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Control of Mycobacterium avium subspecies paratuberculosis on Western Canadian dairy farms: Prevalence, diagnostics and risk factors

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Control of *Mycobacterium avium* subspecies *paratuberculosis* on Western Canadian dairy farms: Prevalence, diagnostics and risk factors

by

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Abstract

Mycobacterium avium subspecies *paratuberculosis* (MAP) causes Johne's disease (JD), a chronic, nontreatable enteritis of ruminants. The pathogen causes substantial losses to the dairy industry and might be associated with Crohn's disease in humans. Eradication of MAP through programs that are solely based on 'test and cull' is ineffective because current tests lack sufficient accuracy for reliable detection of infected cattle. Consequently, current MAP control programs focus on prevention of new infections through implementation of best management practices. The overall aim of this thesis was to evaluate the Alberta Johne's Disease Initiative (AJDI), a management-based MAP control program. Research in this thesis focussed on estimating MAP herd-prevalence, evaluating environmental samples as a diagnostic tool, identifying risk factors for MAP infection, and identifying factors that influenced management improvements. A total of 370 farms participated in the AJDI and were visited annually by their herd veterinarians who conducted risk assessments, collected environmental fecal samples, and discussed management changes. Sixty-eight percent of Alberta dairy farms were MAP-infected and environmental samples collected from lactating cow alleyways and manure lagoons were most frequently culture-positive, suggesting that these samples are important to guarantee high environmental sample accuracy. Furthermore, farms with manure-contaminated cattle and pens, poor feed hygiene, or high purchase rates and low purchase precautions were more likely to be MAP-infected; therefore; improvements in these management areas might be most effective in controlling the spread of MAP. Although most farms subsequently improved management, positive test results and agreed management changes increased the rate of management improvements (which were cost effective). It is noteworthy that the current program overlooks

hygiene of young cattle, because 2% of heifers shed MAP which indicates that management improvements in this area may reduce MAP transmission.

Preface

The work conducted within this thesis comprises a team effort of many people who contributed their expertise. In all chapters, Robert Wolf was involved in design of individual research projects, data collection, data management, data analysis, final reports to the grant agencies, and design of manuscripts. Furthermore, Robert Wolf was involved in writing the grant application for Chapter 7.

The following chapters of this thesis are published in peer-reviewed scientific journals. All authors contributed intellectual property, and written permission for reproduction of the articles in this thesis was obtained from the authors and the publishers.

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List of Symbols, Abbreviations and Nomenclature

Symbol	Definition
AJDI	Alberta Johne's Disease Initiative
β	Beta-coefficient
BCS	Body condition score
CD	Crohn's disease
D	Day
ELISA	Enzyme-linked immunosorbent assay
ES	Environmental samples
M	Month
MAP	Mycobacterium avium subsp. paratuberculosis
MP	Management plan
OR	Odds ratio
PCR	Polymerase chain reaction
RA	Risk assessment
SE	Standard error
Var	Variance
Y	Year

Chapter One: **GENERAL INTRODUCTION**

1.1 The pathogen and Pathogenesis

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of Johne's disease (JD), a chronic progressive enteritis. It is well established that MAP is an intracellular, acid-fast Gram-positive bacterium which can survive for extended periods outside the host (Whittington et al., 2004). Furthermore, MAP can not only infect a wide range of domestic ruminants (Clarke, 1997), but it can also infect various wild ruminants (in Canada), including deer, elk, bison, bighorn sheep, and caribou (Mackintosh and Griffin, 2010). In addition, MAP has been isolated from several non-ruminants, including foxes, badgers, mice, and rabbits (Hutchings et al., 2010).

Susceptibility to MAP infection is believed to be age-dependent, with calves probably requiring a lower infection dose than adults (Clarke, 1997; Sweeney, 2011a). Therefore, most cattle are likely infected before adulthood. One transmission route is *in utero* transmission. It is estimated that 9% of fetuses born from subclinically infected dams and 39% of fetuses born from clinically affected dams are MAP-infected (Whittington and Windsor, 2009). Another route of transmission is through infectious milk and colostrum. In that regard, MAP bacteria were isolated from supramammary lymph nodes and from milk samples obtained carefully to prevent contamination. Therefore, there is evidence that MAP bacteria in milk are not only introduced through fecal contamination during milking, but also through shedding of MAP into the mammary gland (Sweeney et al., 1992). An important transmission route on farms with poor

management, however, is fecal-orally, via contaminated feed, equipment, and pens (Raizman et al., 2004; Sweeney, 1996).

After exposure, MAP is taken up by M-cells and mucosal epithelial cells, and transported through the intestinal wall, where MAP is released and phagocytised by macrophages (Momotani et al., 1988; Ponnusamy et al., 2013). Thereafter, MAP-infected cattle have a prolonged incubation period; the specific duration varies greatly and is likely dependent on a number of factors, including host resistance and pathogen virulence, as well as infection dose (Fecteau and Whitlock, 2010). During incubation, the probability of shedding, as well as production of MAP-specific antibodies occurs only sporadically in the early stages of the disease, but increases over time (Nielsen and Ersbøll, 2006; Weber et al., 2010). The clinical stage of the disease is manifested by non-treatable diarrhoea without loss in appetite, gradual weight loss, and in advanced cases, inter-mandibular edema (Taylor, 1953). However, since production losses start before clinical signs are present (Nielsen et al., 2009), and because of the prolonged incubation, it can be assumed that most infected cattle leave the herd before developing clinical symptoms (Whitlock and Buergelt, 1996).

1.2 Impact of MAP infection

Infection with MAP is common in dairy herds and was detected in most countries where prevalence studies were conducted (Barkema et al., 2010b). Reported apparent herd prevalence estimates for Alberta dairy herds ranged from 20% based on pooled fecal culture to 58% based

on serum ELISA (Scott et al., 2006; Sorenson et al., 2003). Only one study estimated the herd prevalence in Saskatchewan dairy herds; they reported an apparent prevalence of 24% based on serum ELISA (VanLeeuwen et al., 2005). However, most reported studies were conducted with limited numbers of herds and true herd prevalence estimates were not calculated. Therefore, there is a paucity of knowledge regarding true herd prevalence of MAP in Western Canada.

Infected cattle cause economic losses through decreased milk production, lower slaughter weight, and an increased risk of being culled (McKenna et al., 2006). Fecal culture-positive cows have 2 to 12% lower milk production compared to fecal culture-negative cows (Hendrick et al., 2005; Raizman et al., 2009; Wilson et al., 1993). In a Danish study, in the lactation previous to the one in which they became milk ELISA-positive, cows produced approximately 300 kg less milk compared to ELISA-negative herd mates, suggesting that production losses occur in MAP-infected but test-negative cows (Nielsen et al., 2009). Additional direct disease losses in test-positive cattle occur through a 3.0 to 3.2 times higher risk of being culled (Hendrick et al., 2005; Raizman et al., 2009), and through an approximately 59 kg lower live weight at slaughter (Whitlock et al., 1985). Production losses through MAP infection were estimated to be Can$2,472 for a 50-cow herd in Atlantic Canada in 2002 (Chi et al., 2002). Although MAP control is cost effective in other countries, results cannot be generalized, since the design of control programs, prevalence estimates, and economic parameters vary greatly among countries (Groenendaal et al., 2002; Kudahl et al., 2008).

It is noteworthy that MAP is not only a production limiting disease. A century ago, it was noted that JD in cattle has pathology similar to Crohn’s disease (CD) in humans (Dalziel, 1913);

starting with that report, a role for MAP in the etiology of CD has frequently been suggested. Furthermore, several studies reported more frequent isolation of MAP from CD patients than from healthy controls (Feller et al., 2007). Notwithstanding, proof for a causative role of MAP in human CD is still lacking, likely due to the complex multifactorial etiology of CD involving patient genetics, environmental factors, and pathogen characteristics (Barkema et al., 2010a). Should MAP be clearly implicated in the etiology of Crohn's disease, a lower consumption of dairy products is expected, resulting in indirect economic losses for the dairy industry (Groenendaal and Zagmutt, 2008).

1.3 Detection of MAP infected cattle and cattle herds

Tests for detection of MAP infection can be categorized into direct pathogen detection assays and indirect assays that detect an immune response to MAP infection (Tiwari et al., 2006). Among the direct pathogen detection assays, culture of MAP bacteria, direct PCR and acid-fast staining are most common and are usually done on samples of feces, tissue and milk. Among the indirect detection assays, the most common are antibody ELISAs done on samples of serum or milk. Interferon-gamma release assays detect an early cellular immune response to MAP infection, but sample handling is challenging since the assay requires live leukocytes (Jungersen et al., 2002).

The choice of which test to use is dependent on the aim of testing because specific tests measure different target conditions (Nielsen and Toft, 2008). For example, isolation of MAP

from tissues is considered useful to determine MAP infection, because MAP in tissues can be detected before cattle start shedding MAP or produce detectable humoral antibody concentrations (McKenna et al., 2004). Sampling of appropriate tissues, however, can only be done post-mortem or using relatively invasive techniques. In addition, MAP exposure can be measured by indirect detection assays (Mortier et al., 2014a; Mortier et al., 2014c), and infectious animals can be detected with direct detection assays on fecal or milk samples (Mortier et al., 2014b; Sweeney et al., 1992). All direct detection assays have the advantage of high specificity, which makes them ideal for confirming MAP infection in cattle with clinical symptoms, or in cattle testing positive with other tests (Whitlock et al., 2000).

Across all applied tests, diagnosis of MAP in subclinically infected cattle is compromised through low bacterial concentrations, and intermittent shedding and antibody production during incubation (Tiwari et al., 2006). Therefore, although none of the available tests can reliably detect all infected cattle, accuracy of all applied tests increases in later disease stages. Furthermore, test accuracies are only known with high uncertainty, because designs, study populations, and reference standards varied greatly among studies estimating the accuracy of tests for MAP detection (Nielsen and Toft, 2008).

Some testing conditions require detection of MAP infected herds instead of MAP infected cattle (Collins, 2011). This is particularly important for herds in herd status programs that want to provide evidence for a low risk for MAP infection (Mason, 2012). In addition to the use of individual-animal testing (with extrapolation to the herd level), environmental fecal samples, MAP detection in bulk milk, and antibody ELISAs on bulk milk are frequently used to

determine MAP infection status of a herd (Pillai and Jayarao, 2002; Tavornpanich et al., 2008; Van Weering et al., 2007). Environmental fecal samples are composite manure samples collected from adult cow pens and manure storage areas. A commonly used protocol requires collection of 6 environmental samples, each composed of at least 4 sub-samples (Berghaus et al., 2006). Since environmental samples are generally processed using MAP culture (which requires viable MAP bacteria), environmental exposure, especially freeze-thaw cycles, reduces the rate of recovery of live MAP bacteria (Khare et al., 2008; Raizman et al., 2011). However, whether this impacts the accuracy of the test method is unknown. Detection of MAP bacteria or MAP-specific antibodies in bulk milk is an interesting method for MAP detection, since samples can be quickly and easily collected by milk truck drivers. However, PCR on bulk milk as well as antibody ELISAs on bulk milk currently lack accuracy, because MAP bacteria and MAP specific antibodies are highly diluted in bulk milk and MAP shedding is likely lower in milk than in feces (Pillai and Jayarao, 2002; Van Weering et al., 2007).

1.4 Control of MAP on dairy farms

Direct and indirect losses for the dairy industry, as well as the public health concern related to MAP have motivated and are still motivating the dairy industry to control the spread of MAP. Although control strategies may differ in design, 3 common measures can be identified (Franklyn, 2011): 1) prevent exposure of susceptible animals to the infectious agent; 2) prevent

entry of infected animals into herds; and if testing of individual cattle is done 3) eliminate MAP-infected cattle from the herd.

To prevent exposure of susceptible animals to the agent, farm-specific risk factors for MAP transmission need to be identified. The MAP-specific risk assessments (RA) which are available online, are frequently used for this purpose (USDA, 2003). The information collected in the RA can be used to design a herd-specific management plan (MP) in which the identified risk factors are addressed through best-management practices. Implementation of these management practices aims to reduce the risk for MAP introduction and transmission on the farm.

Questions in RAs focus on risk factors with regards to both direct and indirect contact of cows and calves. This reflects current knowledge on pathogenesis of MAP, with calves being most susceptible and the probability of shedding, as well as the concentration of shed MAP bacteria increasing throughout the incubation period (Clarke, 1997; Fecteau and Whitlock, 2010; Sweeney, 1996). However, in recent studies, 12-month-old cattle were infected with MAP and experimentally exposed calves shed as early as 2 months after challenge (Mortier et al., 2014b). Should infection of adult cattle and calf-to-calf transmission frequently occur in infected herds, current RAs may not identify all risk factors for MAP within-herd transmission, and MAP control programs might be ineffective. Furthermore, not all dairy producers implement suggested management practices (Wells et al., 2008), and implementation of new management practices might be dependent on suggestions from the herd veterinarian, and on previous MAP test results

(Sorge et al., 2011). Knowledge on factors serving as drivers for management changes helps to predict which farms will improve their management and to what extent.

Introducing MAP by buying cattle is particularly challenging to control, because most farms purchase animals to enrich the gene pool in a herd and to have sufficient numbers of replacement heifers (Franklyn, 2011; Weber et al., 2006). Suggesting producers keep their herds absolutely closed is likely unrealistic. Regardless, producers need to be educated on the risk of cattle purchase. Furthermore, they should insist on having complete and reliable information on disease history of the seller farm, and they should keep the number of purchased cattle as well as the number of source farms to a minimum, thereby reducing the risk for MAP introduction.

Simulation studies revealed that testing and culling as the only tool does not reduce MAP within-herd prevalence, mainly because of the low accuracy of current tests for detection of subclinically infected cattle, which are the majority of infected cattle (Groenendaal et al., 2002; Kudahl et al., 2007). Therefore, testing individual cattle and culling test-positives is not always part of MAP control (McKenna et al., 2006). However, so-called super-shedders are responsible for a high amount of MAP contamination on a farm (Fecteau and Whitlock, 2010). Because these animals are in advanced stages of JD, they can be reliably detected with any of the current tests (Nielsen and Toft, 2008). Removing those super shedders is the key argument of those who promote individual testing and culling to control within-herd transmission of MAP (Collins et al., 2010; Lu et al., 2008).

Allocation of resources among: 1) prevention of animal exposure, 2) risk reduction of MAP introduction into a herd, and 3) elimination of MAP infected cattle also depends on the

underlying goals of the control effort. In known infected herds, preventing exposure of susceptible animals to the agent, optionally combined with testing and culling of test-positive cattle, has priority. In contrast, for a herd with low risk of MAP infection, reducing the risk for MAP introduction, combined with monitoring herd infection status, is the major focus. These principles are valid for the design of MAP control efforts on individual dairy farms, and also for provincial and federal MAP control programs.

1.5 The Alberta Johne's Disease Initiative

Current Canadian MAP control programs were launched within the context of the Canadian Johne's Disease Initiative (CJDI) which was formed in July 2009 (CAHC, 2009). The CJDI focuses on: 1) education of producers on MAP and its control and creation of awareness among producers; 2) facilitating development of provincial MAP control programs and providing a platform for communication among provinces; and 3) supporting development of research projects with regards to MAP and its control.

The Alberta Johne's Disease Initiative (AJDI) was launched within the CJDI in October 2010. It is a producer-driven MAP control program coordinated by Alberta Milk and the University of Calgary, Faculty of Veterinary Medicine. On-farm work is conducted by specially trained herd veterinarians that visit voluntarily participating farms on an annual basis to collect 6 environmental fecal samples, conduct a MAP-specific RA, and agree with the farmer on a maximum of 3 changes in management that aim to reduce the risk for MAP introduction and

within-herd transmission. Environmental samples and RAs are sent to University of Calgary where samples are cultured and RAs are digitized and stored. Project funding which is shared by the Alberta Livestock and Meat Agency (ALMA) and by Alberta Milk covers administration and laboratory costs and supports on-farm work with Can$200 for each herd visit. In addition, all collected data are made available for research.

1.6 Outline of the thesis

The overall aim of this thesis was to evaluate a management-based MAP control program, the AJDI. The prevalence of MAP on Western Canadian dairy farms is only known with low certainty, because previous studies mainly used ELISAs and only reported apparent prevalence estimates (Scott et al., 2006; Sorenson et al., 2003; VanLeeuwen et al., 2005). Regardless, knowledge on baseline prevalence is essential for monitoring changes in prevalence over the course of a control program. Chapter 2 describes baseline herd prevalence of MAP infection in Alberta and Saskatchewan dairy herds, based on environmental sampling. In addition, sensitivity of an environmental sample set was estimated and apparent prevalence and accuracy were combined in a Bayesian model to estimate true herd prevalence.

Environmental samples are regarded as a relatively accurate tool for detection of MAP-infected dairy farms (Lavers et al., 2013; Lombard et al., 2006). However, knowledge on cheap but accurate sampling protocols is scarce and environmental sampling could be compromised by very low temperatures and frequent freeze-thaw cycles during winter. Chapter 3 demonstrates

the impact of sampling location, herd size, and season on the accuracy of environmental samples. Furthermore, the impact of season and herd size on collection of samples from all requested locations was estimated.

Because no individual testing of cattle is done within the AJDI, implementation of best management practices is the only tool to control MAP. Consequently, knowledge on effectiveness of management practices is essential. Risk factors for MAP infection have mainly been estimated for European and US herds and generalizability may be limited, because management and herd structure differ substantially among populations and locations (Doré et al., 2012; Elliott et al., 2014). Chapter 4 describes the association between environmental sample results and known and suspected risk factors for MAP introduction and transmission on dairy farms, which helps to focus management suggestions onto management practices with evidence for effectiveness in MAP control, and additionally generates hypotheses for future studies to determine causation.

Herds participating in a MAP control program generally improve their management over time (Raizman et al., 2006; Wells et al., 2008). However, management decisions are complex and not all producers implement best management practices. Chapter 5 identifies predictors that impact implementation of new best management practices.

Economics of various MAP control strategies have been reported for Europe and the United States (Dorshorst et al., 2006; Groenendaal et al., 2002). It is unknown whether participation in the AJDI is cost effective and results of previous studies cannot be generalized

since economic parameters differ greatly among countries. Therefore, Chapter 6 describes the net benefit for an Alberta dairy producer by their participation in the AJDI.

Chapter 7 focuses on a potential factor that might have reduced the effectiveness of current MAP control programs that focus on the interruption of MAP transmission mainly between cows and calves. This study estimated the prevalence of MAP infectious young stock on MAP-infected dairy farms and was motivated by high proportions of fecal culture positive calves in a recent infection experiment conducted at the University of Calgary (Mortier et al., 2014b).

In Chapter 8, results of all studies presented in this thesis are discussed, with a special focus on implementation of findings and proposed directions for future research.

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Chapter Two: **HIGH HERD-LEVEL PREVALENCE OF *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* IN WESTERN CANADIAN DAIRY FARMS, BASED ON ENVIRONMENTAL SAMPLING**

2.1 Abstract

Mycobacterium avium subspecies *paratuberculosis* (MAP) causes chronic progressive enteritis in ruminants. The pathogen is present in most countries with modern dairy production, causing substantial economic losses for the industry. The objectives of this study were to estimate dairy herd prevalence of MAP in the Western Canadian provinces of Alberta and Saskatchewan, and to determine whether herd size and housing system (tie-stall versus free-stall or loose housing) affected the risk of a herd testing positive for MAP. Six environmental samples were collected on 360 Alberta farms (60% of registered producers) and on 166 Saskatchewan dairy farms (99%). In total, 47% of the sampled farms in Alberta and 53% of the sampled farms in Saskatchewan had at least 1 environmental sample that was MAP culture-positive and were therefore defined as infected. Sensitivity of environmental sampling was estimated using 3 subsequent annual tests performed on 82 farms. Since laboratory protocols were continuously improved throughout the project, the sensitivity increased over time. Therefore, a mean of the sensitivity estimates weighted on sampling year was constructed; this resulted in sensitivities of 68 and 69% for Alberta and Saskatchewan, respectively. Implementing those estimates in an Approximate Bayesian Computation model resulted in a true herd prevalence of 68% (95% probability interval (PI): 60-80%) for Alberta and 76% (95% PI: 70-85%) for Saskatchewan. Herds with >200 cows had 3.54 times higher odds of being environmental sample-positive and had more positive samples than herds with <50 cows (neither province nor housing system

affected those results). In conclusion, the majority of Alberta and Saskatchewan dairy farms were infected with MAP and larger herds were more often MAP-positive than smaller herds.

2.2 Introduction

Johne's disease (JD) is a chronic progressive enteritis, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), affecting most ruminant species (Fecteau and Whitlock, 2010). The disease is present in most countries with a modern dairy industry and causes substantial losses through decreases in milk production and slaughter value, combined with an increased risk of being culled (Barkema et al., 2010b; McKenna et al., 2006). For Atlantic Canada, the actual financial losses were estimated to be $2,472 for a 50-cow herd with 7% MAP-infected cattle (Chi et al., 2002). A second concern associated with MAP is its potential zoonotic nature. Although controversial, there may be a link between MAP infection in cattle and Crohn's disease in humans (Barkema et al., 2010a). Should this link ever be proven, lost consumer trust as well as a decreased milk price are expected (Groenendaal and Zagmutt, 2008).

The herd prevalence of MAP on Alberta dairy farms is uncertain, as estimates range from 20 to 59% (Scott et al., 2006; Sorenson et al., 2003). Only 1 study estimated a relatively low herd prevalence of 24% for Saskatchewan (VanLeeuwen et al., 2005). It is noteworthy that most estimates were based on testing a subset of cows in a herd using serum ELISA, which has low sensitivity and low specificity (Tiwari et al., 2006). To avoid overestimation of herd prevalence due to poor specificity, the authors defined only herds with at least 2 cows positive on serum

ELISA as MAP-infected. However, this approach only adjusts for a lack in specificity, but not for a lack in sensitivity; therefore, reported herd prevalence estimates are likely an underestimation of true prevalence (Barkema et al., 2010b).

Environmental sampling is another potential testing strategy used to detect herds with cows infected with MAP. It is currently used in prevention and control programs in the United States and in some parts of Canada, including Alberta (Alberta Johne's Disease Initiative) and Atlantic Canada (Mason, 2012; Whitlock, 2010; Wiebe, 2011; Wolf et al., 2014c). Trained sampling personnel collect manure samples from the cows' environment and manure storage areas. The advantages are that it does not require sample collection from individual animals, and it relies on the nearly perfect specificity of bacterial culture, which simplifies true prevalence estimation (Tiwari et al., 2006). The testing method is reliable and the sensitivity, compared to fecal testing of individual cows, is approximately 70% (Aly et al., 2009; Lombard et al., 2006; Raizman et al., 2004).

Herd size is positively associated with risk of MAP infection, with almost all large dairy herds in the USA being reported as infected (Pillars et al., 2009; USDA, 2008; Wells and Wagner, 2000). Possible reasons for this association are differences in management practices such as an increased use of pooled colostrum or using group calving pens in large herds, both of which increase the risk of within-herd transmission of MAP (Pithua et al., 2013; Stabel, 2008), but also more purchased animals in large herds, which increases the risk of MAP introduction (Wells and Wagner, 2000). However, herd size distributions and management practices are area-specific which limits the generalizability of results. Knowing the impact of herd characteristics

such as herd size and housing system on the risk of MAP infection, in combination with local prevalence estimates, will provide individual dairy farmers with valuable information regarding the actual risk of MAP infection on their farm. This information can be used to estimate the importance and cost effectiveness of management practices implemented to reduce the within-herd transmission of MAP. Knowing the herd prevalence in a population can also be used to estimate the risk of MAP introduction into an uninfected herd through the purchase of animals. The objectives of the current study were to estimate herd prevalence of MAP infection in Alberta and Saskatchewan dairy herds, based on environmental sampling, and to determine whether housing type or current herd size influenced the risk of MAP infection for a herd.

2.3 Materials and Methods

2.3.1 Herds

At the beginning of the study (December 2010), the Alberta dairy industry consisted of 597 dairy farms. During the 3 y of the study, 50 farms ceased their dairy operations and 24 new farms started producing milk. The Alberta study population consisted of herds voluntarily participating in the Alberta Johne's Disease Initiative (AJDI). Herds were visited annually by their herd veterinarian to collect environmental samples, followed by a risk assessment and suggested management changes (Wolf et al., 2014c). Farms could join or leave the project throughout the study. Furthermore, 166 of 167 Saskatchewan dairy farms were visited from August 2012 to

November 2013 by either their herd veterinarian (16 farms) or by a single employee of the producer organization Saskmilk who visited 150 farms (Saskmilk, 2012).

Herd size and lactating cow housing (tie-stall versus free-stall or loose housing) of Alberta participants were assessed as part of the AJDI through questions in the annual risk assessment. For farms in Saskatchewan, information on housing was noted by the sample collectors and herd size (in increments of 50 cows) was estimated based on milk quota.

2.3.2 Sample collection

Sample collectors received standardized training through AJDI workshops or one-on-one training sessions. Collectors received sampling kits containing: 6 zip lock bags for mixing subsamples, 6 sample containers (90 ml), and an instruction sheet. Duplicate samples were collected from 3 areas: 1) manure storage (e.g. lagoons, piles, or pits), 2) manure concentration (e.g. alleys and the end of scraper lines), and 3) cow concentration, including sick cow pens (Berghaus et al., 2006). If manure was not accessible in manure storage areas, or if <2 cows were present in the sick cow pens, collectors were instructed to collect additional samples from the remaining areas. Each sample consisted of at least 4 subsamples which were thoroughly mixed inside the zip lock bags, and the fecal mix subsequently transferred into containers. Samples were collected between Monday and Wednesday and shipped to the University of Calgary using Express Mail. When samples were collected after Wednesday, collectors were instructed to keep samples refrigerated and ship them the following Monday.

2.3.3 Laboratory analysis

Upon arrival at the University of Calgary, samples were stored at 4^{o}C for a maximum of 7 d. Sample processing started every Monday using a standardized 3-d decontamination protocol, followed by 48 d of culture using a TREK ESP culture protocol (McKenna et al., 2005). All culture products were analysed with conventional IS900 PCR, with previously described primers (Vary et al., 1990). Over the 3 y of the project, the purchase of new equipment such as a gel reading device and a new thermo cycler likely increased the sensitivity of the laboratory procedures. The laboratory protocol was also modified with the increased use of F57 qPCR for confirmation of inconclusive IS900 results (mainly double bands, faint bands, or bands very close to the target area) from January 2012 onwards (Slana et al., 2008). Before that, laboratory personnel based their decision solely on IS900 results. Thereafter, the case definition was either positive on IS900 conventional PCR or positive on F57 qPCR (performed following inconclusive IS900 outcomes).

2.3.4 Statistical analyses

Test sensitivity and prevalence were estimated using the Excel add in @RISK-6 (Palisade Corp., Ithaca, NY, USA), whereas data management and analysis of predictors for positive environmental sample results were done with STATA 11 (STATA Corp, College Station, TX, USA).

Test accuracy. Environmental sample results of the farms participating in the project for 3 y were used to estimate the sensitivity of environmental sampling (Figure 2-1). It was assumed

that culture of 6 environmental samples per farm would result in 1 false-positive farm out of 100 uninfected farms (specificity: 99%). For sensitivity estimation of environmental testing, the 3-year test result was used as a gold standard. The assumption was made that the herd infection status did not change throughout the duration of the project, which is justified by the endemic nature of the disease, and that the scientific literature apparently does not include any reports of successful eradication of the pathogen from a farm without complete depopulation. Notwithstanding, introduction of MAP infection could have occurred during the 3 y. Herds that had at least 1 positive result in 3 y were considered positive. Beta distributions were constructed with the following parameterization: alpha = herds positive during the specific year of testing; and beta = herds negative during the specific year of testing, but positive during at least 1 of the other years. As it can be assumed that not all infected herds were detected in 3 test events, the default analysis assumed that 20% of the 3 times negative herds would be infected with MAP. The rationale was derived from 2 studies estimating the sensitivity of repeated environmental sampling compared to pooled fecal culture (Khol et al., 2009; Lavers et al., 2013). Khol et al. (2009) collected samples over a 6-mo interval on 26 farms, whereas Lavers et al. (2013) used a 3-mo interval for 32 herds. Means of the 3 sensitivity estimates weighted by the number of herds visited in 2011, 2012 and 2013 were constructed for both provinces.

Two additional sensitivity estimates were calculated to account for uncertainty around the parameter. The first sensitivity estimate (high sensitivity) assumed that none of the 3-times negative herds would be infected with MAP (instead of 20% infected herds among the 3-times negative herds in the default analysis). The second sensitivity estimate (low sensitivity) assumed

that 50% of the 3-times negative herds would be infected. The rationale for this sensitivity analysis was based on a 5-y longitudinal study using pooled fecal culture that concluded only 50% of infected herds could be detected with 3 test events (Kalis et al., 2004).

Prevalence estimation. The apparent prevalence was calculated, including the most recent sample set collected on each participating dairy farm. A sample set was deemed positive if at least 1 environmental sample was positive. Apparent prevalence and accuracy estimates were used to calculate the true MAP herd prevalence in the 2 provinces. The true herd prevalence represented the proportion of herds with at least 1 infected cow and was estimated separately for each of the 3 sensitivity estimates (default, high sensitivity, and low sensitivity). Analysis was performed using Approximate Bayesian Computation (ABC). Compared to traditional Bayesian analysis methods, ABC estimates posterior distributions without specifying a likelihood function and therefore can solve more complex analytical tasks (Turner and Van Zandt, 2012). The most important feature in the present model was incorporation of finite population sizes, which enables prevalence estimation for sample sizes which are close to the population sizes. The advantage of the method is that it results in lower posterior uncertainty (smaller probability intervals) compared to Bayesian analysis using binomial models (Branscum et al., 2004; Su et al., 2004). In Equation 1 (Eq1), the ABC rejection model estimated the number of positive farms using a uniform distribution between 0 and the population size (M). If this number equalled the number of positive farms estimated from a hyper-geometric distribution using the sample size (n), number of positive farms (x) and M, the iteration was accepted; otherwise, it was rejected

(Eq2, Eq3). The last step incorporated test accuracy and translated the outcome into a proportion (Appendix A).

Herd size, housing, and province. The association between the dichotomous environmental sample results (0 positive samples versus ≥ 1 positives) and herd size (<50, 50-100, 100-150, 150-200, >200), housing type (tie-stall yes/no), province (Alberta, Saskatchewan), and sampling year (2011, 2012, and 2013) was analysed using Chi-square tests on contingency tables. Since the majority of tie-stall herds had <100 cows, the association between environmental sampling results and housing would be biased by herd size. Therefore, to determine whether the herd MAP prevalence was different among housing types, a separate analysis was performed including only herds <100 cows. Thereafter, associations between categorized environmental sample results (0 positive samples, 1-3 positives, 4-6 positives) and all predictors were analysed using ordinal logistic regression. Parameters were included using manual forward selection. Criteria for inclusion in the final model were $P < 0.05$ or evidence for confounding (>20% change of any other coefficient if tested coefficient was removed). The validity of the proportional odds model was tested using a Brant test. In case of validity, the same set of predictors can be used to describe an association for 2 different cut points in the outcome. For the present study, this would be the predictor specific odds ratio for a herd having no positive environmental sample versus ≥ 1 positive environmental sample, and the odds ratio for a herd having ≤ 3 positive environmental samples versus > 3 positive environmental samples (Brant, 1990).

2.4 Results

2.4.1 Herd characteristics

Alberta. 360 (60%) Alberta dairy farms participated in the AJDI, including 122 located south of the city of Calgary, 194 between Calgary and Edmonton, and 44 north of the city of Edmonton (participation rates of 74, 55 and 59%, respectively). Overall, 114 (31%) farms had < 100 cows and 21 (6%) housed their cows in tie-stalls. Average herd size was 140 cows and 82% participated in an organized milk recording system (CanWest DHI, Guelph, ON, Canada). Only 17 farms (5%) submitted samples in 2011, 123 farms (34%) submitted their most recent sample set in 2012 and 220 (61%) in 2013 (Table 2-1).

Saskatchewan. There were 166 (99%) Saskatchewan dairy farms sampled, of which 66 (40%) were visited in 2012 and 100 (60%) in 2013. Of these farms, 78 (47%) had <100 cows, 103 (62%) participated in CanWest DHI milk recording, and 24 (14%) housed their cows in tie-stalls (Table 2-1).

2.4.2 Test accuracy

On 82 Alberta farms, environmental samples were collected in 3 consecutive years. Of these farms, 59 (72%) had at least 1 positive environmental sample result during at least 1 sampling event. Furthermore, 19 (32%) of those 59 farms were positive in Year 1, 39 (66%) positive in Year 2 and 48 (80%) positive in Year 3 (Table 2-2). Assuming 20% false-negatives in the 23 repeatedly test-negative farms, the sensitivity of environmental sampling weighted by the

number of farms visited in each year was estimated at 68% (95% probability interval (PI): 60-76%) for Alberta. For Saskatchewan, where a higher proportion of farms was visited in 2013, sensitivity was 69% (95% PI: 60-77%). Assuming 100% sensitivity, 3 y of testing resulted in a sensitivity of 73 and 74% (high sensitivity), whereas assuming 50% of the 3 times negative farms as infected resulted in a sensitivity of 62 and 63% (low sensitivity), respectively, for Alberta and Saskatchewan (Table 2-3).

2.4.3 Prevalence estimation

In total, 47% of the farms in Alberta and 53% of the farms in Saskatchewan had at least 1 environmental sample that was MAP culture-positive and were therefore defined as infected (Table 2-1). The default analysis resulted in a herd prevalence of 68% (95% PI: 60-80%) for Alberta and 76% (95% PI: 70-85%) for Saskatchewan (Table 2-3). The high sensitivity and the low sensitivity analysis resulted in a change of the prevalence estimates of -8 and +8% respectively, compared to the default analysis.

2.4.4 Herd size, housing, and province

Whereas 74 (38%) of 192 herds with <100 cows tested positive, 61 (73%) of 83 herds with >200 cows tested positive, resulting in a significant impact of larger herds ($P < 0.001$, Table 2-1 & 2-4). 14 (31%) of 45 tie-stall herds, and 243 (51%) of 481 free-stall or loose housing herds tested positive ($P= 0.08$). In herds <100 cows, 12 (35%) of 34 tie-stall herds, and 62 (39%) of 158 free-stall or loose housing herds tested positive. The 2 proportions were not significantly

different ($P = 0.67$). The proportion of positive herds increased from 2011, 2012, to 2013 (12, 44, and 54% respectively; $P = 0.001$). There was no evidence for an association between province and testing MAP-positive ($P = 0.20$).

In the ordinal logistic regression model, herd size ($P < 0.001$) and year of sample collection ($P = 0.01$) were associated with the number of positive environmental samples collected on a farm (Table 2-4). Herds with >200 cows had 3.54 times higher odds to have at least 1 positive environmental sample and had a higher number of positive samples than herds <50 cows. Herds tested in 2012/2013 were more likely to test positive and had more positive environmental samples than herds tested in 2011. Province and housing in tie-stalls were not included in the final model. The Brant test for the final model was not significant ($P = 0.27$). Therefore, there was no evidence for a violation of the proportional odds assumption.

2.5 Discussion

In the current study, 68 and 76% of Alberta and Saskatchewan dairy farms, respectively, were estimated to be infected with MAP, whereas large herds were more often MAP culture-positive than smaller herds. To the authors' knowledge, this MAP prevalence study had by far the highest participation rate of any similar study, with > 60% of the population participating in Alberta and 99% participating in Saskatchewan.

Herd-level MAP prevalence estimates were not different between provinces, but seemed to be higher than in other reports (Scott et al., 2006; Sorenson et al., 2003; VanLeeuwen et al.,

2005). In 1 study, the proportion of herds with at least 1 ELISA-positive cow was very close to the prevalence reported in this study (Scott et al., 2006). Therefore, we suspect that using a cut-off of 2 ELISA-positive cows most likely resulted in an underestimation of true prevalence. The substantially lower prevalence estimates by 2 other studies cannot be explained; we suspect that they either were due to a surprisingly large increase in prevalence over the last 10 y, or more likely, an underestimation of the herd prevalence in these studies through systematic error (Sorenson et al., 2003; VanLeeuwen et al., 2005). This new knowledge on prevalence of MAP in Western Canada has important implications for MAP control. A recent study estimated the economic benefit of participation in the AJDI and concluded that participation was not only cost effective for the average producer, but also for herds with low within-herd prevalence (Wolf et al., 2014c). Because almost three quarters of the herds are infected, most herds can expect a positive net benefit through participation in a management-based control program like the AJDI.

Estimation of the true herd prevalence using environmental sampling was recently done in the US and in Southern Chile (Kruze et al., 2013; Lombard et al., 2013). The estimated true herd prevalence of MAP seemed to be higher in the United States and lower in Southern Chile than in Western Canada.

Especially in the case of MAP testing, with limited knowledge on the accuracy of the tests used, true prevalence estimates depend on the assumed accuracy, even more so if only a single test is used (Branscum et al., 2004). Because culture protocols differ among institutions, local information (if available) was emphasized to estimate the accuracy for the protocol using repeated testing on 82 herds. Three years of repeated testing was used as a first step to estimate

the sensitivity of an environmental sampling event. Subsequently, it was assumed that 20% of the 3-times negative farms would be MAP-infected (Khol et al., 2009; Lavers et al., 2013). The resulting sensitivity estimates of 68 and 69% differed slightly between the 2 provinces, due to different sampling weights. The estimates are the basis for the default analysis, which can be interpreted as the most likely true herd-level MAP prevalence in these 2 provinces. It should be noted that sensitivity estimates from this report were very similar to estimates reported in other test evaluation studies (Khol et al., 2009; Lavers et al., 2013; Lombard et al., 2006; Raizman et al., 2004). Hence, the use of sensitivity estimates from the literature would have resulted in similar prevalence outcomes. The first sensitivity analysis (high sensitivity) assumed no false-negatives among the 3-times negative farms; therefore, the estimates can be interpreted as the lowest possible prevalence in the 2 provinces. The second sensitivity analysis (low sensitivity) described the worst-case scenario, assuming 50% of the 3 times negative herds as infected.

The specificity of 6 environmental samples was assumed to be 99%. Other studies used 99.9 or even 100% as specificity priors (Kruze et al., 2013; Lombard et al., 2013). The first reason for the more conservative estimate was the fact that not 1 but 6 samples from a negative herd must be correctly deemed negative to assign the correct herd infection status. Herd specificity decreases with an increasing number of samples (Dohoo et al., 2003b). The second reason was the theoretical possibility that an environmental sample could be contaminated with MAP bacteria by a source other than dairy cattle. Since MAP is an intracellular pathogen that does not reproduce in the environment, possible sources for contamination would be beef cattle and other domestic ruminants such as goats, sheep or farmed elk. The probability for that is

relatively low, since most farms only have dairy cattle (personal communication Alberta Milk and Saskmilk), dairy cattle are generally housed separately from beef cattle and other ruminants, and their manure is stored separately. However, it is still a possibility that a very small number of samples was contaminated through other sources, which also justifies the use of a more conservative specificity prior. Although there is uncertainty in the specificity of environmental sampling, it should be kept in mind that variation of this parameter only results in minor changes in true prevalence estimates, since most herds are infected with MAP and the magnitude of uncertainty around specificity estimates is rather small (a conservative range would be between 96 and 99.9%) (Lombard et al., 2013).

Analysing results of the 82 herds tested 3 times revealed that the proportion of positive farms increased over time. Assuming that MAP is an endemic disease with slow prevalence changes, the number of farms getting newly infected within 3 y should be rather low, which provided evidence for a change in accuracy rather than a change in prevalence. As the laboratory at the University of Calgary was certified to perform MAP cultures throughout the project and the USDA certification does not allow any false-positives, this change in accuracy was most likely due to increased sensitivity, which was attributed to ongoing protocol improvements and purchase of new equipment in the laboratory. Before 2012, laboratory personnel based the case definition solely on conventional IS900 PCR. In the absence of exact alignment of PCR bands with the target amplicon size, samples were conservatively called negative. Since the F57 sequence is highly specific for MAP, F57 qPCR was increasingly used to classify these inconclusive samples (which resulted in a higher number of positives). Further improvements

were acquisition of new PCR machines, a new gel-reading device, and changes in PCR gel composition. These changes likely enabled detection of lower bacterial concentrations. As these protocol changes were implemented simultaneously, a single weighted average was created for sensitivity rather than treating changes in protocol as different tests. These protocol improvements resulted in a sensitivity increase over time from approximately 30% in the first year, to approximately 77% in the third year. In a study done in the USA, reported sensitivity estimates were close to the first year estimates (Smith et al., 2011); therefore, we suspect that there were similar accuracy problems in other laboratories.

Large herds were more likely to be MAP-positive, consistent with other studies (Pillars et al., 2009; Wells and Wagner, 2000). They were also more likely to have > 3 positive environmental samples, which gives evidence for higher within-herd prevalence in large herds, since the number of positive environmental samples is associated with within-herd prevalence (Lavers et al., 2013). Why herd size is such an important risk factor for infection and higher within-herd prevalence is unknown. Perhaps differences in management contribute, as large farms are more likely to have more cows calving concurrently; thereby they would be more likely to have more than one cow in the calving pen and to pool colostrum from several cows. Conversely, those farms would also be more likely to control these risk factors, since control expenses (e.g. colostrum pasteurizers) can be shared among more cows. A second explanation might be that larger herds probably purchase more replacement animals (often from multiple sources), which increases the risk of pathogen introduction. The National Animal Health Monitoring System (NAHMS) Dairy 2002 study suggests that large herds are managed

differently compared to small herds (USDA, 2002), but further research is needed to identify risk factors for MAP infection in large herds, especially in Canada where management and the supply management system likely result in different management practices than the US. Housing system did not significantly predict the outcome if herd size was included in the final model, because almost all tie-stall farms had < 100 cows. Therefore, their lower tendency of having culture-positive environmental samples can be explained by smaller herd sizes.

Although the participation rate in the AJDI was relatively high (60%), not all Alberta dairy farms participated. Some farmers might be more likely to participate because they know that their herd is infected in order to receive advice for control strategies from their veterinarian. In contrast, other farmers might be less likely to participate because they do not want anybody to find out that their herds are MAP-infected. The University of Calgary conducted a survey study among non-participants; preliminary results indicated that farmers have different reasons for non-participation which are positively as well as negatively associated with the disease (Ritter et al., in preparation). Also, average herd size, as well as the proportion of farms participating in the milk recording program (CanWest DHI), were very similar between participants and the total Alberta dairy farm population (Dairy farming in Canada, 2014). Nevertheless, despite the large sample size, a small impact of selection bias cannot be excluded. Dependent on the direction of this selection bias, the herd prevalence in Alberta may be slightly over- or underestimated.

Environmental samples were collected by herd veterinarians and employees of the producer organization Saskmilk. Inter-observer variability could introduce bias. However, the impact of this bias was considered to be low, since all collectors received standardized training

(workshops or personal information sessions) and since sample collection followed a strict protocol outlined in an instruction sheet which accompanied every sampling kit. Results of a study in the US supported the low impact of inter-observer variability by estimating a high repeatability of environmental sampling between collectors (Aly et al., 2009).

Repeated testing of herds over 3 y was used to estimate the sensitivity of environmental sampling. The assumption was made that these herds would not change their infection status within the duration of the project. It is not likely that any infected herds became uninfected within those 3 y; no report of eradication of MAP from a herd without complete depopulation was found in the literature. A larger concern is that uninfected herds may have become infected during that timeframe. Unfortunately, no information on the number of newly MAP-infected herds in a population over a certain timeframe was available. However, the risk of new infections among these herds was likely lower than in average herds because of their participation in the AJDI; they likely used stricter biosecurity measures and caution during animal purchase, which thereby reduced the risk of MAP introduction.

Important potential study limitations included that only environmental samples were collected and no individual testing was conducted could be regarded as important limitations of this study. There is no doubt that repeated testing of all animals in participating herds would have resulted in higher accuracy. However, it was not feasible for a study which included > 500 herds. An alternative approach with similar costs would have been collection of individual fecal samples from 30 cows, with subsequent processing in pools of 5 cows. Despite an increased workload for cow selection and sample collection, the laboratory costs would have been equal.

However, a US simulation study estimated a lower sensitivity for pooled fecal culture of 30 cows compared to 6 environmental samples, likely due to the low number of tested cows within a herd (Tavornpanich et al., 2008). Nevertheless, that study reported a high herd sensitivity for ELISA testing. The lower processing costs, and in case of milk ELISA, the lower costs for sample collection would have allowed us to test more cows within each herd. Regardless, we decided to use environmental samples, since ELISAs for detection of a MAP antibody response likely have lower specificity than environmental samples which complicates true prevalence estimation (Tiwari et al., 2006).

A limitation of the Saskatchewan dataset was that exact herd sizes were not available. Consequently, herd size was approximated using the milk quota. Since none of the herds sell substantial amounts of milk directly to consumers, the potential for bias was low (SaskMilk, personnel communication). It is noteworthy that the ordinal logistic regression used the farm-specific test result as the outcome and not the true infection status of the herd. Hence, some low prevalence farms were misclassified as uninfected. If these misclassifications were herd-size dependent, differential misclassification would have resulted in biased estimates. There is the possibility that environmental sampling is more accurate in large herds than in small herds. In herds with the same prevalence, large herds will, because of the number of cows present, have higher odds that at least 1 cow sheds (compared to small herds), which could result in a higher sensitivity of environmental sampling in large herds. Conversely, the present protocol collected only 6 samples, with 4 subsamples within each herd. It might also be more likely on small farms, that the manure of an infected cow is collected with one of the 24 subsamples; that would reduce

sensitivity in large herds. Considering these arguments and the missing evidence in the scientific literature, it cannot be concluded that the sensitivity of environmental samples was dependent on the herd size. Regardless, further research is needed to address these issues.

2.6 Conclusions

It was estimated that the majority of dairy farms in Alberta and Saskatchewan were infected with MAP. Furthermore, large herds were more likely to test positive than small herds.

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Table 2-1: Associations between herd characteristics and the most recent environmental sample results estimated on 360 Alberta and 166 Saskatchewan dairy farms.

	Alberta				Saskatchewan			
	0 pos.[1] n (%)[2]	1-3 pos.[1] n (%)[2]	4-6 pos.[1] n (%)[2]	Total N	0 pos.[1] n (%)[2]	1-3 pos.[1] n (%)[2]	4-6 pos.[1] n (%)[2]	Total n
Total	191 (53)	92 (26)	77 (21)	360	78 (47)	50 (30)	38 (23)	166
Region								
South of Calgary	72 (59)	35 (29)	15 (12)	122	Not applicable			
Calgary - Edmonton	94 (48)	50 (26)	50 (26)	194				
North of Edmonton	25 (57)	7 (16)	12 (27)	44				
Number of cows								
<50	6 (67)	1 (11)	2 (22)	9	9 (50)	7 (39)	2 (11)	18
50-99	69 (66)	24 (23)	12 (11)	105	34 (57)	14 (23)	12 (20)	60
100-149	73 (53)	39 (28)	26 (18)	138	21 (57)	10 (27)	6 (16)	37
150-200	25 (49)	11 (22)	15 (29)	51	10 (40)	10 (40)	5 (20)	25
>200	18 (32)	17 (30)	22 (39)	57	4 (15)	9 (35)	13 (50)	26
Lactating cow housing								
Free-stall/Loose housing	175 (52)	88 (26)	76 (22)	339	63 (44)	44 (31)	35 (25)	142
Tie-stall	16 (76)	4 (19)	1 (5)	21	15 (63)	6 (25)	3 (13)	24
Year of sample collection					-	-	-	-
2011	15 (88)	1 (6)	1 (6)	17	32 (49)	18 (27)	16 (24)	66
2012	74 (60)	21 (17)	28 (23)	123	46 (46)	32 (32)	22 (22)	100
2013	102 (46)	70 (32)	48 (22)	220				

[1]Number of positive environmental samples during last testing event

[2]Percentages represent row percentages

Table 2-2: Environmental sample results for herds participating in the Alberta Johne's Disease Initiative over 3 consecutive years (n = 82).

Year 1 (represents 2010/2011)	Year 2 (represents 2012)	Year 3 (represents 2013)	Farms n (%)
+	+	+	12 (15)
+	-	+	3 (4)
-	+	+	17 (21)
-	-	+	16 (20)
+	+	-	3 (4)
+	-	-	1 (1)
-	+	-	7 (9)
-	-	-	23 (28)

Table 2-3: Sensitivity and specificity estimates of environmental sampling, and herd prevalences for *Mycobacterium avium* subsp. *paratuberculosis* in Alberta and Saskatchewan dairy farms (median (95% probability interval)).

	Default[2]	High sensitivity[3]	Low sensitivity[4]
Sensitivity Alberta[1]	0.68 (0.60-0.76)	0.73 (0.66-0.80)	0.62 (0.54-0.68)
Sensitivity Saskatchewan[1]	0.69 (0.60-0.77)	0.74 (0.66-0.82)	0.63 (0.55-0.70)
Specificity	0.99 (0.96-1.00)	Default	Default
Prevalence Alberta	0.68 (0.60-0.80)	0.64 (0.56-0.72)	0.76 (0.66-0.90)
Prevalence Saskatchewan	0.76 (0.70-0.85)	0.70 (0.65-0.78)	0.84 (0.76-0.94)

[1]Sensitivity estimates were based on consecutive test results from 82 Alberta dairy farms. Estimates represent weighted averages of the annual sensitivities and varied between the provinces due to different sampling weights.
[2]The default analysis assumed that 20% of the repeatedly test-negative herds were infected.
[3]High sensitivity assumed that all positive farms would be detected with 3 environmental sampling events, hence assumed 3 of consecutive testing as a gold standard.
[4]Low sensitivity assumed that 50% of the repeatedly test-negative herds were infected.

Table 2-4: Final ordered logistic regression model on the association between the number of positive environmental samples (in 3 categories: 0, 1-3, 4-6 positive samples) and herd characteristics estimated on 360 Alberta and 166 Saskatchewan dairy farms.

Parameter	Coefficient	*P*-value	Odds ratio	95% CI
Herd size		<0.001[1]		
<50 cows	Reference			
50-100 cows	-0.26	0.525	0.77	0.35-1.71[2]
100-150 cows	0.09	0.814	1.09	0.50-2.40[2]
150-200 cows	0.49	0.260	1.62	0.70-3.78[2]
>200 cows	1.27	0.003	3.54	1.54-8.17[2]
Year of sample collection		0.011[1]		
2011	Reference			
2012	1.98	0.011	7.26	1.58-33.32[2]
2013	2.20	0.004	9.01	1.99-40.85[2]
Intercept				
0 versus ≥ positives	2.33	-	-	0.67-3.98[3]
≤3 versus >3 positives	3.65	-	-	1.98-5.32[3]

[1]Wald *P*-value for the parameter.
[2]Confidence interval around the odds ratio.
[3]Confidence interval around the coefficient.

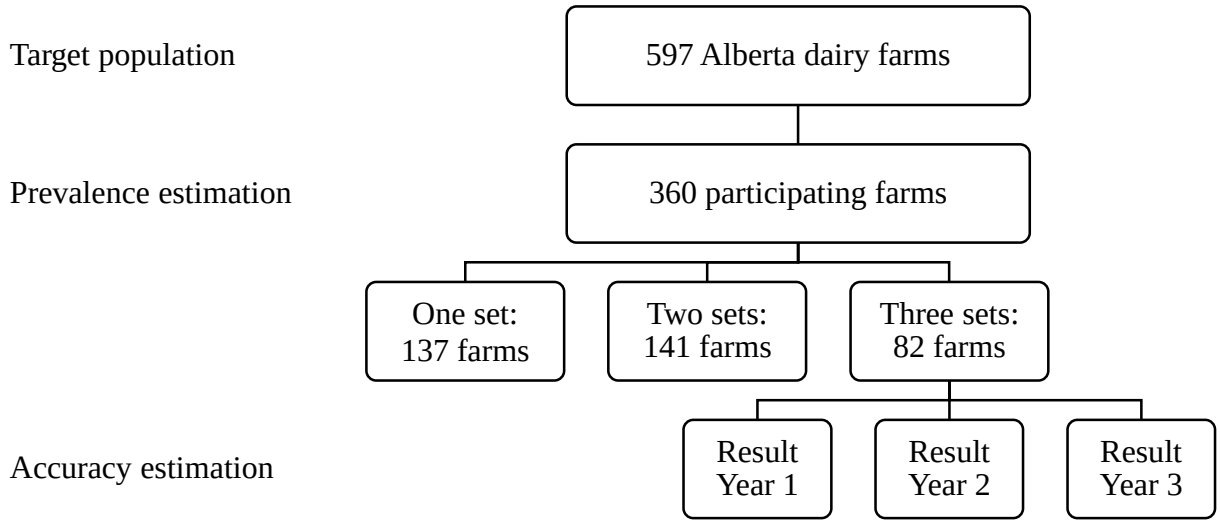

Figure 2-1: Flow diagram illustrating the number of herds in various stages of the Alberta Johne's Disease Initiative and the sample used for estimating herd prevalence of *Mycobacterium avium* subspecies *paratuberculosis*.

Chapter Three: **SAMPLING LOCATION, HERD SIZE AND SEASON INFLUENCE *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* ENVIRONMENTAL CULTURE RESULTS**

3.1 Abstract

Mycobacterium avium subspecies *paratuberculosis* (MAP), the etiologic agent of Johne's disease (JD), a chronic progressive enteritis, is a common pathogen on dairy farms. Environmental sampling is frequently used to detect MAP-infected herds, since it does not require sample collection from individual animals. The objectives were to determine: 1) location-specific odds of MAP-positive environmental sampling results, and whether season or herd size affect results; 2) whether season and herd size impact the odds of collection of samples from certain locations; and 3) whether sample set composition affects the odds of a positive set. Herd veterinarians, producer organization staff, and University of Calgary staff collected 5,588 samples on dairy farms in Alberta and Saskatchewan. Samples from sick cow and calving pens, and samples from dry cow housing had lower odds of testing MAP-positive than lactating cow pen samples (OR = 0.3 and 0.4, respectively). Samples collected from bedding packs and manure piles were less frequently MAP-positive than those collected from alley ways and lagoons, whereas samples collected in spring and summer more often tested MAP-positive than those collected in winter. Sample sets collected in summer more often included samples from all locations than samples collected in winter; therefore, we inferred that collectors had difficulties accessing certain areas in winter. Substitution of sample locations with others had minor impact on the sensitivity of sample sets containing 6 samples. However, set composition had an impact on the sensitivity of sample sets containing only 2 samples. In that regard, whereas sets with 2

manure storage area samples detected 81% of farms with at least 1 positive environmental sample, sets with only dry, sick or calving pen samples detected only 59%.

Environmental samples should be collected from areas where manure from numerous cows accumulates and can be well mixed (e.g. alley ways and manure lagoons). Collection of samples should be performed in spring or summer when locations are easier to access and samples have higher odds for testing MAP-positive.

3.2 Introduction

Johne's disease (JD) is a chronic progressive enteritis, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) (Fecteau and Whitlock, 2010). This disease is present in most countries with modern dairy industry (Barkema et al., 2010b). The bacterium is mainly transmitted fecal-orally, although intrauterine transmission and transmission through infected colostrum have been reported (Stabel, 2008; Whittington and Windsor, 2009). Even before the onset of clinical symptoms, affected animals have lower milk production, increased risk of being culled and decreased slaughter value (Hendrick et al., 2005; Raizman et al., 2009).

Culture of environmental samples is a relatively cheap, accurate and reliable method for detection of MAP-infected herds (Aly et al., 2009; Lavers et al., 2013; Lombard et al., 2006; Raizman et al., 2004). Since the laboratory test method used for these samples is a direct pathogen detection assay, it is commonly assumed that false-positive results are rare (Nielsen and Toft, 2008; Whitlock et al., 2000). Due to this advantage in accuracy and since

environmental sampling does not require sample collection from individual animals, it is used in various regional and national MAP control programs (Whitlock, 2010; Wiebe, 2011; Wolf et al., 2014b). The most commonly used sampling protocol requires 6 samples collected at various locations on dairy farms (Berghaus et al., 2006). Samples collected from lactating cow alleyways and manure storage areas are more frequently MAP-positive than those collected from sick cow pens (Lavers et al., 2013; Lombard et al., 2006; Raizman et al., 2004). It is unknown, however, whether this apparent higher sensitivity is confounded by the origin of a sample within a pen. Sick cow pen samples are often collected from straw packs, whereas lactating cow pen samples are most often collected from alleys. It is also unknown whether the type of manure storage (piles, pits or lagoons) has an impact.

The effect of season on accuracy of environmental samples is controversial. Although a recent field study concluded there was no effect of season (Lavers et al., 2013), and a laboratory experiment concluded that long-term storage of samples at -20ºC did not affect MAP survival (Raizman et al., 2011), another laboratory experiment concluded that short term storage at -20ºC reduced MAP survival (Khare et al., 2008). If sub-freezing temperatures and frequent freeze-thaw cycles impact the survival of MAP bacteria, a seasonal effect can be anticipated for samples collected in certain geographical regions including Western Canada.

The commonly used sampling protocol requires sample collection from manure storage areas and close up, sick or calving pens if ≥ 2 cows are present. However, collection of complete sample sets could be compromised in winter and on small farms. It is currently unknown whether the set composition affects sensitivity of the sample set.

The impact of herd size on the sensitivity of environmental culturing is unknown despite the fact that larger herds are more likely to be infected (Pillars et al., 2009; Wells and Wagner, 2000). It is plausible that the sensitivity of environmental samples is associated with the herd size. In that regard, assuming constant within-herd MAP prevalence, the odds of having at least 1 shedding cow in a pen is higher in large herds than in small herds since numerically, more animals are shedding. This effect should especially be present in dry, close up, sick and calving pens, because they are only populated by a few cows.

The objectives of this study were to determine: 1) location-specific odds of MAP-positive environmental sampling results, and whether season and herd size influence results; 2) whether season and herd size impact the odds of including at least 1 manure storage area sample, and 1 dry, sick or calving pen sample in a sample set; and 3) whether the sample set composition affects the odds of a positive set.

3.3 Materials and Methods

3.3.1 Herds

Environmental samples were collected on 601 dairy farms; the majority (70%) of samples were collected on 360 Alberta dairy farms voluntarily participating in the Alberta Johne's Disease Initiative (AJDI). The AJDI samples were collected annually by herd veterinarians and farms could join and leave the program at any given time. Additionally, environmental samples were collected by University of Calgary personnel as part of other research projects on 75 farms

not participating in the AJDI, and 166 Saskatchewan dairy farms that were visited once either by an employee of the producer organisation Saskatchewan Milk (n = 162 farms), or by their herd veterinarian (n = 4 farms). The study population included MAP-infected as well as uninfected herds. The true herd-prevalence was estimated to be 68% for Alberta and 76% for Saskatchewan dairy farms, respectively (Wolf et al., 2014a).

3.3.2 Sample collection, shipping and processing

Sample collection was described in detail (Wolf et al., 2014a). In short, sample collectors were requested to collect 6 environmental samples, 2 samples in each of 3 predetermined locations: 1) manure concentration samples from lactating cow alleys and the end of scraper lines; 2) manure storage samples from lagoons, piles, or pits; and 3) if a minimum of 2 cows present, cow concentration samples from dry cow, close up, calving or sick cow pens. Additional samples were collected from the other predetermined locations if locations did not qualify or were not accessible. Each sample contained at least 4 sub-samples, resulting in mixed manure rather than manure from single cow pies. Samples were thoroughly mixed in zip lock bags and transferred into 90 ml non-sterile disposable containers. Before April 2011, sampling location was recorded on the containers. Thereafter, a standardized description sheet was used in which type of the animals in a pen (lactating cows, dry cows, or sick cows), pen type (tie-stall, free-stall, or loose housing), and sampling area (cross-over alley, alley, exercise areas like paddocks and milking parlor waiting areas, bedding pack, gutter, manure pit, manure pile, or lagoon) were recorded (Appendix B). The comment section was used to describe sampling locations in detail.

Samples were sent to University of Calgary using express mail and stored at 4ºC. All samples were processed in fresh condition within 7 d after collection using a TREK ESP culture protocol with subsequent IS900 PCR confirmation (McKenna et al., 2005; Vary et al., 1990). Throughout the project, several improvements were implemented in the laboratory protocol, with the most important being the continuously increased use of *F57* qPCR; as of January 2012, *F57* qPCR (Slana et al., 2008) was used on culture products if IS900 PCR resulted in undefined or inconclusive results. These changes likely resulted in improved sensitivity, since inconclusive IS900 PCR results were conservatively called negative, prior to the introduction of *F57* qPCR (Wolf et al., 2014a).

3.3.3 Statistical analysis

Analyses were conducted using STATA version 11 (Statacorp, College Station, TX, USA). Results interpretation assumed nearly perfect specificity of MAP culture (Nielsen and Toft, 2008; Whitlock et al., 2000). Higher odds for positive test results were therefore assumed to be caused by increased sensitivity instead of decreased specificity. The association between the outcomes and independent variables was examined using multilevel logistic regression to adjust for potential clustering within sample collectors, farms and sample sets. The latent variable approach was used to calculate intra-class correlation coefficients, a measure of the proportion of variance explained by various levels in the models (Vigre et al., 2004). Forward selection was used to select variables to include in the final models and 2-way interactions were built to detect effect modification. A *P*-value of ≤ 0.05 was considered significant and a predictor change of

20% was used to detect confounding. Cluster-specific coefficients (β^{SS}) and odds ratios (OR) were presented for location, herd size, and season; they represented the OR of a specific subject experiencing the outcome in case of the presence of the exposure. In addition, population averaged coefficients (β^{PA}) and ORs were calculated using β^{SS} and the sum of the variance components in the various levels (ZU). The general formula for subject i can be written as:

$$\beta^{PA} \approx \beta^{SS} / \sqrt{(1 + 0.346 * \text{variance } (ZU)_i)} \quad [1]$$

The population averaged ORs represent the OR of experiencing the outcome in case of presence of the exposure across all subjects. It is recommended to use cluster-specific ORs for variables that vary at the lowest level of a model (sample locations within a sample set), and population averaged ORs for coefficients that vary at higher levels (herd size does not vary within a sample set, but differs between herds) (Dohoo et al., 2009). The ORs were not presented for project day and its square and cubic terms, since their interpretation would be complicated and not meaningful.

Environmental samples. To identify predictors for environmental culture results, single sample culture result was used as the outcome whereas location, season, and herd size were included as independent variables. The Herd size and season were included as categorical variables. Herd size and season were categorized in 50-cow (< 50, 50- 100, 100 - 150, 150 - 200, > 200 cows) and 2-mo categories, respectively. To adjust for culture protocol improvements over the years, day of sample collection was used as a continuous variable (November 9, 2010 =

project day 1; November 1, 2013 = project day 1,090). Square and cubic terms were included to account for non-linearity of the relationship. Sampling location was categorized into sites and areas within sites. Sites included the following categories: lactating cow pens, manure storage, and dry/sick/calving pens. Areas within sites included alleys/gutters, exercise areas, bedding packs, and tie-stalls for samples collected from lactating cow pens, and dry/sick/calving pens. It included lagoons, manure pits, and manure piles for manure storage area samples.

The first model estimated the impact of site, herd size and season on the overall odds of a sample being MAP culture-positive, included all samples, and used sample site as the independent variable. Dry cow pen samples and sick/calving pen samples were separate categories. Because it can be expected that the impact of season and perhaps herd size on the odds of a MAP-positive sample is different among sample locations (manure storage samples are collected outside the barn, whereas lactating cow pen samples are collected inside; large herds may have more cows in sick cow pens than small herds, etc.), separate models were built for each site. Area of collection was used as the independent variable.

Sampling sites in a sample set. To determine whether herd size and season influenced the odds of collection of samples from certain sites, models were fit on a sample set level. Only sample sets with sampling sites identified for all 6 samples were included in the analysis. Herd size and season were included as independent variables. Sample collector and farm were included as random effects. Three models were constructed for various outcomes: the first model estimated the odds of collection of a complete sample set containing at least 1 lactating cow housing sample, 1 manure storage area sample, and 1 dry cow housing, sick or calving pen

sample. The second model estimated the odds of a sample set including at least 1 manure storage area sample, and the third model estimated the odds of a sample set including at least 1 dry cow housing, sick or calving pen sample.

Test results on sample set level. To determine whether the herd-level culture result was associated with the composition of a sample set, analysis was performed using sample sets with sampling sites identified for all 6 samples. The herd-level environmental sample result (no MAP culture-positive sample versus ≥ 1 positive sample within a sample set) was used as the outcome, whereas ≥ 1manure storage area sample collected (yes/no), ≥ 1 dry, sick or calving pen sample collected (yes/no) were included as 2 independent variables. The analysis was adjusted for day of sample collection. Sample collector and farm were included as random effects.

Sample sets with < 6 samples. The objective was to determine the relative sensitivity of a sample set with less than 6 environmental samples compared to a set with 6 samples. Only sets that included 2 samples collected from lactating cow housing, 2 samples collected from manure storage areas, and 2 samples collected from dry, sick or calving pens were included in the analysis. Of those, only sample sets with at least 1 positive environmental sample were included. Sampling sites were selectively included in the analysis. For example: the relative sensitivity of 2 lactating cow housing samples described the proportion of sample sets with ≥ 1 positive lactating cow housing sample among the sample sets with ≥ 1 positive sample collected at any site. The analysis was repeated for all possible combinations of sampling sites including 2 – 6 samples.

3.4 Results

3.4.1 Environmental samples

A total of 655 sample sets were collected in the AJDI, 124 in other research projects in Alberta, and 166 in Saskatchewan. In total 5,588 samples were collected, of which 5,345 samples included sufficient information to identify their location, whereas 4,201 samples included sufficient information to identify the area of sample collection within the location.

A total of 620 (25%) of 2,430 samples collected from lactating cow housing were MAP culture-positive, whereas 284 (24%) of 1,146 samples collected from manure storage areas, 73 (19%) of 386 samples collected from dry cow housing, and 41 (17%) of 239 samples collected from calving/sick cow pens were MAP culture-positive (Table 3-1). Although only 14% of the samples from herds with <50 cows were positive, 42% of the samples from herds with >200 cows were MAP culture-positive (Table 3-2). Percentages of positive results fluctuated throughout the year, from 18% positive samples in September and October to 31% positive samples in March and April (Table 3-3). The number of collected samples per month increased over time since an increasing number of farms completed their second and third testing event (Figure 3-1).

Among samples taken within the same sample set, samples collected from dry cow housing, and samples collected from calving/sick cow pens had lower odds of testing MAP-positive than samples taken from lactating cow pens ($P < 0.01$; Table 3-4). Among manure storage area samples, samples collected from manure piles had lower odds of testing positive

than samples collected from lagoons ($P = 0.01$). Culture results of lactating cow housing did not differ for samples collected from alley ways/gutters, exercise areas, bedding packs, and tie-stalls ($P = 0.27$; Table 3-5). Dry cow alley way samples had higher odds of testing positive than dry cow bedding pack samples ($P = 0.03$) and calving/sick cow pen bedding pack samples ($P = 0.01$). The odds of testing MAP-positive were higher for all locations for larger herds compared to smaller herds ($P < 0.01$). Among all samples, samples collected between March and June had higher odds of testing positive than samples collected in January and February ($P = 0.01$). Evidence for seasonality was also present for samples collected in lactating cow housings and manure storage areas.

The percentage of variance explained by the sample collector was low for all samples but highest for samples taken in dry, sick or calving pens (10%). The variance explained by farm ranged from 0 to 34%, whereas the variance explained by sample set ranged from 40 to 54%.

3.4.2 Sampling sites in a sample set

A total of 841 (90%) sample sets had sufficient information to identify the sampling site of all 6 samples within a set (Table 3-6). Overall, 423 (51%) of the 841 sample sets were identified as complete sample sets meaning that they included at least 1 lactating cow pen sample, 1 manure storage area sample, and 1 dry, calving or sick cow pen sample. The probability of a complete environmental sample set differed among months of the year and was higher between March and June, than in January and February ($P < 0.01$; Table 6, Figure 3-2). Dry, sick or

calving pen samples were also more often collected on farms with > 150 cows than on farms < 150 cows ($P < 0.01$; Table 3-7).

The percentage of variance explained by the sample collectors differed among various logistic regression models and ranged from 40 to 48%. The percentage of variance explained by the farm ranged from 2 to 5%.

3.4.3 Test results on sample set level

A total of 192 (45%) of 423 complete, and 192 (46%) of 418 incomplete sample sets had at least 1 sample that was MAP culture-positive (Table 3-6). Herd-level result was not associated with the inclusion of manure storage area, and dry, sick or calving pen samples in a sample set (lowest $P = 0.51$, results not shown, because the predictor estimates for herd size, season and project day were very similar to the estimates presented in Table 3-4).

3.4.4 Sample sets with < 6 samples

A total of 219 sample sets included 2 lactating cow housing, 2 manure storage and 2 dry, sick or calving pen samples; 100 (46%) of these sample sets had at least 1 MAP-positive sample. Among the 100 test-positive sets, 81% would have tested positive if only 2 manure storage area samples had been collected whereas 59% would have tested positive if only 2 dry, sick, or calving pen samples had been collected (Table 3-8).

3.5 Discussion

Environmental samples were significantly more likely to be MAP culture-positive if collected from lactating cow pens and manure storage areas compared to dry or calving/sick cow pens, which confirms earlier reports (Lavers et al., 2013; Lombard et al., 2006; Raizman et al., 2004). The area of sample collection was important, as alleyways and manure lagoons were more likely to test positive than bedding packs and manure piles. Selection of locations in a sample set was more important for sampling protocols that included only 2 samples, although it did not have a high impact on the sensitivity of a sample set that included 5 or 6 samples, which gives sample collectors the required flexibility to collect samples on small farms and in winter when certain locations are not available or accessible.

As reported earlier, the odds of testing MAP-positive and therefore the sensitivity of environmental samples were lower for samples collected in dry, calving, and sick pens compared to samples collected in lactating cow housing and manure storage, which may appear counter intuitive since clinical JD cases should be housed in sick cow pens (Lavers et al., 2013; Lombard et al., 2006; Raizman et al., 2004). One explanation is that farmers are aware of the risk of MAP transmission through clinical cases and remove those animals soon after detection, while leaving subclinical, shedding animals in the lactating cow herd. Another explanation is the number of cows whose manure was included in a sample; in that regard, more cows contribute to the manure in a lactating cow pen or manure lagoon than to the manure in a sick cow pen. Considering that most infected herds have low within-herd prevalences and only approximately

30 to 40% of infected cows actually shed the bacterium (Barkema et al., 2010b; Whitlock et al., 2000), chances are high that a sick cow pen sample tests negative, because no cow in the pen sheds MAP. A third reason for lower odds of a MAP-positive test result for dry cow housing, calving, and sick pens is the area of sample collection. Sick cow pens are often straw packs; therefore, a high proportion of these samples are bedding pack samples (76% of the sick cow pen samples versus 6% of the lactating cow pen samples). Because of the dryness of the material (which is even more increased through the generally drier consistency of dry cow manure) and high straw content, proper mixing of subsamples could have been compromised, perhaps reducing odds of positive samples collected from bedding packs. In contrast, this relationship was not evident for lactating cow samples, possibly due to more cows in the pens. However, only a few lactating cow pen samples were collected from bedding packs and an actual association could have been missed due to low sample size.

The lower odds for MAP-positive results of manure pile samples compared to pits and lagoons can be explained with the same reasons as described above. In that regard, in Western Canada, most lactating cows are housed in free-stalls with liquid manure production, whereas dry, calving, and sick cows are often housed on straw packs. Consequently, manure piles only contain the manure of dry, calving and sick cows, reducing the number of cows contributing to a sample. The degree of manure mixture is also reduced, due to the high straw content in a manure pile sample.

Samples collected in spring had higher odds of testing MAP-positive than samples collected in winter. This provided evidence for an impact of season on environmental sample

results, possibly due to differences in temperature and humidity affecting the viability of MAP bacteria in excreted manure prior to sample collection. The use of direct PCR methods instead of culture would likely be an alternative, because PCR does not require viable bacteria in the sample. However, further research is needed to determine the accuracy of direct PCR in environmental samples.

Samples collected from larger herds were more likely to test MAP-positive than samples collected from smaller herds. One reason is the higher risk of MAP infection for large herds compared to small herds due to differences in management and more purchased cattle (Pillars et al., 2009; Wells and Wagner, 2000). Since the impact of herd size was consistent throughout locations, this study did not provide any evidence for an impact of herd size on the accuracy of environmental samples collected at specific locations. However, should larger herds have higher within-herd MAP prevalence, the accuracy of environmental sampling would be higher in those herds, since within-herd prevalence is positively associated with herd sensitivity (Lavers et al., 2013).

The variance components estimated by the different logistic regression models provide evidence for clustering in the dataset. A very low proportion of variance (< 10%) in the odds of a positive sample result was explained by the sample collector, which confirmed high repeatability of results among collectors (Aly et al., 2009). Much variance was clustered within farms (31% for all samples) reflecting differences in within-herd MAP prevalence among farms. However, it was noteworthy that the results for dry cow housing, calving and sick cow pen samples were not clustered within farms, but instead tended to have a higher residual proportion of variance at the

lowest level (54%), perhaps due to lower repeatability of samples collected at these locations, but might also be caused by different predictors included in the final logistic regression model, since it did not include season and project day.

Models estimating the composition of sample sets had a different distribution of variance components; although 40 to 48% of the variance in the outcome was explained by the sample collector, only 2 to 5% was explained by the farm. The high proportion of variance explained by the collector provided evidence that collection of certain samples was a decision made by sampling personnel. One common reason for incomplete sample sets was not collecting manure storage area samples. In particular, half-empty manure pits posed a challenge since the surface could not be reached and entering the pit would have posed an unacceptable safety risk. A possible solution is the use of commercially available golf ball retriever sticks. Many veterinarians and all University of Calgary personnel used these sticks with an attached zip lock bag to collect samples from pits. Fortunately, the risk of cross contamination between samples was low, since sample material did not come in contact with the stick. Solutions like this could have been communicated during follow-up workshops for collectors which would have been expected to increase the number of complete sample sets.

The composition of sample sets did not only depend on the sample collectors, but also on herd size and the season. Sample sets were more likely to be complete if collected in spring and summer, because lagoons and pits could not be sampled in winter since they were covered with ice and snow. Some farms had manure pits inside, but in many cases, additional samples were collected from lactating cow pens. Collection of samples from outside dry cow pens was also

compromised by low temperatures, snow cover and frozen material. An additional factor for the collection of dry, calving, and sick cow pen samples was herd size. Since collectors were instructed to only collect samples from pens containing ≥ 2 cows, the probability was higher on small herds that pens did not qualify.

The composition of sample sets did not have an impact on the odds of having at least 1 positive sample in the sample set with 6 samples. Sets where locations were replaced with others did not have lower odds of testing positive. However, if a lower number of samples were collected within a set, the location of sample collection was more important. Although 81% of the 100 positive sets were detected with 2 manure storage area samples, only 59% were detected with 2 dry cow housing, sick or calving pen samples, making 2 manure storage area samples an apparently cost-effective sampling protocol. The proportion of positive sets was obviously driven by the accuracy of the samples included in a set, since manure storage area samples were more accurate than sick cow pen samples. Therefore, if a set only includes 2 samples, all samples should be collected from locations with a high sensitivity. In contrast, if 4, 5 or 6 samples are included in a set, the impact of the accuracy of a single sample on the accuracy of the whole set decreases, which supports the suggestion that sample locations can be replaced with others if they are not accessible.

The present study provided insight regarding how to optimize a one-time sampling protocol with a maximum of 6 environmental samples. It also provided estimates on the reduction of accuracy if < 6 samples were collected. In addition, the accuracy of repeated testing with 6 environmental samples per test event is well documented (Khol et al., 2009; Lavers et al.,

2013; Wolf et al., 2014a). It is unknown whether the accuracy increases if > 6 samples are collected per set. If so, uncertainty around results of prevalence studies would decrease, and precision of within-herd prevalence estimation would likely increase. There are also knowledge gaps around sampling intervals and number of samples taken per test event if repeated testing is used to monitor progress in control programs, and to increase certainty around freedom of MAP in test negative herds. These questions should be addressed within a longitudinal study incorporating estimation of within-herd prevalence, and collection of a large number of environmental samples in short intervals.

3.6 Conclusions

To ensure high sensitivity of environmental sampling, sample sets should only include samples collected from areas where manure from several cows can be well mixed; those include lactating cow alleys and gutters over sick/calving pen bedding packs and lagoons and pits instead of piles. Samples should be collected in spring and summer instead of in winter, since manure storage areas and outdoor pens are easier to access and culture of environmental samples is more accurate. Replacing sample locations with others had an impact on the sensitivity of sample sets containing only a small number of samples, but had only a minor impact on the sensitivity of a set containing 6 environmental samples.

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Table 3-1: Percentage of *Mycobacterium avium* subsp. *paratuberculosis*-positive environmental sample culture results collected at various locations, provinces and sampling years (n (% positive samples)).

Location	Year of sample collection			Province		Total
	2010/2011	2012	2013	Alberta	Saskatchewan	
Lactating cows						
Alleys/gutters	239 (8)	808 (28)	1,007 (30)	1,583 (25)	471 (30)	2,054 (27)
Exercise areas	16 (0)	32 (28)	33 (21)	59 (14)	22 (36)	81 (20)
Bedding packs	5 (0)	50 (24)	81 (16)	80 (14)	56 (25)	136 (18)
Tie stalls	21 (10)	74 (16)	64 (17)	75 (13)	84 (18)	159 (16)
Manure storage						
Pits	142 (11)	207 (25)	229 (32)	516 (24)	62 (24)	578 (24)
Piles	42 (5)	95 (20)	75 (15)	197 (15)	15 (13)	212 (15)
Lagoons	88 (18)	144 (33)	124 (40)	332 (31)	24 (38)	356 (32)
Dry cows						
Alleys/gutters	9 (0)	18 (44)	73 (26)	90 (24)	10 (50)	100 (27)
Exercise areas	3 (0)	35 (17)	38 (24)	41 (10)	35 (31)	76 (20)
Bedding packs	12 (0)	75 (17)	123 (15)	124 (15)	86 (14)	210 (15)
Calving/sick						
Alleys/gutters	2 (0)	12 (33)	34 (24)	33 (30)	15 (13)	48 (25)
Exercise areas	0 (-)	7 (29)	2 (0)	6 (33)	3 (0)	9 (22)
Bedding packs	13 (8)	59 (10)	110 (18)	129 (12)	53 (21)	182 (15)

Table 3-2: Percentage of *Mycobacterium avium* subsp. *paratuberculosis*-positive environmental sample culture results collected at various sized herds (n (% positive samples)).

Location	< 50 cows	50 - 100	100 - 150	150 - 200	> 200
Lactating cows					
Alleys/gutters	75 (11)	611 (20)	663 (22)	271 (33)	306 (47)
Exercise areas	5 (20)	26 (8)	28 (29)	7 (43)	4 (50)
Bedding packs	5 (0)	43 (12)	42 (21)	9 (22)	14 (36)
Tie stalls	43 (23)	62 (15)	25 (12)	4 (0)	1 (0)
Manure storage					
Pits	12 (8)	139 (20)	218 (25)	88 (27)	95 (28)
Piles	24 (4)	77 (17)	68 (16)	16 (19)	25 (12)
Lagoons	10 (20)	85 (18)	119 (23)	71 (42)	63 (56)
Dry cows					
Alleys/gutters	1 (0)	10 (1)	29 (34)	15 (47)	12 (50)
Exercise areas	7 (57)	1 (0)	18 (11)	10 (30)	8 (25)
Bedding packs	6 (0)	45 (2)	63 (14)	28 (11)	33 (42)
Calving/sick					
Alleys/gutters	2 (0)	10 (1)	10 (20)	11 (36)	15 (33)
Exercise areas	3 (0)	1 (0)	2 (0)	2 (50)	1 (100)
Bedding packs	6 (0)	45 (2)	57 (12)	24 (13)	43 (35)
Total	199 (14)	1,155 (17)	1,342 (21)	556 (31)	620 (42)

Table 3-3: Percentage of *Mycobacterium avium* subsp. *paratuberculosis*-positive environmental sample results collected in various months (n (% positive samples)).

Location/area	Jan - Feb	Mar - Apr	May - June	July - Aug	Sep - Oct	Nov - Dec
Lactating cows						
Alleys/gutters	418 (24)	309 (34)	615 (29)	245 (27)	243 (21)	224 (22)
Exercise areas	18 (33)	5 (20)	18 (6)	17 (12)	12 (17)	11 (36)
Bedding packs	38 (21)	17 (18)	29 (14)	12 (17)	13 (8)	27 (26)
Tie stalls	34 (12)	23 (9)	20 (25)	0 (-)	31 (13)	51 (20)
Manure storage						
Pits	84 (18)	114 (33)	178 (28)	76 (16)	55 (11)	71 (28)
Piles	46 (11)	40 (20)	48 (21)	22 (9)	18 (6)	38 (16)
Lagoons	7 (0)	44 (39)	156 (33)	64 (30)	68 (26)	17 (41)
Dry cows						
Alleys/gutters	14 (29)	27 (44)	33 (15)	8 (25)	9 (0)	9 (44)
Exercise areas	4 (25)	6 (17)	19 (32)	19 (5)	17 (18)	11 (27)
Bedding packs	62 (15)	30 (27)	46 (4)	28 (14)	22 (18)	22 (18)
Calving/sick						
Alleys/gutters	10 (10)	6 (17)	11 (27)	13 (46)	4 (25)	4 (0)
Exercise areas	1 (0)	2 (50)	2 (0)	0 (-)	4 (25)	0 (-)
Bedding packs	44 (16)	40 (25)	43 (16)	22 (0)	9 (11)	24 (8)
Total	780 (20)	663 (31)	1,218 (26)	526 (22)	505 (18)	509 (23)

Table 3-4: Final logistic regression models on the association between the *Mycobacterium avium* subsp. *paratuberculosis* environmental sample culture result and sample location (all samples, manure storage area).

	All samples (n = 4,876)				Manure storage (n = 1,110)		
	Estimate (SEM)	OR[2]	*P*-value		Estimate (SEM)	OR[2]	*P*-value
Location			< 0.01[1]	Area			0.01[1]
Lactating cows	Reference			Lagoons	Reference		
Manure storage	0.04 (0.13)	1.04/1.02	0.73	Pits	0.36 (0.37)	1.43/1.16	0.33
Dry cows	-0.98 (0.20)	0.38/0.61	< 0.01	Piles	-1.43 (0.56)	0.24/0.55	0.01
Calving/sick	-1.37 (0.21)	0.25/0.50	< 0.01				
Herd size			< 0.01[1]				< 0.01[1]
< 50	Reference				Reference		
50 - 100	0.25 (0.66)	1.28/1.13	0.70		1.37 (1.43)	3.94/1.76	0.34
100 - 150	0.65 (0.66)	1.92/1.38	0.33		1.21 (1.43)	3.35/1.65	0.40
150 - 200	1.66 (0.70)	5.30/3.88	0.02		2.50 (1.49)	12.18/2.81	0.09
> 200	2.71 (0.70)	15.03/3.88	< 0.01		3.40 (1.52)	29.96/4.07	0.03
Accuracy change			< 0.01[1]				< 0.01[1]
Project day	-0.01 (0.01)		0.18		-0.01 (0.01)		0.38
Project day^2	2.8e-5 (1.3e-5)		0.03		3.6e-5 (2.4e-5)		0.13
Project day^3	-2.0e-8 (7.9e-9)		0.01		-2.5e-8 (1.46e-8)		0.09
Season			0.02[1]				0.14[1]
Jan. - Feb.	Reference				Reference		
Mar. - Apr.	1.36 (0.44)	3.90/1.98	0.01		1.75 (0.85)	5.75/2.06	0.04
May - June	1.08 (0.45)	2.94/1.72	< 0.01		1.49 (0.84)	4.44/1.85	0.08
July - Aug.	0.29 (0.49)	1.34/1.16	0.56		0.32 (0.91)	1.38/1.14	0.73
Sep. - Oct.	0.08 (0.49)	1.08/1.04	0.88		0.15 (0.94)	1.16/1.06	0.87
Nov. - Dec.	0.76 (0.46)	2.14/1.46	0.10		1.48 (0.91)	4.39/1.84	0.11
Intercept	-5.30 (1.06)		< 0.01		-6.85 (2.26)		< 0.01

Table 3-4: (continued)

Random effects	Variance (SEM)	% Var.	Variance (SEM)	% Var.
Collector	0.60 (0.44)	5	1.2e-18 (1.4e-9)	0
Herd	3.49 (0.81)	31	5.88 (2.63)	34
Sample set	4.55 (0.78)	35	8.16 (2.61)	47
Sample	-	29	-	19

Table 3-5: Final logistic regression models on the association between the *Mycobacterium avium* subsp. *paratuberculosis* environmental sample culture result and sample location (lactating cows, dry/calving/sick cows).

	Lactating cows (n = 2,713)				Dry/calving/sick cows (n = 568)		
	Estimate (SEM)	OR[2]	*P*-value		Estimate (SEM)	OR[2]	*P*-value
Area	Not sign.		(0.27)[1]	Area[3]			0.07
Alleys/gutters				DC alleys/gutters	Reference		
Exercise areas				DC exercise areas	-0.54 (0.73)	0.58/0.74	0.46
Bedding packs				DC bedding packs	-1.55 (0.63)	0.21/0.42	0.01
Tie-stalls				CS alleys/gutters	-0.83 (0.79)	0.44/0.63	0.29
				CS exercise areas	0.34 (1.43)	1.40/1.21	0.81
				CS bedding packs	-1.69 (0.65)	0.18/0.39	0.01
Herd size			< 0.01[1]				< 0.01
< 50	Reference				Reference		
50 - 100	0.17 (0.83)	1.19/1.08	0.84		-0.41 (1.25)	0.66/0.80	0.75
100 - 150	0.32 (0.83)	1.38/1.15	0.70		1.42 (1.28)	4.14/2.21	0.27
150 - 200	1.54 (0.89)	4.66/1.93	0.08		1.88 (1.31)	6.55/2.86	0.15
> 200	2.97 (0.89)	19.49/3.56	< 0.01		3.31 (1.39)	27.39/6.35	0.02
Accuracy change			< 0.01[1]		Not sign.		(0.09)[1]
Project day	-0.01 (0.01)		0.08				
Project day^2	4.1e-5 (1.6e-5)		0.01				
Project day^3	-2.8e-8 (1.0e-8)		< 0.01				
Season			0.22[1]				(0.13)[1]
Jan. - Feb.	Reference						
Mar. - Apr.	1.06 (0.54)	2.89/1.57	0.05				
May - June	0.90 (0.53)	2.46/1.47	0.09				
July - Aug.	0.32 (0.60)	1.38/1.15	0.60				
Sep. - Oct.	-0.16 (0.63)	0.85/0.93	0.80				
Nov. - Dec.	0.75 (0.57)	2.12/1.38	0.19				

Table 3-5: (continued)

Intercept	-5.00 (1.29)	< 0.01		
Random effects	Variance (SE)	% Var.	Variance (SE)	% Var.
Collector	1.09 (0.79)	7	1.17 (0.94)	12
Herd	5.31 (1.37)	33	2.3e-8 (8.5e-3)	0
Sample set	6.50 (1.33)	40	5.2 (2.5)	54
Sample	-	20	-	34

[1]Wald *P*-value for the coefficient; values in parentheses refer to *P*-values for variables not included in the final model.
[2]Cluster-specific odds ratio/approximated population averaged (marginal) odds ratio.
[3]DC: dry cow pens; CS: calving, sick cow pens.

Table 3-6: Locations of environmental sample collection for dairy farms in Alberta and Saskatchewan (# sample sets (% sample sets with at least 1 positive sample)).

LC[1]	MS[1]	DC[1]	CS[1]	Herd size (cows)			Season				Total
				< 100	100 - 150	> 150	Jan - Mar	Apr - Jun	Jul - Sep	Oct - Dec	
+	+	+	+	24 (29)	43 (27)	35 (60)	20 (35)	51 (45)	17 (47)	15 (40)	103 (43)[2]
+	-	+	+	11 (27)	8 (63)	13 (77)	17 (59)	6 (100)	8 (25)	8 (50)	39 (56)
-	+	+	+	1 (0)	0 (-)	0 (-)	1 (-)	0 (-)	0 (-)	0 (-)	1 (0)
+	+	-	+	35 (57)	43 (37)	71 (62)	43 (49)	66 (62)	22 (45)	21 (52)	152 (55)[2]
+	-	-	+	9 (44)	5 (20)	14 (64)	19 (63)	4 (25)	1 (100)	5 (20)	29 (52)
+	+	+	-	63 (29)	48 (40)	43 (49)	34 (47)	67 (40)	38 (29)	29 (34)	168 (38)[2]
+	-	+	-	13 (62)	17 (53)	15 (60)	23 (52)	17 (71)	6 (83)	15 (47)	61 (59)
-	+	+	-	1 (100)	0 (-)	1 (100)	0 (-)	2 (100)	0 (-)	0 (-)	2 (100)
+	+	-	-	80 (33)	88 (39)	48 (56)	53 (30)	75 (49)	46 (37)	46 (41)	220 (40)
+	-	-	-	31 (32)	21 (52)	6 (67)	14 (36)	27 (44)	6 (33)	18 (50)	65 (43)
-	+	-	-	0 (-)	1 (100)	0 (-)	0 (-)	1 (100)	0 (-)	0 (-)	1 (100)
Total				268 (36)	274 (41)	246 (59)	224 (44)	316 (51)	144 (39)	157 (43)	841 (46)

[1]LC: lactating cow pen, MS: manure storage area, DC: dry cow pen, CS: /calving/ sick pens

[2]Complete sample sets with at least 1 lactating cow housing, 1 manure storage area, and 1 dry cow housing, sick or calving pen sample.

Table 3-7: Final logistic regression models for the association between the completeness of an environmental sample set and season and herd size (n = 841 sample sets).

Parameter	Complete sets			Manure storage areas			Dry/calving/sick pens		
	Est. (SEM)	OR[1]	*P*-value	Est. (SEM)	OR[1]	*P*-value	Est. (SEM)	OR[1]	*P*-value
Herd size	Not sign.		(0.24)[2]	Not sign.		(0.91)[2]			< 0.01[2]
< 50 cows							Reference		
50 - 100							0.01 (0.51)	1.01/1.01	0.96
100 - 150							0.13 (0.51)	1.14/1.09	0.80
150 - 200							1.06 (0.55)	2.89/2.05	0.06
> 200							1.01 (0.56)	2.75/1.99	0.07
Season			< 0.01[2]			0.02[2]			< 0.01
Jan. - Feb.	Reference			Reference			Reference		
Mar. - Apr.	1.17 (0.34)	3.22/2.29	< 0.01	0.58 (0.37)	1.79/1.51	0.11	0.95 (0.39)	2.59/1.91	0.02
May - June	1.03 (0.31)	2.80/2.07	< 0.01	0.65 (0.33)	1.92/1.59	0.05	0.50 (0.36)	1.65/1.40	0.16
July - Aug.	0.34 (0.35)	1.40/1.27	0.34	0.86 (0.41)	2.36/1.85	0.04	-0.07 (0.41)	0.93/0.95	0.87
Sep. - Oct.	0.65 (0.34)	1.92/1.58	0.06	1.30 (0.43)	3.67/2.53	< 0.01	-0.44 (0.41)	0.64/0.74	0.28
Nov. - Dec.	-0.45 (0.33)	0.64/0.73	0.16	-0.04 (0.33)	0.96/0.97	0.90	-0.83 (0.39)	0.44/0.57	0.03
Intercept	-0.29 (0.33)		0.33	1.85 (0.40)		< 0.01	0.52 (0.62)		0.40
Random effects	Var. (SE)		% Var.	Var. (SE)		% Var.	Var. (SE)		% Var.
Collector	2.65 (0.94)		43	2.49 (1.00)		41	3.22 (1.13)		48
Herd	0.25 (0.45)		4	0.27 (0.56)		4	0.15 (0.43)		2
Sample set	-		53	-		55	-		50

[1]Cluster-specific odds ratio/approximated population averaged (marginal) odds ratio.
[2]Wald *P*-value for the coefficient; values in parentheses refer to *P*-values for variables not included in the final model.

Table 3-8: Percentage of *Mycobacterium avium* subsp. *paratuberculosis*-positive environmental sample sets with various numbers of samples collected at various locations (n = 100 sample sets with ≥ 1 positive environmental sample).

2 Samples			% pos. sets	3 Samples			% pos. sets
LC[1]	MS[1]	DCS[1]	(95% CI)	LC[1]	MS[1]	DCS[1]	(95% CI)
0	2	0	81 (73 - 89)	1	2	0	87 (71 - 87)
2	0	0	70 (61 - 78)	0	2	1	85 (78 - 92)
0	0	2	59 (49 - 69)	1	1	1	82 (74 - 90)
1	1	0	77 (69 - 85)	2	1	0	86 (79 - 93)
1	0	1	62 (52 - 72)	2	0	1	74 (65 - 83)
0	1	1	72 (63 - 81)	0	1	2	82 (74 - 90)
				1	0	2	72 (63 - 81)
4 Samples			% pos. sets	5 Samples			% pos. sets
LC[1]	MS[1]	DCS[1]	(95% CI)	LC[1]	MS[1]	DCS[1]	(95% CI)
0	2	2	93 (88 - 98)	2	2	1	95 (91 - 99)
1	2	1	90 (84 - 96)	2	1	2	94 (89 - 98)
2	2	0	93 (88 - 98)	1	2	2	96 (92 - 100)
2	1	1	89 (83 - 95)				
2	0	2	81 (73 - 89)				
1	1	2	88 (82 - 94)				

[1]Number of samples collected at LC = lactating cow housing, MS = manure storage, DCS = dry cow, calving and sick pens.

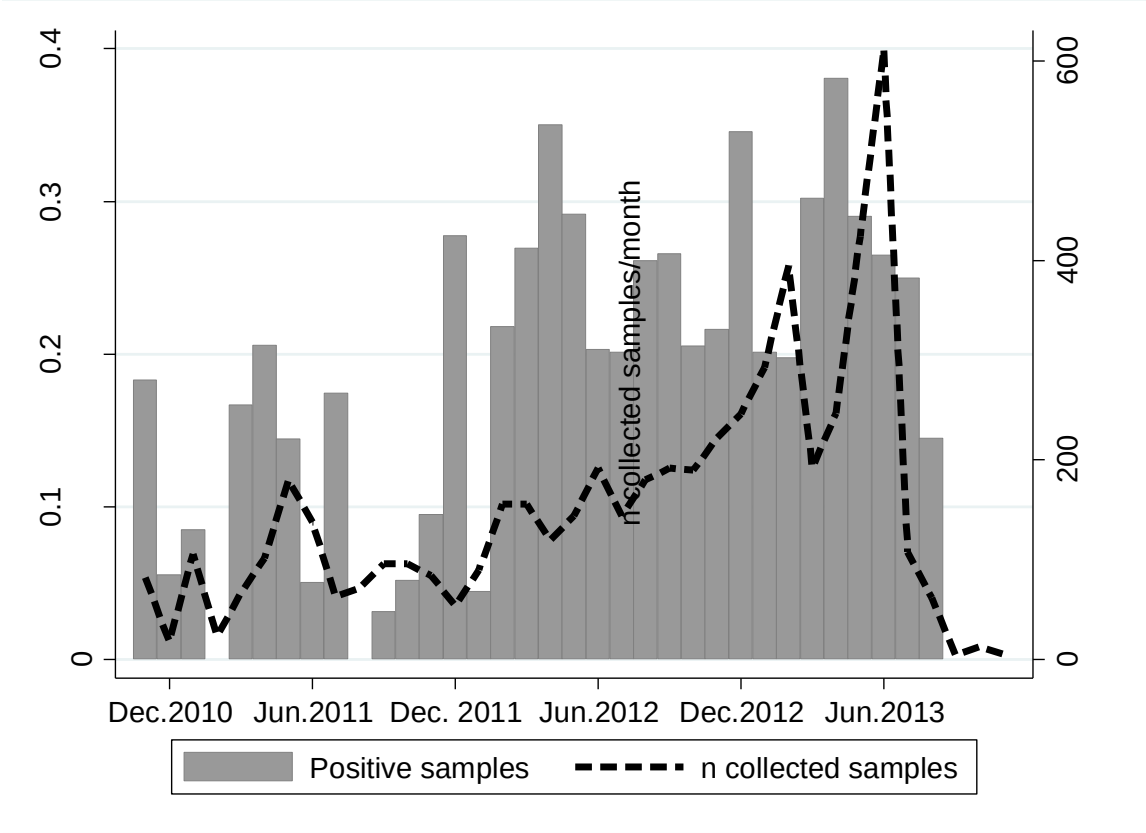

Figure 3-1: Temporal pattern of the monthly proportion of environmental samples testing positive for *Mycobacterium avium* subsp. *paratuberculosis* and the number of samples collected in Alberta and Saskatchewan.

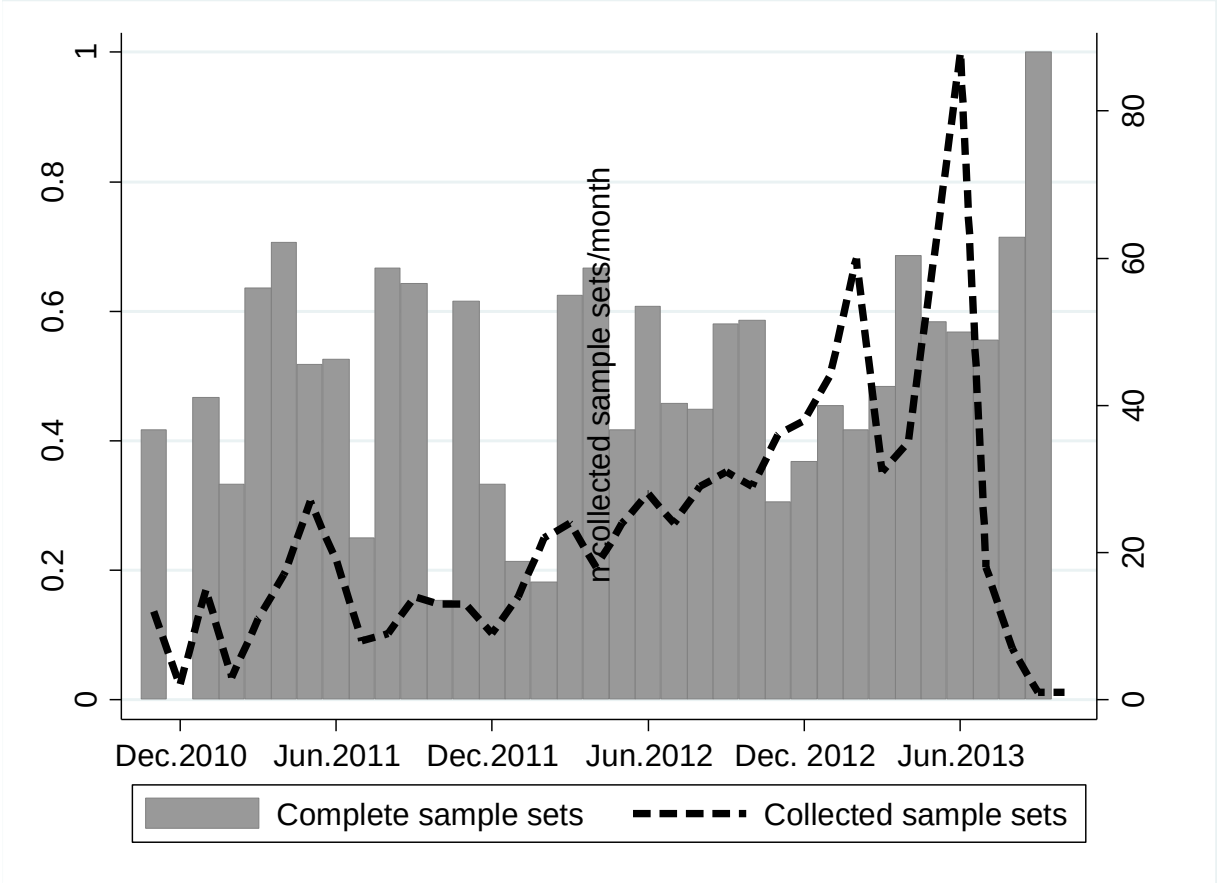

Figure 3-2: Temporal pattern of the proportion of complete environmental sample sets (sample sets with at least 1 lactating cow pen, 1 manure storage area, and 1 dry, sick or calving pen sample), and the number of sample sets collected in Alberta and Saskatchewan.

Chapter Four: **DAIRY FARMS TESTING POSITIVE FOR *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* HAVE POORER HYGIENE AND ARE LESS CAUTIOUS WHEN PURCHASING CATTLE THAN TEST-NEGATIVE HERDS**

4.1 Abstract

Mycobacterium avium subspecies *paratuberculosis* (MAP) is present on most dairy farms in Alberta, causing economic losses and presenting a potential public health concern. The objective of this cross-sectional study was to identify risk factors for Alberta dairy herds being MAP-positive based on environmental samples (ES). Risk assessments (RA) were conducted and ES were collected on 354 Alberta dairy farms (62% of eligible producers) voluntarily participating in the Alberta Johne's Disease Initiative (AJDI). In univariate logistic regression, risk factors addressing animal and pen hygiene, as well as the use of feeding equipment to remove manure, and manure application on pastures, were all associated with the number of positive ES. Furthermore, based on correspondence analysis and factor analysis, risk factors were not independent, but could be summarized as 4 independent factors: 1) animal-, pen- and feeder contamination; 2) shared equipment and pasture contamination; 3) calf diet; and 4) cattle purchase. Using these factor scores as independent variables in multivariate logistic regression models, a 1-unit increase in animal-, pen- and feeder contamination resulted in 1.31 times the odds of having ≥ 1 positive ES. Furthermore, a 1-unit increase in cattle purchase also resulted in 1.31 times the odds of having ≥ 1 positive ES. Herds with an increased score for shared equipment and pasture contamination tended to have a higher odds of > 3 positive ES. Finally, a 100-cow increase in herd size resulted in an OR of 2.1 for being above the cut-off in both models. In conclusion, cleanliness of animals, pens and feeders, as well as cattle purchase practices affected the risk of herd infection with MAP. Therefore, improvements in those

management practices might be effective tools for the control of MAP on dairy farms.

4.2 Introduction

Mycobacterium avium subsp. *paratuberculosis* (MAP) causes Johne's disease (JD), a chronic progressive enteritis that leads to culling or death of the animal (Fecteau and Whitlock, 2010). Unfortunately, MAP is common in countries with modern dairy industry with > 70% of Western Canadian dairy farms regarded as being infected (Barkema et al., 2010b; Wolf et al., 2014a). The high herd prevalence and associated production losses, as well as the potentially zoonotic nature of MAP have led to implementation of control programs in many countries (Barkema et al., 2010a; Ott et al., 1999). Because diagnosis of MAP infection is challenging (Collins et al., 2006), these programs focus on disrupting pathogen transmission. Since cows are responsible for most MAP contamination on a farm and calves are most susceptible (Sweeney, 2011b; Windsor and Whittington, 2010), interruption of direct and/or indirect cow-calf contact is the focus of most programs (McKenna et al., 2006). This is supported by several studies identifying risk factors related to direct or indirect cow to calf contact, including feeding pooled milk or colostrum (Nielsen et al., 2008), poor calving pen hygiene (Ansari-Lari et al., 2009), and group calving pens (Pithua et al., 2013; Wells and Wagner, 2000). In addition, factors related to prolonged survival of MAP in the environment, e.g. application of slurry on crop land, were also identified (Fecteau et al., 2010; Johnson-Ifearulundu and Kaneene, 1999). Nevertheless, despite prior knowledge regarding risk factors for MAP infection, there is a need for additional and

confirmatory studies, since analysed risk factors differed among studies, and management differs among populations of farms within the dairy industry. The Alberta Johne's Disease Initiative (AJDI; Wolf et al., 2014b) assessed a variety of management practices and hygiene measures for cattle in all age groups. With a participation rate of > 60% among eligible producers, the AJDI was one of the Canadian MAP control programs with the highest participation.

The objective of this study was to identify risk factors for Alberta dairy herds being MAP-positive, based on environmental samples.

4.3 Materials and Methods

4.3.1 Herd selection and data collection

The study population for this cross sectional study was comprised of herds participating in the AJDI, a voluntary JD control program available for all dairy producers in the province of Alberta, Canada (Wolf et al., 2014b). Any of the 571 dairy producers in the province could join or leave the AJDI at any time during the project (Agriculture and Agri-Food Canada, Dairy farming in Canada, 2014). Data used in this study were collected between November 2010 and April 2014 by 91 trained herd veterinarians, each of whom had completed a 5-h workshop on conducting AJDI risk assessments (RA) and collection of environmental samples (ES). Participating farms were visited annually for collection of 6 ES following a standardized protocol (Wolf et al., 2014a), and an RA was concurrently done. All ES were processed within 7 d after collection using a TREK ESP culture protocol with subsequent IS900 PCR confirmation

(Forde et al., 2013). The risk assessment contained 34 questions (Appendix C). The first 4 questions dealt with herd characteristics and information on participation in previous JD control programs. The remaining 30 questions were the actual risk factors derived from web resources and following scientific evidence (Doré et al., 2012; Elliott et al., 2014; USDA, 2003), which were divided into 6 sections: general, preweaned heifers, weaned to first calving heifers, calving area, dry cows, and lactating cows. Each section contained questions regarding potential risk factors for fecal-oral transmission of MAP between individuals and for MAP introduction. All questions were closed questions and participants were asked to choose 1 answer per question. Veterinarians were blinded throughout data collection, since ES results were not available when the RA was conducted.

4.3.2 Statistical analyses

Statistical analyses were conducted using STATA version 11 (Statacorp, College Station, TX, USA). Data from the first herd visit of each participant were included. Potential predictors were risk factors assessed within the risk assessment (Appendix C). A causal diagram was used to image potential associations prior to the analysis (Greenland et al., 1999). It visualized that certain risk factors (trade, cattle shows) were associated with MAP introduction into a herd, whereas others (cleanliness, calving management) were associated with within-herd transmission (Figure 4-1). It was noteworthy that within-herd transmission could only occur in MAP-infected herds and influenced MAP within-herd prevalence, which further impacted the number of positive ES. The number of clinical cases and test-positive animals (Q6) was a collider on this

pathway and was therefore excluded from any further analysis (Greenland et al., 1999).

Three approaches were used for data analysis following recommendations for dealing with large numbers of independent variables (Dohoo et al., 1997): 1) univariate analysis to assess crude associations between the outcome and risk factors; 2) correspondence analysis to graphically display associations between the outcome and associated risk factors as well as between risk factors; and 3) multivariate analysis combined with factor analysis to adjust for multicollinearity in the dataset.

4.3.3 Univariate analysis

Logistic regression models were built with 2 outcomes: 1) 0 positive ES vs ≥ 1 positive ES; and 2) < 4 positive ES vs > 3 positive ES. Separate models were built for each of the risk factors included as categorical predictors. A *P*-value < 0.05 overall or for at least one of the categories was considered significant.

4.3.4 Correspondence analysis

Risk factors significantly associated with the outcome in the univariate analysis were included. In addition, the analysis included risk factors associated with the outcome using χ^2 tests ($P < 0.20$) and the known risk factor herd size. To make it simpler to interpret the resulting correspondence plot, the number of categories for all variables was reduced: the number of positive ES were categorized into 3 groups (0, 1 – 3, > 3 positive ES). Risk factors (- = lower 2 scores, + = higher 2 scores) and herd size (< 150 cows, > 149 cows) were dichotomized.

4.3.5 Multivariate analysis

To avoid multicollinearity, the independent variable matrix was summarized using factor analysis. In addition to the RA questions (Q5 – Q34, except Q6), herd size, and housing type were included. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy was used to assess overall suitability of the dataset, as well as suitability of variables to be included in the factor analysis (Dziuban and Shirkey, 1974). A KMO value > 0.7 was interpreted as medium fit of the data. Variables with a KMO < 0.5 (unacceptable fit) were excluded from factor analysis. After assessing which variables should be included in the analysis, the factor analysis was re-run and factors with an Eigenvalue > 1 were retained. Factor loadings > 0.4 after varimax rotation were used to describe the factors (Sieber et al., 1987).

Predicted factor scores were used as independent variables in 2 logistic regression models. The analysis used the same outcomes as the univariate analysis (0 positive ES vs ≥ 1 positive ES and, < 4 positive ES vs > 3 positive ES). A manual backward elimination procedure was used to obtain the final model. Any variables excluded from the factor analysis because KMO < 0.50 were considered additional predictors. A *P*-value < 0.05 was interpreted as a significant association between the predictor and the outcome, whereas a *P*-value between 0.05 and 0.10 was interpreted as a trend.

4.4 Results

A total of 370 herds participated in the AJDI. Sixteen farms submitted only ES and no RAs and were therefore excluded from the analysis. Furthermore, 2 farms submitted incomplete RAs because all cattle in certain age groups were housed on other farms; therefore, these 2 farms were excluded from univariate analysis of questions with missing data and from the factor analysis. There were 225 (64%) of the 354 farms with RAs and ES that had only MAP-negative ES, 80 (23%) had 1 – 3 positive samples, and 49 (14%) had 4 – 6 positive samples. Mean herd size was 139 adult cows (range, 40 to 680). The majority (87%) of herds were in free-stall barns, whereas 6 and 7% of herds were housed in loose-housing and tie-stalls, respectively.

4.4.1 Univariate analysis

Of the 30 risk factors analyzed in univariate logistic regression, 8 were associated with the number of positive ES. All 8 management practices were related to manure contamination: manure contamination of feeding equipment, poor cleanliness of heifers and cows, and application of manure on pastures and crop land used the same year for production of cattle feed were associated with the number of positive ES in univariate logistic regression (Table 4-1 and Figure 4-1).

4.4.2 Correspondence analysis

In addition to risk factors identified in univariate analysis, risk at purchase (Q7.1, $P\ \chi^2 =$

0.10), colostrum pooling (Q10, $P = 0.14$), manure on weaned heifer feed bunks and waterers (Q18, $P = 0.19$), manure contamination of weaned heifers (Q20, $P = 0.10$), and calves born outside the calving pen (Q26, $P = 0.14$) were included in the correspondence analysis. The correspondence plot had a cluster of herds with > 3 positive ES with poor cleanliness of animals, calf buckets and feeders (Figure 4-2). Several risk factors such as shared equipment were closely associated with each other. However, there was no evidence for an association between risk factors and herd size. In addition, there was no evidence for a different risk factor matrix for farms with 0 positive ES and farms with 1 – 3 positive ES.

4.4.3 Multivariate analysis

Herd size, type of lactating cow housing and attendance at cattle shows (Q8) had KMO values < 0.5 and therefore were excluded from the factor analysis. The other risk factors were summarized with 4 factors that had an eigenvalue > 1 (Figure 4-3). The overall KMO value was 0.75, consistent with medium fit of the data, and the 4 factors accounted for 86% of the variance in the data. Factor 1 had factor loadings > 0.4 for manure contamination of water troughs and feed bunks in calf-, heifer-, dry cow-, and lactating cow pens, respectively (Table 4-2). Factor 1 was also associated with manure contamination of cows and heifers and with calving pen contamination. Therefore, it was named Animal-, pen- and feeder contamination. Factor 2 (Shared equipment, pasture contamination) had factor loadings > 0.4 for the use of feeding equipment to remove manure, and with application of manure on pasture and crop land used by cows in the same year. Factor 3 (Calf diet) had factor loadings > 0.4 for feeding of non-saleable

milk and bulk milk to calves, as well as letting calves nurse. Factor 4 (Cattle purchase) had factor loadings > 0.4 for high frequency of cattle purchases and a low level of precaution during purchase. Median factor scores ranged between -0.09 for factor 1 and 0.34 for factor 4. Interquartile ranges of the factors varied between 0.71 for factor 4 and 2.01 for factor 2 (Figure 4-4).

In the final logistic regression, a 1-unit increase in Factor 1 (Animal-, pen- and feeder contamination) resulted in 1.31 times the odds of having ≥ 1 positive ES. A 1-unit increase in Factor 4 (Cattle purchase) also resulted in 1.31 times the odds of having ≥ 1 positive ES. Herds with an increased score in Factor 2 (Shared equipment, pasture contamination) tended to have a higher odds of having > 3 positive ES ($P = 0.096$). A 100-cow increase in herd size resulted in an OR of 2.1 for being above the cut-off in both models (≥ 1, > 3 positive ES). Lactating cow housing, attendance at cattle shows (Q8) as well as Factor 3 (Calf diet) were not significantly associated with any of the ES outcomes (Table 4-3).

4.5 Discussion

Animal-, pen- and feeder contamination with manure, cattle purchase frequency and purchase precautions, as well as the use of feeding equipment to remove manure and application of manure on forage land and pasture increased the risk for MAP-positive ES. Therefore, improvements in those management practices might be effective tools for the control of MAP on dairy farms.

Manure contamination of animals, waterers and feed troughs, as well as calving pens (Factor 1) were positively associated with the number of MAP culture-positive ES. There were reduced odds for ELISA-positive cattle on farms that cleaned calving pens after each use (Johnson-Ifearulundu and Kaneene, 1998), which partially confirmed our findings. The association was biologically plausible, since high levels of contamination likely increased risk of MAP ingestion in infected herds. It would obviously be important to know in which age groups these transmission events occurred, particularly since recent work provided evidence that cattle up to at least 1 y of age were susceptible to MAP and that exposed cattle shed MAP soon after infection (Mortier et al., 2013; Mortier et al., 2014b). However, based on the correspondence plot as well as the factor loadings, it was clear that individual risk factors were correlated. For example, whereas only 6% of farms with visibly clean lactating cows had severely contaminated heifers, 28% of farms with severely contaminated lactating cows had severely contaminated heifers (results not shown). Consequently, risk factors for specific age groups could not be identified, although it was noteworthy that herds with poor hygiene in pens were more likely to be MAP-positive.

In contrast to Factor 1 (Animal-, pen- and feeder contamination), Factor 4 (Cattle purchase) described a risk factor for MAP introduction rather than MAP within-herd transmission. As in a previous study, herds with frequent cattle introductions and low precautions during purchase were more likely to be MAP infected (Nielsen and Toft, 2011). Considering that ~18% of cattle in Alberta are estimated to be infected with MAP (Scott et al., 2006), cattle purchases were regarded as an important route for MAP introduction.

Factor 2 (Shared equipment and pasture contamination) tended to be associated with the number of positive ES. This factor had high loadings on the use of the same equipment for feeding and manure removal, as well as manure application on pastures and crop land used in the same year. Since many of these risk factors were associated with the outcome in univariate analysis, the factor was retained in the final model despite a *P*-value > 0.05. Significance was also supported by prior evidence for the plausibility of this association, since MAP survives in the environment for extended intervals, making contaminated equipment and crop land use after manure application a likely risk factor (Whittington et al., 2004). In addition, in a US study, there was an increased risk for herds testing positive if manure was spread on land where calf feed was grown (Johnson-Ifearulundu and Kaneene, 1999).

Larger herds had higher odds for being MAP-infected than smaller herds, which was previously attributed to differences in management between large and small herds, as described in several publications (Pillars et al., 2009; Wells and Wagner, 2000; Wolf et al., 2014a). It was hypothesized that large herds purchase more cattle, are more likely to pool milk and colostrum, and have > 1 cow at a time in calving and sick cow pens, which was partially supported by findings of the National Animal Health Monitoring System (NAHMS; USDA, 2002). However, the correspondence analysis did not identify a risk factor cluster associated with a specific herd size. Also, herd size was excluded from the factor analysis, because KMO < 0.5 provided no evidence for herd size-dependent risk factors. Notwithstanding, perhaps management practices that were not assessed within the AJDI were herd size dependent and affected the number of positive ES. Potential candidates were management practices related to calf housing, since

infected calves excreted MAP (Mortier et al., 2014b), and calf-to-calf transmission was documented (Van Roermund et al., 2007). However, contacts between infectious and susceptible cattle might be more frequent in large herds, resulting in increased frequency of MAP transmission. Nonetheless, more research is needed, especially since dairy herds keep increasing in size (Barkema et al., submitted).

Included risk factors and their odds ratios were slightly different between the 2 outcomes used in the logistic regression models (0 positive ES vs ≥ 1 positive ES and, < 4 positive ES vs > 3 positive ES). A reason for that is that within-herd prevalence is positively associated with the number of positive ES (Lavers et al., 2013). We inferred that by keeping aside potential misclassification through imperfect accuracy, Model 1 (0 positive ES vs ≥ 1 positive ES) estimated the odds for a herd being infected vs uninfected, whereas Model 2 (< 4 positive ES vs > 3 positive ES) distinguished herds with higher within-herd prevalence from uninfected and low-prevalence herds. Risk factors around disease introduction (Cattle purchase) had higher odds ratios in Model 1, whereas risk factors around within-herd transmission (Animal-, pen- and feeder contamination) had higher odds ratios in Model 2. Although this may have been evidence for different risk factors between disease introduction and within-herd transmission, it is noteworthy that ES did not accurately predict the true within-herd prevalence in a herd.

Many risk factors regarding colostrum and calving pen management were not associated with farms being ES-positive, but were biologically plausible and previously reported. However, these practices should not be neglected in MAP control. Because the present study was observational, the data distribution compromised identification of some known risk factors. For

example, only 9 farms raised their heifers on external rearing operations where they were mixed with young stock from other herds. Unfortunately, it was not possible to determining the association with herds being MAP-infected with this risk factor, although that could be a focus of future research.

The identified risk factors were regarded as important for the control of other infectious diseases and should be included in any biosecurity program. Increased purchase volumes with low precautions also increased the risk for introduction of many other pathogens such as infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus (BVDV), or digital dermatitis (DD, Van Schaik et al., 2002). Animal and pen cleanliness also impacted transmission of other pathogens. Whereas low hygiene standards in calf housing increased the incidence of diarrhea as well as bovine respiratory disease (BRD, Johnson et al., 2011), a ‘quick and dirty’ management style for cows increased both the bulk milk somatic cell count and the incidence of clinical mastitis (Barkema et al., 1999). Therefore, this additional importance of the identified risk factors strengthened the evidence for inclusion of these factors in biosecurity programs.

As in a previous study (Berghaus et al., 2005), some risk factors were correlated, which could have been regarded as a limitation, since this lack of independence compromised the ability to identify specific risk factors for MAP infection. However, we also inferred that management decisions were not made independently, but instead followed a management strategy on a farm, which could be influenced by herd advisers as a whole in order to reduce disease spread.

Inter-observer variability in scoring of risk factors and ES collection was not assessed in the present study, but was mitigated through observer training, since all personnel involved in data collection went through a training seminar explaining standard procedures regarding sample collection and risk assessment conduction. Therefore, this was expected to have reduced the magnitude of this potential bias.

The cross-sectional study design only allowed assessment of associations, but did not allow assessment of causation. Because exposure and environmental contamination were measured concurrently, farms could have changed their management as part of previous control efforts, but could still have had a high within-herd prevalence since management changes were not yet effective. This would result in biased odds ratios, or biologically plausible risk factors would appear to be protective. Fortunately, the potential for this bias appeared to be low, since the majority of farms were not involved in any MAP control programs within the past 10 y and none of the biologically plausible risk factors were inversely associated with the outcome. Notwithstanding, there is still potential for bias, since temporality was not assessed.

Environmental samples were used to assess the outcome with a sensitivity of ~70%, misclassifying some low prevalence herds as negative (Wolf et al., 2014a). Because this happened independent of exposure, non-differential misclassification caused an underestimation of the odds of MAP infection on dairy herds, suggesting that reported odds ratios were rather conservative (Dohoo et al., 2003a).

There was no evidence for violation of internal validity of the study, because herd prevalence and herd size were similar between AJDI participants and none participants (Ritter et

al., 2014a). However, external validity was difficult to estimate, since comparable management data from other dairy populations were not available. Regardless, herd sizes appeared similar to those in some European countries and some US states, and most cows were managed in free-stall barns, a rather standardized housing system. Therefore, results can likely be generalized to mid-sized farms using free-stall barns.

The next step in research on risk factors for MAP infection should be to test reported risk factors in a setting that allows assessment of causality (e.g. randomized controlled clinical trials or cohort studies). It was noteworthy that these studies were previously compromised due to long follow up periods, because infection could only be diagnosed long after exposure. However, since it was recently reported that MAP infection can be detected soon after exposure (Mortier et al., 2014b; Mortier et al., 2014c), this is expected to create new possibilities for future studies with much shorter follow-up periods.

4.6 Conclusions

Poor hygiene of cattle, feed and water troughs as well as calving pens, high purchase frequency, and low precautions during purchase, as well as the use of manure equipment for feeding and spreading of manure on pasture used the same year increased the risk for herds having positive environmental samples. Therefore, addressing these factors should be part of any MAP control efforts on dairy farms.

4.7 References

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Table 4-1: Risk factors associated with number of *Mycobacterium avium* subspecies *paratuberculosis* positive environmental samples (ES) on dairy farms, based on univariate logistic regression ($P < 0.05$).

	0 vs. ≥ 1 ES		< 4 vs. > 3 ES	
	OR	*P*	OR	*P*
Cow manure on milk feeding utensils (Q13)[2]		0.09[1]		0.10
• No manure, utensils washed daily	Reference		Reference	
• Traces of manure, but utensils washed at least weekly	1.0	0.96	1.2	0.65
• Some manure contamination	2.8	0.03	3.1	0.03
• Extensive manure contamination	No observations			
Cow manure in calf water buckets and feed bunks (Q14)		0.74		0.10
• All calf feed and water containers are clean	Not sign.		Reference	
• A scant amount of manure is visible			1.3	0.42
• Manure is clearly visible in calf feeders or water buckets			3.8	0.03
• Extensive manure contamination			No obs.	
Manure spread on heifer forage or pasture used the same year (Q21)		< 0.01		0.04
• Manure is never spread on land used for heifers	Reference		Reference	
• Manure is spread on forage land used to feed heifers	2.8	< 0.01	2.1	0.05
• Manure is spread on land next to heifer pastures	2.9	0.03	2.5	0.14
• Manure is spread on pastures where heifers graze	3.1	< 0.01	3.5	< 0.01
Manure contamination on cows in calving pens (Q24)		0.17		< 0.01
• No visible manure, udder hair is clipped and teats are washed	Reference		Reference	
• Manure present above dewclaws but not on teats or udders	1.2	0.54	0.6	0.32
• Manure present up to the hocks or on teats or udders	1.1	0.83	0.8	0.73
• Manure present above hocks and on teats or udders	3.6	0.04	5.3	0.01

Table 4-1: (continued)

Manure contamination of dry cow feed bunks and water troughs (Q29)		0.48		0.03
• No manure contamination	Not sign.		Reference	
• Trace amounts of manure visible			1.9	0.12
• Manure clearly visible			2.3	0.08
• Extensive manure contamination			17.1	< 0.01
Manure contamination in dry cow forage or on pasture used the same year (Q30)		0.03		0.03
• Feeding equipment not used to scrape manure, manure not spread on pasture or crops	Reference		Reference	
• Feeding equipment not used to scrape manure, manure spread on crops but not pasture	2.4	< 0.01	4.3	< 0.01
• Feeding equipment not used to scrape manure, manure spread on pasture or crop land	2.3	0.03	2.2	0.19
• Feeding equipment used to scrape manure	1.5	0.13	2.5	0.04
Manure contamination of lactating cow feed bunks and water troughs (Q32)		0.07		< 0.01
• No manure contamination	Reference		Reference	
• Trace amounts of manure visible	1.8	0.03	3.1	0.02
• Manure clearly visible	1.7	0.15	6.5	< 0.01
• Extensive manure contamination	4.0	0.04	19.6	< 0.01
Manure contamination in lactating cow forage or on pasture (Q33)		0.01		0.02
• Feeding equipment not used to scrape manure, manure not spread on pasture or crops	Reference		Reference	
• Feeding equipment not used to scrape manure, manure spread on crops but not pasture	2.9	<0.01	4.5	< 0.01
• Feeding equipment not used to scrape manure, manure spread on pasture or crop land	2.4	0.03	2.2	0.19
• Feeding equipment used to scrape manure	1.5	0.15	2.5	0.03
Manure contamination of lactating cows (Q34)		0.09		0.12
• No manure above fetlocks	Reference		Reference	
• Manure below knees/hocks	2.0	0.07	2.3	0.19
• Manure above knees/hocks, but not on flanks	2.0	0.09	2.7	0.15
• Manure above knees/hocks and on flanks	3.4	0.01	5.0	0.02

[1]Number of MAP-positive cultures of 6 collected environmental samples
[2]Question number in Risk Assessment (Appendix C)

Table 4-2: Factor loadings on factors summarizing a risk assessment used as part of a Johne's disease control program on dairy farms (factor loadings > 0.4 are marked in bold).

	Factor			
Risk factors	1	2	3	4
General				
Visitor access (Q5)[1]	0.15	0.16	0.31	0.17
Purchase frequency (Q7)	0.01	-0.03	-0.11	**0.84**
Risk at purchase[2] (Q7.1)	0.01	0.01	-0.02	**0.86**
Preweaned heifers				
Custom heifer rearing (Q9)	0.09	-0.02	0.11	0.02
Colostrum pooling[2] (Q10)	0.04	0.10	0.27	0.04
Feeding non-saleable milk (Q11)	-0.02	0.01	**0.64**	-0.12
Feeding bulk/pooled milk (Q12)	0.02	0.01	**0.65**	-0.17
Cow manure on calf buckets[2] (Q13)	0.32	0.11	0.16	0.06
Manure on calf feed and waterers[2] (Q14)	**0.50**	0.17	0.12	0.05
Proximity to cows/group housing (Q15)	0.21	0.15	-0.11	0.10
Staff contamination (Q16)	0.35	0.15	-0.11	-0.01
Weaned heifers				
Exposure to cow manure/runoff (Q17)	0.19	-0.01	0.03	0.07
Manure on feed bunks and waterers[2] (Q18)	**0.60**	0.08	0.13	0.01
Use of feeding equipment to remove manure (Q19)	0.03	**0.74**	0.01	0.02
Animal contamination[2] (Q20)	**0.60**	0.08	-0.10	0.06
Manure spread on heifer forage or pasture[2] (Q21)	0.09	0.27	0.17	0.01
Calving area				
Number of cows in calving pen (Q22)	0.23	0.03	0.01	0.14
Calving pen contamination (Q23)	**0.47**	0.03	0.17	0.14
Animal contamination[2] (Q24)	**0.66**	-0.01	0.05	0.03
Use of calving area for sick cows (Q25)	0.23	0.12	0.06	0.08
Calves born outside calving pen[2] (Q26)	0.14	0.03	0.07	-0.06
Nursing (Q27)	0.16	0.16	**0.44**	-0.06
Time that calves spent with dam (Q28)	0.14	0.10	**0.46**	-0.01
Dry cows				
Manure on feed bunks and waterers[2] (Q29)	**0.67**	0.13	0.08	-0.01
Shared equipment, manure on pasture grass[2] (Q30)	0.10	**0.90**	0.02	-0.03
Animal contamination (close up cows) (Q31)	**0.68**	0.04	-0.05	-0.07
Lactating cows				
Manure on feed bunks and waterers[2] (Q32)	**0.56**	0.19	0.12	-0.03
Shared equipment, manure on pasture grass[2] (Q33)	0.06	**0.89**	0.03	0.01
Animal contamination[2] (Q34)	**0.57**	0.14	-0.08	0.04

[1]Question number in Risk Assessment (Appendix A)

[2]Associated with number of positive environmental samples in χ^2 test ($P < 0.20$).

Table 4-3: Final logistic regression model on the association between number of *Mycobacterium avium* subspecies *paratuberculosis* positive environmental samples (ES) and factor scores summarizing information of a Johne's disease risk assessment on 352 dairy farms in Alberta, Canada.

	0 vs ≥ 1 positive ES			< 4 vs > 3 positive ES		
	OR	*P*	95% CI	OR	*P*	95% CI
Intercept[1]	-1.61	< 0.001	-2.11 - -1.11	-3.12	< 0.001	-3.81 - -2.43
Factor 1 (Pen and animal contamination)	1.31	0.034	1.02 – 1.68	1.85	< 0.001	1.31 – 2.61
Factor 2 (Shared equipment, pasture contamination)			Not associated	1.35	0.096	0.95 – 1.93
Factor 4 (Cattle purchase)	1.31	0.050	1.00 – 1.72			Not associated
Herd size (in 100-cow increments)	2.08	< 0.001	1.51 – 2.86	2.10	< 0.001	1.49 – 2.99

[1]Values are coefficients (log odds)

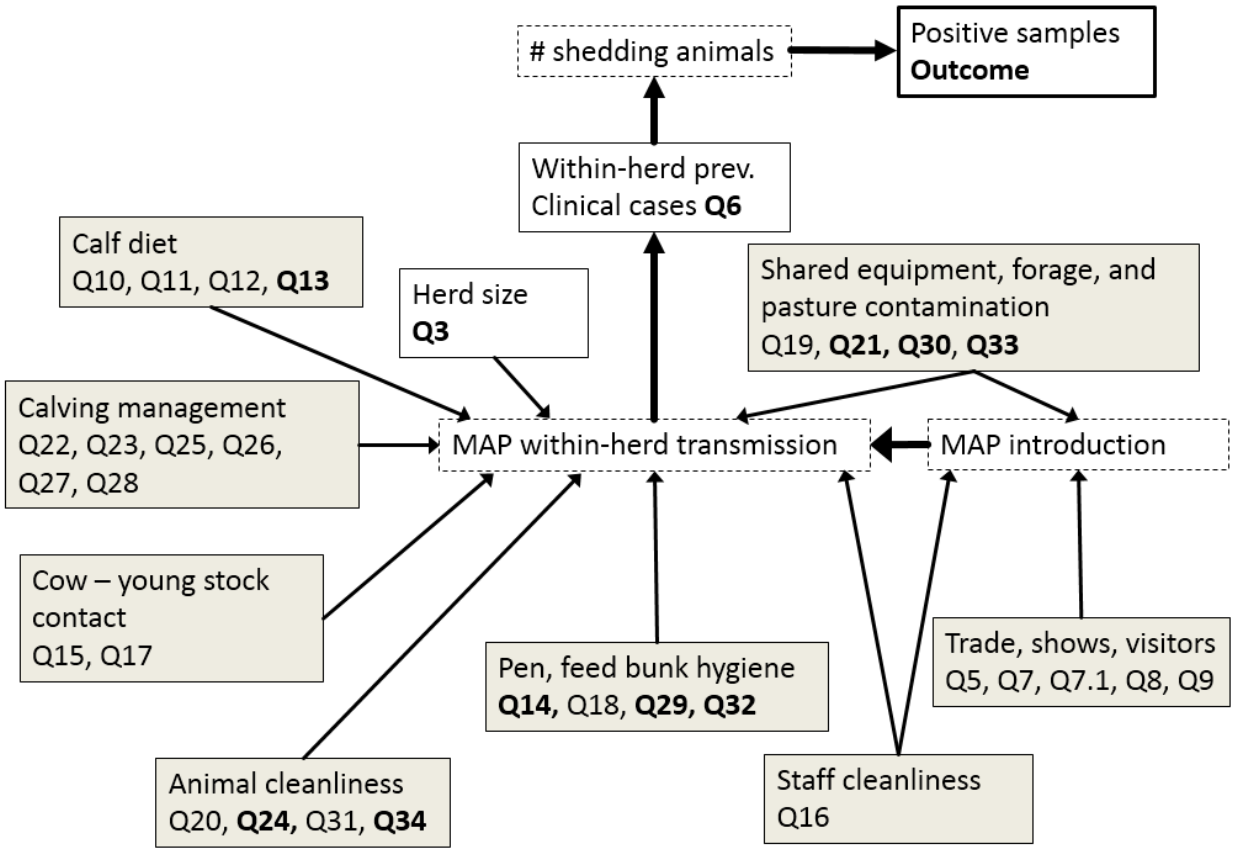
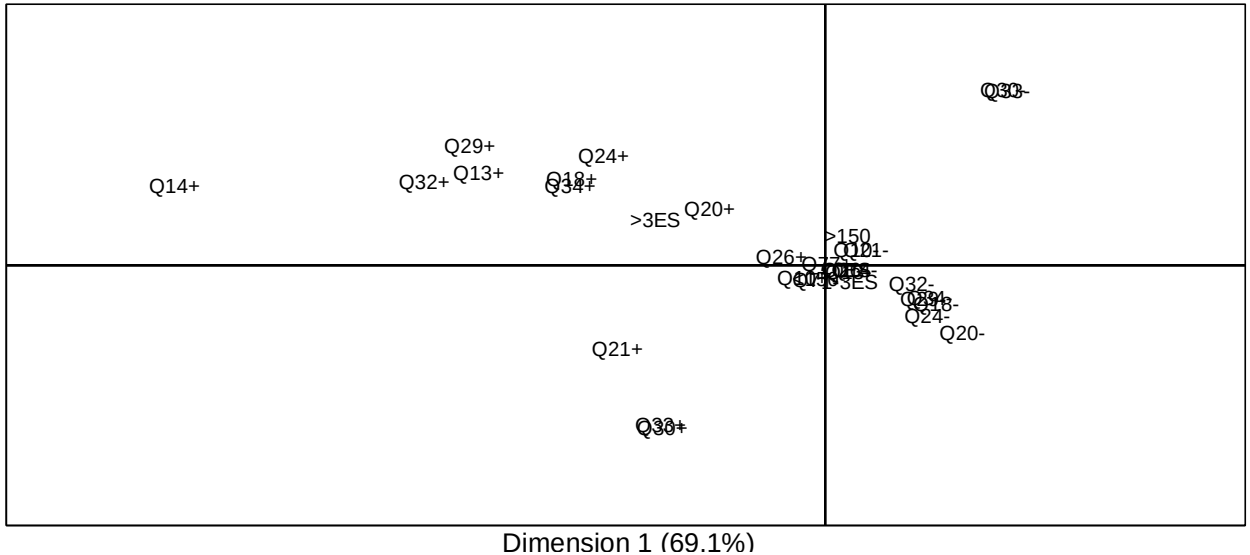


Figure 4-1: Causal diagram illustrating associations between risk factors assessed with questions (Q; see Appendix A) as part of the Alberta Johne's Disease Initiative (AJDI) risk assessment and the number of *Mycobacterium avium* subspecies *paratuberculosis* positive environmental samples. Dashed boxes represent factors influencing the association but not assessed in the AJDI. Risk factors associated with the number of positive environmental samples using univariate logistic regression ($p < 0.05$) are in bold.



General: Risk at purchase (Q7.1)
Calves: Colostrum pooling (Q10)
Calves: Cow manure on calf buckets (Q13)
Calves: Manure on calf feed and waterers (Q14)
Heifers: Manure on feed bunks and waterers (Q18)
Heifers: Animal contamination (Q20)
Heifers: Manure spread on forage or pasture (Q21)
Calving pen: Animal contamination (Q24)
Calvin pen: Calves born outside calving pen (Q26)
Dry cows: Manure on feed bunks and waterers (Q29)
Dry cows: Shared equipment, manure on pasture grass (Q30)
Lactating: Manure on feed bunks and waterers (Q32)
Lactating: Shared equipment, manure on pasture grass (Q33)
Lactating: Animal contamination (Q34)

Figure 4-2: Correspondence analysis on the associations between risk factors, herd size and the number of positive environmental samples (ES). Risk factors were dichotomized (Q..+ = score ≤ 2, Q..- = score > 2 for questions with 4 categories), herd size was dichotomized (< 150, > 149 cows), and number of positive ES was categorized into 3 groups (0 ES, 1 – 3 ES, 4 – 6 ES).

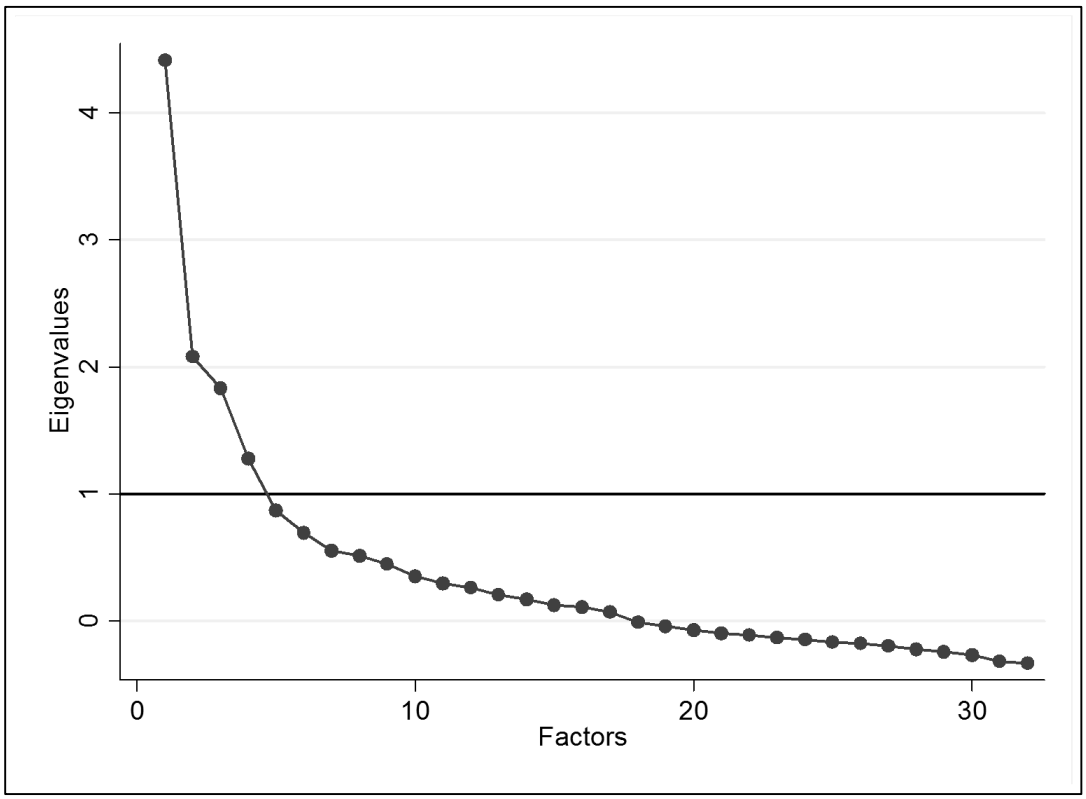


Figure 4-3: Eigenvalues of factors describing questions of the risk assessment used in the Alberta Johne's Disease Initiative.

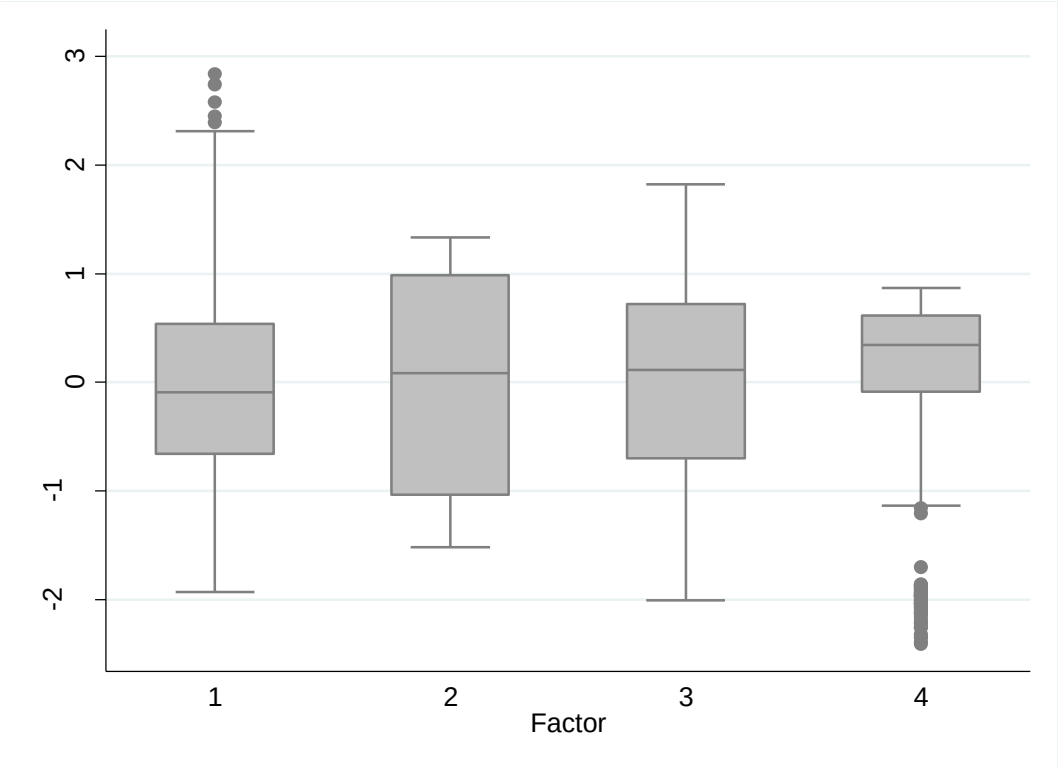


Figure 4-4: Summary statistics on 4 factors describing questions of the risk assessment used in the Alberta Johne's Disease Initiative.

Chapter Five: **FACTORS IMPACTING MANAGEMENT CHANGES IMPLEMENTED ON FARMS PARTICIPATING IN A JOHNE'S DISEASE CONTROL PROGRAM**

5.1 Abstract

Modern Johne's disease (JD) programs aim to control *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection through implementation of management practices that reduce the probability of MAP introduction and within-herd transmission on dairy farms. The success of these programs depends on adoption of suggested management changes by dairy farmers. The objectives of this study were to: 1) evaluate factors as assessed in a Risk Assessment (RA) that motivate producers to make management improvements, and 2) assess whether management improvements were influenced by previously received test results. The RAs measuring on-farm management were conducted annually by herd veterinarians on 370 dairy farms participating in the Alberta Johne's Disease Initiative (AJDI). Each RA consisting of 34 questions was accompanied by a management plan (MP) with no more than 3 proposed management changes that should reduce the risk of MAP introduction and transmission for the herd. The MAP infection status of the herds was assessed through culture of 6 environmental samples (ES). Overall, 76% of farms improved their total RA score between their first and their last RA. In the final multilevel logistic regression model, questions related to a management change that the farmer and the veterinarian had agreed upon had 1.32 times the odds to improve in score compared to questions for which no change was proposed. The odds for improving the score of a question were 1.59 times higher between 1st and 2nd RA than between 2nd and 3rd RA. Farms with > 3 culture-positive ES collected the previous year had 1.31 times the odds for improving the score of a question than ES culture-negative farms.

In conclusion, improvements in management were not randomly distributed within farms participating in a management based disease control program. Instead, knowledge of MAP infection status of a herd, agreed management changes during the previous year, and duration of participation influenced whether management changes were implemented.

5.2 Introduction

Mycobacterium avium subsp. *paratuberculosis* (MAP) is prevalent on dairy farms worldwide (Barkema et al., 2010b), causes economic losses to the industry (Wolf et al., 2014b), and is potentially associated with Crohn's disease in humans (Atreya et al., 2014). After intrauterine, or fecal-oral infection, cattle go through an extended incubation period that can ultimately result in development of Johne's disease (JD), a non-curable enteritis (Sweeney, 2011a). During incubation, infected cattle frequently remain undetected, because immune response and bacterial shedding occur only intermittently (Mortier et al., 2014a; Mortier et al., 2014b), and available tests lack accuracy (Nielsen and Toft, 2008). This compromises the effectiveness of testing and culling for successful eradication of MAP on cattle farms (Franklyn, 2011; Whitlock and Buergelt, 1996). As a consequence, most MAP control programs are not solely based on testing and culling, but encourage implementation of best hygiene management practices to control transmission of MAP between infectious and susceptible cattle (McKenna et al., 2006). These MAP control programs can, however, only be effective if farmers implement management changes that mitigate associated risks for MAP transmission. In 2 US studies, farms

generally improved their management during participation in a MAP control program (Raizman et al., 2006; Wells et al., 2008). Nevertheless, whereas the majority of Ontario dairy farms significantly improved their management during participation in a MAP control program, management on 20% of the farms significantly worsened (Sorge et al., 2011).

Decisions regarding management changes are complex and motivated by expected increases in profit (Edwards-Jones, 2006). Therefore, in a disease control program, farmers that know about the presence of a pathogen are more likely to be motivated to invest in its control (Mueller and Jansen, 1988; Sorge et al., 2011). Furthermore, owners of large herds are described as more progressive than owners of small herds and might therefore be more likely to implement management improvements (Bergevoet et al., 2004; Sayers et al., 2013). The objectives of this study were to: 1) evaluate factors as assessed in a risk assessment (RA) for MAP control that motivated producers to make management improvements, and 2) assess whether management improvements were influenced by previously received test results.

5.3 Materials and Methods

5.3.1 Data collection

Participants in this longitudinal study consisted of the 370 dairy farms (63% of Alberta, Canada, dairy farms) that voluntarily participated in the Alberta Johne's Disease Initiative (AJDI), a management-based MAP control program (Wolf et al., 2014a). Data collection was conducted between November 2010 and April 2014. During that interval, farmers could join and

leave the program at any given time. Herds were visited annually by veterinarians, who were trained during a half-day workshop, to conduct an RA that identified risk factors for MAP infection on the farm (Appendix C). The first 4 RA questions dealt with herd characteristics (e.g. herd size and housing), whereas Questions 5 – 34 were actual risk factors and management practices as related to MAP introduction and transmission and were based on previous suggestions for RA design (USDA, 2003). Questions had categorical scores (1, 2, 3...), and a higher score meant an increased risk. After identifying high-risk areas, veterinarians and farmers decided on a maximum of 3 to be implemented as management improvements, which were recorded in a management plan (MP). To assess whether knowledge on test results influenced decisions on actual management improvements, 6 environmental samples (ES) were collected during herd visits (Wolf et al., 2014a), and processed within 1 week after collection using a standardized liquid culture protocol, with subsequent PCR confirmation conducted on all samples (Forde et al., 2013; Wolf et al., 2014a).

5.3.2 Statistical analysis

Data were entered in Microsoft Access (Microsoft Corporation, 2007), and management suggestions were assigned to associated RA questions. In case of implementation, scores for these questions were expected to decrease. Eight suggestions did, if implemented, not result in a score improvement in any of the RA questions, mainly because the suggested management practices were regarded insufficient for controlling the risk of MAP transmission in a specific area and were not assigned to any questions (e.g.:, not to use milk from test-positive cows to feed

calves, or to disinfect equipment used to remove manure before using it to handle feed). Data analysis was conducted using STATA 11 (Statacorp, College Station, TX, USA), and statistical significance was defined at $P < 0.05$.

The number of suggestions in the MPs between farms in different years of AJDI participation was compared using a Chi-square test on contingency tables. Multilevel logistic regression on question level was used to identify predictors for changes in individual management practices. The outcome was management improvement defined as score reduction in a specific question between 2 consecutive RAs (dichotomous: yes = 1, no = 0), and the predictors were: 1) whether a question-related management change was agreed upon as recorded in the previous MP (dichotomous: yes = 1, no = 0); 2) assigned question category (categorical: cattle introductions, visitor access, calf liquid diet, young stock housing, calving pen, lactating and dry cow pens, feeding hygiene and equipment use, and management of likely infected cattle); 3) year of participation (categorical: management improvements in the 2nd, 3rd and 4th year); 4) mean RA score in the previous year (categorical: 1st, 2nd, 3rd, and 4th quartile of mean RA scores in the dataset); 5) herd size (< 100, 100 – 199, > 199 cows); and 6) number of positive ES in the previous year (categorical: 0, 1 – 3, > 3). The analysis was adjusted for the score in the specific question during the previous year (0 = score 1 and 2, 1 = score > 2). A manual backward selection procedure was used for variable selection, and coefficient changes > 20% were considered as evidence of confounding. To adjust for clustering in the dataset, veterinarian, and farm were included as random effects resulting in a 3-level model. The reported odds ratios (OR) for score reduction were cluster-specific (Dohoo et al., 2009). Population-averaged ORs were not

reported because preliminary analysis revealed that the cluster-specific ORs were very similar to the population averaged ORs (< 1% difference). The latent variable approach was used to estimate intra-class correlation coefficients (ICC), a measure for the magnitude of clustering by veterinarian and farm (Vigre et al., 2004).

5.4 Results

A total of 709 RAs and MPs conducted by 55 veterinarians were received. A total of 137 farms submitted 1 RA, 134 submitted 2 RAs, 92 submitted 3 RAs, and 7 farms submitted 4 RAs. Mean herd size was 139 adult cows, ranging from 40 to 680 cows, with 87, 6, and 7% of herds housed in free-stalls, loose-housing, or tie-stalls, respectively. Of the 709 RAs, 4 had missing observations for some of the questions, because some age-groups were housed off site. Data from these RAs were included in the analysis for questions with available data.

Farmers and veterinarians agreed upon a total of 1,598 management changes (Table 5-1). Fourteen MPs (all from different farms) did not include any suggestions. The number of management suggestions decreased with increasing year of a farm's AJDI participation from a mean of 2.38, 2.14, 2.01 and 1.86 suggestions per farm in Years 1, 2, 3, and 4, respectively ($P < 0.01$). In 8 cases, the suggestion could not be identified due to poor handwriting and was excluded from further analysis. The most frequently suggested changes were to increase the number of calving pens (n = 135; 8% of all suggestions), to separate calves from cows soon after

birth (n = 128; 8%), and to avoid spreading manure on pastures grazed the same year (n = 110; 7%; Table 5-1).

The total (sum) RA score between the first and the last RA improved on 76% (95% CI: 70 – 81%) of participating farms (Figure 5-1). Farms most frequently reduced their scores (risk decrease) in questions around calving pen hygiene (Q23; 25% of risk assessments), purchase rates (Q7; 24%), and use of feeding equipment to remove manure, and manure contamination on pastures and crop land (Q19, Q30, Q33, 23 – 25% dependent on the group of animals) (Table 5-2). Whereas 19% of the questions improved their score between 2 RAs on farms with 0 MAP-positive ES, 21% of the questions improved their score between 2 RAs on farms with > 3 MAP-positive ES (Table 5-3). In the final logistic regression model, farms with > 3 positive ES during the previous year had 1.31 times higher odds for improving the score of a question than ES-negative farms ($P = 0.024$). A question that resulted in a farmer and veterinarian agreed-upon change in management was 1.32 times more likely to improve in score than a question where no change was proposed ($P = 0.001$). The odds for improving the score of a question was 1/0.63 =1.59 times higher after the 1st RA than after the 2nd RA ($P < 0.001$). Questions regarding calf diet (OR = 1.49), young stock housing (OR = 1.48), calving pen (OR = 2.22), lactating and dry cow pens (OR= 2.94), feeding hygiene/equipment use (OR = 2.16), and management of likely infected cattle (OR = 1.66) had higher odds for improvement than questions regarding cattle introductions ($P < 0.001$).

5.5 Discussion

Farms with MAP culture-positive ES were more likely to implement management improvements than culture-negative farms. Questions for which a management change was agreed in the previous year were more likely to reduce their score, and the probability for management improvements decreased with increasing years of participation.

As observed in other MAP control programs, most participating farms improved their total RA scores throughout participation (Raizman et al., 2006; Sorge et al., 2011; Wells et al., 2008). The impact of previously received test results was in agreement with Sorge et al. (2011) who reported more frequent improvements in calving, and colostrum management in herds with MAP ELISA-positive cows compared to herds with MAP ELISA-negative cows (Sorge et al., 2011). A similar relationship was also observed for crop farmers, with those that perceived higher losses caused by a specific pest being more likely to implement a new spraying technique than those that perceived lower losses (Mueller and Jansen, 1988). The underlying driver for this association was obviously farm economics: positive test results made farmers aware of the presence of MAP in their herd and the associated economic losses. That management improvements were dependent on test results has both positive and negative implications. On one hand, implementation of new management practices results in a higher net benefit for MAP infected herds than uninfected herds (Wolf et al., 2014b). On the other hand, environmental sampling detects only 70% of infected dairy herds (Wolf et al., 2014a). Therefore, the motivation for management improvements may be decreased in test-negative, perhaps false-negative herds.

Furthermore, uninfected herds are less likely to make management improvements, which would reduce the risk for MAP transmission in case of future MAP introduction. However, firm conclusions regarding whether herd level testing should be included in a control program cannot be made, because it remains unknown whether positive test results were an additional motivator for management improvements or whether negative results were an additional constraint.

Proposed management changes increased probabilities for management improvements in associated areas, which emphasized the role of herd veterinarians as trusted advisors and partners in disease control on many dairy farms (Ritter et al., 2014b; Sayers et al., 2014). The number of management improvements would likely have been lower without support of the veterinarian, as lack of knowledge among farmers is an important constraint for implementation of biosecurity measures (Alarcon et al., 2014; Sayers et al., 2013). Herd veterinarians knowledge on MAP and its control was further increased through the mandatory AJDI half-day certification workshops that were attended by 91 Alberta veterinarians. These workshops increased awareness for MAP among veterinarians, which may have further increased producer participation in the AJDI to 63%, higher than other MAP control programs (Orpin et al., 2009; Wells et al., 2008).

Implementation of management improvements and number of suggestions made by veterinarians decreased over time, confirming results of a study reporting similar differences in total RA scores between Years 1 and 2, and Years 1 and 4 (Wells et al., 2008). Because the analysis was adjusted for the question score in the previous year, and because perfect management does not exist, we inferred that farmers and veterinarians exhausted options for management improvements. We concluded that changes in RA design over the years, either

through focusing on different areas on a farm, or on different additional diseases, may increase management improvement rates.

Rates of management changes differed between sections of the RA, similar to another Canadian study (Sorge et al., 2011). Odds for management improvements were generally higher regarding hygiene practices for various cattle groups than in management areas regarding cattle introduction. The low odds for improvement in the section cattle introduction was not visible in descriptive statistics and only became obvious when the question score for the specific question during the previous year was included as a covariate. Therefore, the association between management improvements and RA section was confounded by the question score in the previous year, suggesting that differences in management improvements were impacted by baseline management in a population.

The total RA score in the previous year was identified as a predictor for reduction in RA scores indicating that farms with bad management are more likely to improve (Sorge et al., 2011). The present study adds knowledge to this finding by identifying the question score for the individual question in the previous year as a predictor for management improvements in that specific question. However, total RA score, represented by the mean question score in an RA, did not impact management improvements; therefore, we inferred that herds with bad management were not more likely to implement new management practices.

A limitation of the study was the method of risk factor and management assessment, with some risk factors and management practices assessed by veterinarians (e.g., animal contamination), and others assessed by producers (e.g., number of cows that calve outside the

calving pen). Self-reported management can differ from true management, mainly because of misunderstanding the subject, suggesting potential for bias due to producer-assessed management (Sayers et al., 2013). However, all question scores were entered by veterinarians who could clarify questions for producers, which likely reduced potential for bias. Another related concern might be that producers falsely stated that they implemented management practices in order to achieve progress. This was mitigated by the use of herd veterinarians for data collection, who are trusted advisors on the farm. Furthermore, producers received a written statement stating that results were treated anonymously by researchers, and no premiums (e.g., financial incentives) were given for improvements in management. In addition, because management improvements were assessed through comparisons between subsequent RA scores instead of through asking whether changes were made, improvement rates were unlikely to have been overestimated.

Study participants were representative of the Alberta dairy farm population, because herd size and MAP prevalence did not differ between study participants and non-participants (Ritter et al., 2014a). Study results can be generalized to other dairy farm populations because herd sizes and management strategies are likely similar in other Canadian provinces, Europe, and most states in the United States. Adoption rates of best management practices are likely higher in control programs for diseases with more frequent clinical cases like lameness, calf-hood diseases or mastitis, because associated disease losses are more visible to farmers, and therefore more obvious than losses through MAP. Therefore, results of the study can be generalized to control programs on dairy farms targeting infectious pathogens with mainly subclinical occurrence.

5.6 Conclusions

Participation in a management-based MAP control program lead to management improvements on most farms. Farms that received MAP-positive ES results were more likely to implement best management practices than ES-negative farms, which suggests that knowledge of the infection status of a herd is important for management decisions. Management improvements were positively associated with management changes proposed by farmers and veterinarians, underlining the importance of the latter as trusted advisors and partners in disease control. Furthermore, management improvements decreased over years of participation, suggesting that it became more and more difficult to make management improvements, based on the knowledge gained by conducting the same RA over several years.

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Table 5-1: Percentages of farms with high scores (above category 2 of mainly 4 categories) in risk assessment questions during the first year of participation in the Alberta Johne's Disease Initiative and frequencies of proposed management changes recorded in the accompanied management plans (n = 370 farms).

Risk assessment question	% high score[1, 3]	Proposed management change	#[3]	%[2, 3]
Cattle introductions				
Purchase frequency (Q7)	86	Inquire about seller's Johne's disease control program	51	3
Purchase precautions (Q7.1)	85	Inquire about seller's Johne's disease control program		
Cattle purchase (Q7, Q7.1)		Only buy cattle from herds with known MAP status	46	3
Purchase precautions (Q7.1)		Test animals before entering herd	1	1
Cattle purchase (Q7, Q7.1)		Keep a closed herd/Minimize cattle purchases	21	1
Visitor access				
Visitor access (Q5)	71	Put bulls outside for buyer to pick up	64	4
Visitor access (Q5)		Put up biosecurity signs	44	3
Visitor access (Q5)		Restrict visitor access to barn	17	1
Visitor access (Q5)		Provide visitors with clean boots	16	1
Calf diet				
Colostrum pooling (Q10)	58	Buy a pasteurizer	76	5
Feeding of bulk/pooled milk (Q12)	67			
Colostrum pooling (Q10)		Use colostrum replacement or dam's colostrum	55	3
Colostrum pooling (Q10)		Use frozen colostrum labeled with cow ID	62	4
Feeding of non-saleable milk (Q11)	61	Do not feed mastitis or non-saleable milk	7	1
Feeding of bulk/pooled milk (Q12)		Do not feed bulk milk	51	3
Cow manure on calf buckets (Q13)	6	Clean/disinfect calf feeders/buckets/nipples/bottles	10	1
Manure on calf feed and waterers (Q14)	4			
Feeding of non-saleable/bulk milk (Q11, Q12)		Use milk replacer or pasteurized milk for calves	20	1
Not related to any question		Clip the udder prior to obtaining colostrum	3	1

Table 5-1: (continued)

Calf and young stock housing				
Proximity to cows/group housing (Q15)	23	Separate calves into individual pens with no contact	28	2
Proximity to cows/group housing (Q15)		No direct or indirect cow – calf contact	36	2
Exposure to cow manure/runoff (Q17)	74	No direct or indirect cow – calf contact		
Staff contamination (Q16)	59	Clean boots and coveralls for feeding calves	59	4
Custom heifer rearing (Q9)	3	No observations		
Young stock cleanliness (Q20)	54	Keep stalls as clean as possible/more bedding	4	1
Manure on feed bunks and waterers (Q18)	30	Clean waterers/feed troughs more often	1	1
Calving pen				
Number of cows in calving pen (Q22)	77	Only one cow per calving pen	44	3
Calving pen contamination (Q23)	28	Add clean bedding for every calving	19	1
Animal contamination (Q24)	32	Add clean bedding for every calving		
Nursing of calves (Q27)	51	Removal of calves within 30 minutes	128	8
Time that calves spend with dam (Q28)	69	Removal of calves within 30 minutes		
Number of cows in calving pen (Q22)	77	Increase number of calving pens	135	8
Calves born outside calving pen (Q26)	31	Have more cows calve in the designated calving pen	20	1
Calves born outside calving pen (Q26)		Have a calving pen	34	2
Use of calving area for sick cows (Q25)	40	No sick cows in the calving pen	29	2
Lactating and dry cow pens				
Not related to any question		Make a close-up pen for cows ready to calve	17	1
Manure on dry cow feed bunks and waterers (Q29)	22	Clean dry cow drinkers more often	8	1
Dry cow contamination (Q31)	26	More bedding in close-up pen	25	2
Dry cow contamination (Q31)		Keep stalls as clean as possible/more bedding		
Lactating cow contamination (Q34)	28	Keep stalls as clean as possible/more bedding		
Manure on dry cow feed bunks and waterers (Q29)	22	Clean waterers/feed troughs more often		
Manure on lact. cow feed bunks and waterers (Q32)	17	Clean waterers/feed troughs more often		

Table 5-1: (continued)

Feeding hygiene and equipment use				
Not related to a score improvement in any question		Disinfect feeding equipment after handling of manure	65	4
Use of feeding equipment to remove manure (Q19)	56	Separate equipment for manure handling and feeding	85	5
Shared equipment, manure on dry cow pasture (Q30)	52	Separate equipment for manure handling and feeding		
Shared equipment, manure on pasture (Q33)	53	Separate equipment for manure handling and feeding		
Cattle show attendance (Q8)	25	Use trailer for own cattle only	51	3
Equipment use/leftover feed (Q19)	56	Do not feed leftover milking cow ration to heifers	32	2
Manure spread on heifer pasture (Q21)	15	No spreading of manure on pastures used the same year	110	7
Manure spread on cow pasture (Q30, Q33)		No spreading of manure on pastures used the same year		
Not related to any question		Request cleaning of commercial manure haulers	14	1
Management of likely infected cattle				
Not related to any question		On herd health day, check heifers first and then cows	10	1
Previous MAP test results (Q6)	56	Test for Johne's disease more often	46	3
Not related to any question		Don't feed milk from test positive cows to calves	4	1
Not related to any question		Cull test-positive cows	41	3
Not related to any question		Cull old cows rather than young ones	1	1
		Suggestion not identifiable	8	1

[1]Percentage of farms that had a score ≥ 3 for the specified question in the first risk assessment.

[2]Percentage of management suggestions among all suggestions.

[3]Frequencies and percentages are shown for the first description of the variable.

Table 5-2: Question-specific changes in risk assessment (RA) scores on 233 farms participating in the Alberta Johne's Disease Initiative for at least 2 years.

	Change in score between 2 RA (%)		
Question	Worse	Equal	Better
Cattle introductions			
Purchase frequency (Q7)	9	68	24
Risk at purchase (Q7.1)	7	73	19
Visitor access			
Visitor access (Q5)	7	72	20
Calf diet			
Colostrum pooling (Q10)	12	67	21
Feeding of non-saleable milk (Q11)	8	75	17
Feeding of bulk/pooled milk (Q12)	9	69	22
Cow manure on calf buckets (Q13)	10	79	11
Manure on calf feed and waterers (Q14)	9	85	6
Calf and young stock housing			
Custom heifer rearing (Q9)	2	98	0
Proximity to cows/group housing (Q15)	15	70	15
Staff contamination (Q16)	13	64	23
Exposure to cow manure/runoff (Q17)	15	67	17
Manure on feed bunks and waterers (Q18)	16	66	18
Animal contamination (Q20)	19	58	22
Calving pen			
Number of cows in calving pen (Q22)	8	76	16
Calving pen contamination (Q23)	16	58	25
Animal contamination (Q24)	19	63	19
Use of calving area for sick cows (Q25)	19	57	24
Calves born outside calving pen (Q26)	17	60	23
Calves nurse the cow (Q27)	11	66	23
Time that calves spend with dam (Q28)	15	60	24
Lactating and dry cow pens			
Manure on feed bunks and waterers (Q29)	22	61	17
Animal contamination (close up cows) (Q31)	20	63	17
Manure on feed bunks and waterers (Q32)	17	68	15
Animal contamination (Q34)	16	64	20

Table 5-2: (continued)

Feeding hygiene and equipment use			
Show attendance (Q8)	5	82	13
Use of feeding equipment to remove manure (Q19)	13	63	25
Manure spread on heifer forage or pasture (Q21)	10	73	17
Shared equipment, manure on pasture grass (Q30)	11	66	23
Shared equipment, manure on pasture grass (Q33)	12	65	23
Management of likely MAP-infected cattle			
MAP testing history (Q6)	11	69	20

Table 5-3: Changes in risk assessment (RA) question scores for individual questions assessed at 233 farms participating in the Alberta Johne's Disease Initiative for at least 2 years.

	Change in question score (# (%))		
	Worse	Equal	Better
Number of MAP-positive environmental samples			
0	804 (12)	4,591 (69)	1,244 (19)
1 – 3	306 (13)	1,619 (70)	404 (17)
4 – 6	220 (16)	855 (63)	291 (21)
Proposed management change			
No	1,263 (13)	6,559 (69)	1,617 (17)
Yes	77 (7)	656 (61)	348 (32)
RA section			
Cattle introductions	54 (8)	480 (71)	146 (21)
Visitor access	25 (7)	247 (72)	69 (20)
Calf diet	134 (10)	985 (73)	239 (18)
Calf and young stock housing	252 (12)	1,497 (74)	286 (14)
Calving pen	358 (15)	1,493 (63)	523 (22)
Lactating and dry cow pens	306 (18)	1,093 (64)	296 (17)
Feeding hygiene and equipment use	175 (10)	1,186 (70)	337 (20)
Management of likely infected cattle	36 (11)	234 (69)	69 (20)
Year			
2nd RA	938 (13)	4,769 (66)	1,523 (21)
3rd RA	369 (12)	2,290 (75)	414 (13)
4th RA	33 (15)	156 (72)	28 (13)
Mean question score in prev. RA			
1st quartile (low risk)	425 (15)	1,958 (69)	440 (16)
2nd quartile	344 (13)	1,838 (70)	428 (16)
3rd quartile	291 (12)	1,717 (68)	503 (20)
4th quartile (high risk)	280 (11)	1,702 (66)	594 (23)
Herd size			
< 100 cows	355 (11)	2,183 (70)	594 (19)
100 – 199 cows	750 (13)	3,852 (68)	1,047 (19)
> 199 cows	235 (14)	1,180 (68)	324 (19)
Question score in the previous year			
Low risk (< 3)	1,152 (18)	4,633 (73)	539 (9)
High risk (> 2)	188 (4)	2,582 (62)	1,426 (34)

Table 5-4: Final multilevel logistic regression model for improvement in the score of a question between consecutive Johne's disease risk assessments.

	OR	95% CI	*P*
Intercept	-2.70[1]	-2.93- -2.46	< 0.001
Number MAP positive environmental samples			0.029[2]
0	Reference		
1 – 3	0.93	0.77 – 1.14	0.502
4 – 6	1.32	1.03 – 1.68	0.026
Proposed management change			< 0.001[2]
No	Reference		
Yes	1.37	1.64 – 1.61	< 0.001
RA section			< 0.001[2]
Cattle introductions	Reference		
Visitor access	1.28	0.90 – 1.82	0.167
Calf diet	1.49	1.16 – 1.93	0.002
Young stock housing	1.48	1.16 – 1.89	0.002
Calving pen	2.22	1.76 – 2.78	< 0.001
Lactating and dry cow pens	2.94	2.28 – 3.79	< 0.001
Feeding hygiene and equipment use	2.16	1.69 – 2.76	< 0.001
Management of likely infected cattle	1.66	1.17 – 2.37	0.005
Year			< 0.001[2]
2nd RA	Reference		
3rd RA	0.63	0.54 – 0.73	<0.001
4th RA	0.55	0.39 – 0.93	0.025
Mean question score in previous RA	Not significant		(0.473)[2]
Herd size (# cows)	Not significant		(0.149)[2]
Score in that question in the previous year			< 0.001[2]
Low risk (< 3)	Reference		
High risk (> 2)	7.21	6.33 – 8.20	< 0.001
Random effects	Var. (SEM)		% Var.
Veterinarian	0.12 (0.06)		4
Herd	0.29 (0.05)		8
Observation	-		88

[1]Variable describes the log odds for management improvement in the baseline group.

[2]Overall Wald *P*-value for the independent variable.

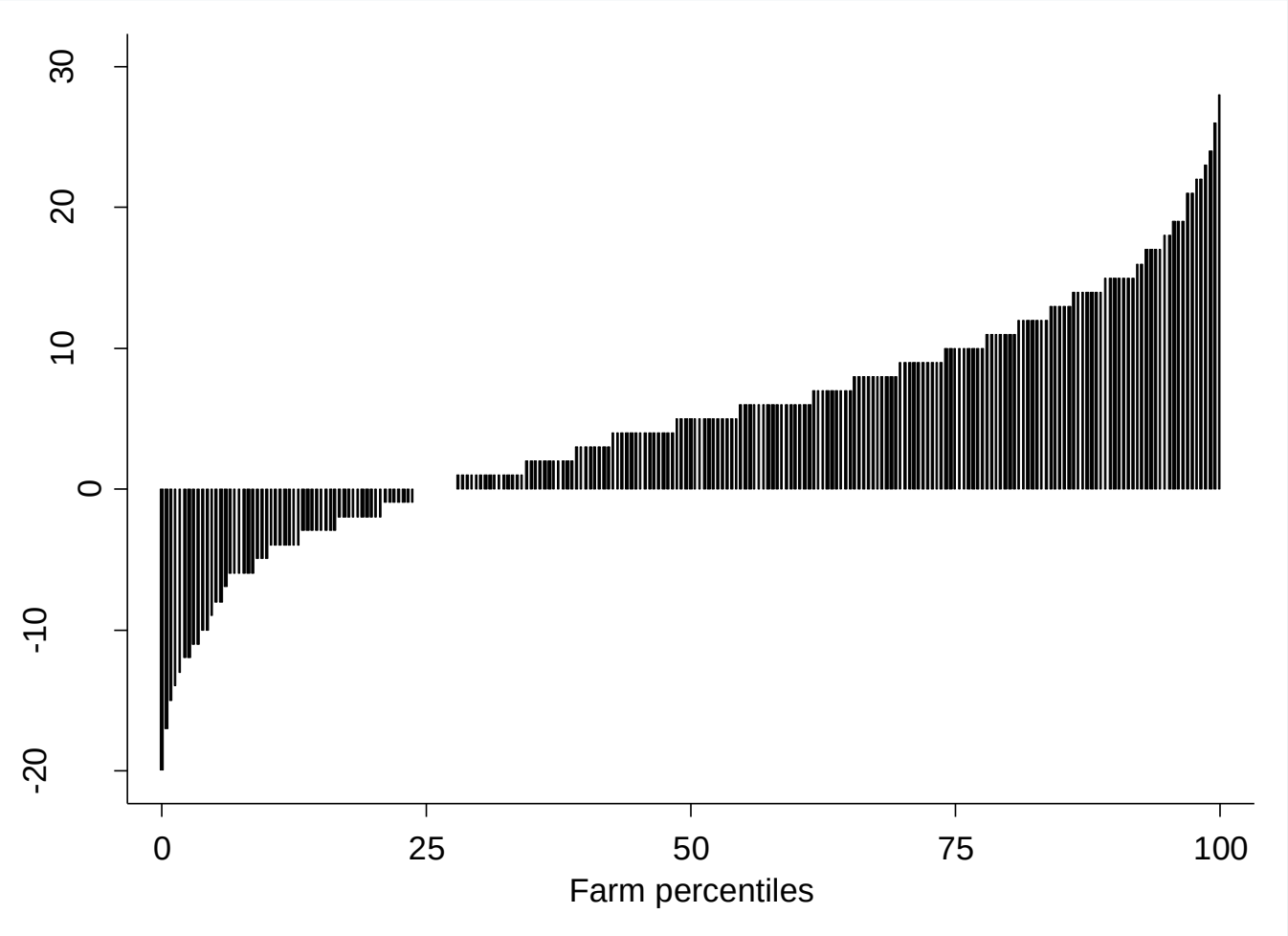

Figure 5-1: Sum of changes in total risk assessment (RA) scores between the first and the last RA conducted on 233 farms participating at least 2 years in the Alberta Johne's Disease Initiative (y-axis = total RA score in first RA – total RA score in last RA; x-axis = farm percentiles; 370 = 100%).

Chapter Six: **ECONOMIC EVALUATION OF PARTICIPATION IN A VOLUNTARY JOHNE'S DISEASE PREVENTION AND CONTROL PROGRAM FROM A FARMER'S PERSPECTIVE**

6.1 Abstract

The Alberta Johne's Disease Initiative (AJDI) is a Johne's disease (JD) control program with the goal of reducing the spread of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) through implementation of best management practices. The objective was to estimate the economic benefit of participation in the AJDI. A decision tree was constructed in which disease prevalence, test characteristics, and probabilities for implementation of best management practices suggested by herd veterinarians were implemented. Analysis was performed using a Markov analysis and input data were assigned using estimates from the AJDI and published data. A cost-effectiveness analysis was performed and the net benefit of participation (from the perspective of a dairy farmer) in the AJDI was calculated compared to no participation. A series of 1-way sensitivity analyses were used to control for uncertainty in input parameters.

Farms participating in the AJDI were estimated to have a net benefit of Can$74 per cow over the course of 10 y. If project costs were covered by the participating farm, the net benefit was Can$27. In addition to the effects on MAP infection, a reduction in calf diarrhea was modelled for farms improving their calf management through the use of pasteurizers. In that case, additional costs outweighed additional revenues compared to the baseline analysis, resulting in a reduced net benefit of Can$19. Participation would not be cost-effective if cows in early stages of MAP infection did not decrease production and if prevalence of MAP infection would not increase on farms with poor management. A limitation of the study, despite high uncertainty in some input parameters, was the lack of knowledge regarding changes in

prevalence on farms with various management strategies. In conclusion, participation in the AJDI was cost-effective for an average Alberta dairy farm.

6.2 Introduction

Johne's disease (JD) is a chronic progressive enteritis caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). In cattle, infection usually occurs in young calves by ingestion of infectious feces. The incubation period is typically 2 to 5 y, but can be as long as 10 y after initial infection. Cattle that develop clinical symptoms suffer from a chronic non-treatable diarrhea that leads to cachexia and ultimately culling or death (Fecteau and Whitlock, 2010). Direct losses for the dairy industry are due to decreased milk production, premature culling and decreased slaughter value of infected animals (McKenna et al., 2006). Annual losses due to JD were estimated at Can$2,472 for a 50-cow herd with a mean MAP within herd prevalence of 7% (Chi et al., 2002). However, in addition to direct losses, there is an unproven association between MAP infection in cattle and Crohn's disease in humans (Barkema et al., 2010; Behr, 2010). Should this association be proven, consumers could reduce consumption of cattle products, which would decrease prices for both dairy and beef products (Groenendaal and Zagmutt, 2008). These factors motivate producers to participate and decision makers to give JD control programs a high priority. In countries with endemic MAP infection, the focus of almost all control programs is to promote implementation of best management practices (BMPs) on dairy farms, with the aim of reducing transmission of MAP and therefore reducing the within-herd prevalence

to a low level, or keeping the herd uninfected (Bakker, 2010, Kennedy and Citer, 2010, McKenna et al., 2006, Whitlock, 2010). Knowing the expected costs and benefits due to participation in a JD prevention and control program is essential for farmers to make an informed decision whether to participate or not.

In previous studies, changes in management were cost-effective, but estimates varied widely (Appendix D). Most of the studies were conducted in the United States where herds are larger and production costs as well as revenues are lower than in Canada. In addition, these studies did not include detailed information on management strategies used and expected changes in management available to accurately estimate all expected costs and benefits that arise through participation for a whole population of farmers. However, the large amount of data collected by Alberta Johne's Disease Initiative (AJDI), with participation exceeding 50% of the approximately 580 Alberta dairy farms, provided a great opportunity to assess accurate data on management, changes in management and the prevalence of the disease in a simulation model. The objective of the study was therefore to determine whether participation in a JD prevention and control program such as the AJDI is cost-effective for a dairy farm. As implementation of best hygiene management practices will also reduce the transmission of other diseases (Johnson et al., 2011), expected additional benefits through reduction of losses caused by other fecal-orally transmitted diseases were also incorporated.

6.3 Materials and Methods

6.3.1 Alberta Johne's Disease Initiative

In 2010, Alberta Milk and the Department of Production Animal Health of the University of Calgary launched the AJDI. The aims of the program were to increase the awareness of JD among dairy farmers, and to decrease the prevalence of MAP infection in the province through implementation of best hygiene management practices (BMPs). The program has 3 components: 1) collection of 6 environmental samples each year to assess the infection status of a herd. These are processed using a commercial liquid culture protocol (TREK diagnostic systems, Cleveland, OH, USA) and subsequent IS900 PCR. The used case definition is positive on IS900 PCR; 2) a risk assessment to analyze strengths and weaknesses in farm management; and 3) a management plan which includes implementation of a maximum of 3 changes in management, agreed upon by the herd veterinarian and the farmer(s), which should reduce the risk of MAP transmission. In contrast to many other programs, the AJDI does not include individual cow testing. Procedures are conducted by specially trained herd veterinarians and the costs for veterinarians' time and sample processing are covered by the project. However, the participating farm is responsible for costs associated with changes in management.

6.3.2 Design

This economic analysis was conducted following Canadian guidelines for economic evaluation of health technologies (Guidelines for the economic evaluation of health technologies:

Canada, 2006). TreeAge Pro (TreeAge Software, Inc., Williamstown, MA, USA) was used to construct a decision tree to evaluate the cost effectiveness of participation in the AJDI versus no participation, from the perspective of an Alberta dairy farmer (Dijkhuizen et al., 1995). The calculation used farm characteristics and economic input data that were preferably recently estimated in Canada (Table 6-1). Farms entered the tree in 1 of 4 management profiles (Figure 6-1). Management profiles reflected the risk of horizontal transmission of MAP between adult infectious and young susceptible animals, with Profiles 1 and 4 having respectively the best and poorest within-herd prevention of MAP transmission. Assignment to the 4 management profiles considered important management in 3 areas, using evidence from previous randomized controlled clinical trials (Pithua et al., 2013; Stabel, 2008) and conditions similar to those reported in previous simulation studies (Dorshorst et al., 2006, Groenendaal et al., 2002). Conditions for assignment included: calving: only 1 cow present in the calving pen at least 75% of the time, and < 10% of the calves born outside the calving pen, and < 50% of the calves nurse the cow; diet: calves are not regularly fed unpasteurized pooled colostrum, unpasteurized bulk tank milk or non-saleable milk; and housing: calves do not have any direct or indirect contact to cows or cow manure. Farms which met the criteria in all 3 areas were assigned to management Profile 1 (low risk), farms which met the criteria in 2 of 3 areas were assigned to Profile 2, farms which met the criteria in 1 of 3 areas were assigned to Profile 3, and farms which did not meet the criteria in any of the 3 management areas were assigned to Profile 4 (high risk). A total of 369 first-year AJDI risk assessments, from 64% of the Alberta dairy farms, were used to assess the distribution of management profiles on Alberta dairy farms (Table 6-2).

The probability of farms changing management profiles was assessed through comparison of management profiles in Year 1 with management profiles in Year 2 on 227 farms participating in the AJDI for 2 consecutive years. Management costs and changes in within-herd MAP prevalence were dependent on the management profile. The tree also incorporated the risk of introduction of MAP infection into previously uninfected herds through purchase of MAP-infected animals. The tree was populated using real-time data from the AJDI, and published data. The databases Scopus (Elsevier, Amsterdam, The Netherlands) and Medline (Atlanta, GA, US) were used to search the scientific literature. Variables were entered in form of distributions to enable probabilistic sensitivity analysis. The weighted averages of estimates from different input sources were used as means of the assigned distributions. The standard deviations were approximated using 25% of the difference between highest and lowest input estimate. If only 1 source was available, the upper and lower limit of the 95% confidence interval (CI) was used as basis for the calculation. If no CI was reported, a conservative range was assigned according to the authors' opinions (authors Robert Wolf, Karin Orsel and Herman W. Barkema have a major MAP research focus, whereas author Fiona Clement is a health economist). Normal distributions were used for normally distributed unrestricted input data, beta distributions (alpha = number of successes; beta = number of failures) were used for proportions, and a log normal distribution was used for the apparent within-herd prevalence at the start of the study.

6.3.3 Comparators

The tree compared farms participating in the AJDI to farms not participating in the AJDI. The tree design was identical for AJDI-participating farms and non-participating farms, with the exception that AJDI-participating farms changed their management profile, whereas non-participating farms did not. As there was no information available on changes in management on farms not participating in a control program, this assumption was necessary.

6.3.4 Benefits

Benefits included revenues through sale of milk and slaughter cows, minus replacement costs. Revenues were reduced by production losses caused by MAP infection. Herd and within-herd prevalence estimates were chosen from 2 peer-reviewed studies (Scott et al., 2006, Sorenson et al., 2003). Environmental sample results from the AJDI were used as an additional source for herd prevalence data. A log normal distribution was used to implement variability of within-herd prevalence among Alberta dairy farms. 45% of the farms were recoded as uninfected. An animal-level MAP prevalence of 14% was chosen as the mean of the distribution (Table 6-3). This resulted in a right-skewed distribution of MAP within-herd prevalence, which represents a high proportion of farms either uninfected or infected with a low within-herd prevalence and a small proportion of so called “problem farms” with a high within-herd prevalence, similar to previous reports regarding Alberta dairy farms (Scott et al., 2006, Sorenson et al., 2003).

The 3 main components of losses due to MAP infection considered in the analysis were: 1) loss in milk production, 2) increased risk of being culled, and 3) decreased slaughter value (Table 6-3). Only studies that used fecal culture as their test method were included as source for production loss estimates (Hendrick et al., 2005, Raizman et al., 2009, Whitlock et al., 1985, Wilson et al., 1993). These losses were assigned to a proportion of MAP-infected cattle equivalent to the sensitivity of fecal culture. Proportionate disease losses (50%) were assigned to infected cattle that were fecal culture negative.

6.3.5 Costs

Costs were management costs that depended on farm management profile. Changes in management suggested by herd veterinarians as part of the AJDI procedures were used to assign costs for various management areas (Table 6-4). As veterinarians suggest different solutions to meet the criteria for each area, a commonly suggested solution with low costs, as well as a commonly suggested solution with high costs was chosen for each area. No costs were assigned to farms in management Profile 4 (high risk). The sum of the costs of all 3 areas was assigned to Profile 1 (low risk). As not all farms in Profiles 2 and 3 met the criteria in the same areas, weighted averages according to criteria met in first year AJDI risk assessments were used to assign costs for Profiles 2 and 3.

6.3.6 Effectiveness

Simulation studies and observational studies were used to estimate the longitudinal change in MAP prevalence dependent on management in the 3 areas. A recent review comparing outcomes of the Dutch JD simulation model, JohneSSim, with the Danish simulation model, PTB-Simherd, was used to retrieve estimations on the expected change in MAP within-herd prevalence for management Profiles 1 and 4 (Nielsen et al., 2011). Additionally, 2 longitudinal studies were considered for estimates on prevalence changes in Profile 1 (Benedictus et al., 2008, Collins et al., 2010). To avoid bias in these studies by wrong assumptions in simulations and by communication of test results to participating producers in observational studies, input studies were considered with equal weights, and very conservative estimates (including zero prevalence increase or decrease) were chosen for subsequent 1-way sensitivity analysis. As no estimates were available for management Profiles 2 and 3, 50% of the prevalence decrease in Profile 1 was assigned to Profile 2, and 50% of the prevalence increase in Profile 4 was assigned to Profile 3 (Table 6-5). The change in within-herd prevalence was incorporated as a factor of the starting prevalence, which was added to the stage-specific prevalence; this resulted in a linear increase or decrease of the within-herd MAP prevalence, on a magnitude dependent on the starting prevalence. For farms changing their management profile, the factor for the prevalence change was adjusted after 2 y, mimicking a delayed response in adult cow within-herd prevalence (due to the nature of the disease).

6.3.7 Modelling

Data were analyzed using a Markov simulation on a herd level (Dijkhuizen et al., 1995). The chosen time horizon of the dynamic simulation was 10 y, with a stage interval of 1 y. Costs and effectiveness were discounted on a value of 5%. The analysis used 5000 iterations with 500 samples. The apparent within-herd prevalence was re-sampled per individual simulated farm (sample) as it was used as a parameter of individual variation among farms. All other distributions were re-sampled per group of iterations, as they were used as parameters of uncertainty. Calculation outputs were exported into Microsoft Excel (2010)®. The net benefit, namely the incremental effectiveness minus the incremental costs, was calculated for each iteration. The net benefit was reported per cow over the duration of 10 y. The mean and the confidence intervals of incremental costs, incremental effectiveness, and net benefit were calculated using Microsoft Excel functions (AVERAGE, CONFIDENCE.NORM). Means and confidence ellipses were presented using the "ellip" command in STATA 11 (STATA Corp, College Station, TX, USA).

6.3.8 Uncertainty and variability

To identify sources of uncertainty, 1-way sensitivity analyses were performed around estimates of all input variables. Results were ranked in accordance to their impact on the mean net benefit, and the most important sources of uncertainty were presented in a tornado diagram designed in Microsoft Excel. The impact of variability in apparent MAP fecal culture within-

herd prevalence was assessed through 1-way sensitivity analysis using prevalence values between 0 and 3% (in increments of 1%).

6.3.9 Scenario analyses

The fecal-oral pathway is the most important transmission pathway for MAP bacteria (Fecteau and Whitlock, 2010). As management-based JD prevention and control programs aim to reduce transmission by this route, it is reasonable to assume that participation in the AJDI reduces the incidence of other fecal-orally transmitted pathogens, e.g. *Cryptosporidium* spp., *Escherichia coli*, rotavirus and coronavirus, coccidia, and *Salmonella* spp. (Johnson et al., 2011). Scenario analysis 1 estimated the additional impact of changes in the 3 management areas on the incidence of other fecal-orally transmitted diseases. Estimates on effectiveness of immediate separation of cow and calf after birth, use of individual calving pens versus multi cow calving pens, and the effect of colostrum pasteurization on the incidence of calf diarrhea (management areas 1 and 2) were based on results of 3 randomized controlled trials (Godden et al., 2012, Pithua et al., 2009, Quigley 3rd et al., 1994, 1995) (Table 6-6). The cost of calf diarrhea was included as a reduction of the benefits in our model. This reduction was composed of treatment costs and animal losses. However, losses in future performance were not considered, as a previous study reported lower first lactation milk production for cows with a history of mild calfhood diarrhea, but did not report lower milk production for cows with a history of severe diarrhea (Svensson and Hultgren, 2008). The costs for area “diet” were assumed to be

Can$47.49/cow/y to simulate the situation that all farms meeting criterion “diet” would use on farm milk pasteurizers.

The second scenario analysis simulated the situation in which project costs were covered by the participating farm instead of by the project. Annual project costs of Can$200 for conducting the risk assessment and sample collection, Can$360 for sample processing (liquid culture and subsequent IS900 PCR), and Can$45 for administrative work were added to the costs for participating farms.

6.4 Results

Mean incremental costs for participation were Can$117 (95% CI: $117-119) and mean incremental effectiveness was Can$191 (95% CI: $190-194) per cow per 10 y. Overall, participating farms had therefore a Can$74 (95% CI: $72-76) higher net benefit per cow per 10 y compared to non-participating farms (Figure 6-2). Most important sources of uncertainty were proportional losses in fecal culture-negative MAP-infected cattle and magnitude of the MAP prevalence increase in management Profile 4 (Figure 6-3). Extreme values in those input parameters yielded a negative net benefit for producers participating in the AJDI. However, net benefit increased with increasing within-herd MAP prevalence upon initiation of the program (Figure 6-4).

Inclusion of the impact on other fecal-orally transmitted diseases (Scenario analysis 1) resulted in a net benefit of Can$19 ($17-22) per cow per 10 y. Mean incremental effectiveness

was Can$200 (95%CI: 198-203) and mean incremental costs were Can$180 (95%CI: 179-182). If AJDI costs were covered by producers (Scenario analysis 2), the net benefit was Can$27 ($25-30).

6.5 Discussion

Participation in the AJDI was cost-effective for an average Alberta dairy farm. Additional costs through implementation of BMPs were outweighed by additional benefits through lower disease costs. Only a small number of iterations resulted in a negative net benefit, which means that there is a small chance that participation would result in a negative net benefit for the average Alberta dairy farmer. This was apparently the first study that incorporated extensive data on baseline management and changes in management, observed within an existing JD prevention and control program. As in all simulations, the outcome depends on the model design, its assumptions, and parameter estimates. Parameter uncertainty was addressed using 1-way sensitivity analysis on all input parameters. There was high uncertainty in magnitude of several parameters; this uncertainty impacted results. This uncertainty was due to limited knowledge regarding pathogenesis of the disease, but was also due to conservatively chosen ranges around estimates of input parameters. Consequently, analysis precision was relatively low. Regardless, the most important part of information from a farmer's perspective, is knowing whether participation results in a positive or in a negative net benefit.

According to this simulation, the uncertainty around the estimates of only 2 parameters in the model had an impact on the farmers' decision. The first of these parameters was proportional production losses in MAP-infected animals in early stage of the disease, i.e., the fecal culture-negative cattle in this simulation. Should these cattle produce the same amount of milk as healthy cattle and should they have no greater risk of being culled as well as no reduction in slaughter value, participation in the AJDI would not be cost-effective. However, this was very unlikely, especially since it was observed in an infection trial recently conducted at the University of Calgary that 18-mo-old steers infected with MAP weighed, on average 39 kg less than uninfected controls housed the same way (Mortier et al., unpublished data). Not all exposed animals had positive test results, which gives evidence that animals are affected by the disease although they are not consistently test-positive. Nielsen et al. (2009) reported decreased milk production starting 300 d before the first positive milk ELISA test result in cows previously ELISA-negative (Nielsen et al., 2009). The authors also reported higher milk production in cows with fluctuating antibody responses, which was regarded as possible misclassification of cattle caused by imperfect test sensitivity and specificity (Nielsen et al., 2009). Therefore, production losses due to MAP infection are generally underestimated due to non-differential misclassification (Dohoo et al., 2003), suggesting that estimates used in the present study are rather conservative. The second parameter impacting the farmer's decision was the within-herd MAP prevalence increase on farms with poor management. In simulation studies, within-herd prevalence increased over time if BMPs were not implemented (Groenendaal et al., 2002, Kudahl et al., 2008, Nielsen et al., 2011). In addition, 2 randomized controlled clinical trials

provided evidence for an association between calving pen design and colostrum source with the risk of MAP infection (Pithua et al., 2013; Stabel, 2008). Nevertheless, apparently no observational study has been published in the peer-reviewed literature on the longitudinal change in within-herd prevalence on farms not implementing any MAP control strategy.

Although the mainly fecal-oral transmission of the disease implies that within-herd prevalence increases if BMPs are not implemented, the lack of proof was the reason for the use of a very conservative approach in sensitivity analysis, simulating a constant within-herd MAP prevalence if BMPs were not implemented (management profile 4). In that unlikely case, participation in the AJDI would not be cost-effective.

Results of a previous study (Dorshorst et al., 2006), which indicated that the within-herd MAP prevalence in the first year was the parameter with the highest impact on the economic results, were confirmed by this study. As it is a parameter of variability among farms rather than a parameter of uncertainty, it was not included in the tornado diagram, but a separate graph was constructed, which enables farmers with test results available to estimate their expected benefit of participation in the AJDI. The apparent fecal culture prevalence was used as a basis to show the results as it is more often available to farmers than the true within-herd prevalence. We concluded that farms with an apparent fecal culture MAP prevalence <0.8% should not expect to derive a positive net benefit from participation in the AJDI.

Based on the economic simulation model, uninfected herds had a negative net benefit, as MAP-associated production losses could not be reduced. For a test and cull based control program, this finding might be close to reality, as such a program would only influence the

prevalence of MAP. However, for a control program based on reduction of disease transmission through management changes, it is an oversimplification. A program which reduces the fecal-oral transmission pathway of MAP should also reduce fecal-oral transmission of other pathogens. This was the rationale for the first scenario analysis, which included additional benefits through reduction of other fecal-orally transmitted diseases. Although the scientific literature is rich on reports estimating the association between young stock health and management (Johnson et al., 2011), most of the studies could not be used as sources of estimates on the effectiveness of management changes on the risk of fecal-orally transmitted diseases. In that regard, most studies were observational and their quantitative estimates, which varied widely among studies, could be biased by confounding through other uncontrolled factors impacting the study participants. Therefore, only estimates from 3 randomized controlled clinical trials were included in the analysis (Godden et al., 2012, Pithua et al., 2009, Quigley 3rd et al., 1994, 1995). Surprisingly, immediate separation of calves from their dams, as well as the use of individual calving pens versus multiple cow calving pens, were not described as effective in reducing calfhood disease, although an observational study reported decreased risk of mortality for calves immediately removed from their dams (Wells et al., 1996). As we aimed to conduct a conservative analysis, we did not simulate a reduction in calfhood diseases for these management practises, again probably underestimating the true net benefit. In contrast, there was strong evidence for the effectiveness of heat treatment of calves' liquid diet for control of calfhood diseases (Godden et al., 2012). To simulate this effectiveness, it was assumed that all farms controlling management area "milk" would purchase an on-farm pasteuriser, that

increased the annual costs in this area from Can$27 in the baseline analysis to Can$47 in the scenario analysis. Although the incremental effectiveness increased through additional revenues (Can$199 to Can$210) caused by increased calf health, the net benefit decreased (Can$73 to Can$17) as a result of increased costs (Can$126 to Can$191). Also this scenario analysis was conducted conservatively, as it did not include any losses in milk production or lower fertility for cows with a history of diarrhea. This decision was made as a previous publication estimated a 344 kg lower milk production in cows with a history of mild diarrhea, but no significant losses in production for cows with a history of severe diarrhea (Svensson and Hultgren, 2008). Assuming cows with a history of diarrhea would have a 344 kg reduction in milk production during their first lactation would result in a net benefit of Can$46 per cow and 10 y (results not shown). Based on the analysis shown, increased investment costs lead to a significant reduction in expected net benefit, emphasizing the need for governmental support or funding from producer organisations to support investments into biosecurity. The second scenario analysis estimated the net benefit after expiration of AJDI project funding in mid-2013. In that case, participation would still be cost effective.

Major limitations of this study were due to extensive knowledge gaps on MAP transmission and prevalence changes over time. The objective was to construct a simple model and to add complexity only in the case of sufficient knowledge available to support it. Consequently, longitudinal changes in within-herd MAP prevalence were modeled as a linear change dependent on the starting prevalence. An alternative approach would be to model various transmission pathways through the use of contact structures and estimates for intrauterine

transmission, as well as transmission through contaminated environment. The approach used could be regarded as an oversimplification; nevertheless, it answered the research question and was less vulnerable to wrong assumptions than an extensive simulation model. Another limitation was that there was insufficient knowledge regarding the effectiveness of the implementation of specific management practices for control of MAP. Consequently, the impact of specific management practices on the MAP within-herd prevalence was not assessed. When assigning costs for changes in management practices, it was assumed that extra labour would be available on farm and no additional personnel had to be hired. This assumption was valid for management practices suggested in the AJDI, as veterinary practitioners are instructed to suggest changes that can be implemented easily with low financial burden and limited extra work, as this will maximize the probability of implementation. No information was available on baseline management and management dynamics on farms not participating in the control program. The assumption that the baseline on AJDI participating farms is representative for dairy farms in the province poses only a minor risk for bias in the analysis as sensitivity analysis showed that variations in baseline management did not have a major impact on net benefit. Assumptions that non-participating farms will keep their management constant over 10 y whereas participating farms change their management repeatedly throughout the years may seem inappropriate. Nevertheless, it still represents a conservative assumption: Management changes on participating farms were modelled bi-directionally. Therefore, farms not only progressed to a better (lower risk) management profile, but also downgraded to a worse (higher risk) management profile according to observations made within the AJDI. Therefore, progress on management profiles on

participating versus non-participating farms was limited to the observed progress minus the observed downgrading, which represented a rather conservative approach.

Results of this study were comparable with those of previous studies in the sense that all studies reported a positive net benefit if BMPs were implemented (Appendix A). With a net benefit of Can$7 per cow per year, the present study resulted in higher estimates than previous simulation studies (Cho et al., 2012, Groenendaal et al., 2002), and lower estimates than an observational study (Groenendaal and Wolf, 2008). Differences of that magnitude can be expected, as all studies considered different populations with different cost and revenue estimates and differences in disease prevalence. In addition, studies varied significantly in their designs and assumptions.

This study was conducted from the perspective of an Alberta dairy farmer. Results are most generalizable to other parts of Canada in which the within-herd prevalence, as well as cost and revenue estimates are similar. It is expected that the net benefits are slightly lower for eastern Canada due to a tendency towards lower herd and within-herd prevalence (Tiwari et al., 2006). It is more challenging to generalize results to outside Canada. The milk price in the United States is lower, thereby reducing the expected net benefit (Geuss, 2013). Conversely, a higher herd MAP prevalence increased net benefit (Lombard et al., 2013). Herd size is another important factor that should be considered. Although average herd sizes are similar between Alberta (145 cows in 2011) and the United States (172 cows in 2010), some areas in the United States such as California or New Mexico include a growing number of very large dairy operations with >1,000 cows (Hoard's dairyman: A 2010 snapshot of U.S. dairying, 2010,

Statistics of the Canadian dairy industry, 2011). For those herds, some investments into biosecurity as well as project costs could be amortized across more cows, which would reduce the burden of these costs. In addition, management of those operations is very different from the management on farms in Alberta, making generalizability of results more challenging. Generalization to Europe is not feasible, as the dairy industry (management and structure) varies significantly among countries. Another challenge is that knowledge on MAP prevalence is limited (Nielsen and Toft, 2009). All these issues in generalizability led to the conclusion, that input of region-specific parameters is required to use the presented model as a supportive tool for dairy farmers and decision makers worldwide. Regardless, a major advantage of the presented model is that most area-specific input parameters can be studied easily and are available online for most dairy populations (The model operated with TreeAgePro is available upon request from the authors).

To fill persistent knowledge gaps, an extensive longitudinal study estimating the association between management and changes in MAP within-herd prevalence should be conducted, as well as estimating production losses of MAP-infected animals. Such a study should be done on several herds representing various management strategies and test results should not be communicated with producers.

6.6 References

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Table 6-1: Farm characteristics and baseline economic data of the average Alberta dairy farm.

Parameter	Estimate	Reference	Model Input
Annual milk production per cow (kg/305 d lactation)	10,126	Statistics of the Canadian dairy industry, 2011	NORMAL(10126;100)[1]
Milk price (CAN$/kg)	0.8	Current milk class price, 2012	NORMAL(0.8;0.1)[1]
Heifer raising costs (CAN$/heifer)	2500	Investing in your dairy herd's future, 2011	NORMAL(2312.5;93.75)[1]
	2125	Mohd Nor et al., 2012	
Live weight of a slaughter cow (kg)	700	Holstein Canada	NORMAL(700;88)[1]
Slaughter value (CAN$/kg live weight)	0.87	Daily cattle report, 2012	NORMAL(0.87;0.1)[1]
Annual culling rate (%)	38	Statistics of the Canadian dairy industry, 2011	BETA(39.3;64.12)[2]
Herd size (milking cows)	145	Statistics of the Canadian dairy industry, 2011	145
Calving interval (d)	422	Norman et al., 2009	422
Annual purchase rate (%/cow present)	0.3	Weber et al., 2006	BETA(63.8;21204.53)[2]
Labour costs (Can$/h)	17.33	Lang, 2010	17.33

[1]NORMAL= Normal distribution (mean; standard deviation)
[2]BETA= Beta distribution (alpha; beta)

Table 6-2: Baseline management and changes in management profiles of farms participating in the Alberta Johne's Disease Initiative (AJDI).

Parameter	Estimate	Input distribution
Farms in management Profile 1 (%)[3]	3	BETA(62.05;2006.28)[1]
Farms in management Profile 2 (%)[3]	15	BETA(54.25;307.42)[1]
Farms in management Profile 3 (%)[3]	40	BETA(38;57)[1]
Farms in management Profile 4 (%)[3]	42	(100 - profile 1+2+3)[2]
Farms improving at least 1 management profile (%)	26	BETA(12.31;35.05)[1]
Among those, farms improving 2 profiles (%)	19	BETA(9.85;41.99)[1]
Farms downgrading at least 1 management profile (%)	11	BETA(6.27;50.69)[1]
Among those, farms downgrading 2 profiles (%)	4	BETA(2.46;58.98)[1]

Data obtained through review of 369 first and 227 second year AJDI risk assessments
[1]BETA= Beta distribution (alpha; beta)
[2]To avoid cumulative percentage >100 through random sampling of all percentages in parallel
[3]Management profiles reflected the risk of horizontal transmission of MAP between adult infectious and young susceptible animals with Profile 1 having the best within-herd prevention of MAP transmission to Profile 4 having the poorest within-herd prevention of MAP transmission. These profiles were assigned according to the management in 3 areas: A: calving, B: diet, C: housing

Table 6-3: Estimates for prevalence, test accuracy and direct costs associated with *Mycobacterium avium* subspecies *paratuberculosis* infection.

Parameter	Estimate (95% CI)	Reference	Model input
Prevalence of infected herds (%)	40[1] (36.4-53.6)	Sorenson et al., 2003	55[3]
	58.8[1] (42.2-75.4)	Scott et al., 2006	
	57 (NA)[2]	AJDI[2]	
True adult cow prevalence (%)	8.1[1] (7.3-9.0)	Sorenson et al., 2003	14.23
	17.5[1] (NA)	Scott et al., 2006	
Losses in milk production (%)	6.2 (1.9-10.4)	Hendrick et al., 2005	BETA(10.95;113.72)[3]
	2.2 (NA)	Wilson et al., 1993	
	12	Raizman et al., 2009	
Increase in risk of culling (hazard ratio)	3.2 (2.5-4.2)	Hendrick et al., 2005	NORMAL(3.08;0.425
	3.0 (1.6-5.8)	Raizman et al., 2009	)[4]
Reduced slaughter weight (kg)	59 (NA)	Whitlock et al., 1985	NORMAL(59;10)[4]
Sensitivity of fecal culture (%)	38 (NA)	Whitlock et al., 2000	BETA(26.58;66.67)[3]
	19.4 (13.3-25.5)	McKenna, 2005	
Percentage of production loss associated with fecal culture-negative, MAP infected cows[5]	50 (0-100)	Assumption	BETA(1.5;1.5)[3]

[1]Based on serum ELISA (herds with 2 or more test positive cows)
[2]Based on results of 2 consecutive years of environmental sampling on 227 farms participating in the Alberta Johne's Disease Initiative
[3]BETA= Beta distribution (alpha; beta)
[4]NORMAL= Normal distribution (mean; standard deviation)
[5]The proportion of these animals in a herd was calculated using the within-herd prevalence and the sensitivity of fecal culture

Table 6-4: Costs for changes in management in 3 areas important for the control of *Mycobacterium avium* subspecies *paratuberculosis* transmission on Alberta dairy farms.

Management area Suggested changes	Annual costs (Can$/cow)	Model input
Calving[1]		NORMAL(10.17;2.59)[7]
Build additional calving pens	10.35[2]	
Remove calves immediately after birth	5.00[3]	
Diet[1]		NORMAL(26.86;10.31)[7]
Pasteurize colostrum and milk	47.49[4]	
Feed only dams colostrum/colostrum/milk replacer	6.23[5]	
Housing[1]		NORMAL(3.50;0.44)[7]
Keep young stock and cows separated	3.5[6]	

[1]Criteria: calving: only 1 cow present in the calving pen at least 75% of the time and < 10% of the calves born outside the calving pen, and < 50% of the calves nurse the cow; diet: calves are not regularly fed unpasteurized pooled colostrum, unpasteurized bulk tank milk or non-saleable milk; housing: calves do not have any direct or indirect contact to cows or cow manure.
[2]Increase the number of calving pens from 2 pens per 100 cows to 4 pens per 100 cows using existing buildings. The costs for installation of 1 calving pen were assumed to be $5,000 on material and 10 h of labour; projected life time: 10 y.
[3]Assuming 20 min extra work per cow and calving.
[4]Initial investment $12,250; projected life time: 6 y; Daily operating costs (energy, maintenance, cleaning): $4.73; Extra labour: 0.5 h/d
[5]Extra work for feeding dams colostrum: 5 min per calving; Heifer calves fed colostrum replacer: 25%; Costs for colostrum replacer per calf: $19.70; Waste milk production per cow and lactation: 42 kg; Waste milk assumed to be free; Daily costs for milk replacer: $1.20
[6]Minor investment into separating housing facilities: Material: $5,000; Labour: 5 h
[7]NORMAL= Normal distribution (mean; standard deviation)

Table 6-5: Expected change in within-herd prevalence of *Mycobacterium avium* subspecies *paratuberculosis* on dairy farms, depending on the management profile.

Parameter	Estimate	Reference	Input distribution
Annual prevalence reduction for herds in Profile 1 (%)	10[1]	Nielsen et al., 2011	NORMAL(0.08;0.009)[2]
	8[3]	Collins et al., 2010	
	6.5[3]	Benedictus et al., 2008	
	10[1]	Nielsen et al., 2011	
Proportionate prevalence reduction in Profile 2 (%)	50	Assumption	BETA(1.5;1.5)[4]
Annual prevalence increase for herds in Profile 4 (%)	20[1]	Nielsen et al., 2011	NORMAL(-0.19;0.007)[2]
	17[1]	Nielsen et al., 2011	
Proportionate prevalence increase in Profile 3 (%)	50	Assumption	BETA(1.5;1.5)[4]

[1]Source reviewed 2 simulation studies with similar outcomes
[2]NORMAL= Normal distribution (mean; standard deviation)
[3]Intervention in source study defined as changes in management and testing and culling of test positive animals
[4]BETA= Beta distribution (alpha; beta)

Table 6-6: Relationship between management practices suggested for control of transmission of *Mycobacterium avium* subspecies *paratuberculosis* and the incidence of calf diarrhea and its associated costs.

Parameter	Estimate	Reference	Model Input
Hazard ratio for scour treatment for calves fed non-heat treated versus heat treated pooled colostrum	1.32 (1.14-1.53)	Godden et al., 2012	NORMAL(1.32;0.39)[2]
Effectiveness of immediate cow-calf separation	NS[1]	Quigley 3rd et al., 1994, 1995	-
Effectiveness of individual calving pens	NS[1]	Pithua et al., 2009	-
Cumulative incidence of pre-weaning diarrhoea (%)	20.48 24.7	Waltner-Toews et al., 1986b Wells et al., 1997	BETA(269;965)[3]
Age at first occurrence (d)	16	Waltner-Toews et al., 1986b	NORMAL(16;2)[2]
Duration (d)	3	Waltner-Toews et al., 1986b	NORMAL(3;1)[2]
Case fatality rate (%)	5.5-7.1	Waltner-Toews et al., 1986a	BETA(14.46;215.13)[3]
Percentage of total heifer rearing costs before weaning	12.3	Gabler et al., 2000	BETA(4.77;33.81)[3]
Daily treatment costs for diarrhoea (Can$) Light case/severe case	40/200[4]	Expert opinion[4]	NORMAL(45.33;5.66)[2,5]

[1]Not significant
[2]NORMAL= Normal distribution (mean; standard deviation)
[3]BETA= Beta distribution (alpha; beta)
[4]Personal communication with an Alberta dairy practitioner and an ex- practitioner currently employed by a major pharmaceutical company
[5]Assuming 10% of the patients would require intensive treatment for 1 d

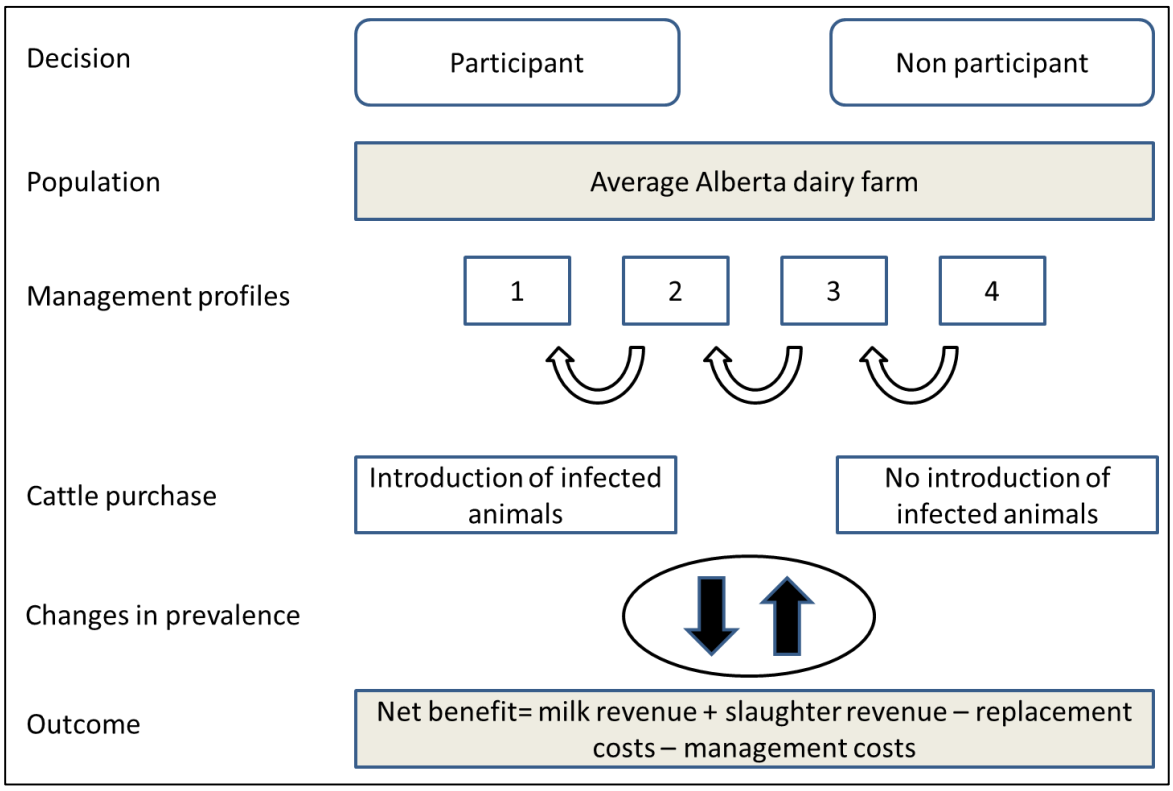

Figure 6-1: Decision tree to assess the economic impact for dairy farms participating in the Alberta Johne's Disease Initiative from a farmer's perspective.

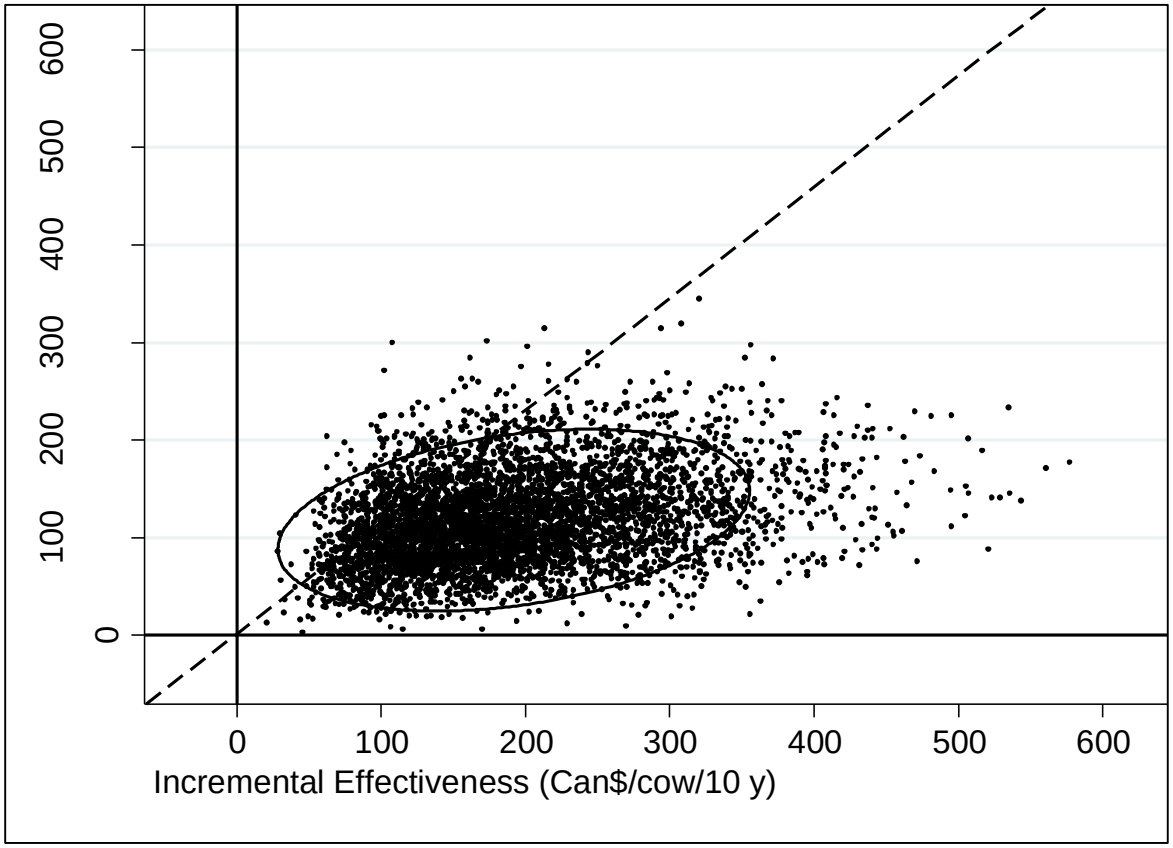

Figure 6-2: Incremental costs and incremental effectiveness for participation in the Alberta Johne's Disease Initiative versus no participation. Iterations below the dashed line resulted in a positive net benefit.

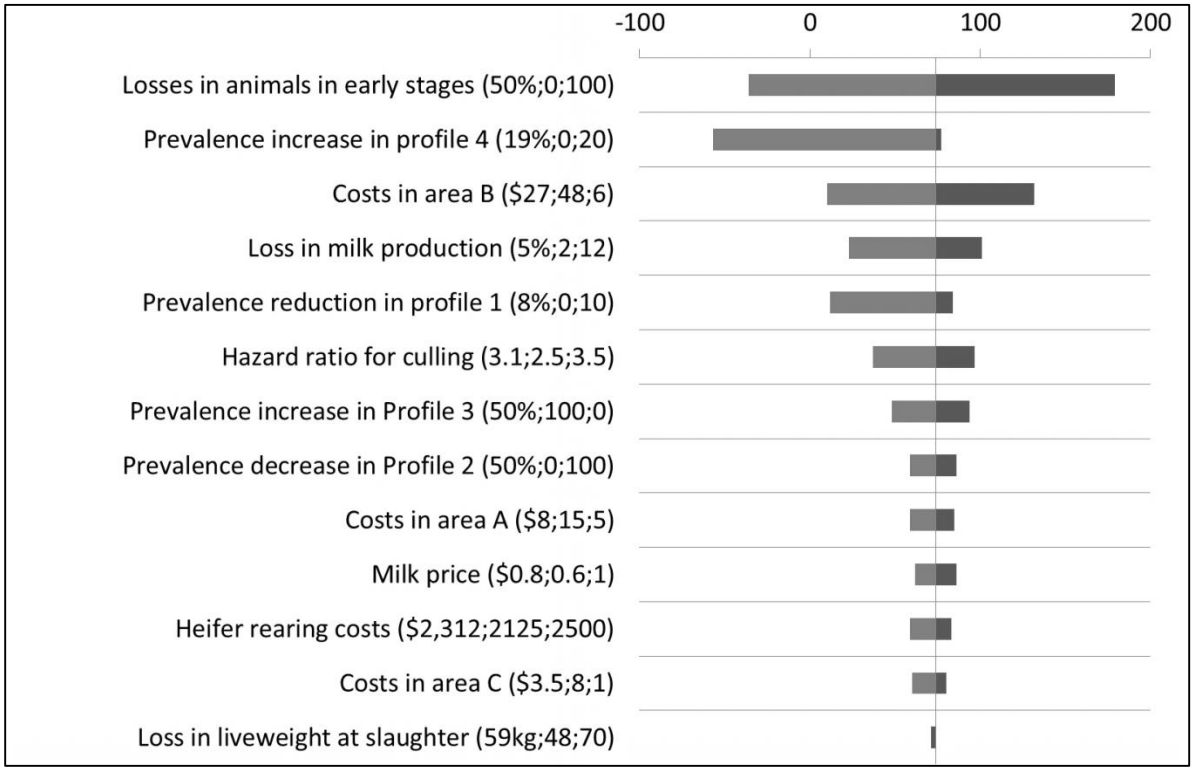

Figure 6-3: Tornado diagram displaying sources of uncertainty in the net benefit (x-axis) around the economic impact of participation in the Alberta Johne's Disease Initiative (default value; lower limit; upper limit).

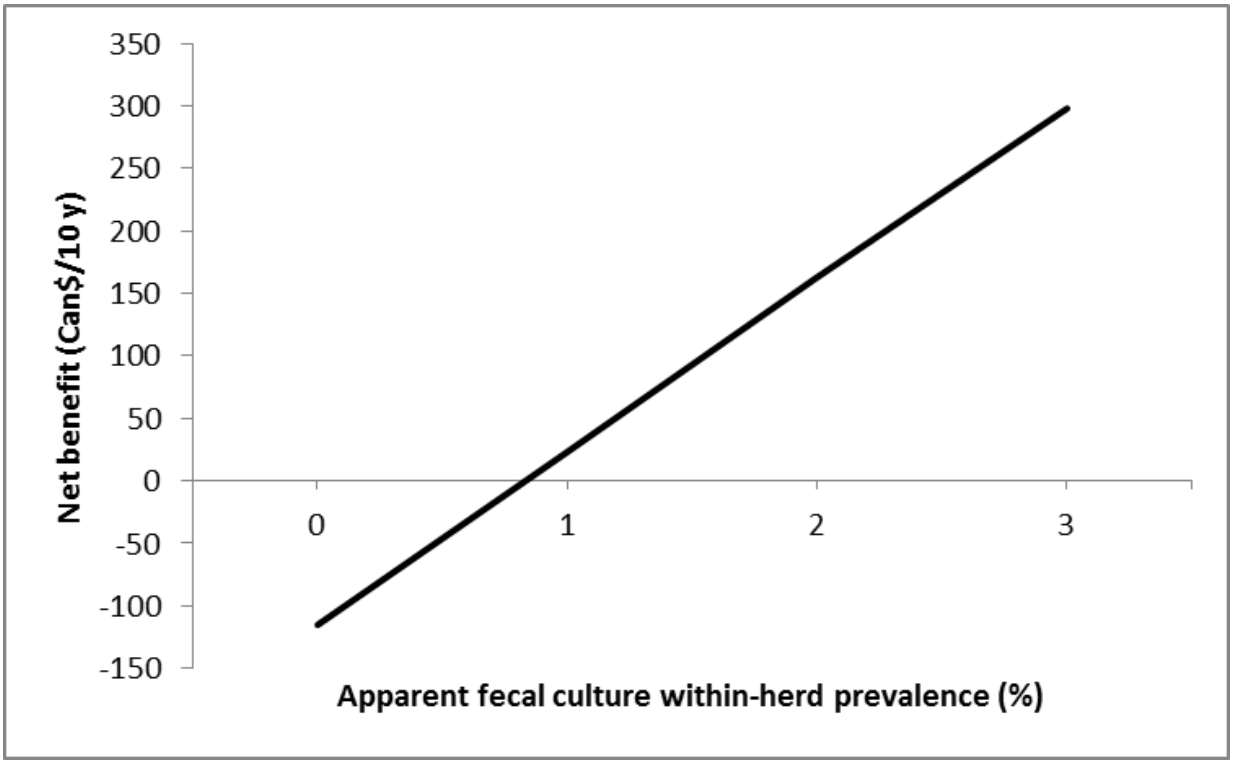

Figure 6-4: Impact of apparent within-herd prevalence on the net benefit for participation in the Alberta Johne's Disease Initiative from the perspective of an Alberta dairy farmer (fecal culture sensitivity: mean: 28%; standard deviation: 5%; fecal culture specificity: 100%).

Chapter Seven: **CALVES SHEDDING *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* ARE COMMON ON INFECTED DAIRY FARMS**

7.1 Abstract

Mycobacterium avium subspecies *paratuberculosis* (MAP) causes Johne's disease, a chronic progressive enteritis. It is generally assumed that calves rarely shed MAP bacteria and that calf-to-calf transmission is of minor importance. The objectives of this study were to estimate: 1) prevalence of MAP-shedding young stock in MAP-infected dairy herds, and identify predictors for test-positive young stock; and 2) proportion of MAP-contaminated young stock group housing and air spaces, and furthermore, identify predictors for test-positive pens. Fecal samples were collected from 2,606 young stock on 18 MAP-infected dairy farms. Environmental fecal samples were collected from all group-housing pens and dust samples were collected from all barns. All individual samples were analysed using IS900 and F57 qPCR; positive fecal samples and all environmental and dust samples were cultured. Overall, 2% of cattle were MAP culture-positive with shedding confirmed in all age groups. Calves < 3 months of age had 1.56 times the odds for testing IS900 PCR-positive than young stock between 6 months and 1 year of age. Furthermore, 14% of collected environmental samples, but none of the dust samples, tested positive. Age of cattle in the pen and the prevalence of shedders in the pen were significant predictors for environmental sample results.

Young stock excreted MAP bacteria in their feces and contaminated their environment. This study provided strong evidence for calves as sources of within-herd transmission of MAP on dairy farms known to be infected with this organism.

7.2 Introduction

Mycobacterium avium subspecies *paratuberculosis* (MAP) causes Johne's disease (JD), a chronic progressive enteritis in ruminants (Fecteau and Whitlock, 2010). The disease is common in dairy herds and causes substantial economic losses through decreased milk production and slaughter value, and increased risk of premature culling (Barkema et al., 2010; Wolf et al., 2014a; Wolf et al., 2014b).

Susceptibility to MAP infection is highest in young animals (Chiodini, 1984; Fecteau and Whitlock, 2010). Cattle get infected *in utero* or through ingestion of infectious colostrum, milk, or feces. After infection, the incubation period is prolonged (typically 2 to 5 years, but up to 10 years). During incubation, frequency of shedding as well as fecal concentrations of MAP bacteria increase (Nielsen and Ersbøll, 2006; Weber et al., 2010). These assumptions regarding susceptibility and bacterial shedding have been implemented into JD simulation models, which are frequently used to design control programs (Groenendaal et al., 2002; Kudahl et al., 2007; Mitchell et al., 2008). Consequently, control programs focus on interrupting direct and indirect contact between likely shedding adult cows and highly susceptible calves (Mason, 2012; Whitlock, 2010; Wiebe, 2011). However, in 2 recent infection trials, a high proportion of calves shed MAP soon after experimental challenge, with some calves shedding as early as 2 months after exposure (Mortier et al., 2014; Santema et al., 2012). Field studies provide inconsistent results, with 2 studies identifying MAP shedding young stock on infected dairy farms (Antognoli et al., 2007; Bolton et al., 2011), but 1 other study reporting no evidence for MAP shedding

calves (Pithua et al., 2010). There are similar inconsistencies with regards to studies estimating the relevance of MAP transmission between young stock. For example, whereas1 transmission trial reported evidence for transmission between young stock (van Roermund et al., 2007), another transmission experiment did not observe any (Santema et al., 2012), and 2 simulation studies regarded transmission between young stock as irrelevant for the spread of MAP (Marcé et al., 2011; Weber and Groenendaal, 2012). Accurate knowledge regarding the importance of transmission routes is essential to design future control programs; the first step is to reduce uncertainty with respect to the occurrence and prevalence of MAP shedding young stock in infected herds. There is, therefore, a need for an observational study conducted on many MAP-infected dairy herds estimating proportions of MAP-shedding young stock in various age groups. Should calves and young stock shed MAP bacteria, it would likely be detected in their environment in environmental fecal samples (Wolf et al., 2014a) or in settled dust (Eisenberg et al., 2010a). This would provide strong evidence for young stock contaminating their environment, possibly causing new infections.

The objectives of the present study were to estimate: 1) prevalence of MAP shedding young stock in MAP-infected dairy herds, and identify predictors for test-positive young stock; and 2) proportion of MAP-contaminated young stock group housing and air spaces, and identify predictors for test-positive pens.

7.3 Materials and methods

7.3.1 Herds

Based on the average herd size of 145 cows in Alberta (Dairy farming in Canada, 2014), it was expected that 10 cattle within an age range of 3 months (i.e. preweaned calves) would be available for sampling at any point in time in each herd, which would result in an overall total of 180 cattle in this age group, a sample size sufficient to detect a minimum prevalence of 2% (Dohoo et al., 2003a). Farms were selected among 360 farms voluntarily participating in the Alberta Johne's Disease Initiative (AJDI, > 60% of Alberta dairy farms participate). Eligible producers had ≥ 1 MAP culture-positive environmental sample during one of the previous AJDI sampling events (Wolf et al., 2014b), and were clients of 1 of 4 veterinary clinics with a major focus on dairy. A total of 20 randomly selected farms needed to be approached to achieve the target sample size of 18 participants. Reasons for refusal of participation were lack of interest in 1 case and fear of disease introduction by sampling personnel in the other case.

7.3.2 Sample collection, shipping and processing

Samples were collected between May 2013 and January 2014. Herd size, history of observed clinical JD, and number of MAP-positive environmental samples collected from adult cow housing and manure storage were available through AJDI records. Fecal samples were collected from the rectum (using lubricated gloves) of all female dairy cattle before first calving, and all male cattle < 30 months of age. The presence of watery diarrhea was recorded.

A single-calf environmental manure sample was collected from each group-housing pen. These samples were composed of four well-mixed sub-samples, preferably collected from alleys, or around waterers (Wolf et al., 2014a). If pens did not have these areas, samples were collected from bedding packs or exercise areas. Samples were not collected if pens were occupied by only 1 animal. Settled dust was collected in barns and sheds (1 sample from each barn) using a commercially available dust swipe (12 x 12 cm) wiping an area ~0.5 m long in areas with settled dust and out of reach for the animals (Eisenberg et al., 2010b). Environmental manure samples and dust samples were not collected if sample collection criteria were not met, e.g., groups maintained on pasture.

Samples were transported to University of Calgary on the day of collection and stored at 4ºC (maximum of 21 days). Laboratory procedures were as described (Eisenberg et al., 2010b; Forde et al., 2013). In short, all individual fecal samples were processed using IS900 and F57 qPCR; a MagMAX total nucleic acid isolation kit (Applied Biosystems, Carlsbad, CA) was used for DNA extraction. Any signal during the 40 cycles was considered positive. All samples positive on at least 1 of the 2 PCR methods were cultured from 13 farms (if enough feces had been collected). Only a subset of PCR-positive samples were cultured from the first 5 farms. A standardized TREK ESP culture protocol with a 3-day decontamination, followed by a 48-day incubation period and confirmation using conventional IS900 PCR, was used (Forde et al., 2013). Only MAP culture was performed on environmental samples. The environmental samples were processed using the same culture protocol as for individual fecal samples. Dust samples were processed with a slightly modified culture protocol, as described (Eisenberg et al., 2010b).

7.3.3 Statistical analyses

Analyses were conducted using STATA Version 11 (Statacorp, College Station, TX, USA). The prevalence of MAP shedding calves was determined using samples from 13 herds where a serial testing scheme was performed; therefore, culture was conducted on PCR-positive samples. An animal was determined as shedding if it was positive on IS900 or F57 qPCR, and subsequently confirmed with positive culture results.

Chi-square tests on contingency tables were used to compare herd size, history of clinical JD, and environmental sample results between study participants and non-participants, within the population of farms participating in the AJDI. The association between test results on 1 side and animal age (< 3 mo, 3 – 6 mo, 6 mo – 1 y, 1 y – 2 y, > 2 y), watery diarrhea at sample collection (yes/no), and the number of positive adult cow environmental samples during the last test event (0, 1 - 3, 4 - 6 positives out of 6 collected samples) as an indicator for the adult cow within-herd prevalence on the other side (Lavers et al., 2013), was first assessed using Chi-square tests on contingency tables. Secondly, to control for confounding by covariates and effects of clustering, associations were also analysed using multilevel logistic regression (Dohoo et al., 2009). Three models were built using either the IS900 qPCR result, the F57 qPCR result, or the culture result as the outcome variable. Farm and pen were included as random effects. Although IS900 and F57 models used data from all 18 herds, the culture model used data from the 13 herds with a serial-testing scheme. A manual backwards selection was used for variable selection and a *p*-

value ≤ 0.05 was considered statistically significant and a predictor change of 20% was used as a threshold to identify confounding. Coefficients and odds ratios were cluster-specific.

The environmental sample culture result was used as the outcome in a model identifying predictors for MAP-contaminated group housing pens. Median age of the animals in a pen (< 3 months, 3 – 6 months, 6 months – 1 year, 1 – 2 years, or ≥ 2 years), the number of animals in a pen (1, 2 – 9, or > 9), and the number of positive adult cow environmental samples during the last test event (0, 1 - 3, or 4 - 6 positives out of 6 collected samples), were considered as predictors in the models. The percentage of IS900, F57 and culture positive cattle in a pen (0, 1 – 20%, or > 20%) were considered as predictors in 3 separate models. Farm was included as a random effect.

7.4 Results

The 18 participating farms had a mean herd size of 156 cows. Whereas 56% of the farms participating in the study had observed clinical JD on their farm, 29% of non-participants had also observed clinical JD (P = 0.03; Table 7-1). Although 11% of the farms participating in the study tested negative on all environmental samples, 55% of non-participants tested negative on all environmental samples (P < 0.01).

A total of 2,606 young stock were sampled in 18 herds. 1,741 young stock were sampled in the 13 herds where serial testing was performed (Table 7-2). Of those, 192 (11.0%) were positive on IS900 qPCR and 44 (2.5%) were positive on F57 qPCR. Furthermore, 34 PCR-

positives were also culture-positive, resulting in a MAP shedding prevalence of 2.0% (95% CI: 1.3 - 2.6%).

Information on age and other covariates was available for 2,599 of the 2,606 cattle in the study. Positive test results were associated with age of cattle, and number of positive environmental samples collected in adult cow housing and manure storage areas (Table 7-3 and Figure 7-1). In the final logistic regression model, calves < 3 months of age had 1/0.64 = 1.56 times the odds of testing IS900 qPCR positive than young stock between 6 months and 1 year of age ($P = 0.05$; Table 7-4). Young stock housed on farms with 1 – 3 positive environmental samples collected from adult cow housing and manure storage had 10.8 times the odds ($P < 0.01$), whereas young stock housed on farms with 4 - 6 positive environmental samples had 7.8 times the odds ($P = 0.02$) of testing IS900 qPCR-positive, respectively, than young stock housed on farms with only negative environmental samples. None of the independent variables significantly predicted F57 or culture results as the outcomes in separate logistic regression models.

Environmental samples were collected from 139 (88%) of 155 group-housing pens. Of these, 20 (14%) samples were MAP culture-positive, whereas 9 (50%) of the 18 farms had positive environmental samples (within-herd environmental sample prevalence ranged from 0 to 43%; Table 7-2). Proportions of culture-positive environmental samples in different subgroups are shown (Table 7-5). In the final logistic regression model, pens with cattle in the age group between 6 months and 1 year had 12.4 times the odds for being environmental culture-positive compared to pens with cattle < 3 months of age ($P = 0.04$; Table 7-6). Pens with an IS900 qPCR

prevalence > 20% tended to have higher odds (OR = 3.8) for testing positive than pens with only IS900-negative cattle ($P = 0.06$). In separate models, neither F57 nor culture prevalence were significant predictors for environmental sample results. Finally, none of the 41 collected dust samples were MAP culture-positive.

7.5 Discussion

Calves and young stock that excreted MAP in their feces were present in all age groups. Calves < 3 months were more likely to shed MAP bacteria than cattle between 6 months and 1 year of age. The highest prevalence of MAP shedding (4%) was in young stock between 3 and 6 months of age. A high proportion of group housing pens was contaminated with MAP; positive test results were associated with age of cattle and the prevalence of MAP-shedding animals in the pen. However, all analysed dust samples were MAP-negative, suggesting a minimal role of dust as a vehicle for MAP in a dairy farm population where young stock and adult cattle are often housed in separate buildings.

Overall, 2% of young stock shed MAP in their feces, confirming results of 2 other studies that reported 3 and 2% MAP culture-positive young stock (Antognoli et al., 2007; Bolton et al., 2011). Although the prevalence estimates in the present study were comparable to those of the 2 other studies, estimates should be compared with caution, since the age distributions of included cattle and laboratory protocols differed among studies, 1 of the previous studies included only 2 large US herds (Antognoli et al., 2007), and the second study preferentially selected cattle from

test-positive dams (Bolton et al., 2011). Our results were different from those of Pithua et al. [18], who did not detect MAP culture-positive calves < 3 months of age, possibly because they used solid culture which has lower sensitivity (Pithua et al., 2010; Whittington, 2010).

The probability of shedding was highest for cattle 3 to 6 months of age, confirming previous findings on shedding patterns after experimental challenge suggesting that a high proportion of exposed cattle shed soon after challenge (Mitchell et al., 2012; Mortier et al., 2014).

Probability of shedding was also associated with adult cow environmental culture prevalence, a proxy for within-herd MAP prevalence (Lavers et al., 2013). One obvious reason is that higher adult cow within-herd prevalence is associated with a higher infection risk and subsequently higher within-herd prevalence in young stock. A second reason would be that premature cattle are exposed to MAP more frequently and to higher doses if they are housed on high-prevalence farms, which would result in higher odds of shedding among infected cattle (Mortier et al., 2014). Therefore, a longitudinal study should be done to investigate shedding patterns in young stock on dairy farms with different within-herd prevalences.

In the present study, MAP contamination was detected in 14% of calf and young stock group-housing pens, whereas 50% of farms had at least 1 environmental culture-positive pen. The proportion of positive samples seemed higher than reported in a study identifying no positive pre-weaning calf pens and only 3% positive post-weaning calf pens (Raizman et al., 2004). Apparent discrepancies in results were attributed to the use of different culture protocols and differences in the study population (including uninfected herds in the previous study).

Regarding adult cow environmental samples, the odds of a positive sample was associated with the prevalence of MAP shedding animals in the pen (Lavers et al., 2013). It was noteworthy that environmental samples from pens with 6 months to 1 year-old young stock more frequently were culture-positive than environmental samples from pens with calves < 3 months, in direct contrast to the association in individual samples. A possible explanation is the pen structure; young stock < 3 months were generally housed on straw packs without alleyways, which forced sample collection from bedding packs. In contrast, pens holding > 6 months old young stock usually had alleyways available for sample collection. Alleyway samples are more often culture-positive than bedding pack samples, perhaps due to increased mixing of manure in alleyway samples (Wolf et al., accepted).

No MAP was isolated from any settled dust samples. A Dutch study used the same protocol and isolated MAP bacteria successfully from young stock housings, but only if they were co-housed with cows (Eisenberg et al., 2010a). However, in the present study, young stock and cows were usually housed in separate barns. It is therefore unlikely that infectious cows contaminated settled dust collected in this study. The amount of MAP excreted by infectious young stock might be too small to contaminate settled dust sufficiently to be detected with current culture methods and dust might be of minor importance for the transmission of MAP, as long as young stock and cows are housed independently.

Low agreement between tests is a reality in MAP diagnostics. Identifying more animals using IS900 PCR compared to F57 PCR (in all but 1 farm) could be explained by the difference in numbers of the target insertion element present in the MAP genome (IS900 - F57; multi copy -

single copy) and also by the presence of the IS900 element in other bacteria (Cousins et al., 1999; Stabel et al., 2004). Furthermore, the high C_T value cut off of 40 cycles may have resulted in false-positive samples in the initial PCR screening. In addition, PCR identifies MAP DNA present in samples, whereas viable MAP must be present for culture (Aly et al., 2010). In conclusion, since the perfect test for identification of MAP shedding calves does not exist at this point, false-positive as well as false-negative results were expected for all 3 tests.

The goal of data interpretation was to minimize the magnitude of misclassification in prevalence estimates. The initial PCR screening was performed to identify samples that potentially contained MAP. Two PCR reactions with different primers were performed, which was a rapid and inexpensive screening method ideal for processing many samples. Furthermore, this parallel testing resulted in higher sensitivity than sensitivities of the two separate tests (Dohoo et al., 2003b). To increase sensitivity even further, any evidence of PCR amplification (C_T values < 40) was called qPCR-positive, which is higher than the cut off of 37 cycles, which is standard in our laboratory (Forde et al., 2013). Culture of any positives was done to increase specificity of the testing scheme. Culturing MAP is almost 100% specific (Whitlock et al., 2000), especially in the present study where cattle were unlikely to be housed in proximity to any high shedders or clinical cases of JD, thereby decreasing the probability of passive (pass-through) shedding. However, as a result of the imperfect sensitivity and the high specificity of the current testing scheme, the estimated prevalence was very likely an underestimation of the true prevalence of MAP-shedding in young stock, since cattle shedding very low numbers of MAP were likely missed.

Collection of fecal samples from the rectum was difficult in some calves, resulting in a small sample, insufficient for subsequent culture. Consequently, 7 PCR-positive samples from the 13 herds with serial testing scheme were not cultured and thus they were designated negative, likely misclassifying a limited number of calves. Young stock > 6 months of age was not available for testing in 1 herd with serial testing scheme, impacting prevalence estimates to a small extent.

The prevalence of infectious cattle was low, thereby reducing the power for detection of associations between test results and independent variables. To mitigate this limitation, results and associations were described for all 3 test methods. Consequently, age and adult cow environmental sample results were significant predictors for IS900 results (13% prevalence), but did not predict F57 and culture results (~2% prevalence).

Samples were stored for a maximum of 21 days, which may have had a minor impact on the accuracy of the initial qPCR screening, since PCR does not require live bacteria. However, subsequent culture needed viable bacteria to become positive, suggesting an impact of sample storage conditions on the accuracy of culture protocols in general. However, the thick cell wall of MAP enables it to survive in the environment for extended intervals (Whittington et al., 2005; Whittington et al., 2004), and it was estimated that MAP can be stored at 4ºC for at least 1 week without substantial loss in culture accuracy (Khare et al., 2008). Therefore, we inferred that storage duration had only a minor impact on the sensitivity of MAP culture, although some samples with low bacterial concentrations were possibly misclassified as negative, which would

have resulted in an underestimation of the prevalence of MAP-shedding calves and in an underestimation of the proportion of MAP contaminated pens.

Participating herds were more likely to have a history of observed clinical JD and were more likely to be culture-positive using environmental samples than non-participating AJDI herds. This was expected due to applied herd selection criteria. Therefore, results can be generalized to MAP-infected dairy farms with similar size and management.

This study provided clear evidence that naturally infected dairy calves can excrete MAP bacteria and contaminate their environment. Because we do not know whether this contamination will result in infection of other calves, the importance of these findings with regards to transmission of MAP remain uncertain. Consequently, a transmission trial is needed to quantify the potential for calf-to-calf transmission in group-housed dairy calves.

In conclusion, excretion of MAP by young stock occurred in MAP-infected dairy herds, with shedders present in all age groups. The odds of shedding were associated with age and positively associated with prevalence of MAP-positive environmental samples of adult cattle housing and manure storage. Shedding of MAP lead to contaminated pens, especially in situations with a higher prevalence of MAP shedding cattle. However, MAP was not detected in settled dust, providing no evidence for the importance of dust as a fomite for transmission of MAP between calves.

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Table 7-1: Herd characteristics for study farms and farms participating in the Alberta Johne's Disease Initiative (AJDI, n (%)).

	Study participants (n= 18)	Other AJDI farms (n= 342)	
Herd size			0.69[1]
< 50	0 (-)	9 (3)	
50 – 99	2 (11)	102 (30)	
100 - 149	8 (44)	130 (38)	
150 – 199	3 (17)	48 (14)	
> 199	4 (22)	53 (15)	
History of clinical Johne's disease			0.03[1]
JD has been observed	10 (56)	98 (29)	
Don't know	4 (22)	74 (22)	
JD has never been observed	4 (22)	170 (49)	
Positive environmental samples			< 0.01[1]
0 positives	2 (11)[2]	188 (55)	
1-3 positives	9 (50)	84 (25)	
4-6 positives	7 (39)	70 (20)	

[1] *P*-value based on Chi-square test on contingency table

[2]These 2 herds had no MAP culture-positive environmental samples at the last testing event, but had positive environmental samples in 1 of the 2 previous samplings.

Table 7-2: *Mycobacterium avium* subspecies *paratuberculosis* (MAP) test results stratified by farm (# of positives (n tested)).

Herd	Cow env. culture[1]	Individuals			Environmental culture
		IS900	F57	Culture	
1[2]	0	0 (34)	0 (34)	0 (0)	0 (6)
2	1	51 (178)	0 (178)	3 (49)	0 (9)
3	1	21 (109)	30 (109)	5 (43)	1 (11)
4	4	11 (153)	2 (153)	2 (12)	1 (11)
5	1	4 (104)	0 (104)	2 (4)	3 (7)
6	2	45 (130)	7 (130)	6 (45)	4 (11)
7	2	4 (121)	0 (121)	0 (4)	0 (8)
8	4	16 (227)	2 (227)	3 (16)	1 (8)
9	4	13 (154)	0 (154)	5 (11)	2 (14)
10	2	7 (135)	2 (135)	1 (6)	2 (4)
11	2	3 (76)	0 (76)	1 (3)	0 (5)
12	0	4 (162)	0 (162)	2 (4)	0 (9)
13	4	13 (158)	1 (158)	4 (12)	0 (8)
14[3]	3	33 (202)	13 (202)	5 (12)	0 (3)
15[3]	4	9 (114)	3 (114)	0 (7)	0 (1)
16[3]	3	55 (221)	9 (221)	2 (19)	4 (11)
17[3]	5	30 (214)	1 (214)	0 (5)	0 (7)
18[3]	4	27 (114)	1 (114)	1 (22)	1 (6)

[1]Number of MAP environmental culture-positive samples out of 6 samples collected at the adult cows' environment and manure storage

[2]Young stock > 6 months of age were on pasture and not available for sample collection

[3]Culture was conducted on a sub-set of PCR-positive samples

Table 7-3: *Mycobacterium avium* subspecies *paratuberculosis* (MAP) test results for individual fecal samples. QPCR and culture results.

	IS900 qPCR[1]			F57 qPCR[1]		Culture[2]		
	n	# Pos. (%)	*P*-value[3]	# Pos. (%)	*P*-value[3]	n	# Pos. (%)	*P*-value[3]
Age group			0.07		0.44			0.43
< 3 mo	378	53 (14)		11 (3)		246	6 (2)	
3-6 mo	319	56 (18)		13 (4)		223	9 (4)	
6 mo -1 y	651	72 (11)		19 (3)		427	6 (1)	
1 – 2 y	1,115	150 (13)		24 (2)		737	10 (1)	
> 2 y	136	15 (11)		4 (3)		102	3 (2)	
Watery diarrhoea			0.85		0.10			0.56
Yes	25	3 (12)		2 (8)		17	0 (-)	
No	2,581	343 (13)		69 (3)		1,724	34 (2)	
Pos. env. samples[4]			<0.01		<0.01			0.60
0	196	4 (2)		0 (-)		196	2 (1)	
1 – 3	1,276	223 (17)		61 (5)		853	18 (2)	
4 – 6	1,134	119 (10)		10 (1)		692	14 (2)	

[1]Data collected from 18 herds (2,606 cattle)

[2]Data collected from 13 herds (1,741 cattle)

[3]*P*-value based on Chi-square test on the contingency tables

[4]Positive environmental samples: stratification according to the number of positive environmental samples collected from adult cow housing and manure storage areas.

Table 7-4: Predictors for *Mycobacterium avium* subspecies *paratuberculosis* IS900 qPCR results on individual fecal samples (n = 2,599). Final multilevel logistic regression model.

	OR	95% CI	*P*-value
Intercept[1]	-4.23	-5.87 - -2.59	< 0.01
Age group			0.09
< 3 mo	Reference		
3 – 6 mo	1.20	0.74 – 1.94	0.46
6 mo – 1 y	0.64	0.41 – 1.01	0.05
1 y – 2 y	0.96	0.64 – 1.44	0.83
> 2 y	0.89	0.44 – 1.79	0.74
Pos. env. samples[2]			0.02
0	Reference		
1 – 3	10.84	1.99 – 59.20	< 0.01
4 – 6	7.80	1.34 – 43.59	0.02
Random effects	Var. (SE)		% Var.
Herd	0.54 (0.24)		13
Pen	0.25 (0.14)		6
Animal	-		81

[1]Estimate describes the coefficient (log odds)

[2]Positive environmental samples: stratification according to the number of culture-positive environmental samples collected from adult cow housing and manure storage areas.

Table 7-5: *Mycobacterium avium* subspecies *paratuberculosis* (MAP) positive culture results for young stock environmental samples.

	n	Positives (%)	*P*-value[1]
Median age in pen			0.23
< 3 mo	31	1 (3)	
3-6 mo	39	5 (13)	
6 mo -1 y	25	6 (24)	
1 – 2 y	38	7 (18)	
> 2 y	6	1 (16)	
IS900 within-pen prevalence[2]			0.06
0	60	5 (8)	
1 – 19%	39	5 (13)	
>20%	40	10 (25)	
F57 within-pen prevalence[2]			0.43
0	105	13 (12)	
1 – 19%	22	4 (18)	
>20%	12	3 (25)	
Culture within-pen prevalence[2, 3]			0.82
0	89	11 (12)	
1 – 19%	2	0 (-)	
>20%	20	3 (15)	
Group size			0.10
2 - 9 animals	72	7 (10)	
> 9 animals	67	13 (19)	
Pos. env. samples[4]			0.08
0	15	0 (-)	
1 – 3	69	14 (20)	
4 – 6	55	6 (11)	

[1]*P*-value based on Chi-square test on contingency table

[2]Percentage of cattle in the pen testing positive on the specified test

[3]Analysis used results from 13 of the 18 herds

[4]Positive environmental samples: stratification according to the number of culture-positive environmental samples collected from adult cow housing and manure storage areas

Table 7-6: Predictors for *Mycobacterium avium* subspecies *paratuberculosis* environmental sample culture results (n = 139). Final multilevel logistic regression model.

	OR	95% CI	*P*-value
Intercept[1]	-4.23	-6.63 - -1.84	<0.01
Age group			0.30
< 3 mo	Reference		
3 – 6 mo	4.71	0.46 – 48.32	0.19
6 mo – 1 y	12.39	1.13 – 136.10	0.04
1 yr – 2 y	8.21	0.82 – 82.43	0.07
> 2 y	11.26	0.43 – 297.04	0.14
IS900 within-pen prevalence[2]			0.10
0	Reference		
0.01 – 0.19	1.05	0.24 – 4.56	0.94
< 0.2	3.78	0.94 – 15.29	0.06
Random effects	Var. (SE)		% Var.
Herd	0.52 (0.88)		13
Pen	-		87

[1]Estimate describes the coefficient (log odds)

[2]Parameter included in the model because of evidence for an association in the descriptive statistics and biological plausibility.

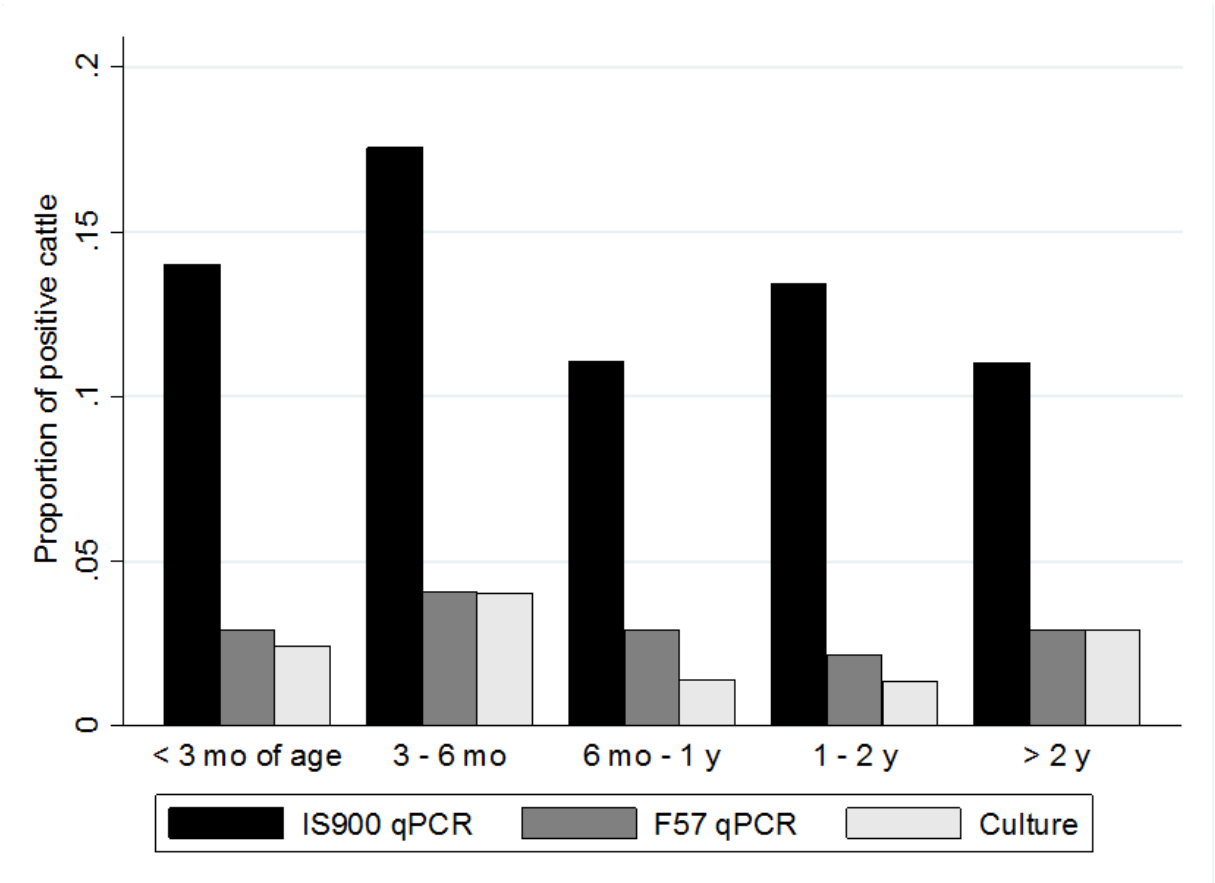

Figure 7-1: Age-specific proportions of cattle excreting *Mycobacterium avium* subspecies *paratuberculosis* in their feces. IS900 and F57 qPCR were conducted on all 18 farms (n = 2,606), whereas subsequent culture was conducted on 13 farms (n = 1,741).

Chapter Eight: **SUMMARIZING DISCUSSION**

8.1 Summary of results

The focus of the research presented in this thesis, was to address some of the major bottlenecks in the Alberta Johne's Disease Initiative (AJDI), a management-based *Mycobacterium avium* subspecies *paratuberculosis* (MAP) control program. The herd-prevalence of MAP in Western Canada was estimated, factors that influence sensitivity of environmental fecal samples were determined, risk factors for MAP-infected herds were explored, and finally factors that influence the implementation of management improvements were investigated. Furthermore, economic benefit of participation in the AJDI and prevalence of MAP-shedding young stock was estimated.

A total of 68% of Alberta and 73% of Saskatchewan dairy herds were estimated to be infected with MAP, higher than previously reported (Scott et al., 2006; Sorenson et al., 2003; VanLeeuwen et al., 2005). This is likely due to low accuracy of test methods used in previous studies, and failure to adjust prevalence estimates for this low accuracy. As reported in other studies, the accuracy of environmental samples depended on sampling site, with samples from lactating cow pens and manure storage being most sensitive (Lavers et al., 2013; Lombard et al., 2006). Additionally, the area of sample collection was a significant driver for the accuracy of a sample, with samples collected from alleyways and lagoons being more accurate than samples from bedding packs and manure piles. In contrast to results of another study (Lavers et al., 2013), more samples were MAP-culture positive in spring than in winter, suggesting an impact of season on accuracy of environmental samples. Season did, however, not only impact the

accuracy of environmental samples; during winter, manure storage area samples were frequently replaced with samples collected in lactating cow pens, suggesting difficulties with sample collection from outdoor sites during winter. However, resulting differences in sample set compositions had only minor impact on the accuracy of a sample set containing 6 samples, but impacted the accuracy of sample sets containing ≤ 3 samples. The risk for MAP infection of a herd was associated with the hygiene level on a farm. On farms with manure-contaminated animals and pens, more environmental samples were MAP-culture positive, which underlines the importance of MAP transmission through contaminated environment (Elliott et al., 2014). Furthermore, frequent cattle purchase from different herds without knowledge on the disease status of the seller herd increased the risk for MAP culture-positive environmental samples. However, as in other MAP control programs, most farms improved their management during participation (Wells et al., 2008). Furthermore, as in Ontario (Sorge et al., 2011), test-positive farms were more likely to make management improvements. Additionally, other factors impacted the rate of management improvements. For example, questions where a management change was agreed between the farmer and his/her veterinarian in the previous year were more likely to improve management than questions where no management change was suggested. The rate of improvements, however, decreased with increasing years of participation. Participation in the AJDI was cost-effective for the average Alberta dairy farmer, and the net benefit through AJDI participation increased with increasing within-herd prevalence, similar to most other MAP control programs in Europe and the US (Groenendaal et al., 2002; Kudahl et al., 2007). Furthermore, the main sources of uncertainty in the economic analysis were magnitude of

prevalence increase on farms with poor management and disease losses in MAP culture-negative infected cattle. Transmission of MAP within young stock groups could be a hazard that is currently overlooked in MAP control programs, as young stock management is not included in the current RA. However, > 2% of young stock shed MAP, and some shedders were < 3 months of age, in contrast to a previous study (Pithua et al., 2010). Furthermore, 14% of young stock group housing pens were MAP culture-positive, much higher than expected considering that a previous study reported only 3% MAP culture-positive environmental samples collected from young-stock pens (Raizman et al., 2004).

8.2 Implications for the necessity of MAP control on dairy farms

The high herd prevalence of MAP suggests that MAP causes substantial economic losses to the Western Canadian dairy industry, which justifies implementation of control strategies that should reduce both herd- and within-herd prevalence of MAP (Chapters 2 and 6). Furthermore, should a link between MAP and human disease ever be proven, established MAP control programs would increase food safety for Canadian consumers, and would also be important for keeping export markets accessible for Canadian dairy products. Regardless, MAP control should be communicated as beneficial for the average producer, representing an opportunity to increase herd productivity (Chapter 6).

8.3 Implications for herd-level testing

Testing herds with only environmental samples did not facilitate removal of infected cattle as it only identified MAP infection at herd level. It was, however, very valuable, because it likely increased awareness for MAP among producers which encouraged implementation of management improvements on environmental culture-positive herds (Chapter 5). Furthermore, environmental testing identified a number of herds with low risk for MAP infection. These herds may present sources for replacement cattle with low risk for MAP infection (Collins, 2011). Farmers need, however, to be aware of the limitations in accuracy of current tests for MAP detection, and test results should be interpreted with caution (McKenna and Dohoo, 2006).

Environmental samples were a quick sampling method to determine MAP infection status of a herd. Because collecting the 6 environmental samples only required approximately 20 minutes, sample collection could be done during the same visit as when the risk assessment was conducted, which would likely not have been possible with a sampling protocol that required sample collection from individual cattle. In addition, as a direct MAP detection method, culture of environmental samples had the advantage of nearly perfect specificity, which cannot be assumed for indirect test methods like serum ELISA (Whitlock et al., 2000). Therefore, herds testing positive were very likely infected, and estimation of the true herd-prevalence was simplified because only the lack of sensitivity had to be considered (Chapter 2).

Although environmental sampling was a useful tool, sets of 6 samples detected only 70% of MAP-infected herds (Chapter 2). A way of increasing the sensitivity of environmental

samples without a cost increase would be to focus on sampling locations with high sensitivity. The most sensitive areas were alleyways of lactating cow pens and manure lagoons (Chapter 3). On farms with only manure piles, or in winter, when manure collection from lagoons was compromised, samples should be collected from indoor manure pits or additional samples should be collected from alley ways. Replacement of sampling locations with others had minor impact on the accuracy of a sample set, as long as ≥ 3 samples were collected , which gives sample collectors the required flexibility to replace locations with others. Sample collection from dry/sick/calving pens could be avoided, because these sampling sites do not always qualify in small herds, and because dry/sick/calving pen samples are less accurate than samples collected from lactating cow pens and manure storage areas (Chapter 3).

It is noteworthy that several herds changed their apparent infection status between testing events (Chapter 2). Because it is unlikely that so many herds changed their true infection status, it is likely that infected herds were not consistently detected. A possible explanation is that in these likely low-prevalence herds, a small number of infected cattle shed intermittently (Van Schaik et al., 2003). Therefore, the concentration of MAP bacteria in the environment varied over time, and shorter sampling intervals may increase the probability for detection of these herds. However, implementation of more frequent sampling events without major cost increases would mean a smaller number of samples per sampling event.

8.4 Implications for MAP control through management practices

Pens and animals were more likely to be contaminated with manure on MAP culture-positive farms than on test-negative farms, suggesting that within-herd transmission occurred more frequently in a less hygienic environment (Chapter 4). This would justify shifting attention in a MAP control program towards cow cleanliness. Improvements in these areas are achievable, as it would frequently only require more bedding and more frequent stall cleaning. Increasing adult cow cleanliness would further result in increased cow health through reductions in incidences of other infectious diseases, e.g. mastitis (Barkema et al., 1999). Results presented in Chapter 7 provided evidence for young stock contaminating their environment with MAP. Additionally, the concept of calf-to-calf transmission was proven in a trial experiment (Van Roermund et al., 2007) Therefore, hygiene of young stock should also be included in a MAP control program. Management practices to improve hygiene of calves would likely result in lower incidence of other calf-hood infections such as rota/corona diarrhea, *Escherichia coli*, *Salmonella* spp., and *Cryptosporidium* spp. (Johnson et al., 2011). These reductions in incidences of other infectious diseases in cows and young stock would further increase economic benefits for farmers participating in a MAP control program (Chapter 6).

Purchase frequency and precautions during purchase were risk factors for introduction of MAP (Chapter 4), which justifies the availability of a herd status program giving farmers the option of purchasing cattle from herds with a low risk of MAP infection (Mason, 2012). However, reduction in cattle purchase rates would decrease the risk for introduction of several

infectious diseases, including MAP (Van Schaik et al., 2002). More frequent use of reproductive technologies like embryo transfer provides an opportunity for farmers to enrich the genetic pool of their herds without introducing live cattle. Another opportunity to decrease purchase would be to decrease demand for externally raised replacement heifers. That could be achieved by inclusion of young stock hygiene management in a MAP control program, which would likely reduce purchase rates through an increase in numbers of successfully raised heifers, especially on farms with very poor heifer management (Johnson et al., 2011).

The AJDI controls MAP prevalence solely through best-management practices. Therefore, identifying weaknesses in management on a farm is only the first step towards MAP control, and management changes need to be made in order to effectively control MAP infections. Unfortunately, identification of weaknesses in management does not always result in management improvements (Chapter 5). Costs for changes in management are certainly a limiting factor for implementation (Edwards-Jones, 2006); examples include avoidance of the use of feeding equipment to remove manure requires purchase of new loaders or at least loader buckets for many farms. This limitation should be addressed through financial support provided by producer organizations or government for investments made to control MAP. Growing Forward 2, which funds conduction of AJDI risk assessments and implementation of management improvements probably leads to increased implementation of expensive management improvements (Mason, 2012). Another limitation for adoption might be that farmers are not aware of MAP causing economic losses in their herd due to lack of clinical signs of JD, which was the reason for a lack of management improvements in test-negative herds

(Chapter 5). In that regard, farmers should be made aware of the high risk that MAP can be introduced into their herds, that MAP can be present on environmental-sample negative herds, and that implementation of best management practices also reduces the incidence of other infectious diseases. Veterinarians and on-farm workshops are possible ways of delivering this information within the AJDI.

8.5 Suggestions for future research

Several important knowledge gaps were filled with the research included in this thesis. However, some persistent questions should be addressed in future research projects.

Herd size was identified as one of the most important predictors for herds testing MAP-positive (Chapter 2), and was still significantly associated with positive environmental sample results after controlling for differences in management practices assessed within the AJDI (Chapter 4). Reasons for that association could be herd-size dependent differences in: 1) management practices that were not assessed within the AJDI, including cattle purchase rates; and 2) group sizes and contact structures. An observational study should be conducted including a systematic selection of small and large herds. Collected data on management, group sizes and group compositions, and trade history should be used as potential explanatory variables predicting the odds for MAP infection of a herd.

In Chapter 3, a standardized 6-sample environmental sampling protocol with annual sample collection was evaluated. However, modifications in number of collected samples per set

and sampling intervals may result in more accurate sampling protocols. Furthermore, the impact of season on the accuracy of environmental samples requires confirmation. A systematic sample of farms with low proportions of MAP-positive environmental samples and MAP-environmental sample culture-negative farms should be included in a longitudinal study and a large number of environmental samples should be collected in short intervals from all age groups including young stock (Chapter 7). Proportions of positive farms should be compared between sampling strategies. The impact of season on the accuracy of samples collected from various locations should be assessed.

Production losses in test negative MAP-infected cattle are only known with great uncertainty (Chapter 6). Furthermore, disease progression is likely dependent on dose of MAP infection (Mortier et al., 2013), which might be associated with within-herd prevalence. However, the impact of within-herd prevalence on production losses is unknown. Therefore, a systematic selection of herds with high and low proportions of MAP-positive environmental samples, a proxy for within-herd prevalence (Lavers et al., 2013), should be included in a longitudinal study. Whole-herd individual-animal testing should be performed at regular intervals. The goal of testing would be to detect MAP infection, and perhaps approximate time of infection. As a test method, fecal culture with its high specificity and possibly higher sensitivity than ELISAs would be most appropriate (Nielsen and Toft, 2008). Interferon-gamma testing should be considered as additional test method, since it measures an early cellular immune response to MAP challenge and might be a good indicator for MAP exposure (Jungersen et al., 2002). Body weight, size, body condition score, and milk production data should be estimated at

regular intervals. Test results should only be communicated with participating farmers after the end of data collection. The primary outcome of the study would be to estimate production losses through MAP infection in different age groups and assess the impact of within-herd prevalence. A secondary outcome would be to describe shedding patterns of cattle in herds with a low and high prevalence of MAP infection.

Farms with manure-contaminated cattle and pens were more likely to be MAP-infected than farms with clean animals and pens, which provided evidence for MAP transmission between group-housed cattle, especially in poor hygienic conditions (Chapter 4). It is suspected that within-group transmission of MAP occurs in different age groups (Espejo et al., 2013; Van Roermund et al., 2007), but it remains unknown whether the rate of MAP transmission differs between age groups. A randomized controlled clinical trial should be conducted including cattle sourced at herds with low risk for MAP infection (AJDI herd status level 4). Calves should be divided into donor and receiver calves. Donor calves should be exposed to an oral dosage of MAP at a predefined age. Donor calves and receiver calves should be divided into 3 groups referring to 3 independent experiments: calves, young stock, and adults. Within each experiment, donors should be housed with receivers for a constant amount of time while they are calves young stock, or cows. After exposure, cattle should be housed individually for a certain amount of time before euthanasia and MAP culture on a high number of tissues. During and after exposure, fecal samples for MAP culture, and potentially whole blood samples for interferon-gamma testing should be collected in regular intervals. Experience gained from a MAP calf-to-calf transmission trial in 2015 should be used to determine sample size, ratio between donors and

receivers, infection dose, and duration of co-housing challenge. The outcomes of the study would be MAP transmission rates in different age groups of cattle. Once transmission rates within the specific age groups are known, it should be assessed in follow-up experiments, whether pen cleanliness impacts the rate of transmission within pens.

8.6 References

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Appendix A: ABC rejection model used to estimate true prevalence using finite population size.

M= Population size

n= Sample size

x= Test positive farms

Se= Test sensitivity

Sp= Test specificity

NA= Reject iteration

Eq1= RiskIntUniform(0,M)

Eq2= RiskHypergeo(n,Eq1,M)

Eq3= IF(Eq2=x,Eq1,NA())

Eq4= Eq3/M

Eq5= (Eq4+Sp-1)/(Se+Sp-1)

Appendix B: Environmental sample description sheet used to record locations of collected samples.

Environmental sample description sheet

(Send with samples)

Veterinarian: ----------

Farm: ----------

Owners name: ----------

Date: ----------

describe sampling location (more than one checkmark per section is possible)

Sample	*Check*	*Type of animals*	*Check*	*Area sampled*
1		Lactating cows		Cross over alley
		Dry cows		Alley
		Sick cows		Exercise area
	Check	*Type of pen*		Bedding pack
		Tie stall		Gutter
		Free stall		Manure pit
		Loose housing		Manure pile
				Lagoon

Comment: _____

Sample	*Check*	*Type of animals*	*Check*	*Area sampled*
2		Lactating cows		Cross over alley
		Dry cows		Alley
		Sick cows		Exercise area
	Check	*Type of pen*		Bedding pack
		Tie stall		Gutter
		Free stall		Manure pit
		Loose housing		Manure pile
				Lagoon

Comment: _____

Appendix C: Prevalence of risk factors on farms participating in the Alberta Johne's Disease Initiative, stratified by the number of Mycobacterium avium subspecies paratuberculosis culture-positive environmental samples.

	# MAP positive environment samples		
General section	0	1 - 3	> 3
Q5. What access do farm visitors have?			
• No restrictions	139	55	32
• Restrictions to mature cattle	17	7	3
• Restrictions to pre-weaned cattle	17	7	2
• Restricted access or required to wear special clothing	52	11	12
Q6. JD history?			
• Yes, JD has been observed in the herd	50	29	28
• Don't know	59	22	12
• JD never observed; no testing done	67	20	7
• JD never observed; testing in past 5 y with negative results	49	9	2
Q7. Did you purchase any animals, including bulls, in last 5 y?			
• Yes, from multiple herds at public auctions	101	45	28
• Yes, from auctions with known contributors	30	10	4
• Yes, but only 1 or 2 animals from a single herd	58	18	9
• Yes, from JD Herd Status farms	0	1	0
• No purchases	36	6	8
Q7.1. What precautions were taken?			
• No precautions	154	65	41
• Seller indicated no JD in the herd, but had never tested	23	6	1
• Purchased animals were tested before delivery	10	2	0
• Purchased animals came from low risk herds (JD Herd Status Program)	3	2	0
• No purchases	35	5	7

Q8. Show attendance; equipment sharing			
• Yes, herd members attend shows and/or hauled in shared vehicles	35	13	9
• Yes, pens are cleaned by custom manure removal operations	23	4	4
• Yes, shows are only attended with herds with lower risk for MAP	6	2	1
• No show attendance, no shared equipment	161	61	35
Preweaned heifers			
Q9. Are any calves raised on custom heifer-rearing operations?			
• Yes, the rearing operation raises heifers from multiple herds	5	1	3
• Yes, but the rearing operation raises only heifers from my herd	1	0	0
• No, all heifers are raised on farm	219	79	46
Q10. What is the source of colostrum?			
• Pooled colostrum from > 1 cow	15	12	8
• Some calves get colostrum from a cow other than their dam	110	39	20
• Calves are only given their dam's colostrum	83	21	16
• Calves are only given pasteurized colostrum or colostrum replacer	17	8	5
Q11. How often is non-saleable milk fed to calves?			
• Non-saleable milk is always (weekly) fed	101	41	27
• Non-saleable milk is often (once or twice a month) fed	30	10	6
• Non-saleable milk is rarely (once or twice a year) fed	19	2	2
• Non-saleable milk is never fed	75	27	14
Q12. What are the sources of liquid diet fed to calves?			
• Bulk or pooled milk	129	45	25
• Milk from individual cows	27	5	7
• Pasteurized milk or milk replacer for < 2 y	19	10	4
• Pasteurized milk or milk replacer for > 2 y	50	20	13
Q13. Is there any cow manure on milk feeding utensils?			
• Extensive manure contamination	0	0	1
• Some manure contamination	8	6	6
• Traces of manure, but utensils washed at least weekly	66	21	14
• No manure, utensils washed daily	151	53	28

Q14. Is there any cow manure in water buckets and feed bunks?			
• Extensive manure contamination	0	0	3
• Manure is clearly visible in calf feeders or water buckets	7	1	4
• A scant amount of manure is visible	49	19	12
• All calf feed and water containers are clean	169	60	30
Q15. How are calves housed?			
• Close proximity to cows	8	2	4
• Group pens until weaning	40	17	13
• Individual pens with contact through partitions	71	19	9
• Individual pens without contact	106	42	23
Q16. Staff hygiene routine when entering calf barn			
• Staff never clean boots or change coveralls	63	20	17
• Staff sometimes clean boots and change coveralls	70	22	15
• Staff always clean boots and sometimes change coveralls	80	32	15
• Staff always clean boots and change coveralls	12	6	2
Weaned heifers			
Q17. Exposure to cow manure or runoff			
• Heifers share pens or pastures with cows	71	31	17
• Heifers are housed near cows, exposure to runoff	94	29	19
• Heifers housed near cows, no exposure to runoff	30	10	7
• Heifers never housed near cows, no exposure to runoff	30	10	6
Q18. Manure contamination of feed bunks and water troughs			
• Manure build up in housing, contaminated feed bunks and water troughs	6	3	5
• Manure clearly visible in feed bunks and water troughs	61	21	11
• Traces of manure visible, feed bunks and water troughs cleaned more than once a month	94	38	23
• No manure visible, feed bunks and water troughs cleaned more often than once a month	64	18	9

Q19. Is feed equipment used for manure, or left over feed given to heifers?			
• Feeding equipment is used to remove manure	98	30	24
• Feeding equipment not used for manure, but cow left over feed fed to heifers < 1 y of age	26	11	9
• Feeding equipment not used for manure, but cow left over feed fed to heifers > 1 y of age	29	15	8
• Feeding equipment is not used for manure, left over feed is never fed to heifers	72	24	7
Q20. To what degree is manure contamination evident on heifers?			
• Manure is present above hocks/knees and on flanks	21	7	10
• Manure is present on hocks/knees or on flanks	91	39	23
• Manure is present up to dewclaws	90	29	13
• No visible manure on animals	23	5	2
Q21. Manure spread on heifer forage or pasture used the same year			
• Manure is spread on pastures where heifers graze	18	9	9
• Manure is spread on land next to heifer pastures	11	6	4
• Manure is spread on forage land used to feed heifers	64	38	20
• Manure is never spread on land used for heifers	132	27	15
Calving pen			
Q22. How many cows are in a calving pen at a time?			
• > 1 cow > 50% of the time	146	53	35
• > 1 cow 25 – 50% of the time	26	9	5
• > 1 cow < 25% of the time	38	7	6
• Never > 1 cow	15	11	3
Q23. How contaminated with manure is the calving pen?			
• Visible manure covering > 2/3rd of the bedding	15	4	5
• Visible manure covering 50% of the bedding	43	19	12
• Visible manure covering 10% of the bedding	122	41	23
• No visible manure	45	16	9
Q24. To what degree is manure contamination evident on cows?			
• Manure present above hocks and on teats or udders	6	2	8
• Manure present up to hocks or on teats or udders	63	19	13
• Manure present above dewclaws, but not on teats or udders	126	52	21
• No visible manure, udder hair is clipped and teats are washed	30	7	7

Q25. Use of calving area for sick cows			
• Frequent use of calving area by non-calving cows or known MAP positive cows	43	17	14
• Occasional use of calving area by non-calving cows	46	11	10
• Rare use of calving area by non-calving cows	69	25	9
• Calving area is never used by non-calving cows	67	27	16
Q26. Calves born outside the calving area in the past year			
• > 10%	53	15	7
• 6 – 10%	23	5	4
• 1 – 5%	93	46	29
• Never	56	14	9
Q27. What percentage of calves nurse their dam?			
• > 50% nurse their dam, are left > 4 h	53	19	15
• 10 – 50% nurse their dam	64	17	14
• < 10% nurse their dam	94	36	17
• None	14	8	3
Q28. How long do calves stay with their dam?			
• < 10% of calves are removed < 30 min	112	31	24
• 10 – 50% are removed < 30 min	42	22	12
• 50 – 90% are removed < 30 min	49	17	8
• > 90% are removed < 30 min	21	10	5
Dry cows			
Q29. Manure contamination of feed bunks and water troughs			
• Extensive manure contamination	2	0	3
• Manure clearly visible	44	15	12
• Trace amounts of manure visible	111	42	26
• No manure contamination	68	23	8
Q30. Manure contamination in dry cow forage or on pasture used the same year			
• Feeding equipment is used to scrape manure	94	31	23
• Feeding equipment not used to scrape manure, but manure spread on pasture or crop land	19	12	5
• Feeding equipment not used to scrape manure, manure not spread on pasture but on crop land	28	13	13
• Feeding equipment not used to scrape manure, and manure not spread on pasture or crop land	84	24	8

Q31. Manure contamination on close up cows			
• Manure above knees/hocks and on flanks	17	4	3
• Manure above knees/hocks but not on flanks	41	14	11
• Manure not above knees/hocks	119	42	26
• Cows are clean above fetlocks	48	20	9
Lactating cows			
Q32. Manure contamination of feed bunks and water troughs			
• Extensive manure contamination	4	1	5
• Manure clearly visible	32	7	13
• Trace amounts of manure visible	114	49	26
• No manure contamination	75	23	5
Q33. Manure contamination in lactating cows forage or on pasture used in the same year			
• Feeding equipment is used to scrape manure	96	30	23
• Feeding equipment not used to scrape manure, but manure spread on pasture or crop land	19	12	5
• Feeding equipment not used to scrape manure, manure not spread on pasture but on crop land	25	14	13
• Feeding equipment not used to scrape manure, and manure not spread on pasture or crop land	85	24	8
Q34. Manure contamination on close up cows			
• Manure above knees/hocks and on flanks	18	9	9
• Manure above knees/hocks but not on flanks	41	15	10
• Manure not above knees/hocks	129	48	27
• Cows are clean above fetlocks	37	8	3

Appendix D: Review of the economic impact of changes in management to control transmission of *Mycobacterium avium subspecies paratuberculosis* (MAP) on dairy farms.

Reference Study location Study design	Losses due to MAP infection	Analysed interventions	Economic outcome
Groenendaal et al., 2002 United States Simulation	Lower milk production Diagnosis and treatment costs Reduced slaughter value Increased risk of being culled	Rearing of heifers off site from day 1. Simulations were conducted with and without improvements in management before the calves were sent to the rearing facility	Net benefit: US$29,905 without improved management and US$ 43,917 with improved management for a 100-cow herd over 20 y
Dorshorst et al., 2006 United States Simulation	Lower milk production Decreased fertility Reduced slaughter value Increased risk of being culled	Better calving hygiene and immediate removal of newborn calves from the dam, colostrum from only 1 dam followed by milk replacer, separation of cows and calves	Although improved colostrum hygiene and feeding only milk replacer yielded a positive net benefit, maternity pen hygiene was not cost effective
Cho et al., 2012 United States Simulation	Lower milk production Reduced slaughter value Increased risk of being culled	Improvements in calf liquid diet management, separation of cows and calves	Net benefit: US$165,621 for a 100-cow herd with an initial prevalence of 10% over 50 y
Groenendaal and Wolf, 2008 United States Observational	Lower milk production Reduced slaughter value Increased risk of being culled	Variety of changes in management implemented on 40 farms; testing of animals (testing costs either included or not included)	Net benefit: US$34 per cow-year if testing costs were excluded, and US$-14 if testing costs were included
Kudahl et al., 2008 Denmark Simulation	Lower milk production Decreased fertility Reduced slaughter value Increased risk of being culled	Better calving hygiene and immediate removal of newborn calves from the dam, colostrum from own dam or colostrum replacer followed by milk replacer, and separation of cows and calves	Farms implementing the intervention had a higher net benefit than farms which did not implement the intervention