed additive may help to reduce the burden of Map on positive farms (Hendrick et al., 2005b; Whitlock et al., 2005b). Monensin sodium belongs to the class of antimicrobials called ionophores and its spectrum of activity includes several Gram-positive bacteria, some *Campylobacter* spp., *Serpulina* spp., and *Mycobacterium* spp., as well as coccidia and *Toxoplasma* (Ipharraguerre and Clark, 2003; Prescott et al., 2000; Liu, 1982). The minimum inhibitory concentration (MIC) for Map was recently reported to be 0.3 micrograms per ml (Brumbaugh et al., 2004). Monensin decreased passive fecal shedding by 55% and tissue infection by 63% in an experimental calf infection model (Whitlock et al., 2005b). The quantity of Map shed in the feces of

infected cows was marginally reduced when monensin was fed (Hendrick et al., 2005b). Further research into the potential use of monensin for Johne's disease control is required.

One of the most commonly attempted Johne's control measures depends on a test and slaughter approach. The potential success of this approach is dependant on factors such as the prevalence of infection, diagnostic test characteristics, and estimated production loss (Collins and Morgan, 1991). It has been suggested that test and cull strategies in isolation from management changes do not reduce the prevalence of Johne's disease in dairy herds (Groenendaal and Galligan, 2003). According to the simulation model they developed, a focus on calf hygiene is the most economical piece of a Map control program in a midsize US dairy farm. This concurred with another study that indicated that a combination of a test and cull program with improved calf management led to the best control of Map (Collins and Morgan, 1992).

2.8.2 Disease Control Recommendations

The principles recommended by the USDA for Johne's disease control for beef producers include: reducing exposure and infection of replacement cattle on farm, identifying and removing the most highly infected cattle, and preventing introduction of infection by screening sources of off-farm replacements (USDA, 1999). The following recommendations, based on first principles of disease control, have been made with a focus on the beef industry (Hansen and Rossiter, 2000; Rideout et al, 2003):

1. Reduce manure build-up in pens and pastures where late-gestation cattle are kept.
2. Keep the calving area clean at all times and maintain a low cow density in these areas.
3. As soon as bonding has occurred, move cow-calf pairs to a clean pasture.
4. Avoid exposing calves to manure build-up by frequently moving location of feed bunks, waterers, and creep-feeders.
5. Once calves are weaned, do not put them on pastures used by cows.
6. Annually test the entire herd and avoid calving-out or raising offspring from any test-positive cattle.
7. Calve first-calf heifers in a separate location from mature cows.
8. Use separate equipment for handling manure and feed.
9. Do not spread manure on land used for grazing, especially for young stock.
10. Purchase replacement animals only from test negative herds and when this is not possible assess herd status through owner and veterinarian statements.

Devising control strategies is important, but little is known on how well they are perceived and utilized by producers. In Australia, it was found that 48% of dairy farmers adopted none of the long-recommended control measures, even though they ranked Johne's disease as their number two calf-hood infection concern (Wraight et al., 2000). Regardless of herd infection status, producers who had tested their herd for paratuberculosis were more likely to be using management strategies, as compared to producers who were not testing (Naugle et al., 2004). The motivation of these producers

to sell breeding stock may have influenced these results, that is, producers with a negative herd status are only likely to test and use specific management strategies if it guarantees that their animals can be sold for a premium. Overall, the value and need for further research and Johne's disease management education programs for producers and veterinarians is clearly indicated.

2.9 Conclusions

The prevalence of *Mycobacterium avium* ssp. *paratuberculosis* in Canadian beef herds is considered very low. There is little doubt that herds infected with Johne's disease may suffer severely. However, the economic loss to the beef industry as a whole is of questionable significance at this time. What has made Johne's disease an issue is the potential zoonotic threat that it presents. The beef industry is in a unique situation, given its low prevalence of Johne's disease to put into motion a strategy to limit further spread of the disease. The control of Johne's disease nationally will be an immense undertaking because of the insidious nature of this disease and the relatively poor performance of tests that are currently available. There is a need to develop best management practices specific to the beef industry with consideration given to the biology and ecology of the disease. Implementing Johne's disease control programs is an important proactive step forward. However, Map has plagued the cattle industry for many years and will likely continue to remain a significant challenge for the foreseeable future.

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CHAPTER 3

SEROPREVALENCE AND RISK FACTORS FOR *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* IN BEEF COW-CALF HERDS IN CANADA

3.1 Introduction

Johne's disease, or paratuberculosis, is a chronic, granulomatous, bacterial enteritis of ruminants that leads to diarrhea, cachexia and eventually death. First described in 1826, Johne's disease remains a disease of importance for all cattle industries, not only because of the economic losses associated with limiting trade and on-farm production, but also because of public health concerns of the potential threat of *Mycobacterium avium* subspecies *paratuberculosis* (Map) being a zoonosis (Chiodini et al., 1984; Collins and Manning, 1995; Hermon-Taylor, 2000; Manning and Collins, 2001). A clear understanding of the epidemiology of Johne's disease is pivotal in the creation of management and control strategies. To date, most research and control efforts have targeted the dairy industry. A survey conducted by the USDA found that 92.2% of beef producers were either unaware of Johne's disease or recognized the disease by name only (United States Department of Agriculture, 1999). Very little is known of the prevalence of Map in Canadian cow-calf herds. Previous cross sectional surveys of beef cattle in Canada have shown individual animal seroprevalence to Map ranging between 0.7% and 1.7% (Waldner et al., 2002; Coté G., 2004; Waldner, 2005; VanLeeuwen et al., 2006). In 1997, the Production Limiting Disease Committee (PLDC) was formed consisting of various members involved in the Canadian cattle industries interested in maintaining both domestic and international markets for the future. In 2003, the PLDC

initiated research focused on the Canadian beef industry. The objectives of this study are to determine the seroprevalence for Map infection in cow-calf herds in Canada and to identify potential risk factors associated with the herd seroprevalence.

3.2 Material and Methods

3.2.1 Study population

The target population consisted of cow-calf herds from the provinces of: Alberta, British Columbia, Ontario, Saskatchewan, New Brunswick, Nova Scotia and Prince Edward Island. Similar surveys were carried out in Manitoba and Quebec independently (Coté G., 2004; VanLeeuwen et al., 2006). In June 2002, promotional materials were sent to various beef industry associations in Canada. Actual herd recruitment was done in 2003 by random sampling of 4,700 cow-calf producers in all participating provinces.

It was estimated that thirty cows per herd were required for testing when assuming an average herd size of 45 cows, an expected within-herd prevalence of 10% for infected herds, an allowable error of 6%, and a level of confidence of 95% (Martin et al., 1987).

3.2.2 Questionnaire survey

Each herd owner was sent a comprehensive, 19-page mail-in questionnaire to gather information on management, biosecurity and demographic factors. The

questionnaire was divided into 6 sections: farm profile, calves and calving, feeding practices, veterinary procedures and vaccination, and farm biosecurity (appendix 10.1).

3.2.3 Serology

Blood samples were collected from randomly selected cows in each herd at the time of pregnancy diagnosis during the fall of 2003. Serum was collected from these samples and stored at -20^{o}C. Serological testing for antibodies to Map was done at the Animal Health Monitoring Lab in Abbotsford, British Columbia, using a commercially available ELISA kit (Paracheck; Biocor Animal Health, Omaha, Nebr.). A study evaluating this ELISA test, using fecal culture as the 'gold standard', reported the sensitivity and specificity to be 28.4%, and 99.7%, respectively (Collins et al., 2005). Test results were expressed as an optical density (OD). A serological sample with an optical density 0.100 or greater above the mean of two negative controls was considered positive, as recommended by the manufacturer.

3.2.4 Data analysis

Questionnaire results were stored in a database (Access® 1997; Microsoft Corporation, Redmond, Washington, USA) and analyzed using SPSS® 14.0 for Windows (SPSS Inc., Chicago, Illinois, USA) and Excel® 2003 (Microsoft Corporation, Redmond, Washington, USA). Individual and herd level seroprevalence was calculated as a proportion of cattle and herds testing positive for Map, respectively, together with

the 95% confidence intervals (95% CI). The individual level true prevalence and its 95% CI were estimated after correcting for test sensitivity and specificity (Dohoo et al., 2003). For the risk factor analysis, Map positive herds were considered to be those herds that had 2 or more seropositive tests. This was done to reduce the likelihood of false positive herds as a result of potential inaccuracies of serological tests for Map.

The identification of management variables associated either positively or negatively with herd infection status to Map was assessed in two steps. Initially, each potentially important risk factor selected from the questionnaire was included in a univariate analysis with herd seropositivity as the dependant variable. Univariate analysis was completed using logistic regression. Variables that were significant at $p \leq 0.25$ in the univariate analysis were then included in a multivariable logistic regression model to determine the main factors associated with herd seropositivity. The model was built following a backwards stepwise likelihood ratio approach as described by SPSS 14.0, using the likelihood ratio statistic for variable removal and the score statistic to select variables for re-entry into the model. The probability for stepwise entry was 0.05 and for stepwise removal was 0.10. Two-factor interactions between the main effects in the model were also tested in a similar fashion and goodness-of-fit was checked using the Hosmer and Lemeshow statistic (Dohoo et al., 2003)

3.3 Results

3.3.1 Seroprevalence

Invitations were extended to 4,700 randomly chosen farmers to participate initially; 280 farmers (6%) responded to the mail-out questionnaire. Of these, 179 farmers (3.8%) actually completed the study. A total of 4,778 cows were tested from the 179 herds, with an average of 27 cows per herd. At least 2 seropositive cows were found in 4.5 percent (95% CI= 1.4 – 7.5) of the herds in the study. The apparent cow prevalence was 0.8% (95% CI= 0.4 – 1.1) with the true cow prevalence calculated to be 1.8% (95% CI= 0.4 – 3.1). The individual seroprevalence at the herd level ranged from 0% to 17.9%. The results by province, herds and cows involved in the study are shown in Table 3.1.

3.3.2 Questionnaire result and statistical analysis

Questionnaire data were missing for 1 herd so that 178 herds were included in the risk factor analysis. A univariate analysis was run on 38 variables of interest from the questionnaire, of which 8 were significantly ($p \leq 0.25$) associated with herd seropositivity to Map (Table 3.3). When these significant variables were entered into a multivariable logistic regression analysis only 4 variables remained in the final model (Table 3.4). For each 1% increase in percentage of calves receiving supplemental colostrum the herd was 1.06 (95%CI = 1.00 – 1.13) times more likely to be seropositive to Map. Herds that

added ionophores to feed were 16.30 (95% CI = 1.22 – 218.48) times more likely to be seropositive to Map than herds that did not use ionophores in the feed. Herds that did not always remove surface manure from maternity pens after each calving were 7.21 (0.631 – 82.51) times more likely to be seropositive to Map than herds that always removed surface manure from maternity pens after each calving although this variable was not statistically significant (P=0.11) in the final model. The presence of a dog on the farm had a protective effect on the herd seropositivity to Map (OR = 0.14; 95%CI=0.02–0.88). This may be more clearly stated by reporting that herds without a dog on farm were 7.42 (95% CI = 1.14 – 48.37) times more likely to be seropositive to Map than herds with a dog present on farm. The model fit the data well (Hosmer and Lemeshow goodness-of-fit χ^2 statistic = 4.5; df=8; p=0.81).

3.4 Discussion

Initially, 4700 Canadian farms were randomly selected to participate in this study. Only 179 (3.8%) of these herds agreed to collaborate and were included. Two major occurrences at that time of this study likely attributed to the poor participation rate. There was both the substantial drought in 2002-2003, as well as the emergence of bovine spongiform encephalopathy in Western Canada which both decreased the likelihood that producers would be willing to participate in a study of this kind.

Participation in this study was completely voluntary and this along with the poor response rate may have introduced a selection bias to the results. Therefore, the findings

of this cross sectional survey should not be considered necessarily representative of the entire Canadian beef cow-calf herd even though it is part of one of the largest studies ever done on beef cattle in Canada.

While a substantial amount of research has been conducted on the epidemiology of Map in dairy cattle, very few studies have been published in beef cattle. Research on cow-calf herds tends to be logistically more difficult due to the standard extensive husbandry practices. Previous cross sectional surveys in Canadian beef cattle have shown individual animal seroprevalence to Map ranging between 0.7% and 1.7% (Waldner et al., 2002; Coté G., 2004; Waldner, 2005; VanLeeuwen et al., 2006). The results of this study are very similar and indicate a low seroprevalence of Map in Canadian cow-calf herds.

The low seroprevalence of Map in these herds was a limiting factor when determining the association between herd seropositivity and management risk factors. The practice of supplementing colostrum was found to be a significant risk factor in this study. It is well documented that Map can be shed in the colostrum of infected cattle (Sweeney et al., 1992; Streeter et al., 1995) and this can be a significant source of infection to young calves while they are most susceptible (Larsen et al., 1975). Map can also survive in the environment for long periods of time (Whittington et al., 2004) and therefore the use of contaminated feeding equipment could potentially act as a vector of transmission to the young calf. The use of dairy colostrum alone was unable to explain the risk associated with supplemental colostrum in this study possibly due to the low

number of case herds. The risk of supplementing colostrum in this study may have been due to the use of colostrum from infected beef cattle on farm. It is also possible that herds with clinical Johne's disease used supplemental colostrum sources more frequently as a preventative management practice. This hypothesis could not be evaluated further in this study as the questionnaire did not ask specific questions on reasons for using supplemental colostrum. These findings suggest that it is imperative to use only Map free colostrum sources and ensure proper cleaning of feeding equipment to reduce the odds of Map transmission to the newborn calf.

The addition of ionophores to the feed was the most strongly associated risk factor identified in this study with an OR = 16.30 (95% CI = 1.22 – 218.48). This was unexpected as previous research has suggested that the addition of ionophores to feed would have a protective effect; that is, calves fed monensin directly had fewer Map culture positive (55%) fecal samples and fewer culture positive (66%) tissue samples (Whitlock et al., 2006). It is possible that Map positive farms would be more likely to add ionophores to the feed in an attempt to treat other potential causes of clinical diarrhea in the herd such as coccidiosis. Management factors that might increase the risk of coccidiosis such as crowding and poor manure management would also increase the risk of Johne's disease. The use of ionophores in the feed would be a response to already having clinical Map in the herd as opposed to being a cause of the disease. Further research is required to fully understand the effect that the use of monensin in the feed has on this disease.

Failure to always remove surface manure from maternity pens after each calving while a positive risk factor for herd seropositivity was not significant in the final model ($p = 0.11$). It has been well documented that manure from Map infected cattle is a significant transmission risk to young calves (Sweeney, 1996) and the lack of statistical significance may have been due to the low number of seropositive herds in this study. It is also possible that in the seropositive cow-calf herds, good maternity pen management may have been recently introduced due to a history of clinical Johne's cattle, resulting in a reduction of the significance of the associated risk between herd seropositivity and poor maternity pen cleanliness found in this study. The practice of maintaining a manure free environment is likely more significant in herds with higher rates of Map infection, as a larger proportion of the manure would be contaminated with viable Map.

The presence of a dog on farm was the only protective variable in the final model. This finding was unexpected as the authors had included this variable to determine if dogs could potentially be a source of transmission through environmental contamination with dog feces. The identified protective effect is difficult to explain but may be due to the effect the presence of a dog has on a potential wildlife source of environmental contamination or this finding may have been due to chance.

Researchers have found other management risk factors associated with herd prevalence for Map in cow-calf herds. The use of a dairy-type nurse cow, the use of running water as a water source, and cattle breed, were all identified as significant risk factors in cow calf herds in Texas (Roussel et al., 2005). Having direct access to a

natural water source was examined as a potential risk factor due to increased likelihood of environmental contamination versus having these areas fenced off and having water pumped into troughs or waterers. Having a pumped vs. direct access to a natural water source during the summer was included in the multivariable analysis in our study due to its significance ($p = 0.24$) in the univariate model but this factor did not remain in the final model. Water source in the winter was not significant at any level. Only 9 herds (5%) used a dairy-type nurse cow in the previous 5 years in our study. None of these herds were seropositive to Map and therefore this risk factor was not included in the analysis. Not enough information was collected on cattle breed to include this as a risk factor into the analysis.

This was a cross sectional study and therefore there are several inherent limitations that need to be considered. Seroprevalence and potential risk factors were only captured at a single point in time. For this reason causal relationships cannot be proven and it is only possible to hypothesize about the relationships between the risk factors and herd seroprevalence of Map in beef cow-calf herds. Further studies would be required to fully determine the effect herd management has on the disease. It would be beneficial to examine the management practices found to be significant in this study in more detail to evaluate exactly what role they contribute to the epidemiology of paratuberculosis in cow-calf herds. Other species may also play a role in the epidemiology of Map and should also be researched further in order to be able to make appropriate recommendations pertaining to them.

In conclusion, it should be restated that at the time of this study there was a low prevalence of Johne's disease in the Canadian cow-calf herd. The industry, however, should remain vigilant in the attempt to keep this disease from spreading: this can be through good management on infected farms and by preventing disease introduction to uninfected farms. Maintaining calf environments free of Map contaminated manure, while ensuring feeding equipment and colostrum sources are uncontaminated with Map should help to reduce the transmission of disease to uninfected animals and contribute to controlling the future impact Map has on the cow-calf industry.

3.5 Acknowledgements

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Table 3.1 Summary of the results of cow and herd level seroprevalence to *Mycobacterium avium* subspecies *paratuberculosis* by province in 2003.

	Animal Level Prevalence				Herd Level Prevalence (≥1 pos.)			Herd Level Prevalence (≥2 pos.)		
Region	No. of cows tested positive	No. of cows tested	Apparent Prevalence % (95%CI)	Estimated True Prevalence % (95%CI)	No. of herds tested positive	No. of herds tested	Prevalence % (95%CI)	No. of herds tested positive	No. of herds tested	Prevalence % (95%CI)
Nova Scotia	0	349	0.00%	0.00%	0	15	0.00%	0	15	0.00%
Prince Edward Island	8	189	4.2% (0-9.0)	14.0% (0-31.0)	4	7	57.1% (17.5-96.7)	1	7	14.3% (0-42.3)
New Brunswick	3	294	1.0% (0-2.8)	2.6% (0-8.8)	1	12	8.3% (0-24.7)	1	12	8.3% (0-24.7)
Ontario	11	1037	1.1% (0-2.1)	2.8% (0-6.4)	5	40	12.5% (2.1-22.9)	3	40	7.5% (0-15.8)
Saskatchewan	5	880	0.6% (0-1.1)	1.0% (0-3.1)	4	32	12.5% (0.9-24.1)	1	32	3.1% (0-9.3)
Alberta	7	833	0.8% (0-1.7)	2.0% (0-5.1)	4	29	13.8% (1.0-26.6)	2	29	6.9% (0-16.3)
British Columbia	3	1196	0.3% (0-0.5)	0% (0-0.7)	3	44	6.8% (0-14.4)	0	44	0.00%
Overall	37	4778	0.8% (0.4-1.1)	1.8% (0.4-3.1)	21	179	11.7% (7.0-16.5)	8	179	4.5% (1.4-7.5)

Serologic results determined by Elisa testing using the optical density cutoff of 0.100 above the mean of two negative controls

Table 3.2 Summary of within herd seroprevalences of *Mycobacterium avium* subspecies *paratuberculosis* positive herds* by province in 2003.

Region	Min.	Median	Max.
Nova Scotia	0	0	0
Prince Edward Island	3.3%	4.8%	17.9%
New Brunswick	10.0%	10.0%	10.0%
Ontario	3.3%	6.7%	17.4%
Saskatchewan	3.3%	4.0%	7.4%
Alberta	3.3%	5.1%	10.0%
British Columbia	3.3%	3.3%	3.4%
Overall	3.3%	4.0%	17.9%

* positive herds defined as => 1 seropositive reactor

Table 3.3 Significant results (P < 0.25) of univariate analysis of the herd odds of seropositivity for Map in Canadian cow-calf herds

Variable	Odds ratio	P value
Dog present on farm	0.15	0.027
Use of ionophores in feed	11.32	0.028
Failure to remove surface manure from maternity pen after each calving Y/N	7.11	0.076
The use of rotational grazing	0.22	0.081
Borrow equipment from neighbours	4.83	0.154
Purebred herd vs. other	3.41	0.164
% of calves that receive supplemental colostrum	1.03	0.199
Direct access to natural water source in summer vs. pumped	0.37	0.237

Table 3.4 Final model of multivariable analysis of the herd odds of seropositivity for Map in Canadian cow-calf herds

Variable	Odds ratio	P value
Use of ionophores in feed	16.30	0.035
Dog present on farm	0.14	0.036
% of calves that receive supplemental colostrum	1.06	0.036
Failure to remove surface manure from maternity pen after each calving Y/N	7.21	0.112

CHAPTER 4

MANAGEMENT RISK FACTORS FOR *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* INFECTION IN COW-CALF HERDS IN WESTERN CANADA

4.1 Introduction

Johne's Disease, caused by *Mycobacterium avium* subspecies *paratuberculosis*, (Map), is a progressive and debilitating disease of livestock affecting beef cattle in Western Canada. It causes a profuse, untreatable diarrhea with high levels of bacterial shedding, dramatic weight loss, and eventually death. Chapter 3 of this thesis assessed the seroprevalence of production limiting diseases in the cattle herds and estimated that 4.5% of cow-calf herds had at least two cattle seropositive to Map and that 0.8% of all tested cattle from cow-calf herds were seropositive to Map. Previous cross sectional surveys of beef cattle in Canada have shown individual animal seroprevalence to Map ranging between 0.7% and 1.7% (Waldner et al., 2002; Coté G., 2004; Waldner, 2005; VanLeeuwen et al., 2006). To date, most research and control efforts have targeted the dairy industry and there is a paucity of evidence based control recommendations for beef cattle herds. While many of these research efforts involving dairy cattle can be helpful when developing disease control recommendations for the cow-calf industry, more specific research is required if success is to be achieved. A survey conducted by the USDA found that 92.2% of beef producers were either unaware of Johne's disease or recognized the disease by name only (United States Department of Agriculture, 1999). Apparently there is a substantial need for education on Johne's disease for producers if significant progress is to be made.

The primary modes of transmission are believed to include oral exposure either through direct exposure through infectious colostrum or milk (Streeter et al., 1995; Sweeney, 1996), or contact with manure from an infected animal either directly or indirectly via a contaminated environment (Manning and Collins, 2001). Calves from cow-calf herds, unlike dairy herds, cannot be isolated immediately after birth and so control efforts must focus on alternate management factors that may be able to reduce the burden of disease to their herds. Cow-calf producers commonly engage in management practices that have the potential to increase the risk of introducing diseases, such as Johne's disease, to their herds. These include activities such as buying cattle from various and potentially unknown sources, inconsistently testing for various diseases in purchased animals, failing to use quarantine procedures, and the use of communal grazing (Sanderson et al., 2000). Management practices that have been found associated with the Map seropositivity of beef herds in Texas have been shown to include: the history of having a dairy-type nurse cow on farm, the use of running streams as a water source, and cattle breed (Roussel et al., 2005).

The objectives of this study were to identify management practices that are associated with a positive paratuberculosis status on cow-calf herds in western Canada and to develop recommendations for producers attempting to control Johne's disease within their herds.

4.2 Methods

Starting in the autumn of 2006, a total of 70 herds were recruited to participate in this research project to determine risk factors associated with Johne's disease in beef cow-calf herds in western Canada. Fifty-eight herds were recruited through contact with local veterinarians throughout western Canada or through contact with the Alberta Beef Producers. An additional 12 case herds were then recruited who had participated in previous Johne's research done at the University of Saskatchewan. A case herd was considered a cow-calf herd from western Canada from which a cow had been diagnosed with Johne's disease within the previous 5 years together with a laboratory confirmation of that diagnosis. A positive test result in the fecal sampling done for this project also resulted in that herd being assigned to the case group regardless of prior history. A herd was considered a control herd if it had no history of any laboratory confirmed diagnoses, nor suspected clinical cases, of Johne's disease within the previous five years. A control herd must also have had all pooled fecal cultures found negative during herd testing for this study.

Individual fecal samples were collected from all cows that were at least two years of age on farms with less than 120 cows and from a representative sample from herds larger than 120 cows in order to help categorize herds as cases or controls. Fecal samples were pooled into groups of five cows, according to age (where possible), and cultured using BACTEC radiometric culture methods. Samples from the initial 58 herds were cultured at the Animal Health Laboratory in Guelph, Ontario while the additional 12 case

herds underwent the same sampling and culture methods at the Animal Health Monitoring Lab in Abbotsford, British Columbia.

Each herd owner was sent a comprehensive 11-page mail-in questionnaire (appendix 10.2) to gather information on management, biosecurity and demographic factors along with a postage paid return envelope. The questionnaire examined the current and past management practices used by the producer using closed and semi-closed questions. An attempt was made to capture seasonal changes in management that occur. The questionnaire was divided into 6 different sections: herd health, feeding management, calving management, farm biosecurity, farm profile, and producer opinions regarding Johne's disease. Questions were developed based on knowledge of common management practices and general disease control recommendations within the industry as well as with consideration of previous paratuberculosis research. Gift certificates for a popular restaurant were offered to the owners that returned the first ten completed questionnaires to motivate owners to promptly complete the surveys. Herd owners were sent reminders at least two times either by phone message or letter following the initial mail out to encourage the return of the completed questionnaires. Subsequent questionnaires were mailed out if a new copy was requested.

Questionnaire results were stored in a database Excel® 2003 (Microsoft Corporation, Redmond, Washington, USA) and analyzed using SPSS® 14.0 for Windows (SPSS Inc., Chicago, Illinois, USA) and Excel® 2003 (Microsoft Corporation, Redmond, Washington, USA). The identification of management variables associated

either positively or negatively with being a Johne's positive herd was assessed in two steps. Initially, each potentially important risk factor selected from the questionnaire was included in a univariate analysis with Case Herd status as the dependant variable. Univariate analysis was completed using a Pearson Chi Square Test for the categorical variables or Logistic Regression for the continuous variables. Variables that were significant at $P \leq 0.2$ in the univariate analysis were further included in a multivariable logistic regression model to determine the main factors associated with being a case herd. The model was built following a backwards stepwise likelihood ratio approach as described by SPSS 14.0, using the likelihood ratio statistic for variable removal and the score statistic to select variables for re-entry into the model. The probability for stepwise entry was 0.05 and for stepwise removal was 0.06. Two-factor interactions between the main effects in the model were also tested in a similar fashion and goodness-of-fit was checked using the Hosmer and Lemeshow statistic which if significant would indicate that the model does not fit the data adequately. Collinearity was assessed for the variables included in the final model.

4.3 Results

Seventy cow-calf herds from western Canada were recruited to participate in this study. The overall provincial distribution of herds is presented in Table 4.1. A mail out questionnaire was sent to each producer participating in this study. Eighty percent of producers (56/70) returned their questionnaire. Four questionnaires were excluded from the final analysis because of missing data and therefore the results from 52 questionnaires

(23 case herds: 29 control herds) were included in the final analysis. The study herds had a mean herd size of 413 cattle and ranged from 25 to 2860 head. A univariate analysis using either a Pearson Chi Square test or logistic regression was run on 37 variables of interest from the questionnaire, only 8 of which were significantly ($p \leq 0.25$) associated with being a case herd (Table 4.2). When these significant variables were entered into a multivariable logistic regression analysis, 3 variables remained in the final model as described in Table 4.3.

Forty-six percent of the herds used some commercial colostrum replacement products on farm. Farms that used these products were 3.96 (95% CI = 1.10 – 14.23, p=0.035) times more likely to be positive for Johne's than herds that did not use these products. Eighty-seven percent of herds had wild deer interacting with their cattle and these herds were 14.32 (95% CI = 1.13 – 181.90, p=0.040) times more likely to be positive for Johne's than herds with no deer. Rotational grazing was used on 75% of the farms. The use of rotational instead of continuous grazing practices had a protective effect on the herd status to Johne's disease (Odds Ratio = 0.20; 95%CI=0.04– 0.93, p=0.039). This may be more clearly stated by reporting that herds using continuous grazing instead of rotational grazing practices were 5.01 (95% CI = 1.08 – 23.25, p=0.039) times as likely to be positive for Johne's disease. Two-factor interactions between the main effects of the model were examined and not found to be significant. The final model fit the data well according to the Hosmer and Lemeshow goodness-of-fit χ^2 statistic = 0.920; df=4; p=0.922.

4.4 Discussion

In total, 70 herds had been recruited to participate in this study. Only 56 (80%) of these herds completed and returned their mail in questionnaire. Participation in this study was completely voluntary and recruitment was largely dependent on local veterinarians and the Alberta Beef Producers. This may have introduced a selection bias to the results if the participating herds were not representative of the general cow calf population in western Canada. It is likely that there are some herds that do not have regular interaction with a veterinarian or with Alberta Beef Producers and this demographic would not be well represented by this study. This study was the first herd level case control study on paratuberculosis undertaken in Canada focused on the cow-calf industry. Research on cow-calf herds tends to be logistically more challenging due to extensive husbandry practices. Beef cattle are not handled as often as dairy cattle and the logistics of sampling cattle for research projects becomes much more difficult. Due to the inherent challenge of diagnostic testing for Map, it is possible that there could be some misclassification bias. In order to reduce this bias, a combination of herd history and herd testing was used when assigning herd infection case and control status.

Other researchers have found other management risk factors associated with herd prevalence for Map in cow-calf herds. The use of a dairy-type nurse cow, the use of running water as a water source, and cattle breed, were all identified as significant risk factors in cow calf herds in Texas (Roussel et al., 2005). Only 2 control herds and no case herds used a dairy-type nurse cow in the previous 5 years in our study and therefore

this risk factor was not included in the analysis. Water source information was collected for both the summer and winter seasons and none of these variables were significant in our study in the univariate analysis and therefore water source was not included in the final analysis. Not enough information was collected on cattle breed to include in this risk factor into the analysis. A previous research study of Saskatchewan community pasture cattle was unable to identify any statistically significant management risk factors associated with the Johne's seroprevalence status of beef cattle (Waldner et al., 2002). The cross-sectional study of Canadian cow-calf herds in Chapter 3 of this thesis identified the use of ionophores, and the use of a colostrum supplementation as risks factors associated with a cow-calf herd being positive for paratuberculosis. In that study the presence of a dog on the farm had a protective effect. These risk factors were included in the analysis of this present study to determine if these factors would still be significant. The use of ionophores and the presence of a dog on farm were not significant at any level in this study. Potential reasons include different populations involved in the study and it is also possible that these factors were significant due to chance, due to the low prevalence of disease which was observed in the previous cross sectional study with very few case herds. Colostrum supplementation was analyzed in this study in two ways. The general practice of supplementation of any source was included as well as the use of specific colostrum sources to determine if there was a association of interest due to the source of colostrum versus the act of feeding the colostrum. While both the percentage of calves receiving supplemental colostrum and the dichotomous factor of whether a commercial colostrum source is used on farm at all were both significant at the univariate level, only the use of a commercial colostrum replacement product was found to be

positively associated with being a case herd (OR = 3.96; 95% CI = 1.10 – 14.23) in the final model at a significant level. There are two possible explanations for this finding. It is possible that the colostrum sources used when preparing these products are not free of Map and that the process of drying the colostrum may not kill all of the viable bacteria. If this were to occur it would be easy to understand how these products could be a source of Map to the newborn calves, the most susceptible animals to infection. There is evidence suggesting that certain methods of pasteurization of colostrum may be effective at eliminating most viable bacteria however the process used to dry colostrum is not the same and may or may not be as effective (Meylan et al., 1996; Godden et al., 2006). While product labels claim freedom from Map, published reports in peer reviewed journals are lacking at this time. An alternate explanation for this positive association is that the use of these products is as part of a Johne's control strategy. If producers know their herd is infected and then make the decision to provide an alternative colostrum source for a calf, they may rely on these commercial products as a safer source of Map free colostrum. If this is the cause of the positive association then the use of commercial colostrum replacer should have started after Johne's was discovered in their herd. The questions used in this questionnaire were not able to determine when these products were first used or why and so it is not possible to determine whether commercial colostrum replacers truly pose a risk or whether they may actually be protective.

The presence of wild deer on the farm was the most strongly associated risk factor with being a case herd (OR =14.32; 95% CI = 1.13 – 181.90). In this analysis, the presence of any deer observed on the farm was included as a potential risk factor to

remove bias due to expected variability of subjective quantification of producer recall. It is possible that deer may play a role in the transmission of Map as there have been previous published reports of various species of deer being infected with this bacterium and shedding Map in their feces (Raizman et al., 2005; Crawford et al., 2006). While it is possible that deer may be a potential reservoir of Map, no deer samples were collected in this study and therefore it is not possible to say these deer were actually infected. It is possible that this positive association is due to an unknown such as geological factors. Climatic and geographic factors have been looked at by some researchers. These findings suggest that higher organic matters in loamy soils, and lower pH in leached sandy soils, both improve the survival of this pathogen (Ward and Perez, 2004). Soil aridity and pH were identified as significant ($p<0.1$) inhibitors to Map survival outside of the host in Alberta (Scott et al., 2007). It is not yet well understood how important these factors are in the epidemiology of this disease and will be looked at in the future to determine what role they may play and these may act as a confounder for a variable such as deer that may prefer to live in an environment that also increases the survivability of Map in the environment. This would increase the risk of exposure of cattle to viable environmental Map sources. Geological factors were not analyzed in this study.

The presence of a dog on farm was not significantly associated with the presence of Map in the herd. The use of rotational grazing practices was protective against being a case herd. Or in other words, the use of continuous grazing instead of rotational grazing was significantly associated with being a case herd (OR = 5.01; 95% CI = 1.08 – 23.25). This is likely due to the fact that with rotational grazing the pasture being grazed

at a particular point in time tends to be in better condition and not be overgrazed. Cattle are more likely to graze near sites contaminated with manure and pick up soil while in an overgrazed pasture. If rotational grazing reduces this risk it is sensible to assume that there could be a benefit as it pertains to Johne's control.

This was a case control study and therefore there are several inherent limitations that need to be considered. Recall bias can be a challenge with this study design and the questionnaire was developed with an attempt to keep it as simple as possible to minimize this issue. Producers were also given adequate time to be able to look at their records prior to completing the survey. Cattle infected with Johne's disease are often latently infected for years prior to becoming clinical or even detectable with laboratory tests. This means that it is possible that control herds may have been recently infected or undetected due to the limitations of the diagnostic tests and therefore introduced a misclassification bias affecting the findings of this study. This would likely lead to an underestimation of the significance of risk factors in this study. Theoretically, a cohort study would be ideal to look for causal relationships between risk factors and disease but due to the low prevalence of Johne's in cow calf herds is Canada and the nature of this disease, specifically the long latent period, this would be a challenging and expensive undertaking. For this reason it is very difficult to prove any causal relationships; however, it is possible to gather evidence to support various hypotheses about the relationships between the risk factors and develop management recommendations based on this evidence.

The following recommendations have previously been developed with the focus on the beef industry based mostly on general disease control principles and on dairy research findings (Hansen and Rossiter, 2000; Rideout et al., 2003):

1. Reduce manure build-up of pens and pastures where late-gestation cattle are kept.

2. Keep the calving area clean at all times and maintain a low cow density in these areas.

3. As soon as bonding has occurred, move cow-calf pairs to a clean pasture.

4. Avoid exposing calves to manure build-up by frequently moving location of feedbunks, waterers, and creep-feeders.

5. Once calves are weaned, do not put them on pastures used by cows.

6. Annually test the entire herd and avoid calving-out or raising offspring from any test-positive cattle.

7. Calve first-calf heifers in a separate location from mature cows.

8. Use separate equipment for handling manure and feed.

9. Do not spread manure on land used for grazing, especially for young stock.

10. Purchase replacement animals only from test negative herds and when this is not possible assess herd status through owner and veterinarian statements.

The protective association of rotational grazing found in the present research would appear to support recommendations 1 and 4. The present study was not able to provide adequate evidence to confirm all of these recommendations due to the relatively small sample size however, we believe that these recommendations are valuable for the

control of many cattle diseases and producers would be wise to consider how they may be used in the management of their own herds.

Future research should be focused on ensuring the safety of commercially prepared colostrum products by providing evidence that it is free of viable Map via peer reviewed research. It would also be helpful to identify the role of various local deer species in the epidemiology of paratuberculosis on cow calf farms. Further study is also required to fully understand the impact various grazing programs have on Map control and to develop recommendations in regard to pasture grazing.

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Table 4.1. Distribution of Cow-calf Herds by Province

Herds Recruited			Herds Used in Analysis		
Province:	No. of Herds:	% of Total:	Province:	No. of Herds:	% of Total:
British Columbia	6	8.6	British Columbia	4	7.7
Alberta	37	52.9	Alberta	25	48.1
Saskatchewan	23	32.9	Saskatchewan	20	38.5
Manitoba	4	5.7	Manitoba	3	5.8
Total	70	100	Total	52	100

Table 4.2 Results of Univariate Analysis of all 37 Variables

Pearson Chi Square used for Univariate Analysis of Categorical Variables

Variable	Odds Ratio	CI low	CI high	p value
Primarily Purebred Herd	3.4	1.09	10.59	0.032
Any Use of Commercial Colostrum Source Used on Farm	3.33	1.09	10.24	0.033
Any Wild Deer Seen on Farm In Past Year	5.52	0.62	49.39	0.094
Any Wild Rabbit Seen on Farm In Past Year	2.59	0.84	8	0.094
Direct Nose to Nose Contact Between Your Herd and Another Cow-Calf Herd	2.46	0.82	7.45	0.107
Rotational Grazing Used	0.39	0.11	1.29	0.117
Any Wild Dogs Seen on Farm In Past Year	0.48	0.14	1.64	0.235
Manure Spread on Pasture for Heifers	0.5	0.15	1.71	0.267
Heifers and Cows Separate Calving Seasons	0.39	0.07	2.15	0.27
Herd Size Increasing	0.56	0.19	1.62	0.28
Feeding Equipment Exposed to Manure	0.58	0.2	1.69	0.315
No On Farm Biosecurity Precautions	0.6	0.2	1.8	0.361
Direct Access to a Standing Water in Summer	0.56	0.16	1.96	0.361
Use Maternity Pen as Hospital Pen	0.59	1.68	2.06	0.404
No Communal Grazing Used	0.76	0.23	2.52	0.655
Feed Directly on Ground	0.72	0.13	3.95	0.708
Calving Location Separate from Winter Feeding Location	0.72	0.13	3.95	0.708
Regularly vaccinate for BVD	0.79	0.23	2.78	0.717
Dogs on Farm	1.52	0.13	17.82	0.739
Borrowed Equipment with Manure Contact from Other Farm	1.2	0.4	3.59	0.739
Never Clean Colostrum Feeding Equip.	0.68	0.06	8	0.759
Use Maternity Pen as Hospital Pen for Cattle with Diarrhea or Dramatic Weight Loss	1.38	0.08	23.36	0.822
Dog/Cat/Wildlife Access to Feed	0.86	0.23	3.28	0.83
Cow-calf Pairs Segregated from Pregnant Cows	0.91	0.29	2.82	0.869
Any Direct Access to a Water Source	0.93	0.22	3.89	0.916
Ionophores Used on Farm	1.05	0.34	3.24	0.932
Heifers and Cows Separate Calving Locations	1.04	0.36	3.06	0.938

Logistic Regression used for Univariate Analysis of Continuous Variables

Variable	Odds Ratio	CI low	CI high	p value
% of Calves that Receive Any Supplemental Colostrum	0.92	0.82	1.03	0.143
% of Calves that Receive Supplemental Colostrum from Dairy Source	0.93	0.82	1.06	0.278
% of Cow Herd Culled in Past Year	44.89	0.01	290381.6	0.395
Cattle Density of Calving Area	0.99	0.98	1.01	0.423
# Annual Vet Visits	1	0.99	1	0.457
Size of Herd	1	1	1	0.483
% Calving in Individual Maternity Pen	1.01	0.98	1.04	0.547
Annual # Cattle Purchased	0.99	0.91	1.08	0.809
% of Deadstock Left for Scavengers	1	0.99	1.01	0.826
Annual # Cattle Sold	1	0.98	1.02	0.889

Table 4.3 Final significant risk factors after multivariable analysis of the herd odds of Johne's disease in Canadian cow-calf herds

Variable	Odds Ratio	95% Confidence Interval	P value
The use of a commercial colostrum replacer on farm	3.96	1.10 - 14.23	0.035
The use of rotational grazing	0.2	0.04 - 0.93	0.039
The presence of wild deer on farm	14.32	1.13 – 181.90	0.04

CHAPTER 5

WILDLIFE AND ENVIRONMENTAL DISTRIBUTION OF *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* ON COW-CALF FARMS IN SASKATCHEWAN

5.1 Introduction

Johne's disease, caused by *Mycobacterium avium* subspecies *paratuberculosis*, Map, is a progressive and debilitating disease of livestock affecting beef cattle in Western Canada. It causes a profuse, untreatable diarrhea with high levels of bacterial shedding, dramatic weight loss, and eventually death. The primary modes of transmission include either direct exposure through the fecal-oral route (Sweeney, 1996), through contact with a contaminated environment or through consumption of infectious colostrum or milk (Giese and Ahrens, 2000). Calves from cow-calf herds, unlike dairy herds, cannot be practically isolated immediately after birth and so control efforts must focus on management such as culling infected cattle and reducing exposure of uninfected cattle to environments contaminated with Map. Map has been shown to be able to survive well in various environmental conditions, including in water sources (Whittington et al., 2004). Research focussed on environmental contamination of cow-calf herds is scarce but is vital for the successful management of this disease. Contaminated environments can also act as a potential source of infection for other species. In studies done in other countries, various wild species were found to either be infected with or spreading the bacteria. European studies have determined red deer, roe deer mouflon, fallow deer (Machackova et al., 2004), rabbits (Greig et al., 1999), foxes, stoats, weasels, badgers, wood mice, Norway rats, European brown hares, jackdaws, rooks, and crows (Beard et al., 2001). In

recent years, Map has been isolated in multiple North American wildlife species including tule elk (Manning et al., 2003), bighorn sheep, a mountain goat (Williams et al., 1979), eastern cottontail rabbit, white-tailed deer (Raizman et al., 2005), bison (Sibley et al., 2007), raccoons, armadillos, opossum, feral cat, hispid cotton rat, norway rat, southeastern shrew, striped skunk, common snipe, european starling, house sparrow (Corn et al., 2005) and coyotes (Anderson et al., 2007). To maximize the effectiveness of disease control efforts, it is important to understand the role of these alternate species in the epidemiology of Map on farms and to consider what, if any, action is required regarding their interaction with cattle.

Multiple regions around the world and within Canada have proactively developed Johne's control programs because of the concern that Johne's disease may be involved with Crohn's disease in humans (Chiodini et al., 1984; Collins and Manning, 1995; Hermon-Taylor, 2000; Manning and Collins, 2001). The process of controlling Johne's disease involves repeated testing of the herd, culling affected animals, and cleaning the environment prior to releasing new animals onto the previously contaminated areas. This is an expensive and difficult process. If the environmental contamination persists, control may not be achieved which equates to economic loss due not only to lost production but also the cost of repeatedly testing the herd, lost use of contaminated areas, and potential lost markets for cattle. Environmental testing has been suggested as an alternative to the traditional herd test methods for the dairy industry and could reduce the cost of testing a herd if it can replace individual animal testing (Raizman et al., 2004).

This was a pilot study to describe the distribution and prevalence of Map in the non-cattle species and the envionment of Map infected cow calf farms in Saskatchewan

throughout the year and to assess the potential use of environmental sampling as a alternative to herd serological and pooled fecal culture on cow-calf farms.

5.2 Materials and Methods

Starting in the summer of 2005, approximately 30 beef cow-calf herds from Western Canada were identified as being actively infected with Johne's disease. Herds were recruited through contact with local veterinarians, and producers were asked if they wished to participate in the study. An actively infected herd was considered a herd that had previously had clinical cases of Johne's disease diagnosed by their veterinarian within the preceeding 2 years, with at least one positive laboratory confirmation. Six herds were selected for more intensive sampling due to a willingness to participate and accessability of cattle for sampling. On these 6 farms, blood samples taken from approximately 100 cattle >2 years of age in the fall of 2005 were tested with a ParaChek ELISA for Map titres by the Animal Health Monitoring Lab of the Abbotsford Agriculture Centre. Pooled fecal cultures (five cattle per culture) were also done on these cattle for comparison with serological and environmental sampling results. Four rounds of environmental sampling were conducted over the span of 1 year (one round of sampling every 3 months). To determine the distribution of environmental contamination, 15 environmental samples were taken per sampling round from similar sites at each selected farm including calving areas, feeding areas, hospital pens, chute systems, pastures and potential water sources. Samples from water samples were collected from livestock waterers, dugouts, ditches, and streams when present. Water

sources and runoff areas were cultured to determine their significance to both on-farm transmission and to broader environmental contamination leading to potential spread to locations downstream.

Each non-water environmental sample was collected by combining approximately 15ml of sample from 4 locations per site for a total of approximately 60ml of combined sample per site. Water samples consisted of 500ml of water collected from a site. The water was centrifuged at 1000g for 30 minutes and the resulting sediment was collected and sent for culture. Biofilm samples were collected from livestock waterers by taking a clean 4 x 4 guaze and wiping the entire interior surface of the waterer.

Wildlife sampling was conducted by the researchers with a focussed two night and one day trapping period during the same time as the environmental samples were being collected. Species of interest included those which are commonly found on cow-calf operations such as rodents and birds but also included other readily accessible wildlife specimens. Domestic non-cattle species including cats, dogs, horses, sheep and poultry also were sampled when available. For each round of sampling, these species were either trapped or collected via convenience sampling on each of the farms. Up to 30 samples (fecal and/or tissue) were taken per species to determine if they are either infected with or shedding Map. Traps were located in areas that were near livestock or livestock feed storage but in areas protected from direct access to cattle to prevent livestock interference with the traps. Rodents were trapped using a combination of kill and live traps. Birds were collected using a combination of a mist net and sparrow cage

trap. All live trapped birds were identified promptly to ensure that endangered species would be released and only species of interest to this study would be collected. All remaining live trapped birds and rodents were promptly and humanely euthanized using a halothane inhalation chamber according to Canadian Council on Animal Care guidelines (CCAC, 2003). Tissue samples were collected in the field using sterile techniques. The sample included the entire intestinal tract along with attached lymph nodes for all species. For birds the entire stomach was also included. Tissue samples were homogenized prior to culture.

Wildlife, environmental and pooled fecal samples were frozen at -20°C until being submitted for culture after each round at the Animal Health Monitoring Lab in Abbotsford, British Columbia where they were stored frozen -80°C until culture. The modified BACTEC 12B culture method was utilized for detecting Map from these samples. All positive cultures were tested using the IS900 polymerase chain reaction (PCR) technique which uses the presence of a Map specific gene to confirm the positive result.

5.3 Results

All six (100%) of herds selected completed all 4 rounds of the required sampling and were included in the analysis. Of the 6 herds, 2 (33.3%; 95%CI 0-74.7) were identified as currently Johne's positive by having at least 2 positive serological tests and 4 (66.7%; 95%CI 25.3-100) of 6 were identified as currently Johne's positive by having

at least one positive pooled fecal culture. Individual herds had between 0.5% and 15.3% of the individual cattle test positive on the ELISA and between 0% and 52.0% of the pooled fecal cultures test positive. Table 5.1 shows the summary of the farm test results.

One of 150 water samples (0.7%; 95%CI 0-2.0) was positive for Map. The 150 water samples were made up of: 48 samples from waterers, 24 biofilm samples, 55 dugout samples, and 23 farm drainage samples. The positive culture was cultured from a dugout sample collected in the summer round of sampling from herd #6. One of 268 environmental samples (non-water) (0.4%; 95%CI 0-1.1) was positive for Map. The single positive culture was from a calving pen sample collected in the fall round of sampling from herd #4. Map was not detected in the any of the samples from the remaining sites. Table 5.2 summaries the culture results of the environmental samples grouped according to the season of sample collection.

Two (33.3%; 95%CI 0-74.7) of the 6 herds were identified as Johne's positive through environmental sampling. The three alternative herd tests had varying agreement. When the ELISA test results were compared to the pooled fecal culture a kappa of -0.20 (95%CI: -0.84-0.44) was calculated. This indicates that the agreement between theses two tests was less than what would be expected due to chance alone. When comparing the environmental testing method to the pooled fecal culture the kappa was calculated to be 0.40 (95%CI: -0.24-1.04) which is considered to be moderate agreement. The kappa calculated when comparing the environmental sampling with the ELISA testing was 0.25 (95%CI: -0.55-1.05) which is considered fair agreement. Table 5.3 compares the ability

of the three separate herd tests to identify a farm with a history of clinical Johne's disease as a positive herd.

A total of 431 wildlife samples were submitted for culture including 374 tissue samples and 57 fecal samples. These were collected from 211 birds, 169 small rodents and a combination of 51 other species (Table 5.4). None of these samples were positive for Map on culture.

5.4 Discussion

The level of environmental contamination was very low on the farms participating in this study with only 2/418 samples collected being positive for Map. This may be due to a small farm sample size or because contaminated sites were not sampled by chance;.however, the negative results are likely because of a combination of multiple factors including a relatively low prevalence of disease in this industry and the extensive management practices of cow-calf farms, which have relatively low cattle density. The only sites from which Map was isolated were a dugout sample and a calving pen sample. The positive dugout sample may be a concern as Map is known to survive for long periods of time in water, so that this could be a potential source of transmission to other animals sharing that water source. The positive calving pen sample may potentially be significant if young calves remain in the pen for a significant period of time as the young calves have an increased susceptibility to infection (Manning and Collins, 2001). There were not sufficient positive environmental sites in this project to study any seasonal

effects. Positive samples were collected in summer and autumn but this is not a significant finding at this level. Positive environment sites were detected in only 2 of 6 herds in this study, one of which had the highest Map seroprevalance in the study of 15%. While reducing the cost of testing, using environmental samples as done in this study does not appear sensitive enough as a herd screening test to be used in place of traditional herd test methods such as serological or pooled fecal testing. The low level of contaminated samples and the low ability of the environmental sampling to identify herds as positive for Map suggest that environmental sampling, as done in this project, is not likely an adequate alternative herd screening method. While the results of environmental sampling had moderate agreement (kappa = 0.40) with fecal pool sampling, it only had fair agreement (kappa = 0.25) with the results of serological sampling using the ELISA. The poor level of agreement between fecal pooled sampling and the ELISA is remarkable; however, some of this is likely a result of the small number of herds in the study and the stage of disease that the individual cattle were in at the time of sampling. Both fecal pooled sampling and the ELISA methods identified herds as positive that were not identified by any other test (Table 5.3). All herds had a recent history of clinical disease prior to the start of this study and it was expected that each herd should test positive by at least one testing method. Further research would be required to determine if environmental culture methods would be more effective as a herd screening test if done at a time with increased cattle densities and stress, such as during the calving season.

None of the wildlife samples were positive for Map and this suggests that these species do not play a signficant role in the maintinence or transmission of Johne's disease

on these farms. It is possible that some of these specimens had a very low number of Map organisms and that the culture methods used were not sensitive enough to identify them. If this is the case it is possible that there may still be a negative impact on the infected animal, but it is unlikely that it would shed enough Map to play a role in the transmission of Map to uninfected cattle. Researchers in other geographical regions have found wildlife species infected with Map to be associated with cattle herds infected with Map, however, these are often associated with areas that have higher regional prevalences of infected cattle (Corn et al., 2005). A limitation in this study was the focus on a small number of collected species of wildlife and other animals. Assuming perfect test parameters, the upper 95% confidence limit for prevalance was calculated for each species based on the number of samples collected. All samples were negative for Map and so we can be 95% confident that the prevalence of Map in these species was below the stated upper limit for prevalence at this time and at these locations (Tables 5.4 and 5.5). Deer and rabbits have been implicated in the epidemiology of Map in other regions (Raizman et al., 2005); however, only a few fecal samples were collected from these species due to their sparcity on the study farms. It is possible that further study focussed on farms with a much higher level of infection or farms with different common wildlife species could show that there may be a more significant role of wildlife under different circumstances.

The results of this study show that the level of environmental contamination of Map on cow-calf herds in Saskatchewan is very low at this time. This suggests that efforts to control this disease should be focussed on other management factors that can

reduce the exposure of susceptible animals to Map such as not keeping infected animals on farm and reducing the movement of potentially infected animals onto the farm. The cow-calf industry has an opportunity to control this disease now before the prevalence of the disease increases in the population and before environmental contamination becomes a more significant challenge.

5.5 Acknowledgements

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Table 5.1 Results of 3 herd screening test methods for Map in 6 cow-calf herds in Saskatchewan

	Serology Results[a]		Pooled Fecal Culture[b]		Environmental Sampling[c]	
Herd #	# Positive	# Collected	# Positive	# Pools	# Positive	# Collected
1	1	111	1	23	0	60
2	1	184	0	20	0	64
3	2	68	0	14	0	66
4	1	84	1	17	0	82
5	19	124	13	25	1	77
6	1	105	1	21	1	69

[a] Parachek ELISA results run on individual mature cattle serology samples

[b] Bactec culture results run on pools of 5 fecal samples collected from individual mature cattle

[c] Bactec culture results run on individual environment samples

Table 5.2 Results of four seasonal rounds of environmental sampling and culture for Map on all 6 farms

	Non Water Source Environmental Samples	Water Source Samples	All Environmental Samples
Fall	1/58	0/41	1/99
Winter	0/71	0/35	0/106
Spring	0/74	0/40	0/114
Summer	0/65	1/34	1/99
Total	1/268	1/150	2/418

Table 5.3 Comparison of the results of three alternative herd test methods for *Mycobacterium avium* ssp. *paratuberculosis*

	Test Method		
Herd #	Pooled Fecal Culture*	Serum ELISA**	Environmental Testing***
1	+	-	-
2	-	-	-
3	-	+	-
4	+	-	-
5	+	+	+
6	+	-	+

* + herd = 1 positive pool (5 samples/pool)

** + herd = >1 positive serological test

*** + herd = 1 culture positive environmental sample

Table 5.4 List of wildlife tissue samples collected and cultured for *Mycobacterium avium* ssp. *paratuberculosis*

Tissue Samples Collected	# of Specimens	Maximum Prevalence (95% Confidence)
Bird specimens		
House Sparrow (*Passer domesticus*)	90	3.2
Dark-eyed Junco (*Junco hyenalis*)	68	4.2
Clay-coloured Sparrow (*Spizella pallida*)	18	15.3
White-throated Sparrow (*Zonotrichia albicollis*)	6	39.3
Yellow-rumped Warbler (*Dendroica coronata*)	6	39.3
Barn Swallow (*Hirundo rustica*)	5	45.0
American Robin (*Turdus migratorius*)	3	63.1
Savannah Sparrow (*Passerculus sandwichensis*)	2	77.7
Black-capped Chickadee (*Poecile atricapillus*)	1	95.1
Brewer's Blackbird (*Euphagus cyanocephalus*)	1	95.1
Gray Partridge (*Perdix perdix*)	1	95.1
Yellow-headed Blackbird (*Xanthocephalus xanthocephalus*)	1	95.1
Rodent specimens		
Deer Mouse (*Peromyscus maniculatus*)	71	4.0
House Mouse (*Mus musculus*)	48	6.0
Richardson Ground Squirrel (*Citellus richardsoni*)	37	7.7
Prairie Vole (*Microtus ochrogaster*)	7	34.8
Meadow Vole (*Microtus pennsylvanicus*)	5	45.0
Norway Rat (*Rattus norvegicus*)	1	95.1
Other specimens		
Pond Snails (*Lymnaea stagnalis*)	3	63.1

Table 5.5 List of wildlife fecal samples collected and cultured for *Mycobacterium avium* ssp. *paratuberculosis*

Fecal Samples Collected	# of Specimens	Upper Limit (95% Confidence)
Bird samples		
Rock Dove (*Columba livia*)	3	63.1
Sharp-tail Grouse (*Tympanuchus phasianellus*)	3	63.1
Domestic Chicken (*Gallus domesticus*)	2	77.7
Domestic Goose (*Anser domesticus*)	1	95.1
Other samples		
Dog (*Canis familiaris*)	12	22.0
Deer (*Odocoileus hemionus* +/- *Odocoileus virginianus*)	11	23.8
Cat (*Felis catus*)	9	28.2
Horse (*Equus caballas*)	8	31.2
Rabbit (*Sylvilagus nuttalli* +/- *Lepus americanus* +/- *L. townsendi*)	4	52.7
Sheep (*Ovis aries*)	2	77.7
Pronghorn Antelope (*Antilocapra americana*)	1	95.1
Elk (*Cervus canadensis*)	1	95.1

CHAPTER 6

DETECTION OF A CLUSTER OF COYOTES INFECTED WITH *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* IN MANITOBA.

6.1 Introduction

Paratuberculosis, or Johne's disease in ruminants, is a chronic, granulomatous, bacterial enteritis that leads to diarrhea, cachexia and eventually death. First described in 1826 (Chiodini et al, 1984), paratuberculosis remains a disease of importance for all cattle industries, not only due to the economic losses associated with on-farm production and limiting trade, but also due to public health concern that *Mycobacterium avium* subspecies *paratuberculosis*, Map, may potentially be associated with Crohn's disease in humans (Herman-Taylor, 2000). A clear understanding of the epidemiology of Johne's disease is pivotal in the creation of management and control strategies. To date most research and control efforts have targeted the dairy industry. In recent years, Map has been isolated in multiple North American wildlife species including tule elk (*Cervus elaphus*) (Manning et al., 2003), bighorn sheep (*Ovis canadensis*), a mountain goat (*Oreamnos americanus*) (Williams et al., 1979), eastern cottontail rabbit (*Sylvilagus floridanus*), white-tailed deer (*Odocoileus virginianus*) (Raizman et al., 2005), bison (*Bison bison*) (Sibley et al., 2007), raccoons (*Procyon lotor*), armadillos (*Dasypus novemcinctus*), one opossum (*Didelphis virginiana*), and one feral cat (*Felis catus*) (Corn et al., 2005). Infection with Map has also been recently identified in a coyote (*Canis latrans*) in Wisconsin (Anderson et al., 2007). The role of wild canids has been identified as a significant risk to the transmission of important diseases to livestock in the past.

Coyotes can act as the definitive host for *Neospora caninum* (Barling et al., 2000), one of the most important causes of abortion in cattle in Canada and internationally (Haddad et al., 2005).

Coyotes have been considered as a potential sentinel species for bovine tuberculosis in wild cervids in Manitoba due to their trophic relationship with those species (Sangster et al., 2007). Coyotes also have frequent contact with cattle of domestic cattle herds with the potential of scavenging on carcasses from cattle that have died of paratuberculosis on the premises. Coyotes have been shown to be capable of becoming infected with Map (Anderson et al., 2007) and it is hypothesized that sampling coyotes collected through trapping and hunting could help to determine the prevalence of disease in the coyote population surrounding Riding Mountain National Park. The objective of this study was to determine whether or not coyotes in this region were infected with Map and to describe the distribution of this infection within this population.

6.2 Methods

Coyote carcasses were collected from hunters and trappers in the fall and winter of 2004-2005 from in and around Riding Mountain National Park in Manitoba in order to determine whether coyotes could be a useful sentinel species for bovine tuberculosis in that region. Retropharyngeal, mesenteric and colonic lymph nodes were collected and stored frozen at -70°C until processed for culture of both *Mycobacterium bovis* and Map. Fecal samples were not collected at the time due to concerns regarding the zoonotic risk

of Echinococcus species for those involved in the project. Histopathological examination of the tissue samples, age and sex determination was done as previously described (Sangster et al., 2007). Tissue samples were cultured by the Canadian Food Inspection Agency at the Mycobacterial Diseases Centre of Expertise in Ottawa. Lymph node samples from individual coyotes were pooled and processed as a single sample.

Specimen processing, decontamination and inoculations were performed in a Microzone Class II Type A biosafety cabinet. Using sterile equipment, tissues were thoroughly examined for visible lesions and a section of any lesion along with surrounding tissue was placed in a disposable 50 mL conical centrifuge tube containing 10 mL 0.067M phosphate buffer. If no visible lesions were observed, a small representative section of each tissue type was excised and placed in the buffer, for a total tissue volume of approximately 10 -15 cubic mm. Each specimen was cultured for Map on Herrold's media with mycobactin and an antifungal cocktail. This media was incubated at 39°C for a maximum of 20 weeks. Smears of isolated colonies were stained with Ziehl-Neelsen method and examined for acid-fast organisms. If the isolate grew only on the Herrold's media, additional plates of Herrold's media were inoculated both with and without mycobactin to determine mycobactin dependency. These were also incubated at 39°C. Isolates that showed mycobactin dependency were confirmed to be Map by a PCR targeting the area of IS900.

6.3 Results

Carcasses of 82 coyotes were collected by trappers and hunters from 18 locations around the RMNP. These carcasses were necropsied at the Western College of Veterinary Medicine. Approximate location data was available for 69 of the carcasses (Figure 6.1). Location information was not available for 13 of the carcasses. Two carcasses were collected as opportunistic samples from within RMNP where trapping and hunting is prohibited. The majority of the coyotes were less than 2.5 years old (Figure 6.2). Age determination was not completed for one coyote as tooth sections were not available. The coyotes collected consisted of 46 females and 34 males. The sex was not determined for the remaining two coyotes.

Tissue samples from three coyotes were culture positive for Map. All three positive cultures were confirmed using IS900 PCR. The infected coyotes were collected from the same location (Figure 6.1) and were among 33 coyotes collected at this site. The prevalence of infection at this site was 9.1% (CI: 5.7-12.5). The prevalence of infection including all sites was calculated to be 3.7% (CI: 2.3-5.1). The infected coyotes consisted of a female less than 2.5 years of age, a male less than 2.5 years of age, and a female 2.5 years of age.

6.4 Discussion

Coyotes are capable of becoming infected with the Map bacteria (Anderson et al., 2007). The three infected coyotes were rather young, with the oldest being 2.5 years. The majority of coyotes (74/81) collected came from the younger age groups and the prevalence in the younger age groups is not significantly different from that of the older age groups. In fact the infection prevalence of the < 2.5 year age group was calculated to be 4.9% (CI: 0-11.6) and the infection prevalence of the 2.5 years of age group was 4.2% (CI: 0-12.3). None of the coyotes infected with Map were greater than 2.5 years of age. There was not enough data to elaborate further on the effect of age nor was there any evidence to suggest that sex is a significant factor according to our data.

At the time of writing, this is the only report of multiple coyotes found to be infected with Map collected from a single location. The distribution of infection indicates an outbreak cluster that had a prevalence of infection of 9.1% (CI: 5.7-12.5). If clustering is ignored the overall infection prevalence of the entire sampled coyote population would be 3.7% (CI: 2.3-5.1). The fact that all infected coyotes did cluster at one location while none of the coyotes from any of the other locations were infected with Map suggests that there is something unique about the coyotes at this site. One hypothesis is that this is indicative of a point source outbreak. The location where these coyotes were collected is in close proximity to a cattle operation and it is conceivable that these coyotes could be exposed to Map either through scavenging on an infected cattle carcass or from an environment contaminated with Map. However, it must be noted that there are cattle

operations in the proximity of other sites near noninfected coyotes as well. It is possible that a cattle operation near the infected coyotes may have a higher infection rate in their cattle or that dead stock are more available for scavenging by wild species at that site. There is no available data on the infection status of the cattle operations and so further conclusions are not possible at this time. The ability of coyotes to become infected implicates them as a species of interest when trying to control Map within a herd, especially if they are able to shed the bacteria. Coyotes move readily from farm to farm and could potentially be a source of inter-farm transmission or a source for re-infection in a herd that is actively attempting to eradicate it. Livestock producers with known Map infections in their herds ought to be careful to prevent the disease transmission between livestock and wildlife including coyotes. Reducing contact of wildlife to Map contaminated material such as manure and infected carcases and preventing wildlife from contaminating livestock areas and feed sources may help reduce the transmission between these species.

Due to the terminal nature of sample collection in this study it was not possible to follow the infected individuals to determine potential outcomes of infection. Further research would be beneficial to: determine the duration of infection, discover any potential pathology that may develop in coyotes, quantify the ability of coyotes to shed Map into the environment potentially transmitting disease to other animals, as well as looking into the long term ecological effects of Map infections on coyote populations.

6.5 Acknowledgements

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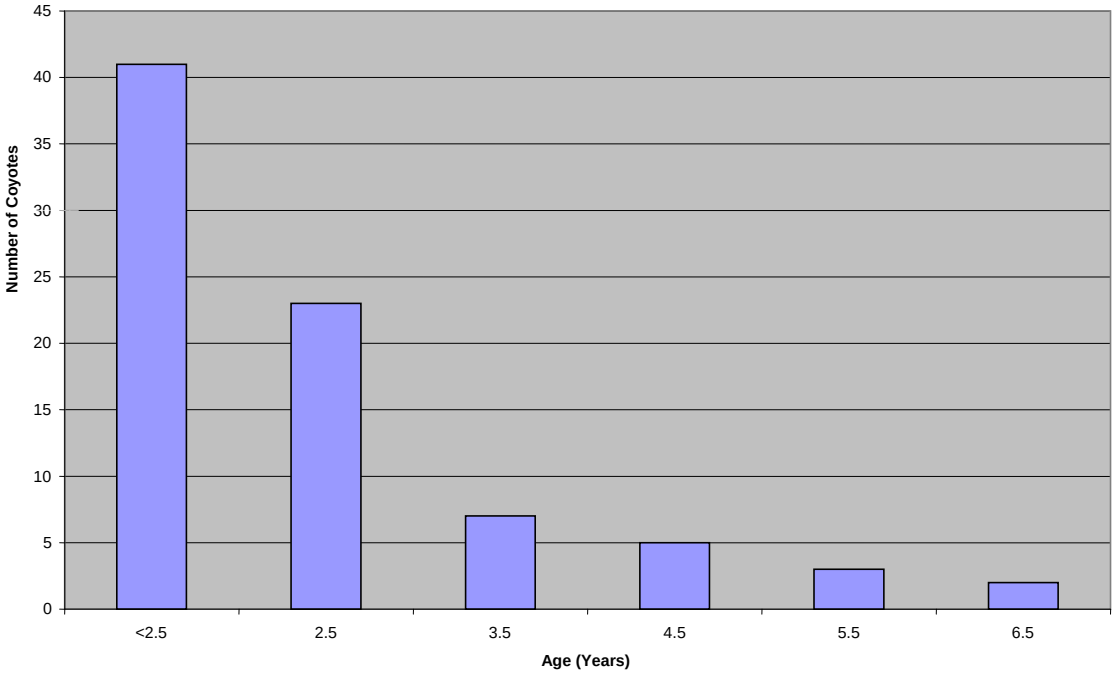
Figure 6.1. Proportion of coyotes infected with *Mycobacterium avium* subspecies *paratuberculosis* collected around Riding Mountain National Park in Manitoba.

Figure 6.2. Age distribution of coyotes collected around Riding Mountain National Park



CHAPTER 7

ENVIRONMENTAL DISTRIBUTION OF *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* ON COW-CALF HERDS WITH A HISTORY OF CLINICAL CASES OF JOHNE'S DISEASE

7.1 Introduction

Johne's disease, caused by *Mycobacterium avium* subspecies *paratuberculosis*, Map, is a progressive and debilitating disease of livestock affecting beef cattle in Western Canada. It causes a profuse, untreatable diarrhea with high levels of bacterial shedding, dramatic weight loss, and eventually death. The primary modes of transmission include either direct exposure through the fecal-oral route, through contact with a contaminated environment or through consumption of infectious colostrum or milk (Giese and Ahrens, 2000). Calves from cow-calf herds, unlike dairy herds, cannot be isolated immediately after birth and so control efforts must focus on culling infected cattle and reducing exposure to the bacteria through environmental sources. Information on environmental contamination associated with cow-calf herds is sparse but may be vital for the successful management of this disease. Map has been shown to be able to survive well in various environmental conditions (Whittington et al., 2004). The level of environmental contamination is dependent on the persistence of the bacteria in the environment as well as the degree of recontamination from cattle or other potential carrier species. Multiple regions around the world and within Canada have proactively developed Johne's control programs due to the concern that Johne's disease may be involved with Crohn's disease in humans (Chiodini et al., 1984; Collins and Manning, 1995; Hermon-Taylor, 2000; Manning, 2001). The process of controlling Johne's disease involves repeated testing of

the herd, culling affected animals, and cleaning up the environment prior to releasing new animals onto the previously contaminated areas. This is an expensive and difficult process. If the environmental contamination persists, control of this disease may never be achieved. This would result in economic losses due not only to lost production but also to the cost of repeatedly testing the herd, lost use of contaminated areas, and potential lost markets for cattle. Environmental testing has been suggested as an alternative to the traditional herd screening methods for the dairy industry and could reduce the cost of testing a herd if it can replace individual animal testing (Raizman et al., 2004).

Water quality has always been important in disease control. Contaminated water acts as a potential source of infection for cattle, other animals and humans as well. Contaminated water could potentially move downstream as well as into the groundwater causing further environmental and health concerns. Studies undertaken outside of Canada (Pickup et al., 2005; Whan et al., 2005; Pickup et al., 2006) have shown the ability of Map to survive in water sources and this potential source of exposure needs to be understood to formulate informed management recommendations. There has been remarkably very little research done on controlling Map infections in beef cow-calf herds under Western Canadian conditions. The vast majority of research on Johnes disease has focussed on controlling the disease in dairy herds. The objective of this study was to determine the distribution of *Mycobacterium avium* subspecies *paratuberculosis* in the environment of cow-calf herds in Western Canada with a history of clinical Johne's

disease and to compare the sensitivities of pooled fecal sampling and environmental sampling on these farms.

7.2 Materials and Methods

Starting in the summer of 2005, 30 beef cow-calf herds with a history of clinical Johne's disease from Western Canada were recruited through contact with local veterinarians. An actively infected herd was considered a herd that had previously had clinical cases of Johne's disease diagnosed by their veterinarian within the last 2 years with at least one positive laboratory test confirmation. Producers were asked if they wished to participate in the study and permission to collect samples from cattle and the environment was obtained. Fecal samples were taken from up to 150 cattle greater than 2 years of age from each farm in the fall of 2005, and were cultured in pools of 5 using the modified BACTEC 12B technique by the Animal Health Monitoring Lab of the Abbotsford Agriculture Centre.

To determine the distribution of environmental contamination, approximately 15 environmental samples were taken from similar sites at each selected farm including calving areas, feeding areas, hospital pens, chute systems, pastures and potential water sources. Water samples were collected from livestock waterers, dugouts, ditches, and streams when present. Water sources and runoff areas were cultured to determine their significance to both on-farm transmission and to broader environmental contamination leading to the potential spread to locations downstream.

Each non-water environmental sample was collected by combining approximately 15ml of sample from 4 locations per site for a total volume of approximately 60ml of combined sample per site. Each sampling location per site was at least 1 meter away from previous sampling location to improve the likelihood of detecting potential contamination. Water samples consisted of 500ml of water collected from a site. From each site, samples were taken both from the undisturbed water source but also after agitating the water in order to stir up and collect recent sediment. Water samples were centrifuged at 1000g for 30 minutes and the resulting sediment was collected and sent for culture. Biofilm samples were collected from livestock waterers by taking a clean 4 x 4 guaze and wiping the entire interior surface of the waterer. Environmental samples were collected during the approximate midpoint of each herd's calving season; this season tends to have increased cattle density as cattle are congregated near calving facilities and contamination was expected to be more likely.

All sampling was conducted by local veterinarians and the expense of sample collection was funded by the researchers, so that there was no expense to the producer. Environmental and pooled fecal samples were frozen at -20°C until being submitted for culture after each round at the Animal Health Monitoring Lab in Abbotsford, British Columbia where they were stored frozen at -80 °C until culture. The modified BACTEC 12B culture method was utilized for detecting Map from these samples. All positive cultures were tested using the IS900 polymerase chain reaction (PCR) technique which uses the presence of a Map specific gene to confirm the positive result.

Calculations were completed according to methods described by (Dohoo et al., 2003) with the exception of the kappa statistics which were done using Win Episcope 2.0.

7.3 Results

Twenty-seven of the thirty herds selected (90%) completed all the required sampling and were included in the analysis. Of the 27 herds, 17 (63.0%; 95%CI 44.4-81.5) had at least one positive pooled fecal culture. Individual herds had between 0% and 60.0% of the pooled fecal samples culture positive with a median of 5.6% positive fecal pool cultures (Figure 7.2).

None of 114 water samples were positive for Map. These included 54 samples from cattle waterers, 26 biofilm samples, 21 dugout samples, and 13 farm drainage samples.

Map was isolated from a very small percentage of the environmental samples (non-water) (Figure 7.1). Fifteen of 243 samples collected (6.2%; 95% CI 3.1-9.2) were positively cultured for Map. Samples collected from within chute systems were the most likely to be contaminated with Map with 4/26 (15.6%; 95%CI 1.2-29.5) positive. Other Map positive sites included: 3/21 (14.3%; 95%CI 0-29.6) of samples from the ground outside of cow feeders, 2/21 (9.5%; 95%CI 0-22.4) of samples inside cow feeders, 2/13 (15.4%; 95%CI 0-35.8) of samples taken from mothering-up pens, 1/7 (14.3%; 95%CI 0-

42.3) from bullpens, 1/10 (10.0%; 95%CI 0-29.6) from turnout pens, 1/18 (5.6%; 95%CI 0-16.4) from calf shelters, and 1/26 (3.8%; 95%CI 0-11.4) from calving pens. Map was not detected in any of the 101 samples from the remaining sites.

Eight of the 27 infected herds (29.6%; 95% CI 12.1-47.2) had at least one environmental sample test positive for Map. Herds had between 0/15 (0%) and 3/9 (33.3%) of non-water environmental samples test positive for Map. Of 17 herds identified as positive by pooled fecal culture, 6 (35.3%; 95%CI 11.9-58.7) were also positive on environmental sampling. Of 10 herds that were negative on fecal pools, 2 (20%; 95%CI 0-46.1) were positive on environmental sampling. The agreement between the two testing methods had a calculated kappa of 0.13 (95%CI -0.17 – 0.43) which suggests only slight agreement.

7.4 Discussion

The level of environmental contamination was extremely low in the study presented in Chapter 5 with only 2/418 samples collected during four rounds of sampling being positive for Map. The farms used in the present study had slightly higher levels of contamination identified. However, none of 114 water samples and only 15/243 (6.2%) of the non-water environmental samples collected during the calving season were positive for Map. This is likely due to a combination of multiple factors including a relatively low prevalence of disease in the cow-calf industry, the extensive management practices of cow-calf farms, and a relatively low cattle density. There was a much lower

rate of environmental contamination than seen in dairy herds (Berghaus et al., 2006; Lombard et al., 2006) and this may be because the animals are less densely populated in cow-calf herds. Cow-calf herds tend to have lower herd prevalence of Johnes disease infection and the herds in this study were no exception. Seasonality is thought not to play a significant role, as no other season had a significantly higher rate of environmental contamination in the study presented in chapter 5. The higher Map positive rates around feeders and chute complexes suggest that these areas are not moved or cleaned frequently enough and may be sites of increased risk of exposure to uninfected cattle and calves. The lack of positive cultures in samples from hospital pens and manure storage samples was unexpected. It may be that owners recognize cattle with clinical Johne's disease and do not allow them into the hospital pens or, perhaps, these pens are being adequately cleaned between cattle and therefore are not as likely have positive samples collected. Failure to identify Map in manure storage areas may be a result of the beneficial effect of composting, as has been previously described (Grewal et al., 2006). The low level of environmental contamination suggests that cattle in cow-calf herds are relatively unlikely to become infected with Map by being exposed to an infected environment. Cow-calf producers who wish to reduce the level of Johnes disease in their herd should focus instead on identifying positive cows and removing them from the herd as soon as possible.

The low level of environmental contamination and lack of any positive wildlife samples on the cow-calf farms that were followed for a year in Chapter 5 made conclusions on significant seasonal effects impossible. The complete lack of positive

samples from the wildlife samples suggests that at this time non-ruminant wildlife do not play a significant role in the epidemiology of Johne's disease on cow-calf farms.

The low level of contaminated samples and the sensitivity of environmental sampling for Map suggest that environmental sampling as done in this project, is likely not an adequate alternative herd test. Environmental sampling only identified 8/27 (29.6%) of the herds with a positive history of clinical Johne's disease and had only slight agreement (kappa = 0.13) with fecal pool sampling. This suggests that using individual animal tests on the entire herd or individual testing of a representative sample of the herd on an annual basis may be the best option to determine the status of a replacement source herd. Using environmental sampling as done in this study, while reducing the cost of testing, is not sufficiently sensitive as a herd test to be used in place of traditional herd screening methods. The infection levels on the tested farms were generally low and this may be the reason that Map was not detected more frequently in the environment. Sites such as chutes, feeders, and mothering up pens were found to be positive on multiple farms, suggesting that if environmental testing was to be used there should be a focus on these sites in order to reduce cost.

It was not possible to calculate the specificity of using environmental sampling as a herd screening method as all included herds had a recent positive history of clinical Johne's disease. Even though some herds were not identified as positive to Map by pooled fecal culture it is unlikely that they were free from infection. To prove freedom of infection the herd would need to have undergone a series of testing over a long period of

time, because of the long incubation period associated with this pathogen and the relative low sensitivities of the available diagnostic tests. The specificity of environmental testing is likely less than 100%. It is possible to have a contaminated environment due to contamination from cattle already culled or other sources and this contamination can persist for long period of time. However, for practical purposes, assuming a specificity of 100% would be an appropriate conservative approach when considering a source herd for the purpose of purchasing replacement cattle without a history of appropriate animal testing.

The sensitivity of pooled fecal culture and environmental sampling could likely have been increased by collecting more samples. It is possible that some positive cattle were not included in the sampling due to the random nature of the sampling methods. It is also likely that some positive environmental sites were not included and the sensitivity would be higher if the numbers of samples had been increased. This would likely not be cost effective, as this would have increased the cost significantly with little return as the sites selected in this study were those expected to have the highest likelihood of contamination. It is likely that some samples with negative cultures were actually false negatives as a result of the low number of Map bacteria in the samples being below the level of detection of the culture methods.

The results of this study show that the level of environmental contamination of Map on cow-calf herds in western Canada is very low at this time. While continuing to maintain a hygenic environment is still strongly recommended, these results suggest that

efforts to control this disease should be focussed on other management factors that can reduce the exposure of susceptible animals to Map, such as not keeping infected animals on farm and reducing the movement of potentially infected animals onto the farm. The cow-calf industry has an opportunity to control this disease now before the prevalence of the disease increases in the population and before environmental contamination becomes a more significant challenge.

7.5 Acknowledgements

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Figure 7.1 Results of sampling sites with at least one bacterial culture positive for *Mycobacterium avium* subspecies *paratuberculosis.*

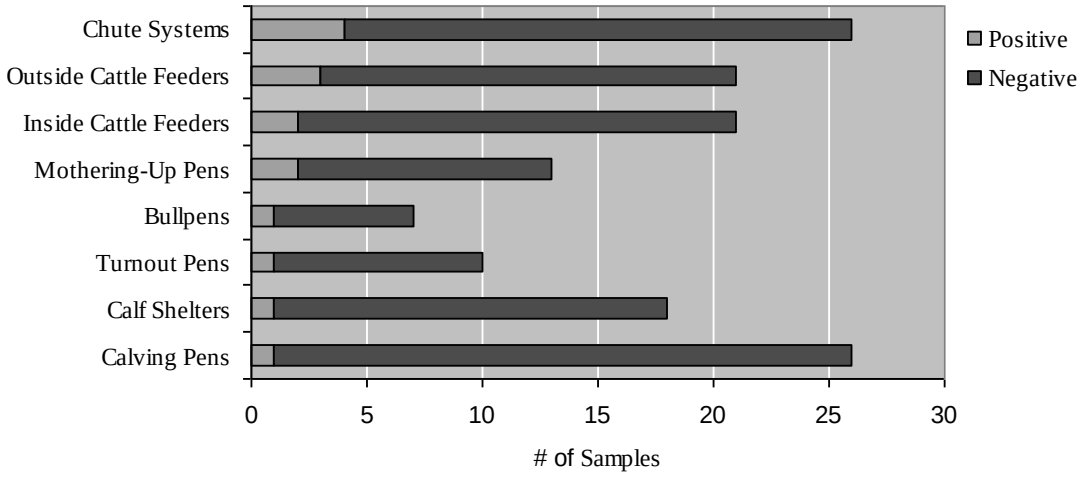
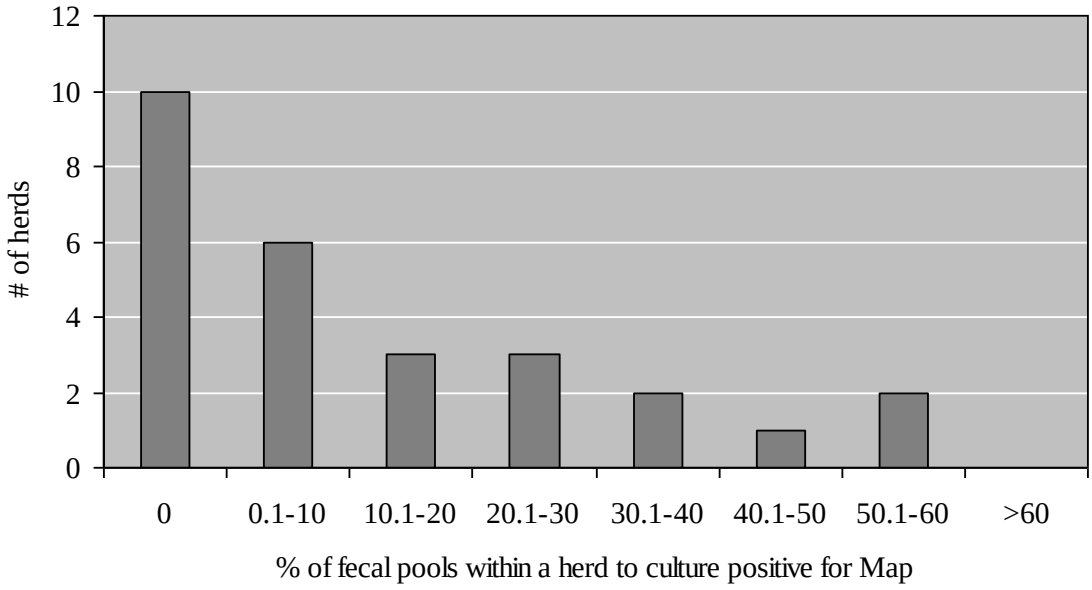


Figure 7.2 Percentage of pooled fecal samples that were culture positive for *Mycobacterium avium* subspecies *paratuberculosis* from cow-calf herds with a history of Johne's disease



CHAPTER 8

RISK OF INTRODUCTION OF *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* INTO COW-CALF HERDS IN SASKATCHEWAN ASSOCIATED WITH THE USE OF FOUR HERD SCREEN TEST STRATEGIES

8.1 Introduction

Johne's disease, caused by *Mycobacterium avium* subspecies *paratuberculosis* (Map), is a chronic, granulomatous, bacterial enteritis that leads to cachexia and death in ruminants. First described in 1826 (Chiodini et al., 1984), Map remains a disease of importance for cattle industries, not only due to the losses associated with limiting trade and on-farm production, but also as a potential zoonotic concern (Chiodini et al., 1984; Collins and Manning, 1995; Hermon-Taylor, 2000; Manning, 2001, Bull et al., 2003; Chiodini and Rossiter, 1996). Most research and control efforts have targeted the dairy industry and very little is known of the impact of Map in Canadian cow-calf herds. In 2003, it was reported that 0.6% of cattle tested from commercial cow-calf herds in Saskatchewan were seropositive to Map antibodies and 12.5 % of cow-calf herds had at least one test positive cow in the herd as reported in chapter 1 of this thesis.

Due to a complicated pathogenesis of disease including a long latent period of infection and a slow immune response there is currently no perfect gold standard test available for detection of Map infection. In the absence of a true gold standard, fecal culture is most commonly used as the standard to which other diagnostic tests are compared. The sensitivity and specificity of diagnostic tests for Map have had variable

results depending on the age of the animal at the time of infection and of sample collection, as well as the type of test and methodology used (Neilsen and Toft, 2008).

Most Map control strategies include recommendations for purchasing replacement cattle from low risk or disease free herds. Identifying herds as low risk or disease free involves examining the herd history of disease as well as some consideration of testing protocols used. Many different testing strategies have been used as herd screening tests in Map control programs including pooled fecal culture, serological antibody detections tests, and more recently, culture of strategically collected environmental samples.

Risk analysis has been used as a tool to attempt to quantify the uncertainty of a testing protocol when no gold standard test results are available (Carpenter et al., 2004). The objective of this study was to use a Monte Carlo stochastic modeling technique to compare the risks associated with using three different herd screening test regimes along with the risk of not using any testing regime utilizing previously collected provincial test data from the cow-calf herd of Saskatchewan.

8.2 Methods and Materials

8.2.1 Model herd and diagnostic approach

It is assumed that a herd free from Map will make a purchase of replacement animals from one farm. The owner chooses one of four herd screen test strategies and

would select the source herd based on a negative herd screen test. The four herd screen strategies compared in this analysis, and their test parameters, were derived from previous research (Table 8.1 and Table 8.2) and consisted of the following options:

1. No Testing
2. ELISA Serology: 30 randomly selected individuals at least two years of age using the Paracheck ELISA as described in chapter 3 and using test parameters from previous research.
3. Environmental Sampling: 15 strategically collected environmental samples with culture and PCR confirmation as described in chapter 7.
4. Pooled Fecal Culture: up to 30 pools of five individual fecal samples each from randomly selected cattle at least two years of age as described in chapter 7.

The four potential outcomes of using a herd screen test on a herd would be made up of true positive, false positive, true negative, or false negative herd infection status. The outcome risk is the probability that the owner would unknowingly select an infected herd as his source herd based on the false negative results of a herd screen test strategy.. In other words the outcome risk is the probability that a herd is infected given a negative test or:

Risk = 1- HNPV (HNPV = Herd negative predictive value).

8.2.2 Serological Tests

The Parachek ELISA was chosen as the serological test for this study because it was the test used in the previous cross sectional study conducted in chapter 3 to determine the seroprevalence of production limiting diseases in the province of Saskatchewan. Test parameters of the various ELISA tests have been reevaluated many times over the years with wide ranging results for both sensitivity and specificity (Nielsen and Toft, 2008). Test parameters had not been previously evaluated for use in the cow-calf herd in Saskatchewan and so for the purpose of this study a uniform distribution was developed (Table 8.1) to describe the test parameters of the ELISA on individual animals by using the minimum and maximum reported values for both the sensitivity and specificity reported in the literature in order to represent the maximum uncertainty (Nielsen and Toft, 2008). Apparent prevalence (AP), herd level sensitivity (HSens), and herd level specificity (HSpec) for the ELISA serology strategy was calculated according to Dohoo et al., 2003.

$$HSens = 1-(1-AP)^n$$

$$HSpec = (Spec)^n$$

$$AP = p*Sen + (1-p)(1-Spec)$$

Individual animal test parameters were derived from previous research (Table 8.1). The Saskatchewan within herd prevalence (p) and number of animals tested (n) were derived from previous research (chapter 3).

p: Beta (x+1,nt-x+1)

x = # of test positive cattle = 5 (data from chapter 3)

nt = total # of cattle tested from test positive herds =107 (data from chapter 3)

8.2.3 Culture tests

Pooled fecal sampling and environmental sampling have both been used as herd screening tests. Both of these testing strategies have previously been evaluated at the University of Saskatchewan in previous research to determine their respective sensitivities in identifying cow-calf herds infected with Map, as shown in chapter 7 of this thesis. Consistent Bactec radiometric culture methodologies were used for both the pooled fecal and environmental samples and all positive cultures were confirmed using a IS900 Polymerase Chain Reaction. The specificity of a positive culture confirmed by PCR was assumed to be 100%. In brief, each test was used in herds that had been previously confirmed to have positive diagnostic tests for Map and were known to have an ongoing history of clinical Johne's disease cases within the previous two years. A summary of the results of that study are found in Table 8.2. Data from Table 8.2 was used to develop a beta distribution, Beta (x+1, nt-x+1), to represent the uncertainty in the tests sensitivity.

8.2.4 Estimation of true herd prevalence

The provincial population data was collected from a previous cross sectional study undertaken by the University of Saskatchewan to determine the seroprevalence of production limiting diseases in the cow calf herd in the Province of Saskatchewan as described in chapter 3. In this study, 4 out of 32 herds had at least one seropositive cow. The average sample size per herd was 27 cattle. Of 880 cattle tested provincially only 5 cattle were seropositive for an apparent provincial prevalence of 0.6% and a mean within herd prevalence of 4.7% in infected herds.

Using Bayesian techniques, the data from previous research as described, was used in combination with the derived diagnostic test parameters to approximate the true herd-level prevalence of Map in the cow-calf herd in Saskatchewan. Assuming no prior knowledge and using the data collected for Saskatchewan in the production limiting disease study described in chapter 3 as the sample population, the normalized posterior herd prevalence probability distribution was formed as described in (Murray and International Office of Epizootics., 2004):

BINOMDIST(A,B,C*D+(1-C)*(1-E),0)

A= # of test positive herds from sample population
B = # of herds tested from sample population
C = series of potential herd point prevalences (0,0.01 …, 0.99,1.00)
D = point taken from derived ELISA Herd Sensitivity Distribution
E = point taken from derived ELISA Herd Specificity Distribution

This distribution, once normalized, represents the simulated probability of the true herd prevalence in Saskatchewan, based on the sample data and the test parameters as derived.

This distribution was used to compare the risks associated with the use of the various herd screening test strategies.

8.2.5 Comparison of Herd Screening Test Strategies

A Monte Carlo stochastic simulation model was constructed in order to compare the three herd screen test strategies along with a no test strategy. The population herd prevalence, Hp, and test parameter inputs used came from the distributions produced in sections 8.2.2-8.2.4 above. For each iteration the risk of selecting an infected herd but not identified as positive (i.e. either not tested or tested with a false negative result) was determined by calculating the herd negative predictive value (HNPV) of the herd test strategy in question and subtracting that value from one, or in other words, the risk equals the probability of a herd being disease positive, P(HD+), given that it is test negative, P(HT-):

$$\text{Risk} = 1\text{-HNPV} = P(HD+|HT-)$$

$$\text{HNPV} = [(1\text{-Hp})(\text{HSpec})]/[(1\text{-Hp})(\text{HSpec})+(\text{Hp})(1\text{-HSens})]$$

In order to directly compare the significance of the differences in risk associated with each test strategy, the following calculation was used for each iteration of the simulation:

$$\text{Risk Ratio (Test A:Test B)} = (\text{Risk: Test A}) / (\text{Risk: Test B})$$

To facilitate the communication of the results, the Reduction in Risks (Table 8.6) were also calculated as:

Reduction of Risk (Test A:Test B) = 1- [Risk Ratio (Test A: Test B)]

If the 95% credible intervals of the Risk Ratio did not include the null value of 1, the tests were determined to be significantly different from each other. If the 95% credible interval was entirely below the null value than the Risk of Test A was significantly lower than the Risk of Test B and therefore the use of Test A was significantly more effective at identifying Map infected herds. Economic factors of testing were not included in this model.

8.2.6 Simulation Parameters

A Monte Carlo model was used to simulate the distributions of the Risk, Risk Ratios, and Reductions of Risk. The numbers described in this paper were simulated using an initial seed number for the Mersenne Twister random number generator fixed at 1. One thousand iterations were used to ensure model stability of all output variables. Minimum and maximum values for all simulated variables were assessed to ensure that no results occurred outside of possible limits such as prevalence values below zero or above 1.

8.3 Results

8.3.1 Input Results

8.3.1.1 Diagnostic Test Parameters

The simulated parameters for the diagnostic tests are summarized in Table 8.3 and were used as inputs into the risk simulations along with the true herd level prevalence distribution.

8.3.1.2 Simulated Population Prevalence

A beta distribution was used to describe the apparent with-in herd prevalence based on previous research as described in section 8.2.2 and was estimated at 5.4% (95%CI: 2.5-9.4). The true herd level prevalence for Map infection was simulated as described in section 8.2.4 and was found to be 12.9% (95%CI: 0.5-42.5). This true herd level prevalence binomial distribution was used in the following risk simulation.

8.3.2 Output Results

8.3.2.1 Risks

Table 8.4 summarizes the risk associated with each herd screen test strategy used in this study. The risk is the probability that a herd is infected given that all the cattle tested with a specific herd screen test strategy were negative (Risk = 1-HNPV). As

expected, using no test strategy has the highest mean probability of a false negative result with a risk of 0.129 which is equal to the true herd level prevalence. The pooled fecal culture strategy had the lowest risk followed by the ELISA sampling and then the environmental sampling with mean risk of 0.061, 0.096, and 0.098, respectively.

8.3.2.2 Risk Ratios and Reduction in Risk

The risks of each herd screen test strategy were compared with each other and the results of the risk ratios (RR) are found in Table 8.5. Not surprisingly, all of the herd screen test strategies performed significantly better than the use of no herd test. Using no test protocol was associated with increased RR of 1.42, 1.74, and 2.59 when compared to environmental sampling, ELISA serology, and pooled fecal culture respectively. The mean probability that the herd was infected given that all the cattle tested were negative when using the ELISA test strategy was not significantly different from either the environmental sampling or pooled fecal culture. Pooled fecal culture performed significantly better than environmental sampling with a mean RR of 0.58. Another way to communicate these results in a more intuitive way is to describe the reduction in risk associated with one test strategy when compared to the risk associated with another test strategy. The use of environmental sampling, ELISA serology, and pooled fecal culture reduced the mean risk of selecting an infected herd given that all the tests were negative, by 28%, 36%, and 59%, respectively, when compared to the no test option as shown in Table 8.6. The use of pooled fecal culture significantly reduced the mean risk of

selecting an infected herd given all negative tests by 42% when compared to the use environmental sampling.

8.4 Discussion

This study has attempted to use Monte Carlo simulation modeling to compare the effectiveness of using various diagnostic testing strategies as a herd screen test for reducing the risk of selecting replacement cattle from a herd that had been misclassified as uninfected although there are infected cattle within the herd. Using this approach enables the modeler to include the uncertainty surrounding all of the variables in the analysis resulting in an output that reflects a more realistic distribution of potential outcomes. It is not surprising that the risk associated with selecting a herd at random has a significantly higher risk associated with it than selecting a herd that has undergone and received negative results with any of the other herd screen test strategies. Although the results of this study are specific to the methodologies of the test strategies used, the findings may be useful when attempting to design a practical approach to purchasing decisions that reduces the risk of introduction of infected cattle. Any plan to instigate Map control should be comprehensive in order to optimize the value of any efforts initiated. Other factors that must be considered include the cost of testing, Map prevalence in the purchaser's herd, individual animal testing requirements, production losses associated with Map infection, as well as other disease control efforts undertaken. The effect of individual animal testing of purchased animals was not included in this simulation and would further decrease the risk of purchasing an animal infected with

Map. The objective of this study was to evaluate the ability of various herd screen test strategies to identify Map infected herds. To optimize the confidence of only purchasing mature cattle uninfected with Map, only test negative cattle should be purchased from herds with at least one negative herd screen test, ideally from herds with no history of clinical disease. If purchasing youngstock, source herds should be herds with at least one negative herd screen test and no history of clinical disease. Parallel or series testing with uncorrelated tests can be used on individual cattle to further increase confidence that the animal is disease negative.

A cumulative summary plot of the results of the performance of the tests is found in Figure 8.1. To assess the significance of the differences in risk between the various test strategies, the risk ratios were calculated and if the 95% credible interval of the risk ratio did not include the null value of 1, the difference was determined to be significant as shown in Table 8.5. Table 8.6 summarizes the reductions in risk associated with the relationships between the various herd screen test strategies. The risks shown in Table 8.4 show the trend of the pooled fecal cultures being the lowest risk herd screen strategy followed by ELISA serology, environmental sampling and finally no testing. While the ELISA serology had the highest mean sensitivity, it also had the widest distribution and a lower specificity which would increase the potential for false positive and false negative results. This led to a higher risk of selecting an infected herd as your replacement source if this strategy were used. The wider distribution also resulted in the lack of significant difference observed between the ELISA serology and environmental sampling strategies. Environmental sampling, while having a significantly higher risk associated with it than

pooled fecal culture but not significantly different risk than ELISA serology, has the benefit of being relatively inexpensive and logistically simple. Environmental testing is also significantly better than not testing at all. For producers who are unwilling to invest much effort or finances, the environmental sampling strategy may be an option worth consideration to reduce some risk. For producers who are more committed to preventing introduction of Map infected cattle, either the ELISA or pooled fecal culture strategies should be encouraged. If a producer is only willing to purchase animals from herd screen test negative herds, then the trend shows that using pooled fecal culture may have some advantage, although the risk associated with pooled fecal was not significantly lower than the risk associated with the ELISA serology strategy. The benefit of using the ELISA strategy is that the results consist of individual animal data while the pooled fecal data only indicate which pool of five animals has at least one infected animal. This information may be useful for the source herd as it enables them to know which animals in their herd caused them to be test positive however, the risk of false positive and false negative results must be considered carefully and test results interpreted appropriately. Ultimately, which herd screen test strategy is most appropriate may depend on who is paying for the testing. The seller would likely be willing to sacrifice a higher risk for more individual data while minimizing potential impacts of false positive results that could reduce their ability to sell replacement animals. A purchaser is likely unconcerned about individual data but would like to optimize the ability of the test to prevent them from purchasing an infected animal.

These results quantify the risks associated with the use of various herd screen test strategies when selecting a source herd prior to purchasing replacement animals and help to explain how to determine which test strategy to use, depending on the scenario. Owners attempting to prevent or reduce introduction of cattle infected with Map should continue to use diagnostic testing of both the herd and the individual cattle to minimize the risks associated with purchasing replacement cattle.

Future research analyzing the risk associated with various testing strategies are required. It would be beneficial to compare a wider variety of test strategies utilizing various sample sizes and at multiple herd prevalences as these parameters have substantial impacts on the risks associated with each strategy. It would be interesting to also add an additional level of complexity to the model by including individual animal testing in series with herd level testing as well as adding a cost benefit analysis to the final results.

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Table 8.1 Input values used for the Pert ELISA parameter distributions for simulation modeling

	Minimum	Median	Maximum
Sensitivity	0.07	0.31	0.80
Specificity	0.90	0.985	1

(parameters from Nielsen and Toft, 2008)

Table 8.2 Results of *Mycobacterium avium* ssp. *paratuberculosis* sampling study used in the culture based test parameter beta distribution for simulation modeling

	No. of infected herds tested positive (x)	No. of infected herds tested (nt)
Environmental Sampling	8	27
Pooled Fecal Sampling	17	27

x and nt = inputs for Beta distributions described in section 8.2.3

Table 8.3 Simulated diagnostic test parameters used as inputs for the final risk model for *Mycobacterium avium* ssp. *paratuberculosis*

Diagnostic Test	Sensitivity			Specificity		
	Mean	Credible Intervals		Mean	Credible Intervals	
		2.50%	97.50%		2.50%	97.50%
Animal Level						
ELISA Serology	0.347	0.148	0.575	0.973	0.941	0.996
Herd Level						
ELISA Serology	0.703	0.409	0.908	0.493	0.160	0.887
Environmental Sampling	0.313	0.179	0.460	1*	1*	1*
Pooled Fecal Culture	0.625	0.467	0.758	1*	1*	1*

*: perfect specificity for environmental and pooled fecal culture was assumed

Table 8.4 Probabilities of a herd being falsely identified as negative for *Mycobacterium avium* ssp. *paratuberculosis* associated with various herd screen test strategies

Test Strategy	Risk	Credible Limits	
	Mean	2.50%	97.50%
No Test	0.129	0.005	0.425
Environmental Culture	0.098	0.003	0.314
ELISA Serology	0.096	0.002	0.354
Pooled Fecal Culture	0.061	0.002	0.227

Table 8.5 Risk Ratio comparisons between various herd screen test strategies for paratuberculosis

Test Strategy	Risk Ratio	Credible Limits	
	Mean	2.50%	97.50%
No Test:Pooled Fecal*	2.59	1.68	3.89
No Test:ELISA*	1.74	1.11	3.10
No Test:Env. Sampling*	1.42	1.16	1.74
Env. Sampling:Pooled Fecal*	1.84	1.16	2.75
ELISA:Pooled Fecal	1.61	0.79	2.56
Env. Sampling:ELISA	1.24	0.78	2.19

* results are considered significant when 95% credible interval does not include the null value of 1

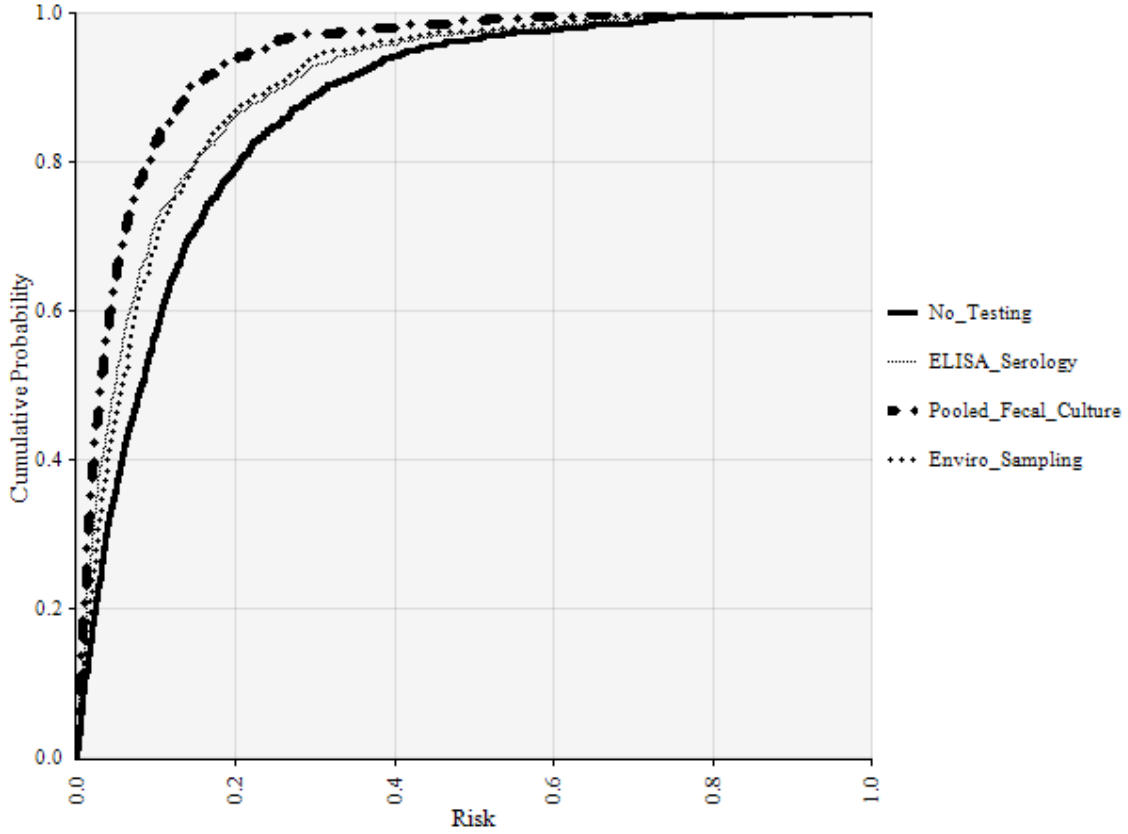
Table 8.6 Reduction in risk of purchasing an animal from a herd infected with *Mycobacterium avium* subspecies *paratuberculosis* using various herd screen test strategies

		Reduction in Risk (Test A:Test B)	
Test A	Test B	Mean	95% Credible Interval (%)
Environmental Sampling	No Testing	28%	14-43*
ELISA Serology	No Testing	36%	10-68*
Pooled Fecal Culture	No Testing	59%	40-74*
Pooled Fecal Culture	Environmental Sampling	42%	14-64*
Pooled Fecal Culture	ELISA Serology	30%	-27-61
ELISA Serology	Environmental Sampling	11%	-29-54

Reduction in Risk (Test A:Test B) = 1-(Risk: Test A)/(Risk: Test B)

*: test strategy A performed significantly better than test strategy B

Figure 8.1 Overlay presentation of cumulative probabilities of risk associated with various Map herd screen test strategies



CHAPTER 9

SUMMARY AND CONCLUSIONS

9.1 Background

Although there has been ongoing research focused on *Mycobacterium avium* subspecies *paratuberculosis* (Map) and Johne's disease for more than a century and much progress has been made in the understanding of the organism, diagnostic options, and disease epidemiology, there remains much we do not know about this complex disease, especially as it relates to the beef cow-calf industry. This thesis, and the research projects associated with it, attempts to fill some of the gaps that exist surrounding the epidemiology of Map infection in the beef cow-calf industry in western Canada. This investigation consisted of broad goals: first, to describe and report the prevalence of Map in the western Canadian cow-calf herd and in the wildlife species commonly associated with those herds; second, to describe the environmental distribution of Map on infected cow-calf farms while evaluating the potential for using environmental sampling as a potential alternative herd screen testing option; and third, to determine which on-farm management activities were associated with Map infection in cow-calf herds in order to affirm or reject commonly suggested control practices based on research in other species or on basic principles of biosecurity.

This study was successful as it described the prevalence of infection in the cow-calf herd, evaluated species commonly found on these farms for evidence of potential

infection, provided evidence for making on-farm Johne's disease control recommendations specific to the cow-calf industry, identified the environmental distribution of Map on herds struggling with Johne's disease, and evaluated the potential use of environmental sampling as a herd screen test while comparing it to other commonly used methods.

9.2 Summary of highlights from each chapter

9.2.1 Prevalence in cow-calf industry study

The objectives of this cross sectional study were to determine the seroprevalence of Map in cow-calf herds in Canada and to identify risk factors associated with a seropositive status of these herds. In total, 4778 cows were tested from 179 herds for antibodies to Map using a commercial ELISA. Overall, 0.8% (95%CI = 0.4-1.1%) of the cattle were seropositive with 11.7% (95%CI=7.0-16.5%) of the herds having a minimum of one positive test result or 4.5% (95%CI=1.4-7.5%) of the herds having a minimum of two positive test results. The true cow prevalence was estimated as 1.8% (95%CI= 0.4 – 3.1). For the risk factor analysis, Map positive herds were considered those herds that had 2 or more seropositive tests. This was to reduce the likelihood of false positive herds due to potential inaccuracies of serological tests for Map. In the final multivariable logistic regression model, the use of ionophores in the feed (OR = 16.3; 95%CI=1.2–218.5), and the practice of supplementing colostrum to calves (OR = 1.1; 95%CI=1.0–1.1) were both positively associated to herd seropositivity, while the presence of a dog on

farm was protective (OR = 0.1; 95%CI=0.2– 0.9). The low number of seropositive herds was a substantial limitation in this study and therefore suggested the need for a case-control study to further explore the significance of on-farm risk factors. It is also important to note that the significant risk factors described are associative and not necessarily causative. It is not possible to determine the direction of the relationship with a cross sectional study, and a longitudinal study design, while challenging to conduct due to the long term nature of this disease, would be beneficial for confirming causation.

9.2.2 Risk factor study

A case control study design was used to assess the possible role of various common cow-calf herd management practices with the status of herd infection with Map. In total, 23 case and 29 control herds, recruited based on herd history from Manitoba, Saskatchewan, Alberta and British Columbia, completed the project and were included in the analysis. A random sample of cows from each herd was selected and fecal cultures for Map were performed in order to confirm the herd's case or control status. The manager of each herd was required to complete a questionnaire on herd management and health history. A multivariable logistic regression analysis indicated that three management factors were significantly associated with herd Map infection status in the final model. Herds that used a commercial colostrum replacement on farm (Odds Ratio =3.96; 95% CI = 1.10–14.23, p=0.035) and herds that had wild deer interacting with their cattle (Odds Ratio = 14.32; 95% CI = 1.13–181.90, p=0.040) were positively associated

with being a herd infected with paratuberculosis. The use of rotational grazing practices was protective (Odds Ratio = 0.20; 95% CI = 0.04–0.93, p=0.039).

Participation in this study was voluntary and therefore not random. This means that the results of this study may not be generalizable to the entire industry. It is likely that producers with prior interest in Johne's disease would be more likely to participate. As with the previous study, it also is important to note that the significant risk factors described are associative and not necessarily causative.

9.2.3 Wildlife and environmental distribution pilot study

The objective of this pilot study was to describe the distribution of Map in wildlife and the environment of six cow-calf farms with a history of Johne's disease in Saskatchewan and to direct future research assessing the potential of using environmental sampling as an alternative herd screening tool. Serum ELISA and pooled fecal cultures (5 fecal samples/pool) were performed on samples from approximately 100 cattle from each farm at the beginning of the study and were compared to wildlife and environmental samples collected quarterly for one year. Of all samples collected, 0.5% of the environmental samples and none (0%) of the wildlife samples collected were Map positive. A third (33.3%) of the herds were identified as positive for Map by both the ELISA and environmental sampling methods, as compared to 66.7% by the pooled fecal culture method. Environmental contamination of Map on these farms was very low. Future research examining the potential for using environmental sampling as a herd test

should likely be focussed during the calving season. This would improve the sensitivity of environmental sampling due to the increased animal density and increased shedding due to stress that would potentially occur at this time period. The low number of herds in this study was a limitation; however, this study was intended for directing future research and was successful for this. The low number of herds did limit the number of wildlife species that were evaluated as only species present on those farms at the times of sample collection could be included. To examine other species a more directed effort would be required to ensure their inclusion.

9.2.4 Coyotes as sentinel species study

Infection with Map has been identified in many ruminant and non-ruminant species. This study utilized 82 coyotes collected around Riding Mountain National Park in Manitoba, Canada for tuberculosis research. Samples of lymph nodes from these animals were collected to estimate the prevalence of Map infection in the coyote population in this region by using culture techniques on tissue samples with PCR confirmation. Only one cluster of three infected coyotes was identified at a single geographical location. The prevalence of Map infection in this cluster was calculated to be 9.1% (CI: 5.7-12.5). The prevalence of infection including all sites, ignoring the effect of clustering, was calculated to be 3.7% (CI: 2.3-5.1).

9.2.5 Environmental distribution study

The objective of this study was to determine the distribution of Map in the environment of cow-calf herds in Western Canada that had a history of clinical Johne's disease, and to compare the sensitivity of pooled fecal sampling and environmental sampling on these farms. Approximately 15 environmental samples were consistently collected from a variety of sites from each of 27 herds. Fecal samples were collected from up to 150 cows per herd and cultured in pools of five. Map was detected using bacterial culture and PCR for confirmation. Sixty-three percent of the herds had at least one Map positive fecal pool. No water samples were positive to Map and 6.2% of the non-water environmental samples were positive. While environmental sampling identified 29.6% of the herds as positive for Map, the methodology used in this study does not appear to be sufficiently sensitive to replace cattle sampling as a herd screening test. These findings led to a simulation modelling study to evaluate how various testing methods would compare in the broader population of cow-calf herds.

9.2.6 Simulation model of herd screen testing study

A Monte Carlo simulation model was developed to determine the risk of selecting a herd infected with Map as the source herd for purchasing replacement animals into a Saskatchewn cow-calf herd when using either environmental sampling, ELISA serology, pooled fecal culture, or no herd screen testing strategies. A source herd that had been selected without any herd screen test had a significantly higher risk of being infected with

Map than herds selected with negative results on any of the other herd screen test strategies. The use of environmental testing had a significantly higher risk than the use of pooled fecal culture. The risk associated with ELISA serology was not significantly different than the use of environmental sampling or pooled fecal culture. The final mean risk of selecting a herd infected with Map, that was not identified as positive via the herd screen test strategy, was 12.9%, 9.8%, 9.6%, and 6.1% for no herd screen test, environmental sampling, ELISA serology, and pooled fecal culture strategies, respectively. These results were based on sampling methodologies and population data from research as described in this thesis. The validity of these results are dependent on the assumption that these sources of information are generalizable to the true population. Findings would change if methodologies were altered or if population data were adjusted via new research or sources of expert opinion.

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9.3 Study limitations

There were some common limitations to these studies that were largely due to the industry involved. The cow-calf industry in western Canada is an extensive industry that often only processes cattle a couple times per year and generally keeps very low stocking densities. This makes it challenging to collect samples and reduces the average producer's willingness to participate in ongoing research. Most cow-calf producers also have a minimal awareness of Johne's disease. These factors combined limit the number of producers that volunteer for research projects. The use of voluntary participation in

this research prevents using a true random selection of participating herds without significantly increasing the costs of the projects. This may reduce how generalizable the findings are to the entire cow-calf population and this must be considered when interpreting results.

Sampling occurred at seasonal intervals or time points, limiting potential temporal extrapolations. The samples for the wildlife and environmental distribution pilot study were collected once every three months over the span of one year. Species present change as seasons change and it is possible that had sampling occurred throughout the entire year other species may have been collected that could have shown evidence of infection with Map. Other environmental samples from 27 farms were collected during the calving season and so the findings may not be valid for other times of year. The simulation model study results may also not be valid for times outside of the calving season.

Risk factor analysis results based on questionnaire data have certain inherent limitations. The primary concern is that of bias. Misclassification bias can occur when herds are incorrectly categorized into the case or control groups. A combination of a producer's lack of awareness of Johne's disease and imperfect tests increases the potential for misclassification. An attempt was made to minimize this via the required herd testing of all control herds to verify their negative herd history. Recall bias is another concern. Some questions asked participants to recall management that had occurred over the previous months to years. The quality of management records can vary

substantially between producers. It is possible that the ability to recall information may be linked to whether or not the herd was dealing with disease issues such as Johne's disease and this could alter the findings. To maximize the quality of data, producers were given time to complete the questionnaires so that they were able to refer to records and complete the questionnaire as accurately as possible.

The lack of a standardized method of comparing diagnostic tests and the long latent period of this particular disease makes comparing the findings in this study to previous research challenging. Further research comparing environmental sampling with other herd screen tests would be useful to determine its full potential.

The long term nature of this infection is the primary limitation to any research focused on Johne's disease. To confidently identify causative relationships associated with Map, a long term longitudinal cohort study would be beneficial but these types of research programs that carry over many years require the stability of both a large time and funding commitment.

9.4 Conclusions

The prevalence of Map infection in Canadian cow-calf herds was relatively low with 4.5% (95%CI=1.4-7.5%) of the herds having at least 2 cows seropositive to Map and, 0.8% (95%CI = 0.4-1.1%) of the national cow herd testing seropositive to Map. This national research supports the few regional studies completed previously (Waldner

et al., 2002; Coté G., 2004; VanLeeuwen et al., 2006; Scott, 2006). It is certain that individual herds infected with Johne's disease can suffer severe losses but these cases appear to remain uncommon. However, the total economic loss to the beef industry as a whole is still unknown at this time. Further research quantifying the financial cost of Johne's disease in the cow-calf industry would help to answer this question. Due to the present low prevalence of Johne's disease in the Canadian cow-calf industry, there is an opportunity to develop control strategies to limit further spread of the disease if this is deemed to be a goal of the industry. For a program to succeed, the epidemiology must be understood. This research gives evidence that at the current level of infection with Map in cow-calf herds in western Canada, other species do not appear likely to become infected or contribute significantly to the transmission of disease. Therefore it is recommended to focus control programs on eliminating clinical cattle which are shedding Map and contaminating the environment and preventing susceptible animals from being exposed to an environment already contaminated. The control of Johne's disease nationally will be an immense undertaking due to the insidious nature of this disease and the relatively poor performance of tests that are currently available. The use of environmental sampling in dairy herds has been found to be economical and effective (Raizman et al., 2004; Berghaus et al., 2006). While using environmental herd screening methods in cow-calf herds may be one additional tool that can be used as an affordable preliminary screening test, it is not presently sensitive enough to be used alone and should only be used in parallel with other more sensitive methods. There is a need to develop best management practices specific to the beef industry with consideration given to the biology and ecology of the disease. This research has identified the use of

commercial colostrum products and the presence of deer as risks significantly associated with the presence of Map infection and the use of rotational grazing as significantly protective. Further research needs to be done to fully understand the direction of these relationships and how these factors can be utilized by producers to minimize the effect of Map on their herds. A longitudinal cohort study would be very helpful in showing causative relationships and ought to be undertaken to optimize evidence based best management practices. Until such studies have been completed, one ought to consider the following previously stated recommendations, based on first principles of disease control, which have been made with the focus on the beef industry (Hansen and Rossiter, 2000; Rideout et al, 2003):

1. Reducing manure build-up of pens and pastures where late-gestation cattle are kept.
2. Keep the calving area clean at all times and maintain a low cow density in these areas.
3. As soon as bonding has occurred, move cow-calf pairs to a clean pasture.
4. Avoid exposing calves to manure build-up by frequently moving location of feed bunks, waterers, and creep-feeders.
5. Once calves are weaned, do not put them on pastures used by cows.
6. Annually test the entire herd and avoid calving-out or raising offspring from any test-positive cattle.
7. Calve first-calf heifers in a separate location from mature cows.
8. Use separate equipment for handling manure and feed.

9. Do not spread manure on land used for grazing, especially for young stock.
10. Purchase replacement animals only from test negative herds and when this is not possible assess herd status through communication with the owner and their veterinarian.

The potential zoonotic risks associated with Map were not investigated in this research however this issue requires further research as well. If Map is eventually conclusively identified as a causative factor for Crohn's disease in humans, public health agencies and consumers will not accept this disease in their food supply. The affected livestock industries will need to be seen to be actively controlling this risk if public confidence in food safety is to be maintained. Implementing Johne's disease control programs is an important proactive step forward regardless of the true relationship between Map and Crohn's disease, for impacts on productivity and food safety perceptions. *Mycobacterium avium* subspecies *paratuberculosis* has plagued the cattle industry for many years and will likely continue to remain a significant challenge for the foreseeable future.

9.5 References

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APPENDICES

10.1 Chapter 3 Questionnaire

Part 1: Herd Inventory Information

1. Which of the following categories best describe the livestock production activities that occur on your farm? (Check all of those that apply)

i) Commercial cow-calf herd ☐
ii) Purebred cow-calf herd ☐
iii) Backgrounding operation ☐
iv) Stocker operation ☐
v) Feedlot ☐
vi) Dairy ☐
vii) Hobby farm ☐
viii) Other livestock operation ☐ (Please specify) _____

2. Is the cow-calf portion of your operation:

i) Your primary source of income ☐
ii) A supplemental source of income ☐
iii) Primarily for other reasons, such as pleasure ☐

3. Considering the hours you work on and off farm, what percentage of your work time is devoted to the cow-calf operation? _____

4. What is the age of the primary person making day-to-day management decisions about the cows on the farm? _____ years.

5. How many full time employees (including family members) work directly in the cow-calf enterprise? _____

6. How many part-time employees (including family members) directly in the cow-calf enterprise? _____

7. What is the area of forage production (in acres) that was both owned and rented as of July 1, 2002? _____ acres.

8. What is the area of pasture (grazing) in acres that was both owned and rentedas of July 1, 2002? _____ acres.

9. As of July 1, 2002, how many of each of the following classes of cattle, regardless of ownership, were present on your operation?

i) Beef cows (including heifers that have calved) _____
ii) Calves that have not been weaned _____
iii) Bulls for use in breeding for your herd _____
iv) Bulls intended for sale (weaned or older)_____
v) Replacement heifers (weaned or older) _____
vi) Steers (weaned or older) _____
vii) Dairy cows _____
viii) Dairy replacement heifers _____

11. Which of the following best describes the breed composition of the majority of the beef cows in this operation?

i) Purebred herd or straight bred herd consisting of only one breed ☐
Please specify breed_____
ii) Purebred herd or straight bred herd consisting of several breeds ☐
Please specify breeds _____
iii) Predominantly British-type crossbred cows ☐
iv) Predominantly Exotic-type crossbred cows ☐
v) Crossbred cows with British and Exotic influences ☐
vi) Other ☐ Please describe:_____

Part 2: Breeding Management

1. Does your breeding management of your cow-calf herd consist of:

i) Natural mating exclusively? ☐
ii) Artificial insemination breeding exclusively? ☐
iii) A mix of natural and Artificial breeding? ☐

2. If artificial insemination is utilized is it:

i) Used exclusively in heifers? ☐
ii) Used exclusively in cows? ☐
iii) Used in both cows and heifers? ☐

3. If artificial insemination is used, are the estrus cycles of the animals synchronized?

i) Yes ☐
ii) No ☐
iii) Some of the time ☐

4. Do you manage heifers separately from cows during the breeding season?

i) Yes ☐
ii) No ☐

5. How many different groups of breeding cattle do you usually manage? _____

6. Which of the following statements best describes your breeding management in 2001?

i) My herd has a defined breeding season from _____ to _____ ☐
ii) My herd has no defined breeding season and are exposed to bulls at all times ☐
iii) My herd consists of multiple groups of cattle which have distinctly different breeding seasons ☐

7. During the 2001 breeding season, what is the number of breeding age females that were exposed to any kind of breeding (natural or AI)? _____

8. During the 2001 breeding season; what is the total number of bulls on your operation that were used for breeding purposes? _____

9. Do you utilize community pastures or communal grazing for any portion of your breeding herd?

i) Yes ☐

ii) No ☐ (Go to Question 16)

10. If Yes, which of the following categories of communal grazing would apply to your herd? (Check all that apply to your herd)

i) PFRA (federal) community pasture ☐
ii) Provincially managed community pasture ☐
iii) Local grazing cooperative ☐
iv) Open range ☐
v) Other ☐ Please describe:_____

11. Do you utilize more than one communal grazing situation for your herd?

i) Yes ☐ Please specify number of pastures: _____
ii) No ☐

12. What would be the total number of other herds that your herd is exposed to during communal grazing? _____

13. What percentage of your cow herd would utilize communal grazing?

i) 0 – 20% ☐
ii) 21-40% ☐
iii) 41-60% ☐
iv) 61-80% ☐
v) 81-100% ☐

14. Do you breed cows at home prior to entering communal grazing?

i) Yes ☐
ii) No ☐

15. Do you **exclusively** use communal grazing situations where no natural breeding occurs? (ie: No bulls present)

i) Yes ☐
ii) No ☐

16. Prior to the 2001 breeding season, did you utilize the services of a veterinarian to perform breeding soundness examinations on your bulls?

i) Yes ☐
ii) No ☐
iii) Occasionally ☐
iv) I don't own any breeding bulls ☐

17. Do you ever test bulls on your farm for Trichomoniasis?

i) Yes ☐
ii) No ☐
iii) I don't know ☐

18. In 2001/2002 did you have your cows checked for pregnancy by:

i) Rectal palpation by a veterinarian ☐
ii) Rectal palpation by a non-veterinarian ☐
iii) Ultrasound evaluation ☐
iv) Visual inspection ☐
v) Not at all ☐

19. If your cows were pregnancy checked, what is the number of cows that were pregnant after the 2001 breeding season? _____

Part 3: Calves and Calving Management

1. How many calves were born alive in your herd as a result of breedings which occurred in 2001? _____

2. How many stillbirths (calves that appeared to be full-term but which were dead at the time of calving) occurred in your herd after the 2001 breeding season? _____

3. How many abortions (calves which were born dead prematurely) occurred in your herd after the 2001 breeding season? _____

4. How many calves were born in each of the previous 12 months?

July, 2001	
August, 2001	
September, 2001	
October, 2001	
November, 2001	
December, 2001	
January, 2002	
February, 2002	
March, 2002	
April, 2002	
May, 2002	
June, 2002	

5. How many of the calves born in the last 12 months have died? _____

6. Which of the following best describes the situation your cows are kept in during the time that the majority of the herd is calving?

i) On pasture or open range ☐ # of acres utilized?
ii) In small paddocks or pastures that allow increased observation ☐
iii) In corrals or dry lot pens ☐
iv) In barns or covered pole sheds ☐
v) Other ☐ Please describe: _____

7. Estimate the total area (in either acres or square feet) the cow herd would occupy when the majority of the herd is calving. _____acres **OR** _____sq. feet

8. What is the maximum number of cows and heifers you put on this area at any one time during calving? _____

9. Is the area where the cows are kept during calving, different from the area where the cows are kept over the winter feeding period?

i) Yes ☐
ii) No ☐

10. What percentage of the cows that calve in your herd would be placed in an individual maternity pen for some period of time? _____%

11. How many individual maternity pens do you have on your farm? _____

12. Do you utilize a maternity pen that may contain more than one cow-calf pair during calving season?
i) Yes☐
ii) No ☐

12. Are maternity pens also used as hospital pens for sick cows during calving season?
i) Yes ☐
ii) No ☐

13. How often do you remove surface manure from maternity pens?
i) Every calving ☐
ii) Every 2-4 calvings ☐
iii) Every 5 or more calvings ☐

14. What type of bedding is used in maternity pens?
i) Straw ☐
ii) Shavings/sawdust ☐
iii) Other ☐
iv) None ☐

15. How often do you add fresh bedding to the maternity pens?
i) Every calving ☐
ii) Every 2-4 calvings ☐
iii) Every 5 or more calvings ☐

16. Do you separate cow-calf pairs from the pregnant cows after calving?

i) Yes ☐
ii) No ☐

17. Do you utilize calf shelters or creep areas for calves?
i) Yes ☐
ii) No ☐

18. What percentage of the calves born in the last 12 months would have received some form of supplemental colostrum? _____%

19. Of all the calves that received supplemental colostrum what percentage would have received it from:

i)	Colostrum milked from own dam	%
ii)	Colostrum milked from other cow in the herd	%
iii)	Pooled colostrum milked from other cows in the herd	%
iv)	Frozen colostrum from dairy cows	%
v)	Commercial colostrums substitute	%

Part 4: Feeding Management

1. During your winter feeding period, please outline how each of the following potential feedstuffs is delivered to your cow herd. If more than one feeding method applies, check more than one answer.

Hay

i) Bale feeder ☐
ii) On ground ☐
iii) Manger ☐
iv) Other ☐ _____

Green feed or baled cereal crop

i) Bale feeder ☐
ii) On ground ☐
iii) Manger ☐
iv) Other ☐ _____

Straw

i) Bale feeder ☐
ii) On ground ☐
iii) Manger ☐
iv) Other ☐ _____

Grain

i) Trough ☐
ii) On ground ☐
iii) Other ☐ _____

Silage

i) Trough ☐
ii) On ground ☐
iii) Other ☐ _____

2. Where is each type of feed stored? (if more than one storage method applies, check more than one answer)

Hay

i) Outdoor stack ☐
ii) Covered stack/loft ☐
iii) Other ☐ _____

Green feed or baled cereal crop

i) Outdoor stack ☐
ii) Covered stack/loft ☐
iii) Other ☐ _____

Straw

i) Outdoor stack ☐
ii) Covered stack/loft ☐
iii) Other ☐ _____

Grain

i) Hopper bottom bin ☐
ii) Door access bin ☐
iii) Outdoor pile ☐
iv) Other ☐ _____

Silage

i) Upright silo ☐
ii) Pit silo ☐
iii) Silage bags ☐
iv) Other ☐ _____

3. Do dogs, cats or wildlife have access to stored grain?

i) Yes ☐
ii) No ☐

4. Have you grazed stubble fields after harvest in the last two years?

i) Yes ☐
ii) No ☐

5. What is the source of water for your cow herd? (If more than one source applies, check more than one answer)

a) Summer

i) Well water ☐
ii) Direct access to Dugout ☐
iii) Water which is pumped from dugout to trough ☐
iv) Natural standing water (pond, slough) ☐
v) Water which is pumped from natural standing water to trough ☐
vi) Natural running water(creek, river) ☐
vii) Water which is pumped from natural running water to trough ☐

b) Winter

viii) Well water ☐
ix) Direct access to Dugout ☐
x) Water which is pumped from dugout to trough ☐
xi) Natural standing water (pond, slough) ☐
xii) Water which is pumped from natural standing water to trough ☐
xiii) Natural running water(creek, river) ☐
xiv) Water which is pumped from natural running water to trough ☐

6. Do you add ionophores to any of your feedstuffs during the year? (Eg: Avatec, Bovatec, Coban, Coxistac, Cygro, Rumensin, Zoamix, Deccox)

i) Yes ☐
ii) No ☐

7. How often is equipment that is used for manure handling (eg: bucket, spreader) also used to handle feed fed to heifers less than 12 months of age?

i) Regularly ☐
ii) Occasionally ☐
iii) Never ☐

8. How often is equipment that is used for manure handling (eg: bucket, spreader) also used to handle feed fed to cows?

iv) Regularly ☐
v) Occasionally ☐
vi) Never ☐

9. Which methods are used to dispose of manure on owned or rented land?

i) Injection ☐
ii) Spread with surface incorporation (eg: plowing, disking) ☐
iii) Spread without surface incorporation ☐

10. Is manure spread on land that is used as pasture for replacement heifers?

i) Yes ☐
ii) No ☐

11. If yes, how many days do you wait after applying manure to a field before heifers are allowed to graze the field? _____

Part 5: Veterinary Procedures and Vaccinations

1. What percentage of the bull calves born on this operation during 2001 were castrated before sale? _____%

2. Which of the following best describes when the majority of calves were castrated?
   i) At or shortly after birth ☐
   ii) At or shortly before turnout to pasture ☐
   iii) At the time of weaning ☐
   iv) Other ☐ _____

3. Which of the following best describes the primary method of castration used on your bull calves?
   i) Surgical castration (with a knife or scalpel) ☐
   ii) Rubber band (Elastrator band) at less than 3 months of age ☐
   iii) Clamp/Burdizzo (crush cords) ☐
   iv) Rubber rings (Callicrate bander or EZE bloodless castrator) at more than 3 months of age ☐

4. If you use surgical castration, do you disinfect the blade between animals?
   i) Yes
   ii) No

5. If dehorning is performed on any calves or cattle on your farm which method would be the primary method employed?
   i) Gougers or spoons ☐
   ii) Saws, Barnes dehorner or guillotine ☐
   iii) Electric dehorner/debudder/hot iron ☐
   iv) Caustic paste ☐
   v) Do not dehorn cattle ☐
   vi) No horned cattle on this operation ☐

6. If you use cutting equipment for dehorning, do you disinfect them between animals?
   i) Yes ☐
   ii) No ☐

7. Do you give young calves (less than 3 months of age) any vaccines for Bovine Virus Diarrhea (BVD)?
   i) Yes ☐ Name of Vaccine if known: _____
   ii) No ☐
   iii) I don't know ☐

8. If Yes, is the BVD vaccine a modified live vaccine or killed vaccine?
   i) Modified live vaccine ☐
   ii) Killed vaccine ☐
   iii) I don't know ☐

9. Do you vaccinate heifers or steers at weaning with a BVD vaccine?
   i) Yes ☐ Name of Vaccine if known: _____
   ii) No ☐
   iii) I don't know ☐

10. If Yes, is the BVD vaccine a modified live vaccine or killed vaccine?
   i) Modified live vaccine ☐
   ii) Killed vaccine ☐
   iii) I don't know ☐

11. Do you vaccinate cows with a BVD vaccine?
   i) Yes ☐ Name of Vaccine if known: _____
   ii) No ☐
   iii) I don't know ☐

12. If Yes, is the BVD vaccine a modified live vaccine or killed vaccine?
   i) Modified live vaccine ☐
   ii) Killed vaccine ☐
   iii) I don't know ☐

12. When are the cows vaccinated?
   i) Prior to breeding ☐
   ii) At pregnancy checking/weaning ☐
   iii) Other ☐ _____

13. Do you use a new needle for every injection?
   i) Yes ☐
   ii) No ☐

14. Do people who do rectal examinations or Artificial insemination change rectal gloves between animals?
   i) Yes ☐
   ii) No ☐

Part 6: Biosecurity

1. During the previous year (July 1,2001 – July 1,2002) how many of each of the following classes of animals were brought onto this operation?

Animal Class	**Number**
Unweaned beef calves	
Unweaned dairy calves	
Weaned beef heifers(not bred)	
Bred beef heifers	
Bred beef cows	
Weaned bulls (all types)	
Weaned steers (all types)	
Dairy cows and heifers	

2. What percentage of the beef animals brought onto the farm were purchased from the following sources?

i) Auction market _____%
ii) Direct from other producers _____%
iii) Through private dealers _____%

3. During the last year (July 1, 2001 –July 1, 2002) how many cows were culled from your operation? _____

4. Have any cows in your herd died or be culled due to diarrhea in the last five years?

i) Yes ☐ (How many? _____)
ii) No ☐

5. Have you purchased an un-weaned Holstein calf in the last 5 years to suckle a cow that has lost her calf?

i) Yes ☐
ii) No ☐

6. Have you purchased an un-weaned beef calf in the last 5 years to suckle a cow that has lost her calf?

i) Yes ☐
ii) No ☐

7. Have you used a Holstein nurse cow for orphaned or twinned calves in the last 5 years?

i) Yes ☐
ii) No ☐

8. During the last year, did any cattle from this operation leave for fairs or shows and return to the premises?

i) Yes ☐

ii) No ☐

9. Do beef cattle on your operation have direct access or fenceline contact with dairy cattle?

i) Yes ☐
ii) No ☐

10. Do beef cattle on your operation share pasture with dairy cattle?

i) Yes ☐
ii) No ☐

11. Is manure from a dairy cattle operation spread on pasture land or crop land associated with your beef cattle operation?

i) Yes ☐
ii) No ☐

12. Do your beef cows, replacement heifers or their feed or water have any physical contact with any of the following species? (Check all that apply)

i) Feedlot cattle ☐
ii) Pigs ☐
iii) Goats ☐
iv) Sheep ☐
v) Chickens/poultry or their litter ☐
vi) Bison ☐
vii) Llamas/alpacas ☐
viii) Horses or other equine ☐
ix) Captive elk or deer ☐

13. In the past 5 years have any of your beef cattle had contact with cattle (dairy or beef) from other herds through any of the following routes:

i) Shared pasture or communal grazing ☐
ii) Contract calving operations ☐
iii) Fenceline contact while on pasture ☐
iv) Contact at fairs/exhibitions ☐
v) Lending cows or bulls ☐
vi) Borrowing cows or bulls ☐

14. In the last year, how many other cattle operations would have potential fenceline contact with your cattle on your home farm? _____

15. Do you have dogs on your farm?

i) Yes ☐
ii) No ☐

a) If yes, how many dogs do you have in each of the following categories?

i) Housedog(s) _____

ii) Free roaming dog(s)_____

b) How many of your dogs are working cattle dogs? _____

c) How many dogs have access to feed storage areas?

d) How many dogs have access to cattle pasture or calving areas?

e) How many litters of dogs have been born on your farm in the last 5 years?

16. In the past 12 months how often have the following animals been seen on the farm?

Coyotes/Wolves: Never ☐ 1-10 times/year ☐ 11-25 times/year ☐ > 25 times/yr ☐

Foxes: Never ☐ 1-10 times/year ☐ 11-25 times/year ☐ > 25 times/yr ☐

Roaming Dogs: Never ☐ 1-10 times/year ☐ 11-25 times/year ☐ > 25 times/yr ☐

17. Estimate the level of rodent infestation on your farm:

i) Low ☐
ii) Medium ☐
iii) High ☐

10.2 Chapter 4 Questionnaire

JOHNE'S DISEASE IN COW-CALF HERDS IN WESTERN CANADA:

Assessing the Problem – Looking for Solutions

UNIVERSITY OF SASKATCHEWAN

A survey to identify risk factors in order to determine recommendations for prevention and control for the beef cow-calf industry.

This questionnaire should be completed by the person who best knows the cow-calf herd.

All answers are strictly confidential.

We greatly appreciate your participation in this important research. Thanks!

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INSTRUCTIONS:

1. Please read all questions carefully before answering.

2. Check the appropriate boxes or fill in the blanks provided.

3. Check only one box per multiple choice question unless instructed to 'check all that apply'.

4. To ensure the accuracy of your answers, please consult your records where detailed answers are required.

Thank you for participating in this research.

If you have not yet received the results from the manure samples that were collected from your herd, they are presently being cultured. Results will be forwarded to your veterinarian when they are completed. If you would like to receive the results directly from us please contact us and let us know.

PART 1. THIS SECTION ASKS QUESTIONS ABOUT THE HERD HEALTH ON YOUR FARM

1. During the last year, how many times has a veterinarian been on your farm and interacted with your cattle herd? _____

2. What is the main health problem for your herd at this time?

3. Do you vaccinate your herd against BVD every year? Yes No

4. Do you add ionophores to any of your feedstuffs during the year? (E.g.: Avatec, Bovatec, Coban, Coxistac, Cygro, Rumensin, Zoamix, Deccox)

Yes No

IF YES → 4.1 What classes of cattle received ionophores? *(Check all that apply)*

Unweaned calves	Breeding cows
Weaned calves	Other - Describe:
Replacement heifers	

5. In the last year how many cows have died or been culled from your operation?_____

6. In the last year have many cows in your herd have died or been culled due to diarrhea?_____

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7. How many cases of Johne's disease have occurred on your farm in the last 5 years? (cattle with chronic weight loss and diarrhea)

	# of Home Raised Cattle	# of Purchased Cattle
Unconfirmed Johne's Cases		
Confirmed Johne's Cases (confirmed by laboratory testing)		

8. In what year did your herd experience its first case of Johne's disease?

Suspected Case (not necessarily confirmed):_____
Case confirmed by testing:_____

9. Why have you tested for Johne's disease prior to this study?

Cattle in my herd had NOT been tested for Johne's disease prior to this study
Cattle with clinical signs of Johne's disease were tested for confirmation
Offspring from dams with clinical signs of Johne's disease were tested
Cattle were tested as part of a voluntary Johne's control program
Purchased Cattle were tested as part of a disease prevention plan
Cattle have been tested prior to sale
Cattle have been tested as part of a research program

10. What tests for Johne's disease have been used on your herd prior to this study? *(Check all that apply)*

ELISA (blood)
ELISA (milk)
Individual Cow Fecal Culture
Pooled Fecal Culture
Polymerase Chain Reaction (PCR)
Post Mortem Exam
Tissue Sample Culture
Unknown
No tests were done prior to this study

11. What actions are taken with cattle in your herd that you suspect have Johne's disease? *(Check all that apply)*

Suspect cattle are tested for confirmation of disease
Test positive cattle are culled
Suspect cattle are culled without testing
Cattle are allowed to calf prior to culling
Suspect cattle are moved onto a separate property
Offspring from positive cattle are tested for Johne's disease
All offspring from positive cattle are culled

PART 2. THIS SECTION ASKS QUESTIONS ABOUT THE FEEDING MANAGEMENT OF YOUR HERD

12. What percentage of your cow herd would utilize communal (shared) grazing (e.g. PFRA)? ____ %

13. How many other herds are exposed to your herd during communal grazing? _____ herds

14. Which of the following categories of communal (shared) grazing would apply to any portion of your breeding herd? *(Check all that apply)*

My herd does not use communal grazing
PFRA (federal) community pasture
Provincially managed community pasture
Local grazing cooperative
Open range
Other (please describe)

15. What type of grazing management do you usually practice?

Rotational Continuous

16. What are the significant sources of water for your cattle herd? *(Check all that apply)*

Water Source	Summer grazing period	Winter feeding period
Well water		
Direct access to standing water (dugout, pond, slough)		
Water which is pumped from standing water to trough/tank		
Direct access to natural running water (creek, river)		
Water which is pumped from natural running water to trough/tank		

17. Do you provide any feedstuffs to your cow herd directly on the ground? Yes No

18. Is equipment that is used for manure handling (e.g.: bucket, spreader) ever used to handle feed fed to cattle?

Yes No

19. Is manure spread on land that is used as pasture for replacement heifers? Yes No

IF YES → 19.1 How many days do you wait after applying manure to a field before heifers are allowed to graze the field? _____days

PART 3. THIS SECTION ASKS QUESTIONS ABOUT THE CALVING MANAGEMENT OF YOUR HERD

20. How many calves were born in each of the previous 12 months?

July, 2005		January, 2006	
August, 2005		February, 2006	
September, 2005		March, 2006	
October, 2005		April, 2006	
November, 2005		May, 2006	
December, 2005		June, 2006	

21. Estimate the size of the area the breeding herd would occupy during the calving season.

(____ sq. feet *OR* ____ acres)

22. What is the maximum number of cows and heifers you would put on this area at any one time during the calving season?_____

23. What percentage of your herd calves in the following situations?

Calving Location	% of Herd
On pasture or open range	
In small pastures that allow for increased observation	
In corrals or dry lot pens	
Group maternity pens (more than 1 cow-calf pair per pen)	
Individual maternity pens	
Other (Please describe)	

24. How often do you remove surface manure from maternity pens? *(insert # in blank)*

After every ___ calvings

25. How often do you add fresh bedding to the maternity pens? *(insert # in blank)*

After every ___ calvings

26. Do heifers and cows calve in different locations? Yes No

27. Do heifers and cows calve at different times?

Yes, Heifers generally calve before the cows calve
Yes, Heifers generally calve after the cows calve
No, Heifers and cows share the same calving season

28. Is the area where the cows are kept during calving, different from the area where the cows are kept over the winter feeding period?

Yes No

29. Do you separate cow-calf pairs from the pregnant cows after calving? Yes No

30. Are maternity pens ever used as hospital pens for sick cows?

Maternity pens are never used as hospital pens
Yes, but never during calving season
Yes, including during calving season

31 Are the maternity pens ever used as a hospital pen for cattle with diarrhea or dramatic weight loss?

Yes No

32 After using a maternity pen as a hospital pen, is this pen always cleaned prior to reusing it as a maternity pen?

Maternity pens are never used as hospital pens
Yes
No

33. In the last 5 years, have you used a dairy breed nurse cow in your herd?

Yes, (How many?_____)
No

34. What percentage of the calves born from in the past year would have received some form of supplemental colostrum?_____ %

IF SUPPLEMENTAL COLOSTRUM IS USED → 34.1 Of all the calves that received supplemental colostrum what percentage would have received it from:

Source	Percentage (%)
Colostrum collected from own dam	
Colostrum collected from other cows in the herd	
Colostrum collected from dairy cattle	
Commercial colostrum substitute (e.g. Headstart)	

35. How frequently do you disinfect colostrum feeding equipment (i.e. bottles/nipples/stomach tube)? *(Check all that apply)*

Never
When it appears dirty
When switching between calves
After each time it is used

PART 4. THIS SECTION ASKS QUESTIONS ABOUT BIOSECURITY ON YOUR FARM

36. What steps are required by the following people prior to entering your facilities? *(Check all that apply)*

	Wash Boots	Change Boots	Change Clothes	No Steps Required
Owners				
Employees				
Visitors				

37. What percentage of the dead stock from your farm is disposed of by the following methods?

Method of Disposal	Percentage (%)
Sent for Rendering	
Sent to Landfill	
Buried on Farm	
Left for Scavengers on Farm (Not Buried)	
Other: Explain	

38. What types of contact is there between your cow-calf herd and the following livestock species? *(Check all that apply)*

Species	Direct Access (nose to nose contact)	Indirect Access (manure and/or runoff access)	No Access to this Species
Cow-calf cattle (different owner)			
Feedlot cattle			
Dairy cattle			
Sheep			
Goats			
Captive elk or deer			
Bison			

39. Do you have dogs on your farm? Yes No

40. Do dogs, cats or wildlife have access to stored feed? Yes No

41. In the past year, how many of the following wild animals are seen on the farm per month?

Wild Species	Approx. # per month	Wild Species	Approx. # per month
Wolves		Rabbits	
Coyotes		Rats	
Foxes		Mice	
Roaming Dogs		Deer	

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42. During the past year (July 1, 2002 to July 1, 2003), did you borrow equipment from other farmers that may have had manure contact (i.e. Foot trimming chute, manure spreader, tractor, cattle trailer)

Yes No

PART 5. THIS SECTION ASKS QUESTIONS ABOUT THE FARM PROFILE OF YOUR FARM

43. Which of the following categories best describe the livestock production activities that occur on your farm? (Check all of those that apply)

Commercial cow-calf herd
Purebred cow-calf herd
Backgrounding operation
Feedlot
Dairy
Other (please specify)

44. As of July 1, 2006, how many of each of the following classes of cattle were present on your operation?

Class of Cattle	# Home Raised	# Purchased	# Owned by others
Beef cows (including heifers that have calved)			
Calves that have not been weaned			
Bulls for use in breeding for your herd			
Bulls intended for sale (weaned or older)			
Replacement heifers (weaned or older)			
Feeder heifers (weaned or older)			
Steers (weaned or older)			
Dairy cows (for milking or nursing)			
Dairy replacement heifers			
Dairy calves			

45. In the last 3 years, how has the number of breeding cows and heifers changed? *(Check only one)*

Increased Decreased Remained the same

46. Approximately how many breeding replacement cattle are sold each year?

Cows: _____ Heifers: _____ Bulls: _____

47. Approximately how many breeding replacement cattle are purchased each year?

Cows: _____ Heifers: _____ Bulls: _____

48. What is the age of the primary person making day-to-day management decisions about the cattle on the farm? _____years.

49. What is the area of pasture (grazing) in acres that was both owned and rented as of July 1, 2006? _____ acres.

50. What is your legal land location? (this information will only be used to collect climatic and environmental data for your farm)

Section:_____Township:_____Range:_____Meridian:_____

PART 6. THIS SECTION ASKS QUESTIONS ABOUT SOME OF YOUR OPINIONS REGARDING JOHNE'S DISEASE

51. How important is Johne's disease to the Canadian cattle industry?

Not very important Very important
Moderately important No opinion

52. How is the importance of Johne's disease changing over time?

Becoming less important Becoming more important
Not really changing No opinion

53. How much per head would you be willing to spend in order to eradicate Johne's disease from your herd? $_____

54. What type of voluntary Johne's control program would you be willing to participate in? *(Check all that apply)*

A program without compensation for Johne's positive cattle and without subsidized testing
A program with compensation for Johne's positive cattle and without subsidized testing
A program without compensation for Johne's positive cattle and with subsidized testing
A program with compensation for Johne's positive cattle and with subsidized testing
I would not participate in any voluntary Johne's control program

55. Would you support a per head check off program that went towards the following? *(Check all that apply)*

Research directed at Johne's disease in beef cattle in Canada
Education programs for beef producers on Johne's control
Subsidies towards reducing the cost of testing for Johne's disease
Compensation towards test positive cattle participating in a Johne's eradication program

56. Prior to this study, how would you describe your knowledge about Johne's disease?

Hadn't heard of it before
Recognized the name but knew very little
Knew the basics of the disease such as the clinical signs
Good knowledge about the disease including clinical signs, prevention methods, tests etc.

57. Where have you received most of your knowledge about Johne's disease?

Your veterinarian	Government sources
Industry magazines	University sources
Fellow Cattlemen	Internet

58. Please feel free to add any additional comments:

Thank you very much for your participation in this study. Please return your completed questionnaire to us using the pre-addressed, postage paid envelope provided. If you wish to discuss any part of this survey, please contact us at the address provided on the front of this questionnaire.

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