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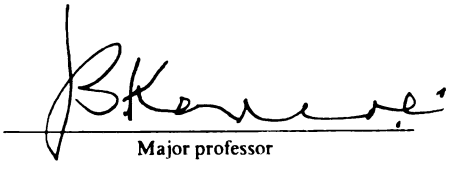

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EPIDEMIOLOGY AND ECONOMIC IMPACT OF SUBCLINICAL JOHNE'S DISEASE ON MICHIGAN DAIRY HERDS

By

Yvette Joyce Johnson-Ifearulundu, DVM, MS

A DISSERTATION

Submitted to
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in partial fulfillment of the requirements
for the degree of

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Department of Large Animal Clinical Sciences
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ABSTRACT

Epidemiology and Economic Impact of Subclinical Johne's Disease on Michigan Dairy Herds

By

Yvette Joyce Johnson-Ifearulundu, DVM, MS

This dissertation describes studies conducted regarding the epidemiology and economic impact of subclinical *M. paratuberculosis* infection on Michigan dairy herds. Two studies were conducted to identify risk factors for *M. paratuberculosis* infection and to evaluate the effect of subclinical paratuberculosis at the herd-level and at the level of the individual animal.

The first study was cross-sectional in design and conducted at a herd-level using a multi-stage stratified random sample of 147 Michigan dairy herds. A random sample of cows from each herd were tested for antibodies to *M. paratuberculosis* using an ELISA. Soil samples were collected and a two-part questionnaire was administered to provide detailed data on herd demographics, management practices, farm productivity and economics. Multivariable statistical methods including logistic regression with random effects, Poisson regression, and linear regression were used to identify management and environmental risk factors for *M. paratuberculosis* infection and to assess the impact of herd infection status on herd productivity.

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The second study was conducted at an individual animal level using a prospective, two group cohort study. A sample of 466 animals from five *M. paratuberculosis*-infected dairy herds was tested using the ELISA and radiometric fecal culture. Milk production and reproductive outcomes were then monitored for 18 months. Multivariable mixed regression modeling with a random effect term to control for herd management was used to compare *M. paratuberculosis* positive animals to their negative herdmates.

Results from the first study showed that application of lime to pastures resulted in a ten-fold decrease in the risk of a herd being paratuberculosis positive. Greater soil iron content and reduced pH were both associated with an increased prevalence of paratuberculosis positive animals. A 10% increase in the paratuberculosis prevalence was associated with a 74 pound decrease in average culling weight. Positive herd paratuberculosis status was also associated with a 3% increase in cow and heifer mortality rate annually.

Results from the second study showed that testing positive on either the ELISA or radiometric fecal culture did not significantly reduce milk, fat, or protein production. ELISA positive cows had a 28 day increase in days open when compared to negative herdmates.

Application of lime to pasture and housing areas may be a useful component in paratuberculosis control programs. Further study of the role of soil type in the epidemiology of paratuberculosis is warranted. Valid assessment of the economic impact of subclinical paratuberculosis must control for the method of case diagnosis and the age of the study population.

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This dissertation is dedicated to Joyce C. Johnson for teaching me to triumph in the face of adversity; and to Harold Sr. and Harold Jr. -- I miss you.

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TABLE OF CONTENTS

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Chap
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SUM

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LIST

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Table

Table

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INTRODUCTION

RATIONALE

Bovine paratuberculosis has been described as one of the most important diseases affecting the cattle industry. First reported in 1895, the disease is characterized by a chronic granulomatous enteritis. To date, there are no drugs approved for use in food animals to cure the disease. Efforts to develop an effective vaccine have failed to produce a vaccine that adequately protects animals from infection and shedding of viable organisms. Clinically ill animals manifest a chronic intermittent diarrhea resulting in productivity losses, weight loss and eventual death. The dramatic losses attributable to the clinical disease have been well documented. Much less information is available about the economic impact of the subclinical carrier. In fact, there is controversy in the literature regarding the magnitude and even the direction of the impact of subclinical paratuberculosis on some indicators of productivity. One obstacle to calculating the cost of the subclinical disease is proper classification of infected animals.

Efforts to prevent the spread of the disease to uninfected herds and to control transmission within infected herds focus on the identification and removal of the subclinical carrier. It is the subclinical carrier that is believed to pose the

greatest risk to the herd because the subclinical animal is difficult to detect and may transmit the organism for months or years prior to the onset of clinical signs. In fact most infected animals in a herd do not proceed to clinical cases. Widespread adoption of these time-consuming and often costly measures will only be achieved if there are effective control strategies that are economically beneficial. Determining risk factors for subclinical infection and estimating economic losses attributable to subclinical infection are important steps in the development of a comprehensive and cost-effective paratuberculosis control program.

PROBLEM STATEMENT

Previous research has indicated that approximately 9% of the cattle presented for slaughter in Michigan were positive for *M. paratuberculosis* infection. While this indicated that paratuberculosis is a problem in Michigan, very little data are available detailing the epidemiology of paratuberculosis among Michigan dairy herds. The prevalence of infected herds and their distribution throughout the state has not been determined. Management-related and environmental risk factors for paratuberculosis in Michigan dairy herds have also not been determined. And lastly the economic impact of paratuberculosis on Michigan dairy herds has yet to be determined. Improved diagnostic tests for the identification of subclinical cases and study designs that evaluate the impact of paratuberculosis on both a herd-level and an individual cow level are two tools

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that make it feasible to further evaluate the epidemiology and economics of paratuberculosis on Michigan dairy farms.

OBJECTIVES

This dissertation, therefore, focuses on the epidemiology and economic impact of the subclinically infected *M. paratuberculosis* positive animals on Michigan dairy farms. The objectives of this study were to:

1) Determine the prevalence of *M. paratuberculosis* positive dairy herds in Michigan
2) Identify management-related risk factors for paratuberculosis on Michigan dairy farms.
3) Identify environmental risk factors for paratuberculosis on Michigan dairy farms
4) Determine the herd-level economic impact of paratuberculosis on Michigan dairy farms.
5) Determine the impact of subclinical paratuberculosis on milk production.
6) Determine the impact of subclinical paratuberculosis on reproductive outcomes.

HYPOTHESES

Specific hypotheses tested are presented in the individual chapters except Chapters 1 and 2 which are literature reviews.

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OVERVIEW

Each chapter in this dissertation is written in a format suitable for publication independently. Thus, each chapter has an abstract, introduction, hypothesis, methods, and discussion (Chapters 1 and 2 are literature reviews and do not have hypotheses or methods). Chapter 1 is a literature review of the epidemiology and economic impact of subclinical paratuberculosis. This chapter describes the current body of knowledge and identifies areas for further research. Chapter 2 is a literature review that analyzes what has been reported about the role of soil pH and iron content in the epidemiology of *M. paratuberculosis* infection. This chapter also identifies areas for further research. These sections provide the background for the following chapters which describe how this study has sought to contribute to the current body of knowledge on the epidemiology and economic impact of subclinical paratuberculosis. Chapters 3 and 4 describe the distribution of paratuberculosis among Michigan dairy herds and identify management and environmental risk factors associated with paratuberculosis. Chapter 5 is an economic study of paratuberculosis positive herds in comparison to negative herds. Chapter 6 is an individual-animal level study of the impact of subclinical paratuberculosis infection on milk production and reproductive outcomes. The dissertation concludes with an overall summary and recommendations for future research.

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Chapter 1

EPIDEMIOLOGY AND ECONOMIC IMPACT OF SUBCLINICAL JOHNE'S DISEASE: A REVIEW

1.1 INTRODUCTION

Johne's disease or ruminant paratuberculosis is considered to be one of the most serious diseases in the cattle industry (McNab *et al* 1991). First described by Johne & Frothingham in 1895, Johne's disease was reported in the U.S. in 1908 (Kreeger 1991; Chiodini *et al* 1984). Clinical cases of bovine paratuberculosis result in chronic intermittent diarrhea that is non-responsive to antibiotic or anthelmintic therapy leading to progressive wasting, emaciation, and death (Sockett 1993; Chiodini *et al* 1984). Annual death losses within a herd may be as high as 3-10% (Kreeger 1991).

Extensive research has been conducted on Johne's disease however, there is no cure for the disease. The vaccine that is currently available does not provide complete protection, and the prevalence of the disease in the US may be increasing despite efforts in several states to implement control programs. While there is a considerable body of literature detailing the economic losses attributable to clinical Johne's disease, efforts to determine the losses to the dairy industry associated with subclinical *M. paratuberculosis* infection have

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been blocked by the difficulty identifying subclinical carriers and assessing the impact of infection on the productivity of these animals. Development of cost effective Johne's disease control programs for dairy producers will require quantifying the losses due to subclinical *M. paratuberculosis* infections and the costs of implementing various aspects of the recommended control strategies.

The objectives this literature study are to: (1) describe the epidemiology of Johne's disease with emphasis on the current knowledge regarding transmission and control of the disease, (2) describe the pathological basis for the reduced productivity attributable to subclinical Johne's disease, (3) identify reported economic losses attributable to *M. paratuberculosis* infection at the national/industry-wide and individual herd level, and (4) identify areas for further research by assessing the limitations in the current knowledge regarding the economic analysis of subclinical Johne's disease.

1.2 EPIDEMIOLOGY

A. Fecal-Oral Transmission

Since chronic infection and a long latency period are characteristic of Johne's disease, subclinical shedders are thought to be the most important means of spreading Johne's disease (Sockett 1993). Once infected, animals may begin shedding organisms at any time but shedding tends to increase with time (Whitlock *et al* 1986). Although clinical cases shed the greatest number of

infectious bacteria, subclinically infected cattle may shed for 6 - 18 months prior to developing clinical signs of Johne's disease (Whitlock *et al* 1986).

M. paratuberculosis infection most often occurs through the fecal-oral route, due to ingestion of contaminated fecal matter. Calves less than thirty days of age are most susceptible to infection and most cattle are infected before they are four months of age (Hagan 1938). Goodger *et al* (1996) reports that hygienic colostrum collection and removal of the calf from the dam within one hour significantly reduce the apparent prevalence of *M. paratuberculosis* infection. Use of the same equipment for handling manure and feed and direct fecal contamination of feed by cattle or from manure run-off have also been implicated in the transmission of Johne's disease (Sweeney 1996). This was reflected in a recent study of management practices and their association with *M. paratuberculosis* infection, in which it was reported that low scores for environmental conditions and manure handling were correlated with an increased prevalence of *M. paratuberculosis* infection (Goodger *et al* 1996). While care of the very young calf is widely known to be associated with prevalence of Johne's disease, recent studies have reported that care of post-weaning calves is also an important element in the transmission of Johne's disease (Collins *et al* 1994; Goodger *et al* 1996).

Susceptibility to the disease decreases as the calves mature even without previous exposure (Larsen *et al* 1975). Larsen *et al* (1975) demonstrated that resistance reaches the level of an adult animal by one year of age. Adult

animals can become infected however, they usually do not progress to clinical disease (Hines *et al* 1995). The mechanism of this resistance remains unknown but it has been hypothesized that the more alkaline pH of the mature ruminant gut may suppress the growth of *M. paratuberculosis* due to increased competition with other microorganisms for available iron (Richards 1989). The solubility of iron increases as pH decreases, making it more readily absorbed by poor iron chelators like *M. paratuberculosis* (Barclay 1985). An alternative theory has also been proposed. Sweeney (1996) suggests that perhaps the mucosal barrier against *M. paratuberculosis* is reduced during the period of time when the gut is "open" for the absorption of immunoglobulins from colostrum. Adult transmission is less common but has been reported (Sherman 1985; Sockett 1993; Kreeger 1991; Rossiter *et al* 1994). Thus, while older animals are more resistant to infection and less likely to develop clinical disease when infected, they can become asymptomatic carriers, periodically shedding the organism.

B. Other Routes of Transmission

Johne's disease has typically been considered an enteric disease however, several studies have demonstrated that paratuberculosis is indeed a generalized infection even in the asymptomatic animal. After ingestion the organism can first be isolated from the supra-pharyngeal and mesenteric lymph nodes and tonsils (Kreeger 1991; Payne & Rankin 1961). *M. paratuberculosis* then localizes in the distal ileum where it is taken up by ileal dome M cells and

transferred to subepithelial and intra-epithelial macrophages (Kreeger 1991; Sockett 1993). The organism multiplies in the macrophages resulting in the formation of multi-nucleated giant cells and spread to regional lymph nodes (Kreeger 1991). Chiodini (1996) speculates that *M. paratuberculosis* may migrate within macrophages from the Peyer's patches to the mesenteric lymph nodes, the thoracic duct, the systemic circulation, and then into the lamina propria. The epidemiological significance of this finding lies in the additional routes of transmission that are now possible.

M. paratuberculosis has been isolated from the supra-mammary lymph nodes of subclinical cows (Sweeney *et al* 1992b). It has been isolated from the colostrum (Streeter *et al* 1995) and milk of asymptomatic fecal culture positive cows (Streeter *et al* 1995; Sweeney *et al* 1992b). Larsen & Kopecky KE (1970) reported the isolation of *M. paratuberculosis* from the semen and genital tract of an infected bull. Recently Sweeney *et al* (1992a) demonstrated that the in-utero transmission of Johne's disease previously reported to occur with clinically affected dams (Seitz *et al* 1989), can also occur with subclinically affected dams. The prevalence of fetal infection was 8.6% in subclinical Johne's positive cows (Sweeney *et al* 1992a). Isolation of *M. paratuberculosis* from the uterine flush fluids of cows with clinical paratuberculosis was reported by Rohde & Shulaw (1990). While this has yet to be reported in subclinical cows, the risk of transmission from harvesting of embryos from infected dams (even if they are not symptomatic) cannot be ignored. Milk, colostrum, semen, and embryos, of

asymptomatic cows now become potential vehicles of transmission. Control programs that once focused on manure management alone must now take these additional risk factors into consideration if they are the be effective.

1.3 CONTROL PROGRAMS

There is currently no approved drug for the treatment of Johne's disease in cattle (St. Jean 1996). Many antimicrobial drugs have been used in the treatment of clinical cases of paratuberculosis, however, none of them eliminate the infection. They only reduce clinical signs and shedding of the organism, the amelioration of clinical signs requires life-long treatment and none of the drugs have been approved for use in cattle. No withdrawal times have been established, meat and milk from animals treated with them cannot be used for human consumption (St. Jean & Jernigan 1991). According to St. Jean (1996) treatment of cattle affected by paratuberculosis is very costly and should be limited to animals of great genetic value or companion animals. Although the risk of transmission of *M. paratuberculosis* infection from artificial insemination or embryo donation is believed to be small - it should still be considered in the decision to treat a Johne's positive animal.

The lack of a practical, effective therapy for Johne's disease means that control of paratuberculosis requires implementation of management practices to prevent both horizontal and vertical transmission within the herd (Brunner 1991). There are two objectives for an effective Johne's disease control program:

identify and cull or isolate *M. paratuberculosis* positive cattle; and reduce exposure of susceptible animals to infectious material (Sherman 1987; Brunner 1991; McCaughan 1989; Collins 1994; Thoen & Haagsma, 1996). According to Whitlock *et al* (1994), the addition of subclinically infected *M. paratuberculosis* carriers into cattle herds is the most important source of infection.

Several states have developed programs for the control of Johne's disease designed to reduce the prevalence of positive animals on infected farms and prevent transmission to uninfected farms. The basic elements of each program are the same (See Figure 1).

Figure 1. Recommendations* for the Control of Johne's disease in the US.

- calving should occur in clean maternity pens
- calves should be separated from dams immediately
- no natural nursing should be allowed
- no fecal contamination of colostrum, milk replacer, milk, or calf feed
- calves should be housed separate from adult cows
- handling of calves should be completed prior to handling of cows
- equipment and utensils used with calves should be kept free of contamination with manure from adult cattle
- drinking water should be kept free of contamination;
- biannual testing of cattle over 2 years of age
- cull culture positive animals and their offspring
- cull animals with recurrent diarrhea and their offspring
- use semen from non-infected bulls
- clean and disinfect areas where infected animals have been
- maintain a closed herd or only purchase from negative herds
- fence manure run-off areas and stagnant pools
- don't spread manure on grazing pasture

* Donch 1992; Brunner 1991; Sherman 1987; Collins & McLaughlin 1989; Whitlock *et al* 1994.

Some control programs include vaccination as a component (Sherman 1987). Use of the vaccine is controversial and there are several restrictions to the use of the vaccine. It can only be used in herds that are fecal culture confirmed positive for Johne's disease and must be administered by a veterinarian after the herds had been certified tuberculosis-free. The vaccine can also only be used on calves less than 30 days of age. However, vaccination does not offer protection from infection, it only helps prevent development of clinical signs and reduces shedding (Sherman 1987; Collins & McLaughlin 1989). Thoen & Moore (1989) reported reduced economic losses in four Iowa dairy herds participating in a control program that included vaccination as one of the elements. In Hungary, Kormendy (1994) demonstrated that vaccination resulted in reduced fecal shedding (from 48% to 1.4% in vaccinated cattle) and an eventual decline in the seroprevalence. In a Dutch study it was reported that vaccination sufficiently reduced the average economic loss of culled cows to be profitable (Kalis *et al* 1994). Recently vaccination has been recommended for the reduction of Johne's prevalence in herds that cannot introduce management changes (Thoen & Haagsma 1996).

In an effort to control the spread of paratuberculosis in infected herds and prevent its introduction into negative herds, voluntary national guidelines have been established. The National Paratuberculosis Certification Program was developed for use by individual states in implementing a Johne's disease-free herd certification program (Sockett 1996). This would provide producers with a

means of insuring that replacement animals purchased from other herds are at low risk for introducing paratuberculosis into the herd.

National interest in controlling Johne's disease has increased in part due to a hypothesized association between *M. paratuberculosis* and Crohn's disease (Chiodini & Rossiter 1996; Van Kruiningen HJ *et al* 1991; Sanderson *et al* 1992). Concern has been further heightened by recent indications that the organism is not inactivated at normal pasteurization temperatures (Grant *et al* 1996; Grant *et al* 1994; Chiodini & Hermon-Taylor 1993) and that store-bought, pasteurized milk may contain genetic evidence of *M. paratuberculosis* contamination (Millar *et al* 1994). A National Johne's Working Group has been established in part to develop guidelines for a National Johne's Disease Control Program (Sockett 1996). Although still a voluntary program, it will establish a uniform national standard for Johne's disease control efforts (Sockett 1996).

Implementing and maintaining a comprehensive paratuberculosis control program can be costly, labor intensive, and prolonged. Four years is the minimum time reported from initiation of a control program to Johne's free status; some herds have remained positive after ten years of implementing control efforts (Sherman 1987) . Justifying the expense and additional management efforts to producers requires an assessment of the impact of Johne's disease on farm productivity and the cattle industry.

1.4 PATHOLOGICAL BASIS FOR REDUCED PRODUCTIVITY IN SUBCLINICAL JOHNE'S DISEASE

Reported economic losses due to subclinical Johne's disease include: reduced feed efficiency (Patterson & Berrett 1968; Patterson *et al* 1967); decreased milk production (Abbas *et al* 1983; Benedictus, *et al* 1987; deLisle 1989; Hutchinson 1988; Wilson *et al* 1993; Wilson *et al* 1995); decreased milk fat and protein (Collins & Nordlund 1991; Sweeney *et al* 1994); reduced slaughter weight at culling (Whitlock *et al* 1985); decreased fertility (Abbas *et al* 1983; Merkal *et al* 1975; Buergelt & Duncan 1978); premature culling (Merkal *et al* 1975; Wilson 1995; Buergelt & Duncan 1978; Benedictus *et al* 1987); and increased incidence of mastitis (Merkal 1975; McNab 1991). Two physiological mechanisms may be responsible for this wide variety of effects - negative energy balance and impaired cellular immunity.

A. Negative Energy Balance

Cows that are infected with *M. paratuberculosis* may have an increased probability of being in negative energy balance due to reduced intestinal absorption of nutrients. This malabsorption begins after infection when, localized granulomatous lesions form in the lymph nodes and lamina propria. Eventually the lesions coalesce forming a granulomatous enteritis which is usually localized within the terminal ileum but may be disseminated throughout the entire gastro-intestinal tract (Sockett 1993; Chiodini *et al* 1984). The granulomatous enteritis and mucosal thickening result in a malabsorption syndrome with a protein losing enteropathy (Kreeger 1991). The association between this pathology and the

observed reductions in feed efficiency, milk production, milk fat and protein production, and slaughter weight are clear. Less obvious however, is the association between negative energy balance and fertility.

Little definitive information is available on the impact of subclinical Johne's disease on reproductive efficiency, however, negative energy balance (inadequate dietary energy intake) can reduce growth and development of corpus lutea and result in a reduction of serum progesterone (Terhune 1993; VandeHaar *et al* in press). In post-partum cows, negative energy balance results in an increased interval to first ovulation, a reduced number of large follicles, and reduced growth of preovulatory follicles (Britt 1994; Terhune 1993). Two studies have stated that there was an increased incidence of infertility among subclinical Johne's disease cases (Merkal *et al* 1975; Buergelt & Duncan 1978) however, they did not quantify the extent of the infertility or attempt to specify its cause. Abbas *et al* (1983) found that calving interval for infected cows were 1.73 months greater than those for noninfected cows. This study was not limited to subclinical cases and did not attempt to identify the source of the increased calving interval.

B. Impaired Cellular Immunity

The pathogenesis for the hypothesized relationship between paratuberculosis infection and increased risk mastitis and premature culling has not been definitively established. It has been proposed that the persistence of disease may be due to inadequate cell-mediated immune responses (Kreeger *et al* 1991; Kreeger & Snider 1992; Kreeger *et al* 1992). Several recent studies

have demonstrated both *in vitro* and *in vivo* that rodents, ruminants, and humans infected with *M. paratuberculosis* have alterations in cell mediated immune responses (Lepper 1989; Singh *et al* 1993; Kreeger *et al* 1991; Kreeger & Snider 1992; Kreeger *et al* 1992; Dalton *et al* 1992; Little *et al* 1994; Veazey *et al* 1994; Stabel 1994). Kreeger *et al* (1991) found that monocytes from infected cattle have a reduced response to antigens. Kreeger *et al* (1991) reported spontaneous release of interleukin-1 by peripheral monocytes of cattle infected with *M. paratuberculosis*. This response has also been reported in association with other granulomatous diseases including sarcoidosis, Crohn's disease, leprosy, and scleroderma (Kreeger *et al* 1991). Dalton *et al* (1992) demonstrated reduced antigen induced suppressor activity in the peripheral blood monocyte cells of patients with inactive Crohn's disease. Singh *et al* (1993) also found that buffalo infected with paratuberculosis or tuberculosis have a significantly higher mean hemolytic complement component C3 level than healthy age-matched buffaloes. This increase in C3 level is thought to be related to granuloma formation. Kreeger *et al* (1992) and Kreeger & Snider (1992) report that cases of chronic paratuberculosis have a reduced capacity to produce interleukin-2. Little *et al* (1994) found a significant increase in the percentage of gamma-delta T-cell receptor bearing cells in sheep with paratuberculosis. Veazey *et al* (1994) conclude that decreased proportions of CD4+ cells and reduced interleukin-2 production or function may be involved in the persistence of *M. paratuberculosis* infections. Stabel (1994) demonstrates that tumor necrosis factor and

interleukin-6 activities are reduced in cattle infected with *M. paratuberculosis*. Each of these studies reveal mechanisms by which cell-mediated immunity may be detrimentally affected by *M. paratuberculosis* infection. In addition to providing a mechanism for maintaining a chronic *M. paratuberculosis* infection, impaired cellular immunity may leave subclinically infected cows at increased risk for other illnesses (Kreeger 1991; Thoen & Baum 1988). Merkal *et al* (1975) also found an increased risk of culling due to health reasons other than mastitis and infertility.

An association between *M. paratuberculosis* infection and reduced immunocompetence may also be the basis for the elevated rate of culling due to mastitis, infertility, and other health problems. Perhaps it is this relationship that is causing the reported increased risk of premature culling in the subclinical paratuberculosis positive cow.

Although it is often reported that Johne's positive animals are at an increased risk for mastitis, the studies that have been conducted have offered conflicting results. Merkal *et al* (1975) demonstrated that culture positive subclinical cows were at a greater risk to be culled for mastitis than their culture negative herdmates. McNab *et al* (1991) reported an increased SCC in ELISA positive cattle. In contrast, DeLisle *et al* (1989) found no difference between culture positive cows and their negative herdmates and Wilson *et al* (1993) found culture positive cows to actually have a reduced incidence of mastitis.

1.5 ECONOMIC LOSSES ATTRIBUTABLE AT THE INDIVIDUAL PRODUCER LEVEL

Identifying the sources of the economic losses attributable to *M. paratuberculosis* infection and determining the pathogenesis of these sign one of the first steps toward determining the economic impact of subclinical paratuberculosis. The next step toward assessing the economic impact of paratuberculosis is quantifying the losses that may be attributed to *M. paratuberculosis* infection. Efforts have been made to calculate the losses due to Johne's disease and most agree that the disease accounts for tremendous losses to the US cattle industry. However, many of the reported losses are due to quantifiable direct costs resulting from clinical disease. A recent nationwide cross-sectional study of dairy cows has reported that in *M. paratuberculosis*-infected herds where at least 10% of the cull cows showed clinical signs of Johne's disease, the average cost to producers was $227 for each cow in the herd. These losses were based on lost milk production, cull cow revenue, and costs of purchasing replacement cows after adjusting for the value of calves born and cows sold as replacements (USDA-APHIS-VS 1997). While these losses are significant, even more devastating may be the indirect costs and losses due to subclinical infection (Jones 1989). Quantifying those losses to the individual producer have proven very challenging.

The wide variation in estimated productivity losses associated with asymptomatic Johne's disease may be attributed to the differing methods for identifying cases and selecting sample populations. Fecal culture; serologic

tests such as ELISA, CF, and AGID; and histopathologic diagnosis may each be revealing a different subset of positive animals. These animals may differ in the stage of disease and thus in influence on productivity. In addition, the lack of sensitivity in many tests results in false negative animals being included in the control group and hence reducing observed differences in productivity. Further complicating matters is the variation in sample populations for these studies. Studies based on production records of cull cattle may not be representative of the economic impact on the productivity of the remaining milking herd. Economic losses in herds known to be infected with Johne's disease not be representative of losses in a random sample of herds both at the herd-level and individual animal level.

A. Reduced Feed Efficiency

Patterson *et al* (1967) demonstrated protein losing gastroenteropathy in Johne's positive cows, reporting that clinical cases of paratuberculosis lost an average of 39 grams of plasma protein more than negative cows. Patterson & Berrett (1968) also revealed that *in vitro* intestinal tissue from clinical cases of paratuberculosis, absorbed histidine at approximately one-half the rate of intestinal tissue from normal cows. These studies indicating a protein losing gastroenteropathy and malabsorption in clinical cases of Johne's disease, however, do not determine the cost of this reduced feed efficiency to producers or establish whether or not a similar effect occurs in subclinically infected animals.

B. Reduced Milk Production

Several studies have reported reduced milk production in subclinical *M. paratuberculosis* test-positive cows. Abbas *et al* (1983) found a 15% (1838 lbs.) reduction in mean annual milk yield in subclinically infected cows when compared to their negative herdmates. Benedictus *et al* (1987) reported a 6% reduction in milk production in the second to last lactation and a 16% reduction in the final lactation prior to culling in subclinically infected cows. Whitlock *et al* (1984) demonstrated that asymptomatic paratuberculosis-infected cows gave 12 pounds a day less than culture-negative herdmates. Wilson *et al* (1993) concluded that beyond the second lactation, paratuberculosis-positive cows produce between 1300 and 2800 pounds less per lactation. It was then determined that the lost milk production costs producers from $80 and $250 per lactation (Wilson *et al* 1995). In a New Zealand prospective study milk production losses ranged from less than statistically significant to a 17% reduction in milk production for the most seriously affected herds (DeLisle 1989). Recently, Nordlund *et al* (1996) reported that ELISA positive cows had a 4% reduction in mature equivalent milk production than their negative herdmates.

C. Decreased Milk Fat and Protein

While a reduction in the quantity of milk produced by subclinically infected cows has been established, only recently has data been gathered to demonstrate a reduction in the quality of milk produced by asymptomatic *M. paratuberculosis*-positive cows. Daily milk fat and milk protein was reported to

be significantly reduced in culture positive subclinical cows when compared to culture negative cows (Sweeney *et al* 1994). Collins & Nordlund (1991) determined that subclinical Johne's disease was associated with a reduction in 305 day mature equivalent protein and fat that costs producers $205 per cow per lactation.

D. Decreased Slaughter Weight at Culling

The reduced carcass weight and slaughter value of clinical cases is apparent and has been documented (Benedictus *et al* 1987). Some investigators also report loss of weight or body condition score in the absence of clinical signs (Hutchinson 1996). In Pennsylvania study of a random sample of cull cows, culture positive animals weighed on average 129 pounds less than culture negative culls. This resulted in a loss of $48 per carcass in lost income at slaughter.

E. Reduced Fertility

Two studies have reported that asymptomatic *M. paratuberculosis* culture-positive cows were at greater risk of being culled for infertility (Merkal *et al* 1975; Buergelt & Duncan 1978). While these studies found a statistically significant increase in the rate of positive animals being culled with infertility cited as the reason for culling they did not quantify the effect on fertility. It has been reported that in a California herd in which the majority of positive animals were asymptomatic, culture positive cows has a calving interval that was 1.7 months longer than negative herdmates (Abbas 1983). There is no data in the current

literature on the financial impact of the reported delay in calving interval. In fact conflicting results have been obtained on the relationship between subclinical Johne's disease and infertility. Using the ELISA to identify *M. paratuberculosis*-infected cows, DeLisle (1989) and NcNab (1991) failed to find any association between calving interval and Johne's infection.

F. Premature Culling

Buergelt & Duncan (1978) reported that many asymptomatic *M. paratuberculosis* culture-positive animals were slaughtered prior to reaching their peak milk production potential resulting in lost genetic potential in the herd. Benedictus *et al* (1987) calculated losses due to premature culling of subclinical culture positive animals. They reported losses due to idle production factors and unrealized future income of British pounds Sterling 316 per average culled animal in addition to losses caused by reduced milk production. Recently, it was reported that subclinical culture-positive cows are at risk for decreased survival in dairy herds. The increased rate of culling resulted losses of approximately $75 per cow per year (Wilson *et al* 1995).

G. Increased Incidence of Mastitis

Two studies have reported an increased risk of mastitis in asymptomatic Johne's positive cows. The risk of culling due to mastitis was found to be 22.6% in culture positive animals, while only 3.6% in culture negative animals (Merkal *et al* 1975). McNab *et al* (1991) demonstrated that lipoarabinomannan-ELISA positive cows had significantly higher somatic cell counts that negative animals

at both the individual animal and herd-levels. The cost to producers of the increased mastitis was not determined. In contrast DeLisle (1989) failed to find any association between Johne's infection status and mastitis. In fact, Wilson *et al* (1993) found subclinical culture-positive cows to be a reduced risk of new and chronic mastitis.

While several factors have limited the ability of investigators to calculate the economic impact of quantifiable losses, many of other potential losses identified may prove even more difficult to quantify and may vary greatly from region to region and farm to farm. For example, the economic costs to a positive herd from lost breeding value (i.e. semen sales, purebred cattle sales, and embryo transfer) and lost trade as states and countries impose restriction on the transport and sale of cattle from *M. paratuberculosis*-infection positive herds may be exceedingly difficult to determine on the individual herd-level (Jones 1989).

1.6 ECONOMIC LOSSES AT THE NATIONAL/INDUSTRY-WIDE LEVEL

A. Determine the Scope of the Problem - Prevalence

Evaluating the impact of paratuberculosis on the US cattle industry requires determining the prevalence of the disease. Johne's disease is known to be widespread in the US. A 1971 survey of the distribution of Johne's disease reported *M. paratuberculosis* positive cows in 47 states (Kopecky, 1973). This study also identified 11 states with widespread distribution of paratuberculosis based on the number of counties with one or more herds identified as positive:

California, Florida, Indiana, Iowa, Maryland, Minnesota, Ohio, Oregon, Pennsylvania, Washington, and Wisconsin (Kopecky 1973). Point and period prevalence estimates from 1.6 - 18% have been obtained in several studies (Riemann *et al* 1979; Abbas *et al* 1983; Whitlock *et al* 1984; Whitlock *et al* 1985; Collins *et al* 1993; Kaneene *et al* 1991; Arnoldi *et al* 1983; Frisby *et al* 1985; Chiodini & Van Kruiningen 1986; Braun *et al* 1990; Merkal *et al* 1987; Richards *et al* 1983). One study of herd prevalence in Wisconsin reported that 33% of the dairy herds had at least one cow that was seropositive for *M. paratuberculosis* (Collins *et al* 1993). Recently research conducted by the USDA has reported a 3.4% prevalence of *M. paratuberculosis*-positive dairy cows in the US using the ELISA (USDA-APHIS-VS1997). That study also reported a 21.6% prevalence of *M. paratuberculosis*-positive herds (a positive herd was defined as one with 2 or more test positive animals and at least 5% of culled cows in the previous year with clinical signs consistent with paratuberculosis) (USDA-APHIS-VS 1997). Several factors can be identified that may account for the large variation in estimates of prevalence among these studies -- uneven geographic distribution of the disease; differing sensitivity of diagnostic tests used; differing prevalence within the test population (i.e. random versus cull cows). Obtaining a true prevalence and distribution of *M. paratuberculosis* infection in the US cattle population will require further study.

B. Regional Estimates of Economic Impact

Despite the difficulty in determining how many cattle are infected with *M. paratuberculosis*, efforts have been made to estimate the economic impact of Johne's disease on the dairy industry. In Pennsylvania, losses due to reduced salvage value and milk production in the last lactation was estimated at $5,859,000 when *M. paratuberculosis* culture positive cows were compared to culture negative cows (Whitlock 1984). Annual losses in New England, due to Johne's disease have been estimated at $15.4 million (Hutchinson 1988). Paratuberculosis is estimated to cost the Wisconsin dairy industry $100 million annually (Sockett 1993). Recently, it has been reported that subclinical disease alone costs the state of Wisconsin $1.85 million per year in reduced milk production (Nordlund *et al* 1996). This figure does not include the costs of other losses directly or indirectly attributable to Johne's disease. These rather staggering calculations of losses due to paratuberculosis may still underestimate the actual economic impact of the disease. Losses are usually based on reduced salvage value at culling and reduced milk yield during the last one or two lactations. However, many of other potential economic costs from paratuberculosis have been identified such as lost future income, increased veterinary costs, decreased feed efficiency, and decreased fertility. Determination of the total economic impact of Johne's disease on the US dairy

and beef industries will require assessment of these factors in addition to lost salvage value and milk yield.

C. Economic Simulation Models

Two economic simulation models have been developed for paratuberculosis. The first, created by Walker *et al* (1988 a,b) predicted the economic impact of Johne's disease under various types of control strategies for an 80 cow, closed herd in Wisconsin. In 1991, Collins & Morgan developed an economic decision analysis model of Johne's disease. This model, which used a microcomputer spreadsheet program has been described as inexpensive, simple and faster than the Walker *et al* model (Collins & Morgan 1991a). It also had the benefits of allowing flexibility in selecting herd size, replacement rates, hygiene level, open or closed herd management, and diagnostic test sensitivity, specificity and cost (Collins & Morgan 1991a). These values were all fixed in the Walker *et al* model (Walker *et al* 1988a). The Collins & Morgan decision analysis model also expressed outcome by the net profit or loss resulting form different actions while the Walker *et al* model expressed outcome by the rate of return to labor and management. The use of net profit or loss as an outcome make the Collins & Morgan model easier to interpret by producers.

Despite is advantages over the Walker *et al* model, the Collins & Morgan model still does not provide a complete economic assessment of the benefit-cost of Johne's disease control strategies. This model is limited by the lack of documentation of some of the costs associated with *M. paratuberculosis*

infection. Collins & Morgan base their calculation of Johne's disease costs on decreased milk production, decreased slaughter weight, increased *M. paratuberculosis*-infection rate among replacements and costs of false positive test results (Collins & Morgan 1991b). Losses associated with paratuberculosis due to mastitis, infertility, reduced feed efficiency, increased susceptibility to other disease and other indirect losses were not included in the model due to lack of documentation (Collins & Morgan 1991b).

The inability to estimate values of these additional sources of economic costs associated with Johne's disease may lead to under-estimation of the costs of the disease and de-valuation of the benefits of control and eradication programs. The determination by the Collins & Morgan model that a test and cull program will be profitable when the herd paratuberculosis prevalence is > 5%, may be overly conservative when the impact of productivity losses not assessed in the model are taken into consideration. Further study to quantify the economic effect subclinical paratuberculosis is essential to the refinement of economic models and decision analysis programs for Johne's disease. Until a more complete assessment of the costs of Johne's disease has been conducted, current models have limited application for advising producers on the most cost-effective strategies for controlling Johne's disease on their farm.

1.7 LIMITATION TO CALCULATING THE COST OF JOHNE'S DISEASE: DISEASE DETECTION

Despite considerable research on Johne's disease since it was first reported in 1895, calculating the cost of Johne's disease at the herd and industry level has proven to be challenging. Difficulty identifying subclinical carriers, calculating herd and regional prevalences, and estimating losses attributable to subclinical paratuberculosis has hampered efforts to determine the true economic impact of Johne's disease. Accurate estimates of the prevalence of Johne's disease are essential to evaluate the economic impact of the disease. The lack of an accurate and efficient survey of a representative population has hampered efforts to determine the prevalence of paratuberculosis (Jones, 1989). Many of the tests available do not have adequate sensitivity or specificity, and are too costly or time consuming for use in a survey to determine the prevalence of Johne's disease. There are three types of diagnostic tests currently in use for the detection of *M. paratuberculosis* infection: bacterial isolation, immunologic assays, and genetic probe tests.

A. Bacterial Isolation

Stained fecal smear has a low sensitivity (Sherman 1985). It can also result in excessive false positives, 54.4% (Kormendy 1990). Only 25 to 35% of cattle found positive by fecal culture were identified by examination of fecal smears (Thoen & Haagsma 1996). The very low sensitivity and specificity of this technique limits its usefulness in diagnosing *M. paratuberculosis* infection.

Cultural isolation of the organism is considered the most accurate method of diagnosing paratuberculosis (Whipple *et al* 1989). Isolation of the organism from a clinical specimen is considered to be 100% specific, however, a very contaminated environment may result in *M. paratuberculosis* transiting through the gut without actually colonizing the gut (Collins 1996; Sweeney *et al* 1992c). Standard fecal culture techniques however, are time consuming and expensive. Culture techniques are not standardized and vary widely across laboratories (Collins 1996). These different techniques may result in differing abilities to eliminate contamination and successfully isolate *M. paratuberculosis* from specimens (Collins 1996). Recent evaluation of standard (HEY) culture with subclinical cases yielded a 45.1% sensitivity (Sockett *et al* 1991) required 12 weeks for results and cost $12 per sample. Recent modification of fecal culture techniques has resulted in radiometric fecal culture. Radiometric culture is more sensitive, faster, and less expensive than standard culture methods. A sensitivity of 54.4% resulted from radiometric culture of subclinical cases. Culture results were obtained in 7 weeks and cost $8 per sample (Sockett *et al* 1991).

Histopathologic examination of tissues has proven to be an impractical diagnostic technique. Pinch rectal biopsy has low sensitivity and results in an excessive number of false negatives (Sherman 1985). Ileocecal lymph node histopathology and culture is also impractical because it is expensive, time

consuming, and results in a scar that may reduce salvage value (Sherman 1985).

B. Immunologic Assays

Intra-dermal and intravenous johnin tests for Johne's disease are *in vivo* tests based on cell-mediated immunity. They have been found to be of low sensitivity and specificity (Kreeger 1991). Excessive numbers of false positive and false negatives have been reported. False negatives are most common with advanced clinical cases (Whitlock 1986). The johnin skin test and intravenous johnin test is no longer recommended (Collins 1996).

Recently a new assay for cell-mediated immunity was developed. Gamma interferon *in vitro* cellular assays have been developed for diagnosis of Johne's disease. These tests have been reported to have better sensitivity with subclinical cases than with advanced clinical cases and should be used in parallel with serology (Wood *et al* 1989). Testing of this new assay is still in the early stages however, according to Collins (1996), recent reports are encouraging and the test may be applicable to cattle, sheep and goats.

There are several serologic tests used most for detecting antibodies to *M. paratuberculosis* in infected animals. Many of these tests have not been widely used under field conditions (Thoen & Haagsma 1996). Three tests that are the most widely used are agar gel immunodiffusion (AGID), complement fixation (CF), and enzyme linked immunosorbent assay (ELISA) (Collins 1996). The limitation to the use of serologic tests is that they may not be able to detect very

early cases of infection that are low-level shedders (Collins 1996; Thoen & Haagsma 1996).

Agar gel immunodiffusion (AGID) is considered a good test for confirming clinical cases of Johne's disease and it is fast and inexpensive with results obtainable in 48 hours (Sherman 1985). However, AGID is less sensitive for subclinical cases, only the heaviest subclinical shedders will test positive (Sherman 1985). A high rate of false positives has also been reported with this test (Kormendy 1990). More recently however, Collins (1996) reports that the specificity of AGID has been increased by the use of improved antigens and removal of cross-reacting antibodies. The United States Department of Agriculture (USDA) has now licensed an AGID with 99% specificity (Collins 1996).

Complement fixation (CF) was thought to be of questionable usefulness due to its moderate sensitivity and specificity (Sherman 1985). A false positive rate of 52.2% was previously reported with the CF test (Kormendy 1991). However, as with the AGID, it has recently been reported that improvements in the test using better antigens and removing nonspecific antibodies have resulted in CF tests with 99% specificity (Collins 1996). Hemagglutination is highly sensitive but of low specificity (Sherman 1985). The lack of specificity is due to problems with cross-reactivity with other bacteria. Florescent antibody tests have been found to lack both the sensitivity and specificity for detecting subclinical cases (Abbas *et al* 1983).

Enzyme Linked Immunosorbent Assay (ELISA) is reported to have the best sensitivity and specificity of all the serologic tests (Kreeger 1991; Collins 1996). The absorbed ELISA has been reported in several studies to have a sensitivity between 47% and 57% and a specificity between 98% and 100% (Milner *et al* 1990; Kaneene *et al* 1992; Collins *et al* 1991). The sensitivity of ELISA varies depending upon the stage of infection of the animal being tested. Sweeney *et al* (1995) demonstrated that the ELISA had a sensitivity of only 15% for very early infections in low fecal shedders. This rose to a sensitivity of 87% in clinical cases (Sweeney *et al* 1995). Overall the sensitivity was reported to be 45% (Sweeney *et al* 1995). The ELISA is relatively inexpensive, rapid, and practical for use on a herd basis.

C. Genetic Probe Tests

Genetic tests based on DNA probes and polymerase chain reactions (PCR) are also being investigated for use in the diagnosis of *M. paratuberculosis* infection. A genetic element unique to *M. paratuberculosis* deemed *IS900* is detected in clinical specimens by PCR amplification (Collins 1996; Whipple *et al* 1992). Recent study of a DNA probe test yielded a 33.5% sensitivity with subclinical cases and 100% specificity (Sockett *et al* 1991). False positives have been reported to occur due to contamination of the specimen with gene products created in the laboratory (Collins 1996; Thoen & Haagsma 1996). The lower sensitivity has been attributed to the greater number of organisms required for detection than with fecal culture (Whipple *et al* 1992). Results were obtained

within one to 3 days and the cost per sample was $25 (Sockett *et al* 1991; Collins 1996; Thoen & Haagsma 1996).

Assessment of diagnostic tests for Johne's disease currently available indicate that the radiometric fecal culture and absorbed ELISA offer the most promising recent advances in the search for a rapid, inexpensive, practical test for the diagnosis of both subclinical and clinical infection. The availability of cost-effective, simple tests that have adequate sensitivity for diagnosing subclinical cases of Johne's disease will help in determining the true prevalence of infection by encouraging testing of representative populations, thereby reducing sampling bias. Many previous estimates of prevalence were based on samples from cull cows. Those animals may not provide a representative sample of the cattle population (Jones 1989).

1.8 SUMMARY OF ECONOMIC LOSSES ATTRIBUTABLE TO SUBCLINICAL JOHNE'S DISEASE

Investigators have identified several potential routes of transmission for *M. paratuberculosis* infection: fecal-oral; in utero; milk and colostrum; and perhaps semen and embryos. The importance of many of these routes of transmission has not bee established however they only complicate efforts to control the spread of paratuberculosis. Current control efforts have been described as costly, time consuming, and frustrating. Difficulty identifying subclinical carriers has impeded efforts to determine the national prevalence of the disease and to assess the economic impact of the disease at the individual herd and industry-

wide levels. The economic impact of subclinical Johne's disease has not been adequately assessed. Productivity of subclinically infected animals is thought to be reduced due to an increased risk of negative energy balance and impaired cellular immunity. The economic effects are manifest through reduced feed efficiency, reduced milk production, reduced milk fat and protein content, decreased slaughter weight, infertility, premature culling, and increased incidence of mastitis and other health problems. However these economic effects have not been quantified and many studies offer conflicting results. The difficulty in establishing the economic effects of subclinical Johne's infection may be due to the low sensitivity of diagnostic tests resulting in the inclusion of false-negative cases in the control groups.

1.9 AREAS FOR FURTHER RESEARCH

The real value in determining the economic impact of Johne's disease on dairy and beef production is not simply to assess the impact of the disease on the industry. Calculating the costs of paratuberculosis to the individual producer provides a means of analyzing the cost-effectiveness of control and eradication programs (Jones 1989). Aspects of the control program can be tailored to meet the specific needs of individual producers and aid in management decision making. A speaker at a recent producer and practitioner symposium on Johne's disease expressed some of the concerns facing producers regarding current testing and control programs for Johne's disease.

"1. the producer who openly attempts to control paratuberculosis on his premises and not spread it to other farms is being asked to carry and unjust burden due to current regulations by comparison to enterprises where it goes knowingly or unknowingly undiagnosed.
2. the producer is not being offered advice on programs that are compatible with reality or economic survival. Thus the cure is worse than the disease.
3. that there is not currently sufficient economic incentive to have a test negative population as opposed to what would fairly be called 'status unknown'." (Knight - Wisconsin Breeders Association 1988).

A detailed assessment of the costs of both clinical and subclinical Johne's disease and a cost-benefit analysis of each component of a Johne's disease control program will address these producer concerns. It will provide an economic incentive for producers to determine their paratuberculosis disease status and to take steps to control it while maximizing productivity and profitability. However, before a benefit-cost analysis of Johne's disease control efforts can be conducted, there are several areas in which more research is needed.

(1) Determining the scope of the problem at the herd-level. Assessment of the costs and benefits of control efforts requires that each producer be aware of the prevalence of *M. paratuberculosis* infection in his/her herd. Further prospective study of the within-herd epidemiology of Johne's disease will offer information regarding current losses and projected future losses as prevalence increases within a herd. Some research in this area has indicated that prevalences in some herds can become quite high in the absence of appropriate control measures (Collins & Morgan 1991a; Collins & Morgan 1992).

(2) Nine areas of potential reductions in productivity in the subclinically infected cow have been identified: reduced feed efficiency; reduced milk production; milk protein and fat; reduced slaughter weight; increased infertility; increased incidence of mastitis; lost genetic potential; premature culling losses; and the increased risk of other infectious diseases. However, some of these associations have yet to be confirmed and none of them have been adequately quantified for use in economic assessments of herd-level impact of subclinical Johne's disease.

(3) Three additional herd-level costs of subclinical Johne's disease must also be assessed: the cost of lost revenue as more states and countries impose trade restrictions on cattle that are not from herds certified Johne's disease free; the cost of implementing a routine testing program; and the costs of control efforts.

In addition to these areas of further research needed to assess the economic impact of Johne's disease at the herd level, further research is also needed to assess the economic impact of subclinical Johne's disease at the national/industry level. Despite over 100 years of research investigators have been unable to determine the economic impact of subclinical paratuberculosis at the national level. Until recently, lack of cost-effective accurate diagnostic tests for subclinical Johne's disease has hampered efforts to determine the national prevalence of the disease. Many previous studies are based upon slaughter based prevalences, this population may not be representative of the national

herd, and thus may introduce bias in the calculation of the apparent prevalence of paratuberculosis. Recent improvements in diagnostic capabilities has made it possible now to determine the prevalence of the disease in a representative sample of cows. However, when designing protocols for paratuberculosis screening sample size calculations must be adjusted for the relatively low sensitivity of the tests available. Alternatively it has been recommended that the apparent prevalence obtained by ELISA testing be doubled to estimate the true prevalence of infection to adjust for the sensitivity of the test (Collins 1996).

(4) National screening for paratuberculosis using a combination of assays including serologic, culture, cell-mediated immunity and perhaps genetic probes is essential to determine the scope of the disease in the US and form the basis for developing a national control program.

This assessment may become even more crucial if the hypothesized association between *M. paratuberculosis* infection and Crohn's disease in humans is confirmed. The concern that current pasteurization temperatures may not be sufficient for the inactivation of *M. paratuberculosis* could indicate that Johne's disease poses a potential health risk both for people working in contact with cattle and for consumers of dairy products. Determination of the national prevalence of *M. paratuberculosis* infection is paramount to assessing the threat that Johne's disease poses to the cattle industry and the public health.

Chapter 2

RELATIONSHIP BETWEEN SOIL TYPE AND *MYCOBACTERIUM PARATUBERCULOSIS*

2.1 INTRODUCTION

Paratuberculosis (Johne's disease) is an economically devastating, chronic wasting disease of ruminants caused by *Mycobacterium paratuberculosis*. Extensive research has been conducted on paratuberculosis since it was first described by Johne and Frothingham in 1895. However, more than 100 years of work has failed to produce a cure or reliable vaccine. Recent advances in diagnostic tests have improved efforts to identify subclinical carriers of the disease; however, control strategies have proven to be costly, labor intensive, and, often, frustrating. It has been reported that a minimum of 4 years compliance in a paratuberculosis control program is required before a herd can be declared disease free (Sherman 1987). Some herds have tested positive for *M paratuberculosis* after 10 years of control efforts (Sherman 1987). The disappointing progress in the reduction of the prevalence of *M paratuberculosis* infection has led to a search for different measures to control paratuberculosis, including vaccine development and adjustment of soil pH.

Efforts to develop a protective vaccine have been largely disappointing. Current vaccines reduce fecal shedding, but do not eliminate the infection (Kormendy 1994; Wentink *et al* 1994; Larsen *et al* 1978). In addition to the incomplete protection provided by vaccination, lesions at the site of vaccination and hypersensitivity to avian and mammalian tuberculin are disadvantages of the current paratuberculosis vaccines (Larsen 1973).

Environmental factors that may prove to be fundamental in the control of paratuberculosis are soil type and pH. If a relationship between soil type and paratuberculosis can be demonstrated, perhaps producers can ameliorate the efficacy of existing control measures by including the adjustment of soil characteristics (in areas containing calf hutches, pastures, exercise lots, and cattle housing areas) into current control regimens. The fundamental question is, "Does the literature contain information that supports the hypothesis that regional soil pH is related to the prevalence of paratuberculosis?"

In epidemiology, criteria for determining causal associations have been established (Hill 1965). The basic elements include strength of association, consistency, specificity, temporality, biological gradient, plausibility, coherence, experimental evidence, and analogy. With respect to each of these criteria, we reviewed the literature regarding the associations between soil type and *M paratuberculosis.*

2.2 STRENGTH OF ASSOCIATION

The strength of association criterion is used to compare the rate of disease in the group with the exposure of interest to the rate of disease in the unexposed group. A strong association would be reflected by a much higher rate of disease in the exposed group when compared with the unexposed group. Weak associations are more likely to be caused by bias or confounding, whereas strong associations are more likely to be causal (Rothman 1986). Results of several studies (Smythe 1935; Jansen 1948; Kopecky 1973; Kopecky 1977; Richards 1989; Vandegraff 1994) indicate that there may be an association between the prevalence of paratuberculosis and regional soil pH. However, these studies do not provide information on associated risk ratios or whether the observed difference in disease prevalence is statistically significant. There is not evidence in the literature indicating a strong association between regional soil type and prevalence or paratuberculosis.

2.3 CONSISTENCY

Consistency, as a criterion, refers to the repeated observation of the association in different populations and circumstances (Rothman 1986). Several studies (Smythe 1935; Jansen 1948; Kopecky 1973; Kopecky 1977; Richards 1989; Vandegraff 1994) provide evidence of an association between high prevalence of clinical paratuberculosis and acidic soils that are deficient in calcium. In England, cases of clinical paratuberculosis did not develop along the

southwest coast where the soil is aeolian sand with a high calcium carbonate content, but clinical disease did develop in an adjacent region with acidic soils deficient in lime (Smythe 1935). In the Netherlands, it was reported that the prevalence of clinical paratuberculosis was high in a region with calcium deficient and acidic soil (Jansen 1948). A survey (Kopecky 1973) was conducted to determine the distribution of bovine paratuberculosis in the United States. This study identified 11 states (Wisconsin, Indiana, Ohio, Maryland, Pennsylvania, Florida, Iowa, Minnesota, Oregon, Washington, and California) with widespread distribution of paratuberculosis (Kopecky 1973). Further analysis of Wisconsin cattle with paratuberculosis between 1971 and 1975 was done to compare the locations of farms on which cattle with paratuberculosis had been reported to the Wisconsin Department of Agriculture with soil maps from the Wisconsin Geological and Natural History Survey (Kopecky 1977). Kopecky (1977) concluded that clinical paratuberculosis persisted in regions with acidic soils, but not in regions with alkaline calcareous soils; in these areas, the disease is self-limiting. Richards (1989) reviewed anecdotal evidence from Italy and several regions of the United States that demonstrated an association between soil pH and paratuberculosis.

Despite a high prevalence of paratuberculosis in several parts of Australia, Vandegraff *et al* (1994) reported that the prevalence of *M paratuberculosis* infection in individual ruminants in South Australia was < 0.5% and the herd prevalence was approximately 1.5%, as measured by postmortem

bacteriologic culture of ileocecal tissue. It is speculated that the low prevalence may be attributed, in part, to the alkaline soils and relatively low rainfall (Vandegraff 1994).

The association between a high prevalence of paratuberculosis in regions with acidic soils is reported consistently in the United States, Western Europe, and Australia. To the authors' knowledge, there have not been any studies that report the opposite relationship or that failed to find any relationship between soil pH and prevalence of paratuberculosis. However, this may be attributed to a lack of epidemiologic studies seeking to find an association between the prevalence of paratuberculosis and the regional soil type. The literature primarily consists of ecologic data and anecdotal observations. Epidemiologic studies that control for potential bias and confounding are necessary before the criterion of consistency can be assessed adequately.

2.4 SPECIFICITY

Specificity refers to a risk factor leading to a single outcome, and as a criterion, specificity has been described as useless and misleading (Rothman 1986). As an example, smoking has been identified as a cause of lung cancer, emphysema, and cardiovascular disease. Alteration of soil pH could alter the risk of several diseases caused by organisms found in the soil, such as anthrax. Therefore, lack of specificity would not be indicative of a lack of a causal relationship between soil pH and paratuberculosis.

2.5 TEMPORALITY

Fulfillment of the temporality criterion requires that the risk factor precede the outcome (Rothman 1986). In this case, ruminants must be exposed to acidic soil prior to infection with *M paratuberculosis*. The only study that assessed soil pH and incidence of paratuberculosis in a prospective manner was conducted by Richards (1989). He reports results of consecutive ELISA in a Missouri dairy herd in which the incidence of ruminants with positive test results for *M paratuberculosis* declined dramatically within 3 years after implementation of a control program that included application of limestone and hydrated lime to calf hutches, barn floors, and manure accumulations (Richards 1989). Although results of this report imply a temporal association, it would be necessary to have a matched control herd, using the standard control protocol alone, for comparison. Alternately, 2 cohorts on the same farm could be used to compare results and quantify any effects that the hydrated lime may have on the prevalence of cattle testing positive for paratuberculosis. Other reports of an association between low soil pH and high prevalence of paratuberculosis have been made on the basis of the results of cross-sectional studies in which the prevalence of disease and soil pH of the region were assessed at a single time. This type of study design does not permit establishment of a temporal association between the risk factor (exposure to acidic soil) and the outcome of interest (risk of infection with *M paratuberculosis*). Thus, information in the literature also fails to meet the criterion of temporality.

2.6 BIOLOGIC GRADIENT

The biologic gradient criterion refers to a dose-response relationship. In this case, we would expect to see a trend of increasing prevalence of paratuberculosis as soil pH decreases. Jansen (1948) reports that the rate of isolation of *M paratuberculosis* from bovine fecal samples increased with decreasing soil pH across several municipalities in the Netherlands. The report does not give sufficient details regarding soil sampling methods or the selection of herds to assess whether the results were representative of herds in the Netherlands. Although the results of this particular study seem to indicate a biologic gradient, they do not provide sufficient evidence to support this criterion because of the potential for bias in the soil sampling methods, herd selection, and poor sensitivity of the diagnostic method (microscopic examination of fecal smears).

2.7 PLAUSIBILITY

Fulfillment of the criterion of plausibility requires that the proposed causal relationship is biologically sound. On the basis of this criterion, there is the greatest amount of credible information in the literature. The role of environmental pH and its relationship to iron availability and bacterial growth have been studied extensively, with respect to *Mycobacterium* spp and other organisms.

Iron is an essential trace element for most bacteria (Robins-Browne & Prpic 1985). Iron availability and sequestration has been reported to be associated with bacterial survival and virulence (Payne 1980; Weinberg 1993; Fiss *et al* 1994; Neilands 1981). Iron transport, however, can pose a problem because at neutral pH, Fe^{3+} forms insoluble colloidal hydroxides (Davis 1980). Thus, many bacteria and fungi produce and excrete soluble siderophores and specific membrane receptors for iron transport (Neilands 1981). The siderophores form tight, soluble complexes with iron that are then taken up by the bacterial membrane receptors. Iron is then released into the cell by hydrolysis of the chelator (Neilands 1981; Davis 1980).

The solubility of iron increases as pH decreases (Barclay 1985). Thus, iron is more readily available to all microorganisms, including *M paratuberculosis*, in an acidic environment. Competition with microorganisms that are more efficient in their uptake of iron may inhibit growth of *M paratuberculosis* at a high environmental pH. Lambrecht and Collins (1993) have demonstrated that mycobacteria are not able to take up iron when iron is chelated to siderophores and unrelated microorganisms.

Northern surface waters and organic coniferous forest soils naturally have a low pH and are rich in organic matter, which supports growth of *Mycobacteria* spp (Iivanainen *et al* 1993; Iivanainen 1995). Whereas acidic soil is thought to enhance growth of *Mycobacteria* spp, alkaline soil is believed to suppress growth of *M paratuberculosis*. Richards (1989) has proposed that the relationship

between pH and iron availability is the key to inhibition of *M paratuberculosis* in alkaline environments.

Most *Mycobacteria* spp produce 2 siderophores, mycobactin and exochelin (Fiss *et al* 1994; Snow 1970). Bacteria differ in their ability to chelate iron. In low numbers, *M paratuberculosis* is a particularly poor iron chelator (Richards 1989). *Mycobacterium paratuberculosis* is unable to produce mycobactin, which makes it unable to sequester iron outside of the host (Snow 1970). This is manifested in vitro by the dependence of *M paratuberculosis* on culture medium with high concentrations of iron or mycobactin supplementation (Barclay & Ratledge 1983). Results of previous studies (Barclay & Ratledge 1983; Lambrecht & Collins 1992) have demonstrated that alteration of medium pH or iron concentration can influence growth of *M paratuberculosis* in the absence of mycobactin. Lambrecht and Collins (1992) report that optimal growth develops in vitro at a pH between 5.5 and 6.0.

Although the role of pH, mycobactin, and exochelins in vitro has been researched extensively, only recently have studies been conducted elucidating the mechanism of iron acquisition by intracellular bacterial pathogens. Results of research by Byrd and Horwitz (1989; 1991) on *Legionella pneumophilia* indicate that intracellular multiplication of the organism is iron dependent and that the host defends itself against *L pneumophilia* by limiting iron availability through the action of interferon gamma-activated monocytes and by activating mononuclear phagocytes. They also reported that chloroquine and ammonium chloride inhibit

intracellular multiplication of *L pneumophilia* by limiting the availability of iron (Byrd & Horwitz 1991).

Research on intracellular pH and iron acquisition by *Mycobacteria* spp in vivo is equally intriguing. As previously cited, the optimal pH for growth of *M paratuberculosis* in vitro is 5.5 to 6.0, and this may be consistent with the in vivo environment. It has been reported that the pH of the phagocytic vacuole is 4.5 to 6.0 (Lambrecht & Collins 1992; Chicurel *et al* 1988). At this low pH, the host iron binding compounds transferrin, lactoferrin, and ferritin may provide sufficient iron for the growth of *M paratuberculosis* in the absence of mycobactin (Lambrecht & Collins 1992; Momotani *et al* 1986; Momotani *et al* 1988).

Information in the literature indicates that in the in vitro and in vivo settings, environmental pH and iron availability are crucial factors influencing growth of *M paratuberculosis*. Thus, it is quite plausible to propose that survival, growth, and transmission of the organism in the freestate are also influenced by environmental pH and iron availability. Indeed, the criterion of plausibility has been met.

2.8 COHERENCE

To meet the criterion of coherence, the proposed cause-and-effect relationship should be consistent with what is known about the pathophysiology and epidemiology of the disease. The evidence of biological plausibility previously cited can also be viewed as coherent evidence with respect to the

pathophysiology of paratuberculosis. In fact, Rothman (1986) states that there is a fine distinction between plausibility and coherence. The epidemiologic evidence of coherence is not as strong.

It has been speculated that the role of pH in the microenvironment of *M paratuberculosis* may explain the mechanism of resistance to infection that develops with increasing age in ruminants (Richards 1989). Results of several studies have revealed that cattle are at greatest risk of infection with *M paratuberculosis* during the first 4 months of life, with the greatest risk being in the first month (Hagan 1938). Cattle greater than 9 months old are more resistant to infection, even if they have not had previous exposure (Larsen *et al* 1975). The mechanism of increased resistance with age is unknown.

Richards (1989) proposes that the increased risk of infection in young ruminants may be the result of a low pH in the rumen of the immature animals. As the ruminant matures, the rumenal pH increases concurrently with an increased resistance to infection with *M paratuberculosis*. If this line of reasoning proves to be true, this would indeed be coherent evidence supporting the role of environmental pH as a risk factor for paratuberculosis. However, several other factors that Richards does not discuss may be fundamental to the age-related resistance to infection with *M paratuberculosis*.

Work by Momotani *et al* (1988 a & b) indicates that in calves, dome epithelial M cells in the ileum phagocytize *M paratuberculosis*. The immature ileum of calves may be at great risk for infection with *M paratuberculosis*,

because mucosal lymphoid tissue occupies 8.6% of the small intestine of calves, approximately two-thirds of which is in the ileum (Momotani *et al* 1988b). In addition, Momotani *et al* (1988b) also demonstrated that uptake of *M paratuberculosis* is enhanced in sera with antibodies to *M paratuberculosis*, thus suggesting that antibodies from an infected dam may facilitate infection of offspring. The mechanism of susceptibility attributable to the influence of M cells in the gastrointestinal tract of calves seems more plausible than that proposed by Richards (1989). However, the 2 mechanisms may work in concert. Perhaps more important than the coherent evidence that is available is the lack of conflicting evidence. Although lack of coherent evidence may not indicate lack of a causal relationship, conflicting information provides strong evidence that the proposed association is not a causal relationship (Rothman 1986). Information in the literature does not offer conflicting information that would violate the criterion of coherence.

2.9 EXPERIMENTAL EVIDENCE

To the author's knowledge, there have not been controlled experiments to determine whether exposure to acidic soil increases the risk of infection with *M paratuberculosis* or the risk of developing clinical disease after infection. Currently, therefore, the criterion of experimental evidence has not been met.

2.10 ANALOGY

The criterion of analogy essentially determines whether the proposed causal relationship is comparable to some established causal relationship. Two examples in the literature provide evidence that soil pH and type can influence the risk of bacterial disease. In the first example, results of several studies (Minett & Dhanda 1941; Van Ness & Stein 1956; Hugh-Jones 1975; Lindeque & Turnbull 1994; Dragon & Rennie 1995) indicate that soil type and pH affect the incidence of anthrax in a region. Minett and Dhanda (1941) demonstrated that the growth of *Bacillus anthracis* was enhanced by neutral to slightly alkaline soil that was rich in nitrogen and calcium. This information was then used to develop a map of US regions that may be at high risk for outbreaks of anthrax on the basis of soil type (Van Ness & Stein 1956). Reports of outbreaks from 1953 and 1954 support the theory (Van Ness & Stein 1956). The association between outbreaks of anthrax and alkaline soils in areas of low lying depressions with high soil moisture content and high concentrations of organic matter was also demonstrated in England and Wales (Hugh-Jones 1975) and Namibia (Lindeque & Turnbull 1994). It has been hypothesized that the association between anthrax and alkaline soils may actually be the result of the high calcium concentration in the soil. Alkaline pH is characteristic of calcareous soils. Dragon and Rennie (1995) theorize that the high calcium concentration in the soil buffers the calcium supply available to *B anthracis* spores, extending their viability and facilitating germination.

The second example of analogous causal relationship is taken from literature of plant pathology. Fusarium wilt is a plant disease caused by *Fusarium oxysporum*, which was prevalent where plants were grown on acidic soils, but did not develop on alkaline soils. Scher and Baker (1982) demonstrated that *Pseudomonas putida* and its siderophore pseudomonin were found in alkaline soils, but not in acidic soils. Pseudomonin forms more stable complexes with iron than siderophores produced by *Foxysporum*; thus, *P putida* is able to acquire iron at the expense of *F oxysporum* and inhibit fusarium growth in alkaline soils. It was also demonstrated that iron concentration in soil tends to increase as pH decreases and that production of pseudomonin by *P putida* decreases as pH decreases (Scher & Baker 1982; Becker *et al* 1985).

Hoper *et al* (1995) proved that the ability of soil type to suppress fusarium wilt of flax plants was enhanced by the reduction of iron availability in the soil. In their experiment, a reduction of EDTA-extractable iron resulted from increasing the soil pH and exchangeable calcium by the addition of montmorillonite or illite. The density of fluorescent pseudomonads was positively correlated with soil suppressiveness, resulting from the increased competition for nutrients.

The anthrax and fusarium wilt examples provide evidence of associations between risk of bacterial disease and soil type that are analogous to the proposed relationship between paratuberculosis and low soil pH. Although the fusarium wilt example relates to a phytopathogen, it may be the more relevant analogy, because the proposed mechanism for inhibition of *M paratuberculosis*

at high soil pH is the same as the known mechanism for inhibition of *F oxysporum*. For this reason in particular, current literature on other diseases such as anthrax and fusarium wilt clearly offers examples that meet the criterion of analogy.

2.11 DISCUSSION

There has been little research on the association between acidic soils and high prevalence of paratuberculosis. The literature does supply evidence that the following 4 Hill's Causal Criteria (Hill 1965) have been met: coherence, plausibility, analogy, and consistency. However, with the exception of strong evidence supporting plausibility, most of the other evidence is in the form of anecdotal reports regarding paratuberculosis and in vitro research with *M paratuberculosis* and other organisms.

To the best of our knowledge, studies to date have not offered any quantitative epidemiologic analysis of the nature of the risk of paratuberculosis posed by low soil pH. Although Kopecky (1977) and Richards (1989) report an association between acidic soils and a high prevalence of paratuberculosis, there has not been a determination of the amount of risk introduced by acidic soil; hence, the strength of this association is not established. Establishment of a biological gradient is an important element of causal association. Barclay (1985) reports that there is a gradient with respect to iron solubility and pH. However, there have not been reports that indicate that growth of *M paratuberculosis* in

soil develops along such a gradient in response to soil pH or iron availability. There is no evidence on whether the relationship between soil pH and the disease follows a linear trend or develops at some threshold value of pH.

The determination of a temporal relationship is crucial in defining causal associations. The risk factor must precede the outcome. Kopecky (1977) established a pattern of association based on cross-sectional data. A prospective study is needed to demonstrate that exposure to low soil pH precedes infection with *M paratuberculosis*. Richards (1989) does provide prospective data from a *M paratuberculosis* infected herd that was tested during a 3-year period in which control measures, including application of lime, were implemented. However, the effect of lime application alone can not be evaluated, because several control measures were implemented simultaneously, and there was not a control herd for comparison.

Specificity is another criterion that has not been addressed. Several confounders may be influencing the apparent relationship between soil pH and prevalence of paratuberculosis. In the soil, soluble iron, and calcium concentrations, other bacterial and fungal species, other organic matter as well as soil type and moisture content may affect soil pH and the growth of *M paratuberculosis.* The influence of these confounders makes it difficult to determine whether there is any specificity in the relationship between soil pH and growth or whether several soil factors may influence growth of *M paratuberculosis* similarly.

Last, there is no experimental evidence demonstrating that changing soil pH alone can alter the prevalence of *M paratuberculosis* infection or clinical paratuberculosis. It has not been determined whether alkaline soils prevent exposure to *M paratuberculosis*, colonization of the gastrointestinal tract by *M paratuberculosis*, or progression to clinical disease.

To answer these questions, field-based epidemiologic studies need to be designed and conducted to measure the strength of association between soil pH and paratuberculosis, while controlling for other risk factors. In addition, a prospective assessment of control programs with and without the application of lime should be undertaken to determine the efficacy of these measures in controlling the disease. Controlled experiments need to be conducted to determine whether the organism is found in the environment and the gastrointestinal tract of ruminants living on alkaline soil. Asymptomatic ruminants may serve as a source of infection if moved to farms that have acidic soil.

It is intriguing to think that soil pH may be the key to controlling paratuberculosis and that the incorporation of a simple inexpensive measure, such as application of crushed limestone or hydrated lime, into existing control measures may succeed where other expensive and time-consuming methods have failed. Although the literature does not provide sufficient evidence to establish a causal relationship, the strength of the evidence supporting the biological plausibility of this association demonstrates that this relationship

warrants further studies. When the strong evidence of biological plausibility is considered in light of coherent evidence, strong analogous evidence, and consistent, albeit anecdotal, evidence, it becomes clear that field-based epidemiologic and controlled experimental studies should be conducted to determine whether a causal relationship does exist between soil type and risk of paratuberculosis.

Chapter 3

DISTRIBUTION AND ENVIRONMENTAL RISK FACTORS FOR PARATUBERCULOSIS IN MICHIGAN DAIRY HERDS

3.0 STRUCTURED ABSTRACT

Objective - To determine the prevalence of *M. paratuberculosis* infection in dairy herds in Michigan and identify soil-related risk factors associated with such prevalence.

Design - Cross-sectional study

Sample Population - A multi-stage stratified random sample of 121 Michigan dairy herds

Procedure - Veterinarians, animal health technicians, and senior veterinary students were trained in the collection and handling of blood and soil samples and administration of the questionnaire. Blood samples were collected from a sample of cows (aged 2 years and above) at each farm. A systematic random procedure was used to select the cows to be tested. Blood samples were tested for antibodies to *M. paratuberculosis* using the IDEXX ELISA test kit. Soil samples were collected from pastures and exercise lots for those farms in which cattle were exposed to pastures or unpaved exercise lots. Soil samples were

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tested for pH and available iron content. A pre-tested questionnaire was administered in-person to collect data regarding farm management practices and productivity.

Results from the complete dataset were used to determine the prevalence and distribution of paratuberculosis infected dairy herds in Michigan. Analysis of the associations between *M. paratuberculosis* infection and soil-characteristics were conducted on the subset of completed questionnaires from farms in which cattle spent time on soil and soil samples were collected. Multivariable logistic regression was used to analyze risk factors associated with a herd being positive for *M. paratuberculosis* infection. Multivariable Poisson regression was used to analyze risk factors associated with the sample prevalence of *M. paratuberculosis* ELISA positive animals.

Results -Samples were collected and testing conducted on 121 farms -- 80 positive herds (with $\geq$ 1 test positive animal) and 41 negative herds. Herds with only one positive animal were eliminated from the study to reduce the risk of misclassification of herds due to false positives. Twelve herds were dropped from analysis because of missing data. The resulting dataset used for statistical modeling included 46 positive herds and 37 negative herds. Thus, 55.4% of the herds sampled had $\geq$ 2 cows that tested positive for *M. paratuberculosis* using the IDEXX ELISA. Adjusting the sample prevalence for the distribution of herd size strata in Michigan yielded a statewide prevalence of 54.0%. Tests were

conducted for 3,886 animals, 267 of those tested were positive. Thus, the prevalence of positive animals was 6.9%.

The logistic regression model identified two soil-related risk factors significantly associated with an increased risk of a herd being paratuberculosis positive. For every part per million increase in soil iron content there is a 1.4% increase in the risk of a herd being paratuberculosis positive (O.R. = 1.014; C.I. 1.003 - 1.026; p = 0.0128). The application of lime to pasture areas in 1993 resulted in a herd being approximately ten times less likely to be paratuberculosis positive (O.R. = 0.063; C.I. 0.008 - 0.472).

The Poisson regression model identified 3 soil-variables associated with an increased number of paratuberculosis positive animals in a herd. A one-tenth unit increase in the soil pH was associated with a 5% decrease in the number of positive animals (O.R. = 0.95; p = 0.0019; C.I. = 0.922 - 0.982). A ten part per million increase in soil iron content was associated with a 4% increase in the number of positive animals (O.R. = 1.004; p = 0.0015; C.I. 1.002 - 1.007). Application of lime to pasture areas was associated with a 72% reduction in the number of positive animals (O.R. = 0.282; p = 0.0001; C.I. = 0.147 - 0.539).

Conclusions - The 54% state-wide prevalence of paratuberculosis positive dairy herds in Michigan obtained in this study was greater than expected, while the 6.9% prevalence of paratuberculosis positive animals was not in excess of anticipated levels. Thus, paratuberculosis is widely distributed throughout Michigan dairy herds but at a low prevalence in most herds.

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After controlling for potential confounding factors such as farm management practices, productivity, and soil texture and drainage, the prevalence of both *M. paratuberculosis* positive herds and positive animals was associated with acidic soil and increased soil iron levels. Application of lime to pasture areas was associated with a reduced risk of paratuberculosis. This may be due to the alkalinizing effect of lime on pasture soils. The reduction in iron availability that occurs at an alkaline pH may diminish the viability of *M. paratuberculosis* in the external environment, by decreasing the amount of sequestrable iron available.

Clinical Relevance - The association between soil iron, soil pH, and liming of pasture areas with a reduction in both the herd paratuberculosis prevalence rate and the prevalence of positive animals is the most clinically relevant finding of this study. If alkalinization of soil through the application of lime or other materials reduces the transmission of paratuberculosis, then this simple, low-cost practice will be an important component of paratuberculosis control programs. However, more research is needed to clearly establish the nature of the reported association. Prospective studies and controlled experiments are necessary to establish a cause and effect relationship. Clinical trials are needed to develop a soil management protocol for paratuberculosis control.

3.1 INTRODUCTION

Paratuberculosis has been described as one of the most important diseases affecting the cattle population worldwide (McNab *et al* 1991). Efforts at determining the total economic impact of the disease have been hampered by difficulty determining the scope of the problem (Johnson-Ifearulundu & Kaneene 1997a). Studies conducted in various areas of the US have reported paratuberculosis prevalences ranging from 1.6-20% of the regional cattle population being positive for paratuberculosis, with dairy cattle having a greater prevalence (Braun 1990; Whipple 1991; Thoen & Baum 1988; Kreeger 1991). A herd prevalence study in Wisconsin reported 34% of randomly selected dairy herds had at least one animal that tested positive for paratuberculosis (Sockett 1993; Collins *et al* 1994). A slaughter-based sero-prevalence study reported that 9% of the culled dairy cows presented for slaughter in Michigan tested positive for paratuberculosis (Kaneene *et al* 1992), indicating that paratuberculosis is indeed a problem in the Michigan dairy industry.

In addition to determining the scope of the paratuberculosis problem, research has also been conducted to identify management and environmental risk factors for paratuberculosis in an effort to improve the effectiveness of paratuberculosis control programs. While several reports have identified management practices that are associated with an increased risk of paratuberculosis (Goodger *et al* 1996; Thoen & Baum 1988; Sherman 1985) much less information is available regarding environmental risk factors for

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paratuberculosis. One potentially important environmental risk factor is soil pH. It has been hypothesized that paratuberculosis is more prevalent in regions with acidic soils. The proposed mechanism by which soil pH is believed to influence the prevalence of paratuberculosis is via modulation of soil iron availability (Johnson-Ifearulundu & Kaneene 1997b).

Soil iron availability may be an important risk factor for paratuberculosis because of its essential role as a bacterial micro-nutrient. *M. paratuberculosis* may be particularly sensitive to environmental iron levels because of its relatively poor capacity for iron-uptake. As an inefficient iron-chelator, *M. paratuberculosis* competes less effectively with other bacterial species for the sequestration of available iron when it is present in limited quantities. Thus, a higher soil iron content may favor the survival of *M. paratuberculosis* in the environment, increasing the risk of transmission to a susceptible host. Iron availability is, however, influenced by both iron content and soil pH.

The aim of this study was to determine the prevalence of *M. paratuberculosis* infected dairy herds in Michigan and to identify soil pH and iron content characteristics associated with an increased prevalence of *M. paratuberculosis* infected herds.

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3.2 HYPOTHESES

The specific hypotheses tested were:

1) greater soil iron content is associated with an increased prevalence of *M. paratuberculosis* infection on dairy farms and this relationship is mechanistically mediated via the soil pH;

2) acidic soil pH is associated with an increased prevalence of *M. paratuberculosis* infection on dairy farms.

3.3 MATERIALS AND METHODS

A. Study Design and Herd Selection

A cross-sectional approach was used to determine the prevalence of *M. paratuberculosis* infected herds in the state. A case-control study was used to identify soil characteristics and management risk factors associated with positive herds. Because dairy herds vary in size and their distribution within the State of Michigan, a multi-stage sampling procedure was used to select a representative sample of herds that will take account of these differences. The Michigan Department of Agriculture has identified 9 agricultural districts within the state (see Appendix A). These districts are designed in part to demarcate regions within the state that are comparable in climate, soil characteristics, and agricultural production patterns. Using these established boundaries, the state was stratified into the 9 agricultural districts. Within each district, herds were

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divided into five size categories based on the number of adult cows -- lactating and dry: (10-49, 50-99, 100-199, 200-399, and 400+ head of cows). A sample proportionate to the number of herds in each agricultural district and herd-size category was selected, using a simple random procedure. This selection was accomplished jointly with the Michigan Agricultural Statistical Service department, which has all names, locations, and sizes of dairy herds in Michigan. Because the serologic test to be used cannot differentiate between an *M. paratuberculosis* vaccinated animal from one that has subclinical paratuberculosis, all herds that practiced vaccination (about 1%) were excluded from the study. Due to the sensitive nature of data regarding herd *M. paratuberculosis* infection status, a low response rate was anticipated, to ensure an adequate sample size for statistical analysis, a total of 1000 Michigan dairy farms were contacted and invited to participate in the study. Data was collected from each farm once during the summer and fall of 1996. Data collection included sampling of cows, sampling of soil, and completion a questionnaire detailing herd productivity data and management practices over the past three years.

B. Training of Data Collectors

Blood and data collection were accomplished by veterinarians, animal technicians, and senior veterinary students following completion of a special training program. The training program consisted of a four hour seminar detailing the goals and objectives of the research project, and providing

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instruction on the proper use of the survey instruments and collection, handling, and shipping of blood samples.

C. Calculation of the number of herds to sample

The formula in Equation 1 was used to calculate the number of herds required to determine the prevalence of *M. paratuberculosis*-infected dairy herds in Michigan. Since Michigan and Wisconsin are located within the same region of the US with similar climates and dairy management practices, it was estimated that the prevalence of paratuberculosis positive herds in Michigan would be approximately the same as the prevalence in Wisconsin. Therefore, it was estimated that 34% of the herds in Michigan were infected -- based on work in Wisconsin (Collins *et al* 1994). The sample size was calculated to determine the prevalence of *M. paratuberculosis* infected dairy herds within 7.5% of the actual prevalence (see Equation 1) and a 5% probability of Type I error.

Equation 1:

$$\text{Minimum number of herds to sample} = [P_h(1-P_h)Z_{(1-\alpha/2)}{}^2]/e^2$$

where, P_h is the estimated prevalence of *M. paratuberculosis* positive herds; e is the maximum acceptable error rate; and α is probability of Type I error (0.05). From this calculation it was determined that 154 herds were required to estimate the prevalence of *M. paratuberculosis* infected herds in Michigan.

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D. Calculation of the number of animals to test per herd

Research in Wisconsin estimated that the prevalence of positive cows in a positive herd was 20% (Collins *et al* 1994). To ensure adequate power to detect herds that had a lower within-herd prevalence, the prevalence of positive cattle in an infected herd was estimated to be 10%. The general formula used to detect the presence of *M. paratuberculosis* infection in a herd of infinite size is expressed in Equation 2.

Equation 2:

$$n_{c\infty} = [\log \alpha]/[\log(1-P_c)]$$

where $n_{c\infty}$ is the minimum number of cattle per herd to test to detect a single positive animal, assuming herd size approaches infinity; P_c is the estimated prevalence of positive cattle in a positive herd; and α is the probability of Type I error (0.05). Using this equation it was calculated that 29 cattle per herd should be tested for a 95% probability of detecting a positive animal in a herd with a prevalence of $\geq$10% *M. paratuberculosis* infected animals.

Due to the low sensitivity of the IDEXX Antibody ELISA (IDEXX Laboratories, Inc., Portland, Maine) (64%) for the detection of subclinically infected animals (Kaneene *et al* 1992), the minimum required number of cows per herd required for sampling was increased by a factor of 1.64. The resulting minimum number of samples required per herd was 48 animals for a herd of infinite size. This was then adjusted for small herds using the method described by Cochran (1977). This calculation is seen in equation 3.

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Equation 3:

$$n_{fin} = [\, n^*_{c\infty} \,] / \{1+[(n^*_{c\infty} -1)/N]\}$$

where, n_{fin} is the minimum number of cattle to test to detect at least one positive animal, in a small herd; $n^*_{c\infty}$ is the minimum number of cattle per herd to test to detect a single positive animal, assuming herd size approaches infinity, adjusted for test sensitivity; and N is the median total herd size in the classification stratum.

Following the selection of participating herds, a systematic random procedure was used to select a sample of cows that were two years and older. The first cow selected for testing was selected randomly and the remaining cows were selected systematically (i.e. every third or fourth cow after the first) until the required sample size had been reached based upon the farm's herd-size stratum classification. Animals that were less than two years old were excluded from the study because the serologic test to be used is only recommended by the manufacturer for use in such animals.

E. Case Definitions

A case was defined as a cow that tested positive for *M. paratuberculosis* infection by the IDEXX antibody ELISA. A positive herd was defined as one in which there were at least two cases of *M. paratuberculosis* infection identified by the IDEXX Antibody ELISA when sampled as a part of this study. A control herd was one in which none of the animals tested positive by IDEXX Antibody ELISA.

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To minimize the risk of false classification of herds, those herds in which only one animal tested positive for *M. paratuberculosis* infection were eliminated from the case-control analysis.

F. Specimen Collection and Laboratory Testing

Ten ml of blood were collected from each selected animal via the middle coccygeal vein using a 20-gauge, 1-in. needle and a 10-ml serum separator Vacutainer tube (Corning Glass Works, Corning, NY). Samples were centrifuged, and serum harvested and stored at -70°C in cryotubes until laboratory testing was conducted.

Serum samples were tested for antibodies to *M. paratuberculosis* with a commercial IDEXX Antibody ELISA test kit using the manufacturer's recommended protocol (Collins *et al* 1993). The sensitivity and specificity of the test are 64% and 96% respectively (Anderson *et al* 1991; Kaneene *et al* 1992).

G. Collection of data relating to possible risk factors for *M. paratuberculosis* infection

To collect data on potentially confounding farm management and productivity-related risk factors, a 2-part pre-tested questionnaire was administered in person to participating farmers (see Appendices B and C). The management data were collected at the time of blood sampling, and included: herd size; number of pounds of milk sold in 1995; number of cattle added to the herd annually; number of cattle culled from the herd annually; number of acres of pasture in use; pasture rotation interval; frequency of scraping barn/drylot; methods of manure disposal; use of maternity pens for calving; use of maternity pens as

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housing for sick cows; frequency of cleaning of maternity pens; number of hours before calf and dam are separated after parturition; whether farmers use individual calf hutches or not; whether hutches are cleaned and moved after each use or not; age of calf at removal from hutch; frequency of calf-pen cleaning; and farm-related income, assets, and expenditures.

M. paratuberculosis infection typically occurs during the first 1 to 6 months of age while clinical signs or detection may not occur until 2 to 5 years of age (Hagen 1938; Larsen *et al* 1975). Due to the long incubation and latent periods for paratuberculosis, 1996 management practices may not have reflected those of the time at which infection actually occurred. In 1996, the average Michigan dairy cow was 44 months of age (3.67 years) (Michigan Dairy Herd Improvement Association, 1996). Thus, management practices of approximately 3 years prior to the study were a better indicator of risk factors affecting the infection of cows in the present milking herd with *M. paratuberculosis* than prevailing practices. Current practices may have been altered post-infection, perhaps in response to recent clinical cases of disease. To address this issue, risk factor data were collected for calendar years 1993 and 1996 and stratified by animal management category: lactating cows, dry cows, and heifers. Data from 1993 were used in the case-control study of management-related risk factors. Data related to cattle purchases and sales were collected for calendar years 1993 - 1996. Herd productivity data were collected for calendar years 1994 and 1995.

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H. Collection and Laboratory Testing of Soil Samples

As a component of the evaluation of environmental risk factors several soil samples were collected from those herds in which the cattle spent time on pasture or an unpaved exercise lot. Quarter-cup, top-soil samples were collected from several sites identified by the producer as pasture land or unpaved exercise lot. Samples were placed in specimen cups and labeled with a herd number and location number. The locations sampled were recorded on state soil survey maps.

Samples were submitted to the Michigan State University Soils Testing Laboratory for pH and iron content analyses. Soil samples were diluted with a distilled water in a 1:1 dilution and pH testing was conducted using a pH meter (Orion Instruments, Boston, Mass.). Iron content analysis was conducted by the standard method (N.C.R. 1998), in which .1N HCL was used for iron extraction, and an atomic absorption spectrophotometer (Varian Instruments, Sunnyvale, CA) was used for analyses.

3.4 DATA ANALYSIS

The t-test was used to test for significant associations between herd *M. paratuberculosis* infection status and continuous risk factors. Pearson's χ^2 was used to test for significant associations between herd *M. paratuberculosis* infection status and categorical risk factors. Since management, environmental, and productivity data were collected for several years and many variables were

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stratified by cattle management group, the complete dataset comprised 250 variables for each farm.

Data were collected from the stratified random sample of Michigan dairy farms. The entire dataset was used to determine the prevalence of *M. paratuberculosis* infected herds and to obtain descriptive data for comparison to state averages. Analysis of soil pH and iron content data were conducted for the subset of farms on which the cattle were routinely exposed to soil -- either through being on pasture or on an unpaved exercise lot. To adjust for potential correlations between farms located on similar soils, a fixed effect term for soil type was included in the multivariable logistic regression model. The soil type variable was based on the texture and drainage classification scheme of Michigan soils. Four strata were created: well-drained loams - parent drift clay (soil type 1); sands (soil type 2); intermediate and mixed drained loams (soil type 3); and wetlands (soil type 4).

Descriptive data presented in this report reflect the analyses of the variables selected for inclusion in the multivariable regression models. Variables excluded from the multivariable analyses included those variables which were deemed not to be biologically relevant to the association being modeled; those variables with an excess number of missing responses (>50%); and those variables that would not converge in the univariable models.

Logistic regression was used to model the risk of a herd being paratuberculosis positive in association with the management and soil

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characteristics listed in Tables 1 and 2. Thirty-two biologically relevant covariates were included in the full multivariable logistic regression model. The 4 soil type strata were forced into the model. No interactions were included. Backward eliminating procedure was used to model the relationship between herd paratuberculosis status and environmental and management practices. Variables were eliminated from the model with $p>0.90$ and less than 60 observations.

Poisson regression was used to model the risk of the herd sample prevalence of test positive animals in association with the management and soil characteristics listed in Tables 3 and 4. Thirty-seven biologically relevant covariates were included in the full multivariable Poisson regression model. Since farms located within the same region and with the similar soil characteristics were more likely to exhibit correlations in management practices and herd paratuberculosis status, the data were stratified on farm soil type. To adjust for potential over-dispersion caused by these spatial correlations, the dataset was aggregated by soil type a dispersion parameter was estimated by the Person Chi-Square statistic using SAS (SAS version 6.12, Cary N.C.:SAS Institute, Inc. 1997). The resulting inflation of the variance adjusted for the anticipated intra-regional correlations. No interactions were included. Backward eliminating procedure was used to model the relationship between herd paratuberculosis prevalence and environmental and management practices.

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Variables were eliminated from the model with p>0.90 and less than 60 observations.

3.5 RESULTS

A. Prevalence and distribution of paratuberculosis positive dairy herds in Michigan

Of the 1000 Michigan dairy producers asked to participate in the study, 426 (43%) replied by returning a response card. Of those responding: 210 agreed to participate in the study, 172 refused, and 44 indicated that they were no longer eligible to participate because they had sold their herd. Samples were collected from 147 farms. Data from 26 farms was eliminated from the study for failure to complete the questionnaire. Samples were collected and testing conducted on 121 farms -- 80 positive herds (with $\geq$ 1 test positive animal) and 41 negative herds. Herds with only one positive animal were eliminated from the study to reduce the risk of misclassification of herds due to false positives. There were 26 farms in which only one animal tested positive for antibodies to *M. paratuberculosis*. Twelve herds were dropped from analyses because of missing data required for multivariable modeling. The resulting dataset used for statistical modeling included 46 positive herds and 37 negative herds. Thus, 55.4% of the herds sampled had $\geq$ 2 cows that tested positive for M. paratuberculosis using the IDEXX ELISA.

The sample prevalence was adjusted for the herd size distribution across the state of Michigan since the mean herd size in the sample was greater than

the statewide mean herd size obtained by MASS. Using the MASS herd size distribution as the standard, the sample prevalence rate was adjusted using the rate standardization method described by Rothman (1986). Adjusting the sample prevalence for the distribution of herd size strata in Michigan yielded a statewide prevalence of 54.0%. Tests were conducted for 3,886 animals, 267 of those tested were positive. Thus, the prevalence of positive animals was 6.9%. See Table 3.5 for the geographic distribution of paratuberculosis positive herds.

Three additional herds were removed from the analysis of soil associated risk factors because the producers indicated that the cows did not spend time on pasture or unpaved exercise lots, thus no soil samples were collected. Removal of these herds from analysis resulted in a sample size of 80 herds for case-control multivariable modeling. The herd prevalence rate in this sample was 58% (46 positive, 34 negative). The prevalence of positive animals in the case-control study subset was 6.9% (177 / 2553).

B. Descriptive and univariable analyses

The mean herd size for this sample was higher than the state mean as reported by Michigan Agricultural Statistics: the state mean for 1995 was 70 milk cows while in this sample the mean was 125 cows for paratuberculosis negative herds and 116 cows for paratuberculosis positive herds (p=.66). The reported median annual milk production per cow of 19,187 for negative herds and 16,657 pounds for positive herds (mean = 18,437 and 16,296 respectively; p=0.14) was similar to the state average of 17,071 pounds per cow in 1995. While the

number of producers included in this study who reported a history of clinical cases of paratuberculosis (20%) was below the herd prevalence obtained by researchers in Wisconsin (34%), the prevalence of herds that contained at least one test positive animal much higher at 66%, even after removing those herds with only a single positive animal, the prevalence remained high (55% - 46/83). Despite the high prevalence of *M. paratuberculosis*-infected herds, the within-herd prevalence on most of the positive farms was low. The median prevalence of positive animals on positive farms (≥ 2 test positive animals) was 8% (mean 12%).

Tables 3.6 - 3.8 summarize the univariable analyses of soil characteristics and pasture management. No significant difference in pasture soil pH (p=0.720) or iron content (p=0.241) was found in association with the application of lime to pasture soils (Table 3.6). However, the failure to find any difference may have been due to low numbers of observations in the 'No lime use' category (this analysis was restricted to farms that only had pasture soil samples). There was also no significant difference in soil pH (p=0.201) and iron (p=0.715) (Table 3.7) or in the application of lime (p=0.974) (Table 3.8) across soil type strata.

C. Association between soil-related risk factors and *M. paratuberculosis* prevalence

The final logistic regression model of the association between soil-related risk factors and risk of herd paratuberculosis infection status is presented in Table 3.9. Two soil risk factors were significantly associated with herd paratuberculosis status. For every part per million increase in soil iron content

there was a 1.4% increase in the risk of a herd being paratuberculosis positive (O.R. = 1.014; C.I. 1.003 - 1.026; p = 0.0128). The application of lime to pasture areas in 1993 was associated with a herd being approximately three times less likely to be paratuberculosis positive (O.R. = 0.063; C.I. 0.008 - 0.472). The remaining covariates were as expected, related to herd paratuberculosis history and manure handling.

The final Poisson regression model of the association between soil-related risk factors and risk of herd sample paratuberculosis prevalence is presented in Table 3.10. Three soil risk factors were significantly associated with herd sample paratuberculosis prevalence. A one-tenth unit increase in the soil pH was associated with a 5% decrease in the number of positive animals (O.R. = 0.95; p = 0.0019; C.I. = 0.922 - 0.982). A ten part per million increase in soil iron content was associated with a 4% increase in the number of positive animals (O.R. = 1.004; p = 0.0015; C.I. 1.002 - 1.007). Application of lime to pasture areas was associated with a 72% reduction in the number of positive animals (O.R. = 0.282; p = 0.0001; C.I. = 0.147 - 0.539). The remaining 12 variables were related to pasture use and management, manure management, and calf rearing.

3.6 DISCUSSION

A. Prevalence and distribution

The adjusted statewide prevalence of 54% obtained in this cross-sectional study was greater than expected. A recent cross-sectional study in Wisconsin reported a herd prevalence of 34% using the IDEXX ELISA. Several factors may account for an actual greater paratuberculosis prevalence in Michigan. However, sample bias may also account for this greater than expected prevalence. Producers that were aware of a problem with paratuberculosis in their herd may have been more willing to participate in this study because of increased awareness of the disease. Producers with positive herds may also have perceived a greater benefit from participating in the study because it offered an opportunity to receive free testing of some of their cattle. Producers already desiring to have their herd tested would receive a greater benefit from having the free testing than a producer who was not planning to do so. One indication that this sample may not have been biased was that prevalence of positive animals (7%) did not exceed anticipated levels, based on results of previous abattoir-based prevalence studies in Michigan, reporting a 9% prevalence of test positive animals presented for slaughter. Additionally the sample was geographically representative of the distribution of herds throughout the state and herd size and productivity per cow was also similar to state averages reported by MASS.

B. Soil-related risk factors

The results of the multivariable modeling approaches indicated that when controlling for herd management practices, productivity and soil type, acidic soil pH was associated with an increased prevalence of *M. paratuberculosis* positive animals; greater soil iron content was associated with increased risk of herd infection and increased numbers of infected animals within a herd; application of lime to pasture areas was associated with a reduction in the risk of both herd infection and the number of infected animals.

The results of this study provide evidence in support of the anecdotal reports in the literature concerning an association between paratuberculosis and acidic soils. This study is unique because it is an epidemiological study in which soil from a random sample of herds was tested to establish the association between soil characteristics and prevalence of paratuberculosis. Controlling for farm management practices, productivity, and soil type made it possible to assess the impact of soil pH and iron content while holding these potential confounding covariates constant.

The association between soil iron content and paratuberculosis is biologically plausible and offers a mechanism by which soil pH may influence viability of *M. paratuberculosis*. Lastly, the association between application of lime to pasture areas and the prevalence of paratuberculosis provides a practical application for this information that is based on a biological relationship.

The association between application of lime to pasture areas and reduced risk of paratuberculosis can be interpreted in light of the association between environmental iron, pH, and *M. paratuberculosis*. This may be due to the alkalinizing effect of lime on pasture soils. The reduction in iron availability that occurs at an alkaline pH may diminish the viability of *M. paratuberculosis* in the external environment, by decreasing the amount of sequestrable iron available.

To the authors' knowledge this is the first study reported in the literature that provides epidemiological evidence of an association between soil characteristics and prevalence of paratuberculosis. The role of soil iron content and pH in the maintenance and transmission of *M. paratuberculosis* is an intriguing question that requires further investigation. Identification of regional environmental factors that affect the prevalence of paratuberculosis can be helpful in planning regional control programs. Resources and efforts can be targeted toward regions that are at increased risk. Producers can be made aware that they are in a high risk region and take appropriate prevention and control measures.

The finding that may have the greatest impact however, is the association between liming of pasture areas and a reduction in both the herd paratuberculosis prevalence rate and the prevalence of positive animals. If alkalinization of soil through the application of lime or other materials reduces the transmission of paratuberculosis, then this simple, low-cost practice will be an important component of paratuberculosis control programs. However, more

research is needed to clearly establish the nature of the reported association. Prospective studies and controlled experiments are necessary to establish a cause and effect relationship. Clinical trials are needed to develop a soil management protocol to reduce paratuberculosis transmission in positive herds and to prevent its introduction into negative herds.

Table 3.1. Univariable Analyses of Categorical Risk Factors in the Full Logistic Regression Model of Soil-related Risk Factors and 1993 Management Covariates for Dairy Herds Tested for *M. paratuberculosis* in Michigan, USA Tested in 1996 Using the IDEXX ELISA (46 Positive, 34 Negative Herds)

		Number of Herds by *M. ptb* test status		Mantel-Haenszel	
Variable		**Positive**	**Negative**	**X^2**	**p**
Soil Type	1: Well drained loams	29	21	0.001	0.988
	2: Sands	7	6		
	3: Mixed drained loams	3	2		
	4: Wetlands	7	5		
Pasture - Dry cows		27	24	1.18	0.277
Scrape Barn	Milk Cow	44	32	0.09	0.757
	Heifers	34	23	0.37	0.543
Exercise Lot	Milk Cows	34	16	5.94	0.015
	Dry Cows	30	18	1.21	0.271
	Heifers	33	15	6.14	0.013
Scrape Exercise Lot	Milk Cows	32	25	0.15	0.700
	Heifers	31	23	0.001	0.981
Maternity Pens		41	31	0.09	0.764
Wash Teats		38	26	0.46	0.500
Wash Udder		14	6	1.68	0.194
Same equip. feed & manure		11	11	0.69	0.406

Table 3.1 (cont'd)

Variable		Number of Herds by *M. ptb* test status: Positive	Negative	Mantel-Haenszel: X^2	p
Common feed site for adults/calves		2	5	2.59	0.107
Feed grown where manure spread	Calves	25	18	0.02	0.901
	Cows	28	18	0.50	0.481
History of Johne's + signs		19	10	1.18	0.277
History of Johne's test + cows		14	3	5.39	0.020
Manure Slurry pit		20	7	4.52	0.033
Manure pack		22	15	0.11	0.744
Manure outside, no cows		19	10	1.18	0.277
Manure outside, with cows		7	7	0.39	0.535
Haul manure		11	2	4.61	0.032
Gutter in barn		9	14	4.40	0.036
Scrape barn		38	28	0.001	0.976
Lime on pasture		4	10	5.74	0.017

Table 3.2. Univariable Analyses of Continuous Risk Factors in the Full Logistic Regression Model of Soil-related Risk Factors and 1993 Management Covariates for Dairy Herds Tested for *M. paratuberculosis* in Michigan, USA Tested in 1996 Using the IDEXX ELISA (46 Positive, 34 Negative)

Variable	*M. ptb* Test Status	Mean	Std Dev	Quartiles	T[1]	p
pH	Positive	72.28	7.62	68, 71.8, 76	-1.71	0.091
	Negative	69.31	7.74	63.5, 67.1, 74.5		
Iron (ppm)	Positive	133.19	83.39	79.3, 108.3, 195	-2.86	0.006
	Negative	90.19	50.45	52.9, 84.1, 124		
Age (months) calves join adults	Positive	17.61	6.92	12, 18.5, 24	-0.764	0.447
	Negative	16.41	6.93	12, 16, 23.5		

1 - Student's T-test

Table 3.3. Univariable Analyses of Categorical Risk Factors in the Full Poisson Regression Model of Soil-related Risk Factors and 1993 Management Covariates for Dairy Herds Tested for *M. paratuberculosis* infection Status in Michigan, USA Tested in 1996 Using the IDEXX ELISA (177 cases and 2,376 non-cases from 46 positive and 34 negative herds)

		Number of Herds by *M. ptb* test status		Mantel-Haenszel	
Variable		**Positive**	**Negative**	**X^2**	**p**
Soil Type	1- Well drained loam	27	19	0.21	0.651
	2 - Sands	7	6		
	3 - Mixed drained loam	3	2		
	4 - Wetlands	5	5		
Pasture	Dry Cows	23	22	1.47	0.225
	Heifers	28	19	0.41	0.521
Rotate Pasture	Milk Cows	29	24	0.31	0.576
	Dry Cows	22	16	0.04	0.840
Scrape Barn	Milk Cows	40	30	0.08	0.781
	Dry Cows	32	25	0.04	0.846
	Heifers	32	22	0.50	0.478
Exercise Lot	Milk Cows	31	16	4.38	0.036
	Dry Cows	27	18	0.49	0.486
	Heifers	29	15	3.65	0.056
Wash dam's teats		35	24	0.77	0.380
Wash dam's udder		12	6	0.94	0.333
Calves housed individually		36	29	0.40	0.525

Table 3.3 (cont'd)

Variable		Number of Herds by *M. ptb* test status		Mantel-Haenszel	
		Positive	Negative	X^2	p
Common feed site for calves and adults		1	5	4.22	0.040
Feed grown where manure was spread	Calves	21	16	0.001	1.000
	Cows	25	17	0.30	0.585
Cattle left and returned from shows		13	9	0.07	0.793
Johne's signs w/i 3 yrs		18	9	1.68	0.195
Johne's test + cows w/i 3 yrs		13	3	4.92	0.027
Manure in slurry pit		18	7	3.53	0.060
Lagoon		8	6	.001	0.974
Manure pack		20	15	.004	0.950
Manure outside, no cows		17	10	0.66	0.417
Manure outside, with cows		6	7	0.71	0.399
Spread solid manure		37	30	0.67	0.413
Haul manure		9	2	3.26	0.071
Gutter in barn		8	13	4.10	0.043
Fertilizer on pasture		9	9	0.44	0.509
Lime on pasture		4	9	4.28	0.039

Table 3.4. Univariable Analyses of Continuous Risk Factors in the Full Poisson Regression Model of Soil-related Risk Factors and 1993 Management Covariates for Dairy Herds Tested for *M. paratuberculosis* infection Status in Michigan, USA Tested in 1996 Using the IDEXX ELISA (177 cases and 2,376 non-cases from 46 positive and 34 negative herds)

Variable	*M. Ptb* Test Status	Mean	Std Dev	Quartiles	Student's T	p
pH * 10	Positive	72.36	7.81	68, 72.2, 76	-1.52	0.134
	Negative	69.57	7.90	64.3, 67.3, 75		
Iron (ppm)	Positive	131.50	79.61	79.3, 108.3, 195	-2.56	0.013
	Negative	92.48	51.17	51, 88, 129		
Time Calf with Dam (hours)	Positive	7.32	6.86	3, 5.5, 8.5	2.12	0.042
	Negative	20.16	33.77	2, 9.3, 24		
Age Calves join Adults (months)	Positive	17.55	6.70	12, 18, 24	-0.95	0.346
	Negative	16.03	6.98	12, 15, 23.8		

Table 3.5. Distribution of M. *paratuberculosis* Positive Herds and Cows Diagnosed by IDEXX ELISA on Dairy Farms in Michigan by Agricultural District from Herds Sampled in 1996 (N=121 herds)

Agricultural District*	Prevalence of + Herds No. + / No. Tested (%)	Mean No. + Animals per Herd
1	1/7 (14.3)	1.65
2	6/8 (75.0)	9.63
3	4/5 (80.0)	4.81
4	4/11 (36.4)	1.96
5	9/13 (69.2)	5.13
6	19/20 (94.12)	13.04
7	13/21 (61.9)	3.47
8	14/28 (50.0)	6.50
9	7/8 (87.5)	9.10

(* See Appendix A for Map of Agricultural Districts.)

Table 3.6. Univariable Analyses of Pasture Soil Iron Content and pH by Application of Lime to Pasture Soil for Dairy Herds (in which only Pasture Soil was Sampled) Tested for *M. paratuberculosis* in Michigan, USA. Tested in 1996 Using the IDEXX ELISA (N = 55 pasture-only herds)

Soil Property	Lime Use	N	Mean	Std Deviation	Student's T-test	p
Iron (ppm)	Yes	8	76.90	49.88	1.19	0.241
	No	47	106.93	68.36		
pH	Yes	8	67.68	5.76	0.36	0.720
	No	47	68.61	6.89		

Table 3.7. Univariable Analyses of Soil Iron Content and pH by Soil Type for Dairy Herds Tested for *M. paratuberculosis* in Michigan, USA. Tested in 1996 Using the IDEXX ELISA (N = 55 pasture-only herds)

Soil Property	Soil Type*	N	Mean	Std Deviation	Kruskal-Wallis X^2	p
Iron (ppm)	1	56	119.31	124.99	1.36	0.715
	2	16	116.42	97.08		
	3	7	113.05	67.90		
	4	13	92.19	89.21		
pH	1	56	70.76	124.97	4.63	0.201
	2	16	70.59	97.06		
	3	7	78.34	67.89		
	4	13	70.89	89.20		

* 1 - well drained loams (parent clay drift); 2 - sands; 3 - intermediate to mixed drained loams; 4 - wetlands.

Table 3.8. Univariable Analyses of Soil Type by Application of Lime to Pasture Soil for Dairy Herds Tested for *M. paratuberculosis* in Michigan, USA. Tested in 1996 Using the IDEXX ELISA.

Soil Type*	Lime Use		Mantel-Haenszel	
	Yes	No	X^2	p
1	43	9	0.001	0.974
2	12	2		
3	4	1		
4	10	2		

* 1 - well drained loams (parent clay drift); 2 - sands; 3 - intermediate to mixed drained loams; 4 - wetlands.

Table 3.9. Final Logistic Regression Model of Soil-related Risk Factors and 1993 Management Covariates for Dairy Herds Tested for *M. paratuberculosis* infection Status in Michigan, USA Tested in 1996 Using the IDEXX ELISA (177 cases and 2,376 non-cases from 46 positive and 34 negative herds)

Variable	Estimate	p-value	Odds Ratio	Wald 95% Confidence Limits	
				Lower	Upper
Soil 2 (Sands)	0.05	0.950	1.05	0.210	5.28
Soil 3 (Intermediate /Mixed Drained Loams)	1.77	0.160	5.89	0.496	70.04
Soil 4 (Wetlands)	0.17	0.846	1.19	0.213	6.62
Iron (ppm)	0.01	0.013	1.01	1.003	1.03
Exercise lot for milkers	1.36	0.042	3.90	1.053	14.48
Use of same equipment for feed and manure	-1.81	0.019	0.16	0.036	0.74
Johne's test + Animals w/i 3 yrs	1.99	0.030	7.35	1.217	44.35
Manure slurry	1.76	0.012	5.79	1.472	22.80
Use of Gutter cleaner	-2.28	0.003	0.10	0.022	0.48
Lime on pasture	-2.77	0.007	0.06	0.008	0.47

Table 3.10. Final Poisson Regression Model of Soil-related Risk Factors and 1993 Management Covariates for Dairy Herds Tested for *M. paratuberculosis* infection Status in Michigan, USA Tested in 1996 Using the IDEXX ELISA -- Aggregated by Soil type and Adjusted for Over-dispersion (177 cases and 2,376 non-cases from 46 positive and 34 negative herds)

Variable	Estimate	p-value	Odds Ratio	Wald 95% Confidence Limits	
				Lower	Upper
Soil pH (*10)	-0.050	0.0019	0.951	0.922	0.982
Soil iron (ppm)	0.004	0.0015	1.004	1.002	1.007
Pasture (dry cows)	-0.563	0.0117	0.569	0.368	0.882
Rotate Lactating Cows on Pasture	-0.647	0.0055	0.524	0.331	0.827
Scrape Barn (Lactating Cows)	1.323	0.0056	3.756	1.473	9.574
Scrape Barn (Dry Cows)	-1.513	0.0001	0.220	0.114	0.424
Scrape Barn (Heifers)	0.771	0.0117	2.162	1.188	3.936
Wash dam's udder	1.346	0.0001	3.840	2.392	6.164
Common feeding site for calves and adults	-1.358	0.0087	0.257	0.093	0.709
Feed Calves where manure was spread	0.533	0.0066	1.704	1.160	2.503
History of Johne's signs w/i 3 yrs	0.647	0.0033	1.910	1.240	2.941

Table 3.10. (Cont'd)

Variable	Estimate	p-value	Odds Ratio	Wald 95% Confidence Limits	
				Lower	Upper
Outdoor manure storage	0.931	0.0001	2.536	1.676	3.838
Fertilizer on pastures	-0.586	0.0327	0.556	0.325	0.953
Solid spreading of manure	-2.052	0.0001	0.129	0.066	0.252
Lime on pastures	-1.266	0.0001	0.282	0.147	0.539

Chapter 4

MANAGEMENT-RELATED RISK FACTORS FOR *M. PARATUBERCULOSIS* INFECTION IN MICHIGAN, USA DAIRY HERDS

4.0 STRUCTURED ABSTRACT

Objective- This study was undertaken to determine specific management practices within the broad categories of: pasture and feed management, manure management, maternity hygiene, calf rearing, and replacement purchasing practices, that are associated with increased risk of paratuberculosis infection.

Design- Cross-sectional study

Sample Population- A multi-stage stratified random sample of Michigan dairy farms was asked to participate in the study.

Procedure- The study was conducted from June through December 1996 to identify management-related risk factors for herd-level *M. paratuberculosis* infection. Data were collected from 121 participating herds. A two-part questionnaire was administered to gather data on current and previous management practices and herd productivity. A random sample of cows aged 24 months and greater was selected from each herd and tested for antibodies to *M. paratuberculosis* using the IDEXX Antibody ELISA (sensitivity 64%, specificity

96%). A positive herd was one in which two or more animals tested positive for antibodies to *M. paratuberculosis*. A negative herd was one in which no animal tested positive. Herds in which only one animal tested positive were dropped from statistical analysis to reduce the risk of including false positive herds in the statistical analyses.

Results- Herds with only one positive animal (26) and incomplete datasets (12) were dropped from the analyses. There were 46 herds with two or more test positive animal. The sample prevalence of herds with 2 or more test positive animals was 55% (46/83). A multivariable logistic-regression model was used to evaluate the results. The variable "use of an exercise lot for lactating cows" was associated with a three-fold increase in risk of a herd being positive for *M. paratuberculosis* infection (O.R. = 3.01, C.I. = 1.03-8.80); "cleaning of calf hutches or pens after each use" was associated with a three-fold reduction in the risk of a herd being positive for *M. paratuberculosis* infection (O.R. = 0.28, C.I. = 0.08-0.89); "application of lime to pasture areas in 1993" resulted in a ten-fold decrease in risk of a herd being positive for *M. paratuberculosis* infection (O.R. = 0.10, C.I. = 0.02-0.56).

Conclusions- The application of lime to pasture areas may be a useful pasture management practice for the reduction of *M. paratuberculosis* infection within dairy herds.

4.1 INTRODUCTION

Clinical paratuberculosis (Johne's disease) is thought to have an important economic impact on the dairy industry. No recent estimates of losses to the dairy industry are available. A previous study indicated that paratuberculosis cost the U.S. dairy industry approximately 1.5 billion dollars annually (Merkal 1984). Losses attributable to clinical paratuberculosis are thought to be as a result of: reduced milk production (Benedictus *et al* 1987); increased calving interval (Abbas *et al* 1983); reduced slaughter weights (Whitlock *et al* 1985); shorter life expectancy, lost potential breeding value, infertility, and increased incidence of mastitis (Buergelt & Duncan 1978). Milk production in clinical cases has been reported to be reduced by 7.8-25% (Buergelt & Duncan 1978; Abbas *et al* 1983; Whitlock *et al* 1985; Wilson *et al* 1993). While the economic effects of clinical paratuberculosis have been documented, limited work has been reported on economic effects due to the subclinical stage of this disease. It is critical to conduct in-depth studies on the economic consequences of subclinical *M. paratuberculosis* infection for two reasons. First, in *M. paratuberculosis* infected herds, most animals remain subclinical (few of them eventually become clinical) for a long time -- but during that period, they can transmit the organism to other animals. Secondly, if it can be shown that cows that are subclinical are less productive than normal cows, dairy producers will have an economic incentive to participate in control programs aimed at reducing this infection in their herds.

Several studies have been conducted to determine the prevalence of paratuberculosis in various regions of the U.S. These studies yielded prevalences ranging from 1.6-20% of the cattle population being positive for *M. paratuberculosis* infection, with dairy cattle having the higher prevalence (Braun *et al* 1990; Whipple *et al* 1991; Thoen & Baum 1988; Kreeger 1991). A recent survey in Wisconsin found that 34% of randomly selected herds had at least one animal that tested positive for *M. paratuberculosis* infection (Sockett 1993; Collins *et al* 1994). Several management practices have been identified as potential risk factors for the introduction and spread of *M. paratuberculosis* infection in a dairy herd including: addition of cattle raised off the farm; pasture and manure management; calf hygiene and housing; and feeding of waste milk to calves (Goodger *et al* 1996; Thoen & Baum, 1988; Sherman 1985).

No epidemiological study has been conducted to determine the prevalence and associated risk factors for *M. paratuberculosis* infection in the Michigan dairy cattle population. The aim of this study was to identify management practices that are associated with dairy herds being at increased risk for *M. paratuberculosis* infection.

4.2 HYPOTHESIS

There are specific management practices within the broad categories of: pasture and feed management, manure management, maternity hygiene, calf rearing, and replacement purchasing practices, that are associated with dairy herds being at increased risk for *M. paratuberculosis* infection.

4.3 MATERIALS AND METHODS

A. Study Design and Herd Selection

A cross-sectional study was used to determine the herd prevalence of *M. paratuberculosis* infection at the state level. A case-control study was used to identify management factors associated with positive herds. Because dairy herds vary in size and their distribution within the State of Michigan, a stratified multi-stage sampling procedure was used to select a representative sample of herds. Initially, the State was stratified into the 9 agricultural districts (see Appendix A). Within each district, herds were divided into five size categories (10-49, 50-99, 100-199, 200-399, and 400+ head of adult cattle). A sample proportionate to the number of herds in each district and size category was selected using a simple random procedure. This selection was accomplished jointly with the Michigan Agricultural Statistical Service department, which has all names, locations, and sizes of dairy herds in Michigan. Since the serologic test to be used cannot differentiate between an *M. paratuberculosis*- vaccinated animal from one that has subclinical paratuberculosis, all herds that practice

vaccination (about 1%) were excluded from the study. Due to the sensitive nature of data regarding herd *M. paratuberculosis* infection status, a low response rate was anticipated, to ensure an adequate sample size for statistical analysis, a total of 1000 Michigan dairy farms were contacted and invited to participate in the study.

B. Training of Data Collectors

Blood and data collection were accomplished by veterinarians, animal technicians, and senior veterinary students following completion of a special training program. The training program consisted of a four hour seminar detailing the goals and objectives of the research project, and providing instruction on the proper use of the survey instruments and collection, handling, and shipping of blood samples.

C. Calculation of the number of herds to sample

The formula in equation one was used to calculate the number of herds required to determine the prevalence of *M. paratuberculosis*-infected dairy herds in Michigan. It was estimated that 34% of the herds in Michigan were infected -- based on work in Wisconsin (Collins *et al* 1994). The sample size was calculated to determine the prevalence of *M. paratuberculosis* infected dairy herds within 7.5% of the actual prevalence (see Equation 1) and a 5% probability of Type I error.

Equation 1:

Minimum number of herds to sample = $[P_h(1-P_h)Z_{(1-\alpha/2)}{}^2] / e^2$

where, P_h is the estimated prevalence of *M. paratuberculosis* positive herds; e is the maximum acceptable error rate; and α is probability of Type I error (0.05). From this calculation it was determined that 154 herds were required to estimate the prevalence of *M. paratuberculosis* infected herds in Michigan.

D. Calculation of the number of animals to test per herd

Research in Wisconsin estimated that the prevalence of positive cows in a positive herd was 20% (Collins *et al* 1994). To ensure adequate power to detect herds that had a lower within-herd prevalence, the prevalence of positive cattle in an infected herd was estimated to be 10%. The general formula used to detect the presence of *M. paratuberculosis* infection in a herd of infinite size is expressed in Equation 2.

Equation 2:

$$n_{c\infty} = [\log \alpha] / [\log(1-P_c)]$$

where $n_{c\infty}$ is the minimum number of cattle per herd to test to detect a single positive animal, assuming herd size approaches infinity; P_c is the estimated prevalence of positive cattle in a positive herd; and α is the probability of Type I error (0.05). Using this equation it was calculated that 29 cattle per herd should be tested for a 95% probability of detecting a positive animal in a herd with a prevalence of $\geq$10% *M. paratuberculosis* infected animals.

Due to the low sensitivity of the IDEXX Antibody ELISA (IDEXX Laboratories, Inc., Portland, Maine) (64%) for the detection of subclinically infected animals (Kaneene *et al* 1992), the minimum required number of cows per herd required

for sampling was increased by a factor of 1.64. The resulting minimum number of samples required per herd was 48 animals for a herd of infinite size. This was then adjusted for small herds using the method described by Cochran (1977). This calculation is seen in equation 3.

Equation 3:

$$n_{fin} = n^*_{c\infty} /\{1+[(n^*_{c\infty} -1)/N]\}$$

where, n_{fin} is the minimum number of cattle to test to detect at least one positive animal, in a small herd; $n^*_{c\infty}$ is the minimum number of cattle per herd to test to detect a single positive animal, assuming herd size approaches infinity, adjusted for test sensitivity; and N is the median total herd size in the classification stratum.

Following the selection of participating herds, a systematic procedure was used to select a sample of cows that were two years and older. The first cow selected for testing was selected randomly and the remaining cows were selected systematically (i.e. every third or fourth cow after the first) until the required sample size had been reached based upon the farm's herd-size stratum classification. Animals that were < two years old were excluded from the study because the serologic test to be used is only recommended by the manufacturer for use in such animals.

E. Case Definitions

A case was defined as a cow that tested positive for *M. paratuberculosis* infection by the IDEXX antibody ELISA. A positive herd was defined as one in

which there were at least two cases of *M. paratuberculosis* infection identified by the IDEXX Antibody ELISA when sampled as a part of this study. A control herd was one in which none of the animals tested positive by IDEXX Antibody ELISA. To minimize the risk of false classification of herds, those herds in which only one animal tested positive for *M. paratuberculosis* infection were eliminated from the case-control study.

F. Specimen Collection and Laboratory Testing

Ten ml of blood were collected from each selected animal via the middle coccygeal vein using a 20-gauge, 1-in. needle and a 10-ml serum separator Vacutainer tube (Corning Glass Works, Corning, NY). Samples were centrifuged, and serum harvested and stored at -70°C in cryotubes until laboratory testing was conducted.

Serum samples were tested for antibodies to *M. paratuberculosis* with a commercial IDEXX Antibody ELISA test kit using the manufacturer's recommended protocol (Collins *et al* 1993). The sensitivity and specificity of the test are 64% and 96% respectively (Anderson *et al* 1991; Kaneene *et al* 1992).

G. Collection of data relating to possible risk factors for *M. paratuberculosis* infection

A 2-part pre-tested questionnaire was administered in person to participating farmers (see Appendices B and C). The management data were collected at the time of blood sampling, and included: herd size; number of pounds of milk sold in 1995; number of cattle added to the herd annually; number of cattle culled from the herd annually; number of acres of pasture in

use; pasture rotation interval; frequency of scraping barn/drylot; methods of manure disposal; use of maternity pens for calving; use of maternity pens as housing for sick cows; frequency of cleaning of maternity pens; number of hours before calf and dam are separated after parturition; whether farmers use individual calf hutches or not; whether hutches are cleaned and moved after each use or not; age of calf at removal from hutch; frequency of calf-pen cleaning; and farm-related income, assets, and expenditures.

M. paratuberculosis infection typically occurs during the first 1 to 6 months of age while clinical signs or detection may not occur until 2 to 5 years of age (Hagen 1938; Larsen *et al* 1975). Due to the long incubation and latent periods for paratuberculosis, 1996 management practices may not have reflected those of the time at which infection actually occurred. In 1996, the average Michigan dairy cow was 44 months of age (3.67 years) (Michigan Dairy Herd Improvement Association, 1996). Thus, management practices of approximately 3 years prior to the study were a better indicator of risk factors affecting the infection of cows in the present milking herd with *M. paratuberculosis* than prevailing practices. Current practices may have been altered post-infection, perhaps in response to recent clinical cases of disease. To address this issue, risk factor data were collected for calendar years 1993 and 1996 and stratified by animal management category: lactating cows, dry cows, and heifers. Data from 1993 were used in the case-control study of management-related risk factors. Data

related to cattle purchases and sales were collected for calendar years 1993 - 1996. Herd productivity data were collected for calendar years 1994 and 1995.

As a component of the evaluation of environmental risk factors several soil samples were collected from those herds in which the cattle spent time on pasture or an unpaved exercise lot. Locations of soil sample collection were indicated on state soil survey maps.

4.4 DATA ANALYSIS

Data from questionnaires and laboratory results were put into a personal computer database program and data analysis was conducted using the SAS software program (SAS version 6.12 , Cary N.C.: SAS Institute, Inc. 1997). Descriptive statistics were computed for the risk factors and the dependent variables of interest. The t- test was used to test for significant associations between herd *M. paratuberculosis* infection status and continuous risk factors. Person's χ^2 was used to test for significant associations between herd *M. paratuberculosis* infection status and categorical risk factors. Since management, environmental, and productivity data were collected for several years and many variables were stratified by cattle management group , the complete dataset comprised in excess of 250 variables for each farm. Descriptive data presented in this report reflect the analyses of the variables selected for inclusion in the full logistic regression model. Variables excluded from the multivariable logistic regression analyses included those variables

which were deemed not to be biologically relevant to the association being modeled; those variables with an excess number of missing responses (>50%); and those variables that would not converge in the univariable models. No variables were forced into the model and no interactions were included. Thirty-eight risk factors (Table 4.1) were included in the full logistic regression model. Backward eliminating procedure was used to model the relationship between herd *M. paratuberculosis* infection status and management practices.

4.5 RESULTS

A. Description of sample population

Of the 1000 Michigan dairy producers asked to participate in the study, 426 (43%) replied by returning a response card. Of those responding: 210 agreed to participate in the study, 172 refused, and 44 indicated that they were no longer eligible to participate because they had sold their herd. Samples were collected from 147 farms. Data from 38 farms was eliminated from the study of missing responses to questionnaire items. The total number of farms with one or more test positive animals was 72, there was a 66% prevalence of herds with $\geq$ 1 test positive animal in the sample population. Herds with only one test positive animal were eliminated from the multivariable analyses. There were 26 farms in which only one animal tested positive for antibodies to *M. paratuberculosis*. Removal of these herds from analysis resulted in a sample size of 83 herds for

multivariable modeling. The herd prevalence rate in this sample was 55% (46 positive).

B. Descriptive and univariable analyses

The mean herd size for this sample was higher than the state mean as reported by Michigan Agricultural Statistics: the state mean for 1995 was approximately 70 milk cows while in this sample the mean was 125 for negative herds and 116 for positive herds. The reported median annual milk production per cow of 19,186 pounds for negative herds and 16,657 for positive herds (mean =18,437 and 16,296 respectively) was similar to the state average of 17,071 pounds per cow in 1995. While the number of producers included in this study who reported a history of clinical cases of paratuberculosis (20%) was below the herd prevalence obtained by researchers in Wisconsin (34%), the prevalence of herds that contained at least one test positive animal much higher at 66%, even after removing those herds with only a single positive animal, the prevalence remained high (55%). Despite the high prevalence of *M. paratuberculosis*-infected herds, the within-herd prevalence on most of the positive farms was low. The median prevalence of positive animals on positive farms ($\geq$ 2 test positive animals) was 8% (mean 12%). Results from testing 83 herds with a total of 2,502 animals tested revealed 141 positive animals (5.6%).

C. Multi-variable analysis of management risk factors associated with herd *M. paratuberculosis* infection status

The full multi-variable logistic regression model included 38 variables (Table 4.1). The final, reduced model (Table 4.2) included five variables: "use of an exercise lot for the milking cows in 1993"; "washing of the dam's udder prior to calving"; and a "history *M. paratuberculosis* infection test-positive animals within the past three years" were each associated with an increased risk of positive herd infection status; "application of lime to pasture areas in 1993" and " cleaning of the calf hutches or pens after each use" were associated with reduced risk of herd infection.

4.6 DISCUSSION

A. Introduction of *M. paratuberculosis* into a herd versus transmission within a herd

In this study, five risk factors were identified that were associated with herd *M. paratuberculosis* infection status. However only one of these risk factors appears to be directly related to the introduction or presence of an infected animal on the farm -- "history of test positive animals on the farm within the past 3 years". The remaining 4 risk factors initially seem to be more closely related to transmission of *M. paratuberculosis* infection within an infected herd. However, it is difficult to differentiate those risk factors responsible for introduction of the

disease into the herd from those responsible for maintenance and transmission of the organism within the herd. There are two reasons for factors affecting transmission of *M. paratuberculosis* infection to appear significant in modeling approaches designed to study herd infection status rather than herd prevalence. The first and perhaps more important reason is inherent in the study design. In determining the number of animals to sample in each herd for a 95% probability of detecting at least one positive animal, the prevalence of test-positive animals in an infected herd was assumed to be 10% in this study. Therefore, in herds with a lower prevalence of infected animals the probability of failing to find a positive animal in an infected herd is increased. Herds in which one or two positive animals have been introduced but whose management practices prevent transmission to 10% or more of the herd have a greater probability of testing negative in this study. Removal of the herds in which only one animal tested positive from the multivariable analysis may also bias the sample so that herds classified as positive were more likely to have a higher within-herd prevalence. The within-herd prevalence of test positive cows was low in this study. Even after removal of the single positive cow herds, the median within-herd sample prevalence was 8% and the mean was 12%. Thus, potential for misclassification of some herds as negative when actually they are very low prevalence positive herds exists.

The second reason that management practices related to transmission may also be important in evaluating herd status is related to the distinction

between introduction of *M. paratuberculosis* into the farm environment and actual infection of susceptible animals. In addition to being introduced into a herd through the purchase of an infected animal, *M. paratuberculosis* may also enter a farm environment through a fomite such as contaminated feed, manure, equipment, or supplies. Whether or not the organism is then introduced into the dairy herd depends upon whether the organism has an opportunity to colonize a susceptible host. The management practices that are most often considered risk factors for within-herd transmission of *M. paratuberculosis* infection can in these instances also serve as risk factors for introducing an environmental contaminate into susceptible herd members and thus altering the herd infection status.

B. Use of an Exercise Lot as a Risk Factor for Herd Infection

Use of an exercise lot for lactating cows was associated with a three-fold increase in risk of a herd being positive for *M. paratuberculosis* infection (O.R. = 3.01, C.I. = 1.03-8.80). It is biologically plausible that use of an exercise lot increases the risk of herd *M. paratuberculosis* infection. In this study, an exercise lot was defined as an outdoor area for cattle in which grazing does not occur. It includes a dirt lot or a paved area. So defined, an exercise lot is generally an area in which cattle congregate at a much higher density than in a pasture. Manure removal from this area may be infrequent or incomplete. Both soil and paved surfaces would prove challenging to clean sufficiently for the elimination of *M. paratuberculosis*. Contact with contaminated manure from the exercise lot by cattle and farm workers may result in the introduction of the

organism to areas such as calf hutches and maternity pens; thereby bringing the organism to those animals that are most susceptible to infection. Thus, introduction of *M. paratuberculosis* into an exercise lot through contaminated feed, worker boots, loading and hauling equipment may provide an environmental reservoir for the organism resulting in infection of susceptible hosts as the organism is disseminated throughout the herd-environment.

C. Washing of cows' udder as a risk factor for herd infection

Washing of cows' udder prior to parturition was associated with a 9-fold increase in the risk of a herd being paratuberculosis-positive (O.R. = 8.66, C.I. = 1.87 - 40.08). Initially one would expect that practices such as udder washing prior to parturition would be a protective measure by removing the source of contaminant. However, in this study this practice proved to increase the risk of herd infection. Udder washing may actually be an indicator variable for poor cow hygiene. Only 22% (18/83) of the herds in the sample engaged in this practice. This is in contrast to teat washing prior to parturition which 83% (69/83) of herds reported practicing and was not significantly associated with herd *M. paratuberculosis* infection status. The need to wash udders may indicate an excessive amount of fecal contamination that is not being adequately removed by washing. In fact moistening dried fecal matter in the udder are may actually enhance teat contamination and facilitate infection of neonatal calves prior to removal from the dam.

D. Cleaning of calf hutches or pens after each use as a protective measure

Cleaning of calf hutches or pens after each use was associated with a three-fold reduction in the risk of a herd being positive for *M. paratuberculosis* infection (O.R. = 0.28, C.I. = 0.08-0.89). Calf hygiene practices have previously been identified as risk factors for the transmission of *M. paratuberculosis* infection. Although several calf rearing practices were evaluated in this study only cleaning or moving of calf hutches or pens was significantly associated with herd *M. paratuberculosis* infection status. This age group is most susceptible to infection from *M. paratuberculosis* and thus key to preventing infection of these animals is minimizing contact with contaminated manure, feed, and equipment.

E. Application of Lime to Pasture As a Protective Measure

Application of lime to pasture areas in 1993" resulted in a ten-fold decrease in risk of a herd being positive for *M. paratuberculosis* infection (O.R. = 0.10, C.I. = 0.02-0.56) While the biological plausibility of this relationship may be less apparent, in the authors' opinion, it is one of the more intriguing results of this study. Several anecdotal reports in the literature have claimed that application of lime to pasture and cattle housing areas seemed to be associated with a reduction in clinical cases of Johne's disease (Jansen 1948; Kopecky 1977; Richards 1989a; Richards 1989b;). Reports have also indicated that geographic regions which have natural limestone deposits also have a reduced prevalence of paratuberculosis (Jansen 1948; Richards 1989a).

The mechanism behind the proposed protective effects of lime are not known, however, it is believed that the elevation in environmental pH caused by the lime, reduces the ability of *M. paratuberculosis* to compete with other micro-organisms for available iron. Iron is essential for many of the biochemical reactions of bacterial species. Bacteria differ in their mechanisms for iron uptake and thus differ in their iron uptake efficiency. The solubility of iron is reduced at elevated pH. Several studies (Richards 1989a; Richards 1989b; Payne 1980; Lambrecht & Collins 1992; Snow 1970; Barclay & Ratledge 1983) have reported that *M. paratuberculosis* is a particularly poor chelator of iron. Thus the elevation in pH caused by application of lime may inhibit iron uptake by *M. paratuberculosis* making it less viable in the environment (Richards 1989a; Richards 1989b; Johnson-Ifearulundu & Kaneene 1997). Application of lime to pasture areas would perhaps reduce risk of herd infection by preventing *M. paratuberculosis* contaminating the pasture environment from surviving and infecting susceptible animals within the herd.

F. Limitations

The external validity of this study beyond the study sample depends upon how representative this sample was of dairy farms in the state and the region. The geographic distribution of herds sampled is representative of the distribution of dairy herds throughout the state. Additionally, the mean herd size and annual milk production per cow were similar to state averages reported by MASS. This sample was clearly skewed toward farms with larger herds. This may be due to

larger farms perceiving a greater economic benefit to participating in the study and receiving tests for a larger number of animals in their herd. Awareness and concern about paratuberculosis may also be greater in larger herds. Prior knowledge of paratuberculosis within the herd could bias participation in the study in either direction. Producers that were already planning to sample their herd may be encouraged to participate, while those that are reluctant to have others discover that their herd is infected may be reluctant to participate.

The cross-sectional nature of this study does not permit one to may cause and effect assertions about the results obtained. The disease patterns indicated only reflect associations. Collection of data on management practices of 3 years prior to sampling of the cows helps to address this issue somewhat, however it does not demonstrate any temporal association between risk factor and outcome because this was a random sample of herds whose Johne's status in 1993 was not determined.

It is possible that risk factors identified were simply indicator variables for some as yet unknown management practice that actually causes an increased risk of herd *M. paratuberculosis* infection. However, the associations revealed are biologically plausible. They are not inconsistent with the results of others and they provide an interesting basis for further research. Prospective studies at both the herd and individual animal level are needed before a causal relationship can be claimed.

4.7 CONCLUSION

Paratuberculosis has been a frustrating problem for dairy producers for more than 100 years. Efforts to treat the disease or develop a fully protective vaccine have failed. Until an effective cure or preventive is found, disease detection and farm management practices are the best weapons in the battle against this disease.

Previous research has indicated broad management categories that pose risk factors for herd *M. paratuberculosis* infection status giving a score to overall management categories (i.e. calf rearing, maternity hygiene, manure management, etc.) (Goodger *et al* 1996). This study identifies specific management practices associated with *M. paratuberculosis* infection within each of those broad categories. In addition, the development of multi-variable models that use data regarding management practices at or around the time that current test positive animals were actually infected minimizes the impact that recently altered practices may have on the assessment of patterns of risk and disease.

Use of an exercise lot in 1993 was associated with a 3-fold increase in the risk of a herd being infected with *M. paratuberculosis*. Cleaning of calf hutches and pens was associated with almost a 3-fold reduction in the risk of a herd being *M. paratuberculosis* infection positive. Additionally, application of lime to pasture areas was associated with a ten-fold reduction in risk of a herd being classified as paratuberculosis positive (having 2 or more ELISA positive animals). Improved hygiene and management of exercise lots and calf rearing

areas - perhaps with the addition of crushed limestone to those areas in addition to pasture, may be important features in efforts to prevent *M. paratuberculosis* infection within dairy herds.

Table 4.1. Univariable Analyses of Categorical Risk Factors in the Full Logistic Regression Model of 1993 Management-related Risk Factors for Herd Paratuberculosis- Test Status in Michigan, USA Dairy Herds Tested in 1996 using the IDEXX ELISA (N = 83; 46 positive herds).

Risk Factor	Frequency by herd *M. ptb* -test status		χ^2	p-value
	Pos.	Neg.		
Farm location in the eastern region of the state (agricultural districts 3,6,8,9)	27	15	2.70	0.100
Use of pasture for lactating cows	12	15	1.95	0.162
Use of pasture for dry cows	19	22	2.70	0.100
Use of pasture for heifers	22	20	0.32	0.573
Rotation of pasture for lactating cows	38	31	0.02	0.887
Rotation of pasture for dry cows	30	21	0.62	0.431
Rotation of pasture for heifers	28	23	0.01	0.904
Scraping of barn(s) housing lactating cows	45	36	0.02	0.876
Scraping of barn(s) housing dry cows	39	30	0.20	0.654
Scraping of barn(s) housing heifers	37	27	0.65	0.421
Use of an exercise lot for lactating cows	34	17	6.77	0.009
Use of an exercise lot for dry cows	30	20	1.07	0.302
Use of an exercise lot for heifers	28	18	1.24	0.266
Use of a maternity pen for parturition	40	34	0.52	0.472
Washing of cows' teats prior to parturition	42	27	4.91	0.027
Washing of cows' udder prior to parturition	14	4	4.65	0.031

Table 4.1 cont.

Risk Factor	Frequency by herd *M. ptb* -test status		χ^2	p-value
	Pos.	Neg.		
Cleaning of calf hutches/pens after each use	19	21	1.96	0.161
Use of common equipment for transporting feed and manure	11	12	0.74	0.380
Sharing of a common feed or water source between calves and adult cows	3	5	1.15	0.283
Use of feed grown on fields where manure has been spread for feeding calves	22	19	0.10	0.750
Use of feed grown where manure was spread for feeding cows	22	21	0.66	0.418
History of paratuberculosis-test positive animals (within the past 3 years)	13	4	3.83	0.050
Use of a slurry for manure storage	22	10	3.75	0.053
Use of a lagoon for manure storage	11	9	0.00	0.965
Use of an indoor manure pack	24	20	0.03	0.865
Outside manure storage with no cattle access to manure	19	12	0.69	0.406
Outside manure storage with cattle access to manure	7	9	1.09	0.296
Inside manure storage with no cattle access to manure	1	1	0.02	0.876
Other manure storage	10	12	1.20	0.273
Soil injection of liquid manure slurry into fields	3	1	0.68	0.419
Spreading of solid manure on fields	41	34	0.18	0.672

Table 4.1 cont.

Risk Factor	Frequency by herd *M. ptb* -test status		χ^2	p-value
	Pos.	Neg.		
Hauling of manure off the farm for disposal	12	3	4.48	0.034
Use of gutter cleaner in barn(s)	7	12	3.44	0.064
Use of alley scraper in barn (s)	40	33	0.10	0.756
Use of water flushing system for manure removal from barn(s)	6	2	1.37	0.241
Incorporation of surface applied manure within 24 hours of application	27	16	1.96	0.161
Application of fertilizer on fields used as pasture	7	8	0.57	0.451
Application of lime on fields used as pasture	4	11	6.13	0.013

Table 4.2. Comparison of Michigan, USA dairy herd geographic distribution in sample tested in 1996 with state-wide herd geographic distribution from Michigan Agricultural Statistical Service (1996).

Agricultural District	Study Population		MASS State-wide Distribution	
	Number	Percent	Number	Percent
1	8	9.6	233	5.0
2	6	7.2	284	6.1
3	4	4.8	219	4.7
4	7	8.4	270	5.8
5	7	8.4	592	12.7
6	10	12.0	778	16.7
7	13	15.7	713	15.3
8	22	26.5	1136	24.4
9	6	7.2	433	9.3
Total	83	99.8	4658	100.0

Table 4.3. Comparison of Michigan, USA dairy herd-size distribution in sample tested in 1996 with state-wide herd-size distribution from Michigan Agricultural Statistical Service (MASS) 1996.

Number of Cows in Dairy Herd	Study Population		MASS State-wide Distribution	
	Number	Percent	Number	Percent
10 - 49	1	1.4	2223	47.7
50 - 99	11	13.0	1522	32.7
100 - 199	29	35.0	713	15.3
200 - 399	30	36.1	172	3.7
≥ 400	12	14.3	28	0.6
Total	83	99.8	4658	100.0

Table 4.4. Univariable Analyses of Continuous Risk Factors for Herd Paratuberculosis Test Status in Michigan, USA Dairy Herds Tested in 1996 using the IDEXX ELISA (N = 83; 46 positive herds).

Risk Factor	Herd *M.ptb* infection status	n	Mean	S.D.	Median	t	p
Average dairy herd size (1995)	Positive	36	116.1	73.2	95.5	0.44	0.66
	Negative	27	124.7	81.5	103.0		
Annual milk production (cwt) per cow (1995)	Positive	24	163.0	53.0	166.6	1.49	0.14
	Negative	19	184.4	37.4	191.9		

Table 4.5. Final Reduced Logistic Regression Model of 1993 Management-related Risk Factors for Herd Paratuberculosis Test Status in Michigan, USA Dairy Herds Tested in 1996 using the IDEXX ELISA.

		Odds Ratio	
Risk Factor	**Wald χ^2 p-value**	**Odds Ratio**	**95% Confidence Interval**
Use of an exercise lot for lactating cows	0.0438	3.013	1.03 - 8.80
Washing of cows' udder prior to parturition	0.0058	8.659	1.87 - 40.08
Cleaning of calf hutches/pens after each use	0.0309	0.275	0.08 - 0.89
History of paratuberculosis test-positive animals on the farm (within the past 3 years)	0.0145	6.714	1.46 - 30.90
Application of lime to pasture	0.0085	0.103	0.02 - 0.56

Chapter 5

A HERD-LEVEL ECONOMIC ANALYSIS OF THE IMPACT OF JOHNE'S DISEASE ON LABOR EXPENDITURE, CULLING WEIGHT, AND MORTALITY IN MICHIGAN DAIRY HERDS

5.0 Structured Abstract

Objective- This study was undertaken to conduct an economic evaluation of the herd-level impact of Johne's disease (paratuberculosis) on labor expenditure, culling weight, and mortality in Michigan dairy herds.

Design- Cross-sectional

Sample Population- A multi-stage stratified random sample of Michigan dairy farms was asked to participate in the study.

Procedure- Data were collected from 121 participating herds. A two-part questionnaire was administered to gather data on management practices, herd productivity, labor use, and expenditures. A random sample of cows aged 24 months and greater was selected from each herd and tested for antibodies to *M. paratuberculosis* using the IDEXX ELISA. A positive herd was one in which at least two animals tested positive for antibodies to *M. paratuberculosis*. A negative herd was one in which no animal tested positive. Multivariable linear regression modeling was used to evaluate the data.

Results- A 10% increase in the paratuberculosis test positive rate was associated with a 74 pound decrease in average culling weight (p= 0.047). Herds that were paratuberculosis positive had an associated 3% increase in mortality rate compared to herds that were paratuberculosis negative (p= 0.044). Paratuberculosis positive herds had an associated annual labor increase of 23 hours per cow, however this difference was not significant (p= 0.225)

Conclusions- For a herd of average size and cull rate, the reduced mean culling weight associated with herd paratuberculosis prevalence represented a loss of approximately $1032 annually each 10% increase in the prevalence of paratuberculosis test positive animals. The losses associated with the increased mortality rate in *M. paratuberculosis* positive herds were $4,400 to purchase replacements.

5.1 INTRODUCTION

Paratuberculosis has had a substantial economic impact on the US dairy industry. It is for this reason that paratuberculosis is considered one of the most serious diseases affecting dairy cattle (McNab *et al* 1991). Direct quantifiable losses due to clinical disease have been documented (Benedictus *et al* 1987). Losses due to reduced productivity and salvage value, treatment costs, idle production facilities and replacement costs can be calculated for an animal that has undergone a period of chronic diarrhea and weight loss prior to culling or death (Sherman 1985). Economic simulation models have been developed based on these documented losses to estimate the potential economic benefit and cost associated with paratuberculosis control strategies at various herd prevalences (Walker et al 1988a,b; Collins & Morgan 1992). Collins and Morgan (1992) conclude that a test and cull program will be profitable for herds with a paratuberculosis prevalence of 5% or greater. The authors indicate however, that losses associated with paratuberculosis due to mastitis, infertility, reduced feed efficiency, increased susceptibility to other disease and other indirect losses were not included in these models due to lack of documentation (1992). Thus, while these models improve the ability to assess the economic impact of paratuberculosis, they may under estimate the cost of disease and the benefits of a control strategy because not all of the costs associated with

paratuberculosis could be included in the model (Johnson-Ifearulundu & Kaneene 1997a).

Determining indirect costs and productivity losses attributable to both clinical and subclinical disease is more challenging. However, it is these more insidious losses that are potentially more economically devastating to the paratuberculosis positive herd (Sherman 1985; Jones 1989) The cost of labor and feed are the two greatest variable costs for dairy producers (Nott & Ruesink 1995). A number of studies have evaluated the economic impact of Johne's disease using individual animal modeling approaches. Because health and other management practices are generally applied on a whole herd basis, it was felt that additional information may be obtained by evaluating the economic impact of paratuberculosis using a herd-level modeling approach. The objective of this study, therefore, was to provide a herd-level analysis of economic losses associated with paratuberculosis on Michigan dairy herds.

5.2 HYPOTHESES

Several hypotheses were developed to assess the economic impact of herd paratuberculosis status:

1) Paratuberculosis positive dairy herds have a lower net farm income than negative herds;

2) Paratuberculosis positive herds have lower farm labor efficiency than negative herds;

3) Paratuberculosis positive dairy herds expend a greater number of hours of labor per cow than negative herds;

4) Paratuberculosis positive dairy herds have a lower average cull cow weight than negative herds;

5) Paratuberculosis positive dairy herds have a greater mortality rate than negative herds.

5.3 MATERIALS AND METHODS

A. Study Design and Herd Selection

A cross-sectional approach was used as the design for this study. Because dairy herds vary in size and their distribution within the State of Michigan, a multi-stage sampling procedure was used to select a representative sample of herds that would take account of these differences. The sample size required to determine the prevalence of paratuberculosis positive dairy herds in Michigan was calculated based on an estimated 34% positive herd prevalence rate (Collins & Sockett 1993). The maximum acceptable error in the calculated true prevalence was $\pm$7.5% with 95% probability. Thus, there was a 95%

probability that the calculated prevalence was within 7.5%, of the actual prevalence of paratuberculosis positive herds in the state. The minimum required sample size was 154 herds. Initially, the State was stratified into the 9 agricultural districts (see Appendix A). Within each district, herds were divided into six size categories (10-29, 30-49, 50-99, 100-199, 200-399, and 400+ head of adult cattle). A sample proportionate to the number of herds in each district and size category was selected using a simple random procedure. This selection was accomplished jointly with the Michigan Agricultural Statistical Service which has all names, locations, and sizes of dairy herds in Michigan. Since the serologic test to be used cannot differentiate between an *M. paratuberculosis* vaccinated animal and one that has subclinical paratuberculosis, all herds that practice vaccination (about 1%) were excluded from the study.

Following the selection of participating herds, a systematic random procedure was used to select a sample of cows that were two years and older. The number selected from each herd was proportionate to the size of the herd. Animals that were less than two years old were excluded from the study because the serologic test to be used is only recommended by the manufacturer for use in animals two years or older.

B. Training of Data Collectors

Blood and data collection were accomplished by veterinarians, animal technicians, and senior veterinary students following completion of a special

training program. The training program consisted of a four hour seminar detailing the goals and objectives of the research project, and providing instruction on the proper use of the survey instruments and collection, handling, and shipping of blood samples.

C. Blood Collection and Laboratory Testing

Ten ml of blood were collected from each selected animal via the middle coccygeal vein using a 20 gauge one inch needle and a 10 ml serum separator vacutainer tube (Corning Glass Works, Corning, NY). Samples were centrifuged, and serum harvested and stored at -70°C in cryotubes until laboratory testing was conducted.

Serum samples were tested for antibodies to *M. paratuberculosis* with a commercial IDEXX ELISA test kit (IDEXX Laboratories, Inc., Portland, Maine) using the manufacturer's recommended protocol (Anderson *et al* 1991) The sensitivity and specificity of the test has been reported to be 64% and 96% respectively (Kaneene *et al* 1992; Collins *et al* 1994).

D. Case Definitions

A case was defined as a cow that tested positive for antibodies against *M. paratuberculosis* by the IDEXX ELISA. A positive herd was defined as one in which there were at least two animals with a positive titre to *M. paratuberculosis* by the IDEXX ELISA when sampled as a part of this study. A control herd was one in which none of the animals tested positive by IDEXX ELISA.

E. Collection of management and economic data

A two part pre-tested questionnaire was administered in person to participating farmers (see Appendices B and C). Economic data were obtained at the time of collecting the blood samples. The data collected included: herd inventories and market values for dairy cattle on 1995 and 1996; use of bST; mortality rate; replacement purchases from 1993 -1995; inventory, weight, price received, and reason culled for all cattle culled during the 90 days prior to interview; number and value of beef cattle on the farm (if any); and farm sales, farm expenses, labor, and inventory for 1995.

5.4 DATA ANALYSIS

Data from questionnaires and laboratory results were input into a database using the R-base software program and data analysis was conducted using the Statistical Analysis System (SAS) software program (SAS version 6.12, Cary N.C.:SAS Institute, Inc. 1997). Because 20% of the 121 farms participating in the study refused to provide any financial data, a comparison was made to determine whether they differed from those who provided financial data. The comparison was done in relation to 1) number of dairy cows in 1996, 2) number of replacement heifers purchased in 1995 as a proportion of herd size in 1995, 3) proportion of animals testing paratuberculosis positive, 4) herd paratuberculosis status, and 5) use of bovine somatotropin (bST). The t- test (continuous variables) and the Chi-square test (categorical variables) were used

to determine if there were significant differences between those farms that provided economic data and those that did not (see tables 1 and 2). The t- test was also used to determine whether herd paratuberculosis status was associated with a significant difference in the number of pounds of milk produced per cow and the expenses per cow.

Multivariable modeling was conducted on the subset of farms from which adequate data were obtained, this subset consisted of 62 herds (39 positive herds and 22 negative herds). Poor response to questionnaire items about farm depreciation expenses (13 herds) and farm inventory (9 herds) made evaluation of the first two stated hypotheses infeasible. Thus, the three remaining outcomes of interest (dependent variables) were: 1) number of hours of labor per cow annually; 2) average cull cow weight; 3) and overall mortality rate. The mortality rate was calculated as the number of dairy cow and first calf heifer deaths (not due to slaughter) divided by the number of dairy cows. Multivariable linear regression analyses were conducted to determine the influence of the proportion of animals testing paratuberculosis positive on the average number of hours of labor per cow, the average cull cow weight, and the mortality rate, controlling for 4 biologically important covariates (see table 3).

As previously stated sample size calculations were conducted to determine the number of herds required for estimation of the prevalence of paratuberculosis positive herds. Thus, while the sample size determined was statistically valid to estimate the proportion of herds infected with *M.*

paratuberculosis, we suspected that it might not have been sufficient to test the stated hypotheses. Therefore, power analysis was conducted for each of the three outcomes of interest. The type II error rate for each of the multivariable linear regression models was determined using the method described by Cohen (1988).

5.5 RESULTS

A. Descriptives and Univariate analyses

Of the 147 randomly selected dairy herds in the state, 77 (63.6%) were positive for *M. paratuberculosis*. Adjusting this true sample prevalence for the distribution of herd size strata in Michigan yielded a statewide true prevalence of 64.8%. Tests were conducted for 3,886 animals, 267 (6.9%) of those tested were positive.

A total of 121 farms completed the sample collection and management questionnaire. The median herd size in this sample was 97.5 adult cows (mean 136.2, range 22 - 1057). The median value for average daily milk yield per cow was 17,722 pounds. Approximately 35.6% (47/132) of the responding producers reported having had a previous history of cattle in their herds manifesting clinical signs of paratuberculosis, and 18.3% (24/131) reported having had an animal test positive for paratuberculosis within the past 3 years.

Of the 121 farms completing the sample collection and management questionnaire, a subset of 97 (80%) chose to complete some portion of the

economic questionnaire. Univariate comparison of those producers who refused to provide financial data with those who complied, showed that the average dairy herd size and number of replacement heifers purchased in 1995 was not significantly different (see table 1). In addition, no statistically significant differences were found in bST use or herd paratuberculosis status between those that provided economic data and those that refused (see table 2). The t-test failed to find any significant difference between herd paratuberculosis status in the number of pounds of milk sold per cow (negative herds mean = 121 cwt; positive herds mean = 122 cwt; $p = 0.897$) or the expenses per cow (negative herds mean = $1705; positive herds mean = $1730; $p = 0.857$). The mean value of culled cows sold for slaughter in this sample was $0.31 per pound.

B. Multivariable and Power Analyses

Interpretation of the multivariable linear regression model indicated that positive herd paratuberculosis status was associated with an increase of 23 hours of labor per cow annually (see table 4). This difference was not significant at the $p<0.05$ level. Power analysis revealed that an effect size (f^2) of approximately 0.23 was the minimum required to attain statistical power of 0.80. This corresponds to an unadjusted model R^2 of 0.1870 in this model. The unadjusted model R^2 of this model was 0.3253. The power of the F test for the linear regression model for average hours of labor per cow was calculated to be approximately 98%.

A 10% increase in the paratuberculosis test positive rate was associated with a 74 pound decrease in average culling weight (p=0.047), see table 5. Positive herd paratuberculosis status was associated with a 3% increase in mortality rate (p=0.044), see table 6.

5.5 DISCUSSION

The 74 pound reduction in average culling weight found in association with a 10% increased prevalence of paratuberculosis positive animals in this study, was biologically plausible. A protein-losing enteropathy (Patterson *et al* 1967) and intestinal malabsorption (Patterson *et al* 1968) have been reported in association with paratuberculosis. Enteropathy and malabsorption can result in reduced feed efficiency and weight gain. While these losses are apparent in the clinical animal, they may also occur in the subclinical animal, thus increasing the magnitude of the loss to the infected herd. Indeed in previous studies, decreased slaughter weight at culling has been reported in both the clinical (Benedictus *et al* 1987) and subclinical (Hutchinson 1996) animal.

The economic impact of this reduction in average culling weight can be calculated based on the 1995 average herd size and slaughter value reported in this sample (136 head and $0.31 per pound, respectively) and the 1995 average cull rate reported by the Michigan Dairy Herd Improvement Association of 33% (Michigan DHIA 1995). Thus a 136 cow herd culling 33% of its cows would

experience a $1032 annual loss in income from the sale of cows for slaughter for each 10% increase in the herd paratuberculosis prevalence.

This study also found a 3% increase in herd mortality rate associated with paratuberculosis. This association was anticipated due not only to mortality directly caused by Johne's disease but also to the increased risk of secondary disease. Annual death losses from clinical paratuberculosis may be quite high. Kreeger (1991) reports that annual death losses may range from 3-10% in an infected herd. An impairment of cellular immunity in paratuberculosis infected animals has been proposed as the mechanism for increased incidence of secondary disease in *M. paratuberculosis* positive animals (Kreeger *et al* 1991; Kreeger *et al* 1992 a,b; Thoen & Haagsma 1996). Previous studies have reported an association between *M. paratuberculosis* infection and impaired cell mediated immunity (Lepper 1989; Singh *et al* 1993; Kreeger *et al* 1991; Kreeger & Snider 1992; Kreeger *et al* 1992; Dalton *et al* 1992; Little *et al* 1994; Veazey *et al* 1994; Stabel 1994). This impairment is believed to be the mechanism for increased rates of premature culling and culling due to secondary disease reported in subclinically infected animals (Kreeger 1991; Thoen & Baum 1988; Merkal *et al* 1975). If subclinically infected *M. paratuberculosis* positive animals are at greater risk of other diseases, positive herds may experience greater mortality rates than negative herds due to both clinical paratuberculosis and an increased incidence of secondary diseases in the subclinically infected paratuberculosis positive animal.

The value of the loss represented by this increase in mortality rate can be estimated by the cost of purchasing replacements due to the increased death losses. For a 136 cow farm a 3% increase in mortality rate represents 4 deaths. Total replacement costs for these animals was approximately $4,400 (DHIA 1995).

Table 5.1. Univariable Analyses Comparing Continuous Variables for Herds Providing Economic Data with Those Refusing in Michigan, USA Dairy Herds Tested for Herd Paratuberculosis Test Status in 1996 using the IDEXX ELISA.

Variable	Economic Data Provided	N	Mean	Std. Dev.	T - test T	T - test p
Number of Dairy Cows in 1996	No	17	186.2	136.2	1.5	0.141
	Yes	79	127.3	150.6		
Proportion of Replacement Heifers Purchased in 1995 to Number of Dairy Cows in 1995	No	13	0.13	0.30	1.1	0.274
	Yes	72	0.04	0.07		
Proportion of animals positive for Johne's	No	40	8.7	10.8	1.2	0.217
	Yes	81	6.4	8.7		

Table 5.2. Univariable Analyses Comparing Binary Variables for Herds Providing Economic Data with Those Refusing in Michigan, USA Dairy Herds Tested for Herd Paratuberculosis Test Status in 1996 using the IDEXX ELISA.

		Economic Data Provided		Chi-Square	
Variable	**Level**	**No**	**Yes**	**Value**	**p**
Herd Johne's Disease Status	Negative	14	38	1.6	0.213
	Positive	26	43		
bST Use	Negative	12	60	0.1	0.706
	Positive	5	20		

Table 5.3. Risk Factors in the Multivariable Models Analyzing the Economic Outcomes Associated with Herd Paratuberculosis - Test Status in Michigan, USA Dairy Herds Tested in 1996 using the IDEXX ELISA.

Risk Factor	Type	Range
Proportion of animals positive for Johne's	Continuous	0-.46
bST Use	Binary	0,1
Average Annual Milk Production per Cow (cwt)	Continuous	86.9 - 290.9
Cull Rate (%)	Continuous	0.4 - 39.4
Dairy herd size	Continuous	22.0 - 1057.0
Interaction Term: Proportion of Tests Positive for Johne's * Cull rate	Continuous	0.0 - 6.3

Table 5.4. Linear Regression Model for Number of Hours of Labor per Cow Associated with Herd Paratuberculosis- Test Status in Michigan, USA Dairy Herds Tested in 1996 using the IDEXX ELISA.

Risk Factor	Parameter Estimate	Standard Error	p-value
Herd status	23.05	18.64	0.225
bST use	3.44	21.24	0.872
Average annual milk production per cow (cwt)	-0.13	0.29	0.646
Dairy herd size	-0.36	0.17	0.045
Cull rate	114.83	90.22	0.213

N = 36; F = 2.989, p = 0.0258; Model R^2 = 0.3253; Model adjusted R^2= 0.2165

Table 5.5. Linear Regression Model for Average Weight of Culled Cows Associated with Herd Paratuberculosis- Test Status in Michigan, USA Dairy Herds Tested in 1996 using the IDEXX ELISA.

Risk Factor	Parameter Estimate	Standard Error	p-value
Proportion of animals positive for Johne's	-7.38	3.56	0.0470
bST use	246.02	50.68	0.0001
Average annual milk production per cow	0.83	0.72	0.2603
Dairy herd size	-1.12	0.43	0.0134
Cull rate	-533.56	292.12	0.0777
Interaction Term[a]	71.97	36.82	0.0600

a = Interaction term = (Proportion of tests Positive for Johne's) x (Cull rate)

N = 36. Model F value = 5.726; p= 0.0005.
Model R^2 = .5338; Model adjusted R^2=.4406.

Table 5.6. Linear Regression Model for Cow and Heifer Mortality Rate in 1995 Associated with Herd Paratuberculosis- Test Status in Michigan, USA Dairy Herds Tested in 1996 using the IDEXX ELISA.

Variable	Parameter	Standard Error	p-value
Herd Johne's status	0.0315	0.0150	0.044
bST use	0.0251	0.0171	0.154
Average annual milk production per cow	-0.0003	0.0002	0.173
Dairy herd size	-0.0001	0.0001	0.584
Cull rate	0.1614	0.0728	0.034

N = 36. Model F value = 4.986; p= 0.0018.
Model R^2 = .4457; Model adjusted R^2 = .3563.

Chapter 6

THE EFFECT OF SUBCLINICAL *M. PARATUBERCULOSIS* ON MILK PRODUCTION AND REPRODUCTIVE OUTCOMES IN MICHIGAN DAIRY COWS

6.0 STRUCTURED ABSTRACT

Objective - To determine the effect of subclinical *M. paratuberculosis* infection on mature equivalent milk, protein, and fat production and on days to first service, number of services per confirmed pregnancy, and days open in Michigan dairy herds.

Design- A prospective two-group cohort study

Sample Population- A sample of local Michigan DHIA participating herds with a history of cows positive for *M. paratuberculosis* diagnosed by fecal culture.

Procedure- Participating herds were enrolled in the study and their herd productivity was monitored for 18 months. All cows aged 24 months and greater were tested for *M. paratuberculosis* infection using the IDEXX ELISA and radiometric fecal culture techniques. Test negative cows were re-tested at the conclusion of the monitoring period to reduce misclassification of infected animals as negative controls. DHIA and producer production records were monitored. Multivariable regression models were used to analyze the impact of

paratuberculosis infection status on milk production and reproductive performance.

Results- Subclinical paratuberculosis test positive status had no statistically significant effect on mature equivalent milk, fat, or protein production. ELISA positive cows had a 28 day increase in the number of days open (p=0.015).

Conclusions- The results of this study concur with the findings of other studies, reporting that the magnitude and direction of the association between subclinical paratuberculosis infection and milk production depends upon the parity of the animal, the stage of disease, and the stage in lactation being monitored. Those studies reporting a positive association between subclinical paratuberculosis infection and milk production have theorized that high producing cows are at greater risk of infection and that these animals may continue to produce milk at or in excess of herd averages until the disease progresses to a level that impairs productivity.

The lack of a consistent relationship between days open and paratuberculosis status when fecal culture is used as the diagnostic method instead of ELISA may be due to increased misclassification due to false positive fecal cultures.

Clinical Implications - Assessment of the impact of subclinical paratuberculosis on milk production must consider the average parity of the sample population. In herds that have an average parity of 2 or less, subclinical M. paratuberculosis infection may have little impact on milk production. In high paratuberculosis

prevalence herds, the ELISA may be a better indicator of the infection status of an individual animal because fecal culture may result in false positives due to the level of environmental contamination.

6.1 INTRODUCTION

Several studies have been conducted to determine the impact of subclinical *M. paratuberculosis* infection on milk production (Abbas *et al* 1983; DeLisle & Milestone 1989; McNab *et al* 1991; Buergelt & Duncan 1978; Nordlund *et al* 1996; Sweeney *et al* 1994; Wilson *et al* 1993; Merkal *et al* 1975; Spangler *et al* 1988). In culled cows, Benedictus *et al* (1987) reported a 6% reduction in milk production in the second to last lactation and a 16% reduction in the final lactation prior to culling in histopathologically positive, subclinically infected cows. Buergelt and Duncan (1978) reported no significant difference in milk production between culled, asymptomatic culture or histopathologically positive cows and negative culled herdmates.

Abbas *et al* (1983) found a 15% (1838 pound) reduction in mean annual milk yield in fecal culture positive subclinically infected cows when compared to their negative herdmates. In a New Zealand prospective study, milk production losses ranged from less than statistically significant to a 17% reduction in milk production for the most seriously affected herds (DeLisle & Milestone 1989). Recently, Nordlund *et al* (1996) reported that ELISA positive cows had a 4% reduction in mature equivalent milk production than their negative herdmates.

In contrast McNab *et al* (1991) reported that LAM-ELISA positive cows had a statistically significant increase in milk production when compared to their

negative herdmates after adjusting for herd management practices and intra-herd correlation. Similarly, Wilson and colleagues (1993) found that, depending upon the parity of the cow and the stage of lactation, fecal positive animals may have higher milk production than their negative controls.

Only recently has data been gathered to demonstrate a reduction in the quality of milk produced by asymptomatic *M. paratuberculosis*-positive cows. Daily milk fat and milk protein was reported to be significantly reduced in culture positive subclinical cows when compared to culture negative cows (Sweeney *et al* 1994). Collins & Nordlund (1991) and Nordlund *et al* (1996) determined that ELISA positive subclinical Johne's disease was associated with a reduction in 305 day mature equivalent protein and fat that costs producers $205 per cow per lactation.

Previous studies have failed to consistently demonstrate a change in milk production associated with subclinical *M. paratuberculosis* infection. These studies have differed in the method of diagnosis, the stage of infection evaluated, and the control group used for comparison. In addition herd-level productivity, management practices, and herd prevalence may also be important factors effecting the impact of subclinical *M. paratuberculosis* infection on milk production.

Few studies have assessed the impact of subclinical *M. paratuberculosis* infection on reproductive outcomes. The biological mechanism for reduced fertility in paratuberculosis-positive cows is based upon the relationship between

energy balance and reproduction. Cows that are infected with *M. paratuberculosis* may be at an increased risk of being in negative energy balance due to reduced intestinal absorption of nutrients.

The granulomatous enteritis and mucosal thickening that are characteristic of paratuberculosis result in a malabsorption syndrome with a protein losing enteropathy (Kreeger 1991; Patterson *et al* 1967; Patterson & Berrett 1968). It has been reported that negative energy balance can reduce growth and development of corpora lutea and result in a reduction of serum progesterone (VandeHaar *et al* 1995; Terhune 1993) In post-partum cows, negative energy balance results in an increased interval to first ovulation, a reduced number of large follicles and reduced growth of preovulatory follicles (Terhune 1993; Britt 1994). It has not been determined whether the intestinal lesions in the subclinically infected animal reduce intestinal function sufficiently to cause negative energy balance. However, it is reasonable to presume that since non-infected cows are prone to negative energy balance in the early post-partum period through peak lactation, cows that have even a mild reduction in intestinal function due to subclinical enteritis are even more susceptible to negative energy balance or are at an increased risk of severe negative energy balance during the early post-partum to peak lactation period. Early post-partum cows are energy deficient because in a process known as 'goal oriented nutrient partitioning' nutrients are shifted away from other tissues and organs to the mammary gland in support of milk production. Hence milk production takes priority over

reproduction (Lucy *et al* 1992). While the first and second ovulatory follicles develop during the pre-parturient dry period at a time when the cow is in positive energy balance, the third, fourth, and fifth ovulatory follicles develop while the cow is in negative energy balance in the early post-partum period (Britt 1994). The pre-ovulatory follicles that develop in the face of negative energy balance produce less estradiol resulting in reduced expression of behavioral estrus, which can lead to reduced estrus detection (Lucy *et al* 1992).

While the first and second post-partum corpora lutea produce levels of progesterone that are not influenced by the cow's energy balance, the third through fifth corpora lutea are from follicles that are dysfunctional and produce less progesterone (Britt 1994). Decreased progesterone has been associated with reduced first conception rates (Fonesca *et al* 1983; Villa-Goday 1988).

These impairments in the function of pre-ovulatory follicles and corpora lutea that are associated with negative energy balance can be expected to result in increased days to first service and increased number of days open. Thus cow subclinically infected with *M. paratuberculosis* may be at increased risk of having a delay first post-partum service and in subsequent conception. Thus, the reproductive efficiency of the herd is reduced to do an increased number of days open while, the overall conception rate however, may remain unaffected.

Two studies reported an increased risk of premature culling due to infertility among cows with subclinical paratuberculosis (Buergelt & Duncan 1978; Merkal *et al* 1975). However, the extent of the infertility was not quantified

nor was the cause specified. Abbas and colleagues (1983) found that calving intervals were 1.73 months greater in *M. paratuberculosis* infected cows than in negative cows. However, this study was not limited to subclinical cases. In contrast, De Lisle & Milestone (1989) and McNab *et al* (1991) failed to find any association between subclinical *M. paratuberculosis* infection and calving interval. Days to first service and days open may be more sensitive indicators of reproductive dysfunction secondary to subclinical paratuberculosis than culling due to infertility or actual calving interval.

Quantifying the economic impact of subclinical paratuberculosis is a vital component of efforts to develop and evaluate cost effective Johne's disease control programs. Due to the controversy in the literature regarding the impact of subclinical *M. paratuberculosis* infection on milk production and reproductive outcomes, this study was designed to investigate this relationship further.

6.2 HYPOTHESES

The specific hypotheses tested were:

1) Subclinical *M. paratuberculosis* infected cows have a lower 305 day mature equivalent milk than their *M. paratuberculosis* negative herd-mates.

2) Subclinical *M. paratuberculosis* infected cows have a lower 305 day mature equivalent fat than their *M. paratuberculosis* negative herd-mates.

3) Subclinical *M. paratuberculosis* infected cows have a lower 305 day mature equivalent protein than their *M. paratuberculosis* negative herd-mates.

4) Subclinical *M. paratuberculosis* infected cows have a greater number of days open post-partum than their *M. paratuberculosis* negative herd-mates.

5) Subclinical *M. paratuberculosis* infected cows have a greater number of days to first service post-partum than their *M. paratuberculosis* negative herd-mates.

6) Subclinical *M. paratuberculosis* infected cows require a greater number of services per confirmed pregnancy than their *M. paratuberculosis* negative herd-mates.

6.3 MATERIALS AND METHODS

The experimental design for this study was a prospective two group cohort to assess milk production and reproductive outcomes in cows testing positive for *M. paratuberculosis* infection compared with herdmates that test negative. The prospective nature of the study allowed determination of the *M.*

paratuberculosis status of control animals over time and it ensured that the cases were indeed positive prior to the time for which the productivity was being assessed.

A. Case definition

To compare and contrast the results of this study with previous studies, several case definitions and respective control groups were determined based upon the results of ELISA and radiometric fecal cultures (see Table 6.1). To evaluate the impact of subclinical infection alone, cases were limited to test positive animals that were not demonstrating clinical signs of paratuberculosis (chronic or intermittent diarrhea or loss of weight that was unresponsive to anthelmintic or antimicrobial treatment, for a duration of 30 days or more without inappetence) at the time of testing. Control animals also had to be free of clinical signs of paratuberculosis. Animals that developed clinical paratuberculosis during the study period were eliminated from the data analyses.

B. Sample Size and Statistical Power

Calculation of the number of animals that must be enrolled in the study was based on considerations of power to detect a significant difference in 305 day mature equivalent milk. Given a desired probability of Type I error of 0.05% and Type II error of 20%, and assuming an intra-herd correlation of 0.04, the minimum difference in mature equivalent milk to be detected was 1000 pounds. The standard deviation of mature equivalent milk was approximately 3000 pounds. Using the method described by Donner (1992), the required sample

size was estimated to be 120 infected animals and 120 uninfected. To control for possible loss to follow-up and the conversion of some test negative animals to positive status, the goal was to obtain 3 negative animals for each case resulting in a total desired sample size of 120 infected animals and 360 uninfected animals.

C. Selection of Herds and Animals

Participating farms were selected from paratuberculosis positive, Holstein Dairy Herd Improvement Association herds. Participants were referred by private veterinary practitioners and Michigan Department of Agriculture Veterinary Medical Officers. For this project, a paratuberculosis positive herd was defined as a herd that had been classified as *M. paratuberculosis* infected prior to the study. Infected herd status was based on having at least one animal positive on fecal culture. In addition to paratuberculosis positive status, participating producers were required to use artificial insemination or hand-mating so that exact breeding dates can be determined and recorded. Records were maintained by the farm manager and collected by the investigators during quarterly farm visits.

For each herd enrolled in the study, all female animals in their first lactation or 24 months of age and older were tested. Collection of DHIA milk production data and reproductive records began at the time of initial testing. Herds were monitored for a period of 18 months. A randomly selected subset of the animals that tested negative at the initial testing were re-tested at the

conclusion of the 18 month observation period. Those animals that remained negative were classified as the control animals for comparison to their positive herdmates. Due to the low sensitivity of the ELISA and fecal culture and the chronic nature of paratuberculosis, any animal that tested positive for *M. paratuberculosis* infection at any time during the study was classified as a case.

The true prevalence for each herd was determined by adjusting the sample apparent prevalence (See Equation 1) for the sensitivity (69%) and specificity (99.7%) of the ELISA and radiometric fecal culture, when used in parallel (See Equation 2).

Equation 1

$$\text{Sample apparent prevalence} = \frac{\text{Number of Test Positive Animals}}{\text{Number of Animals Tested}}$$

Equation 2

$$\text{True prevalence} = \frac{\text{Apparent Prevalence + Specificity - 100\%}}{\text{Sensitivity + Specificity - 100\%}}$$

D. Sample Collection and Handling

Blood and feces were collected concurrently from the selected animals by the investigators. Ten ml of blood were collected from each cow via the middle coccygeal vein using a 20 gauge inch needle and 2 ten ml Vacutainer tubes (Corning Glass Works, Corning New York). After clotting, and within 12 hours,

the samples were centrifuged, and serum harvested and stored at -70°C in a sterile cryotube.

Approximately 20 grams of feces were collected from each cow via rectal palpation using a disposable plastic obstetrical glove, placed in a plastic specimen cup, sealed and labeled. Fecal samples were then mailed to the Johne's Testing Center at the University of Wisconsin in Madison, Wisconsin for radiometric fecal culturing, via overnight express in Styrofoam coolers with cold packs.

E. Laboratory Testing

Serum samples were tested for antibodies to *M. paratuberculosis* via the commercial IDEXX ELISA test kit (IDEXX Laboratories, Inc. Portland, Maine) using the manufacturer's recommended protocol (Collins *et al* 1993; Anderson *et al* 1991) Radiometric fecal culturing was conducted at the Johne's Testing Center at the University of Wisconsin. The laboratory followed the protocol described in recent studies (Collins *et al* 1990). Briefly, BACTEC medium was supplemented with mycobactin J, egg yolk suspension, vancomycin, amphotericin B, and nalidixic acid in a radiometric broth. Decontaminated fecal specimens were filter concentrated and placed in the vial containing the radiometric culture medium. Vials were read weekly on a BACTEC 460. All positive growth vials were subcultured on plate media and confirmation of *M. paratuberculosis* isolation was confirmed by IS900 Genetic Probe (IDEXX Laboratories, Inc.). The sensitivity of fecal culture was enhanced by using the

radiometric technique because it has been reported to detect as few as ten viable organisms per gram of feces (Collins *et al* 1990) in contrast to traditional fecal culture techniques which are reported to require 100-1000 viable colony forming units per gram of feces (Sanfleban 1990; Whipple & Merkal 1985). The use of the ELISA and radiometric fecal culture in parallel has been reported to result in 69% sensitivity, 99.7% specificity, 96.2% positive predictive value, and 96.7% negative predictive value (Collins *et al* 1993).

F. Collection of Milk Production and Other Reproductive Data

General management data of the farm and study animal identification was conducted at the start of the study. The study animal identification form established the animals' age, lactation number, and current reproductive status. Individual cow health and reproductive data were collected quarterly during the study period. Participating farmers were asked to sign permission forms to provide milk production data directly from DHIA to the investigators quarterly.

6.4 DATA ANALYSIS

Data were maintained in a computerized database system and statistical analyses were conducted using SAS (SAS version 6.12, Cary N.C.:SAS Institute, Inc. 1997). Negative and positive cows were compared using the Mantel-Haenszel Chi-square test for parity group (1, 2, 3, $\geq$4) and the t-test for continuous variables (305 ME milk, fat, and protein; days in milk; days open; days to first service; and number of services per confirmed pregnancy). Due to

lack of reliable data on days to first service and number of services per confirmed pregnancy these outcomes were not evaluated.

A multivariable regression modeling approach was used to analyze the relationship between *M. paratuberculosis* infection status and the outcome variables of interest: mature equivalent milk, protein, and fat; and days open. Individual animal test *M. paratuberculosis* test status was the main fixed effect of interest. Since herd members were more likely to be similar with respect to the outcomes of interest, herd was also included in the models as fixed effect covariates. Parity and days in milk were included as covariates in the regression model since they were also known to effect both milk production and reproductive outcomes.

Data on other potential confounding cofactors such as occurrence of diseases including clinical mastitis, metritis, lameness, ketosis, hypocalcemia, displaced abomasum, retained placenta, and dystocia were collected however under-reporting of disease occurrence precluded inclusion of these data in the model (2% [11/533] of the study animals had any reported disease event during the study period) .

In only one of the herds sampled (herd 3) was the herd prevalence low enough to have a ratio of 3 negative animals to each positive animal. A random sample of negative controls stratified on parity were matched to the cases within each herd. The resulting sample consisted of 81 negative and 116 positive cows. Herd 2 was not included in the analysis of reproductive outcomes

because of a lack of reliable data regarding breeding dates. Individual animals from all herds were dropped from the multivariable analyses if there were missing data for the variables of interest. The sample used to evaluate the impact of subclinical *M. paratuberculosis* infection on reproductive outcomes consisted of 40 negative animals and 84 positive animals.

6.5 RESULTS

A total of 533 animals from 7 herds were tested for paratuberculosis. When a case was defined as an animal that was positive on either the radiometric fecal culture or the ELISA, the overall sample apparent prevalence was 41.8% (223 test positive animals / 533 animals tested). Adjusting for the 69% sensitivity and 99.7% specificity of the ELISA and radiometric fecal culture -- when used in parallel, (See Equation 1) results in a calculated true prevalence of 59.9%.

After withdrawal of the herds that no longer participated in DHIA, the sample of herds used for statistical analysis had apparent and true prevalences of 46.5% (198 test positive animals / 426 animals total) and 63.8% respectively. There were 81 negative animals and 116 positive animals used in the statistical analysis. See Table 6.2 for the farm prevalence of test positive animals from the herds used in the multivariable statistical analyses. Tables 6.3 and 6.4 report the univariable analysis of two covariates included in the multivariable models, days in milk and parity by *M. paratuberculosis* test status. Tables 6.5 - 6.7 summarize

the multivariable analysis of 305 ME milk, fat , and protein by *M. paratuberculosis* test status. No significant differences in milk , fat, or protein production were found.

Table 6.8 reports the multivariable analysis of days open by test status. ELISA positive cows had a 28 day increase in days open and this difference was significant (p=0.015). No other significant differences in days open by *M. paratuberculosis* test status was found.

6.6 DISCUSSION

This study did not identify a statistically significant difference in milk, protein, or fat production between *M. paratuberculosis* infected cows and negative controls using any of the six possible classifications of test positive status. In fact, the differences in milk production found seem to indicate a positive association between positive *M. paratuberculosis* test results and milk, fat, and protein production. These results are consistent with results reported by Wilson *et al* (1996) and McNab *et al* (1991).

Wilson and colleagues (1993) report that mature equivalent milk overall was not statistically different between fecal culture positive and negative cows. When stratified by age and stage of lactation they found that *M. paratuberculosis* infected heifers in early lactation had a higher mature equivalent milk production than negative cows. In contrast however, they found that *M. paratuberculosis* infected cows in their second lactation or higher and greater than 100 days in

milk had a significantly lower mature equivalent milk production. McNab et al (1991) reports that at the individual cow level, LAM-ELISA positive cows had significantly higher milk production as measured by the current breed class average and individual cow average kg. of milk produced per year of life since 2 years of age.

Some studies that have reported a reduction in milk production for subclinically infected cows have also indicated that the observed reduction in milk production does not occur across all lactations and that younger animals may even demonstrate higher milk production than their negative herdmates. Benedictus and colleagues (1987) found that subclinical *M. paratuberculosis* positive cows (diagnosed by histopathological examination at necropsy) had a 6% reduction in milk production in the second to last lactation and a 16% reduction in the final lactation prior to culling. Additionally they report that the earlier production of these animals was greater than the herd average (Benedictus *et al* 1987).

McNab and colleagues (1991) have theorized that their results and those of Benedictus *et al* (1987) may be an indication that cattle with a higher production potential may be at an increased risk of later being culled due to paratuberculosis. This is supported by the findings of Buergelt and Duncan (1978), although on average they reported, a reduction in 305 ME milk in the last lactation prior to culling for subclinically infected cows diagnosed by culture and histopathology. They also reported, that young cows subclinically positive for *M.*

paratuberculosis were being culled from the herd prematurely, with excellent milk production at the time of culling (Buergelt & Duncan 1978).

The findings of this study indicate that the key to the inconsistent results reported in the literature regarding subclinical *M. paratuberculosis* infection and milk production may not be in the method of diagnosis but in the parity of the cows in the study. Six different definitions of positive infection status were used to evaluate the impact of subclinical infection on milk production. Using each of these criteria, the difference in milk production between test positive and test negative cows was not significant and the association between testing positive and milk production was positive. Table 6.8 indicates that there was no significant difference in *M. paratuberculosis* test status across parity groups of cows in this sample. However, it also reveals that 59% of the cows in the study were in their first or second lactation. This is consistent with statewide reports that in 1996, the average Michigan dairy cow was 44 months of age (3.67 years) (Michigan Dairy Herd Improvement Association, 1996). This would mean that the average Michigan dairy cow would have a parity of approximately two. If significant reductions in milk production occur primarily at later lactations, herds with an average parity of 2 (the typical Michigan dairy herd) may not demonstrate losses in milk production due to subclinical *M. paratuberculosis* infection.

The characteristic long latency and incubation periods for paratuberculosis may be the biological mechanism driving this seemingly paradoxical relationship. If animals that have a greater production potential are

also at greater risk of infection, these animals (even while infected) can be expected to express greater than average milk production during the first and perhaps second lactations during the very early stages of infection, when they may still test positive without manifesting clinical signs. As the infection progresses, in later lactations one can expect to see a decline in milk production. However, in those herds that are maintaining an average herd parity of 2, many of these subclinically infected animals would be culled prior to experiencing any decline in milk production. While these results indicate that initially subclinically infected animals are not experiencing a decline in milk production, the lost genetic material and future production potential of these animals must be considered when these animals are in a more advanced stage of infection and are believed to be at increased risk of premature culling due either to clinical paratuberculosis or to secondary diseases associated with subclinical *M. paratuberculosis* infection.

While the method of defining a case did not alter the association between milk production and *M. paratuberculosis* test status, it did alter the association between days open and *M. paratuberculosis* test status. The variation of the results across diagnostic techniques in this study, may provide an explanation for a failure to find consistent results in the literature regarding the impact of subclinical paratuberculosis on reproduction. Previous studies have differed in diagnostic technique and study population (culled cows or cows within the herd). Some have defined positive animals on the basis of culture or histopathology

(Abbas *et al* 1989; Buergelt & Duncan 1978) and others on the basis of ELISA alone (DeLisle & Milestone 1989; McNab *et al* 1991). In this study, the 2 diagnostic tests were employed in parallel to allow the results to be compared and contrasted by diagnostic method.

When a case was defined as ELISA positive and a control was defined as ELISA negative (without regard to any fecal results) *M. paratuberculosis* test positive status was associated with a significant increase in the number of days open after parturition (28 days, p =0.015). Conversely, in the two case criteria in which a case was defined as radiometric fecal culture positive (either culture positive and ELISA negative or culture positive with no regard to ELISA results), days open was actually negatively associated with *M. paratuberculosis* test status, although these differences were not statistically significant. Thus, when fecal culture alone or ELISA and fecal culture in parallel were used to identify cases and controls the effect of *M. paratuberculosis* test status on days open was not significant, and in some cases the direction of the association was reversed. This may signify that ELISA is a better indicator of infection status when the objective is to evaluate productivity of infected animals in highly infected herds.

In high paratuberculosis prevalence herds, the risk of false positive fecal results may be quite high. The false positive rate with fecal culture is increased in herds with high prevalence because the environment is very contaminated and animals may culture positive due to organisms simply transiting through the gut

while actual infection has not occurred. In this circumstance, the ELISA may be a better determinant of infection status since a positive result indicates that an immune response has occurred to the agent, this may be more clearly associated with actual infection rather than just passage of *M. paratuberculosis* through the intestinal tract.

The increase in days open is an indication that perhaps reduced estrus expression or an increased post-partum anestrus period are occurring in the subclinically infected ELISA positive animal. This may be due to a negative energy balance secondary to *M. paratuberculosis* infection. Negative energy balance due to a reduction in feed efficiency and perhaps malabsorption are only plausible mechanisms for reduced reproductive performance if an animal is truly infected. The transiting of *M. paratuberculosis* through the intestinal tract of an animal would not be expected to impact energy balance or reproductive performance. Thus, animals that may culture positive to *M. paratuberculosis* without actually being infected will minimize or even change the direction of differences between those classified as cases and those classified as controls.

Table 6.1. Case definitions and respective control groups for comparison of 305 day M.E. milk production, 305 day M.E. fat production, 305 day M.E. protein production, and days open in prospective cohort study of the effect of *M. paratuberculosis* test status on milk production and reproductive outcomes in paratuberculosis positive dairy herds in Michigan, USA monitored from May 1996 to November 1997.

Comparison Number	Case Definition			Control Group		
	ELISA Result		Fecal Result	ELISA Result		Fecal Result
1	Positive	**and**	Positive	Negative	**and**	Negative
2	Positive	**and**	Negative	Negative	**and**	Negative
3	Negative	**and**	Positive	Negative	**and**	Negative
4	Positive	**or**	Positive	Negative	**and**	Negative
5	Positive		-	Negative		-
6	-		Positive	-		Negative

Table 6.2. Prevalence of *M. paratuberculosis* test positive animals* by herd from a prospective cohort study of the effect of subclinical M. paratuberculosis infection on milk and reproduction outcomes in Michigan dairy herds monitored from May 1996 to November 1997.

Herd Number	Number Animals Tested	Number test (+) for *M. paratuberculosis* infection	Herd Prevalence (%)
1	47	30	63.8
2	44	14	31.8
3	126	20	15.9
4	77	62	80.5
5	172	86	50.0

* positive status based on a case definition of an animals testing positive on either the radiometric fecal culture or the ELISA.

Table 6.3. Univariable Analysis: **Days in Milk** by *M. paratuberculosis* test status from prospective cohort study of the effect of *M. Paratuberculosis* test status on milk production and reproductive outcomes in paratuberculosis positive dairy herds monitored from May 1996 to November 1997 in Michigan, USA.

Case Definition	Status	#	Mean Days in Milk	Quartiles	Kruskal-Wallis X^2	p
ELISA + and Culture +[a]	+	17	282.00	281, 305, 305	13.50	0.0002
	-	59	168.83	49, 161, 293		
ELISA + and Culture -	+	39	187.97	93, 209, 288	0.81	0.3669
	-	59	168.83	49, 161, 293		
ELISA - and Culture +	+	51	187.47	77, 201, 284	0.73	0.3932
	-	59	168.83	49, 161, 293		
ELISA + or Culture +	+	107	202.67	96, 242, 295	3.58	0.0584
	-	59	168.83	49, 161, 293		
ELISA +[a]	+	56	216.52	126, 271, 305	5.08	0.0242
	-	110	177.47	65, 177, 284		
Culture +[a]	+	68	211.10	116, 275, 305	4.17	0.0410
	-	98	176.45	69, 188, 288		

a- Statistically significant at $p \leq 0.05$.

Table 6.4. Univariable Analysis: **Parity** by *M. paratuberculosis* test status from prospective cohort study of the effect of *M. Paratuberculosis* test status on milk production and reproductive outcomes in paratuberculosis positive dairy herds monitored from May 1996 to November 1997 in Michigan, USA.

Case Definition	Status	Parity				Mantel-Haenszel	
		1	2	3	4+	X^2	p
ELISA + and Culture +	+	7	5	4	1	0.28	0.596
	-	26	10	14	9		
ELISA + and Culture -	+	14	9	10	6	0.20	0.655
	-	26	10	14	9		
ELISA - and Culture +	+	15	12	16	8	1.19	0.275
	-	26	10	14	9		
ELISA + or Culture +	+	36	26	30	15	0.48	0.488
	-	26	10	14	9		
ELISA +	+	21	14	14	7	0.22	0.639
	-	41	22	30	17		
Culture +	+	22	17	20	9	0.29	0.592
	-	40	19	24	15		

Table 6.5. Model Summaries of the relationship between *M. paratuberculosis* Test Status and **305 ME Milk** from multivariable linear regression models including days in milk and parity, with herd included as a fixed effect from prospective cohort study of the effect of *M. Paratuberculosis* test status on milk production and reproductive outcomes in paratuberculosis positive dairy herds monitored from May 1996 to November 1997 in Michigan, USA.

Case Definition	Parameter Estimate	Std. Error	P	Adjusted Model R^2	Model F	Model P
ELISA + and Culture +	1421.42	1217.82	0.247	0.3887	8.94	0.0001
ELISA + and Culture -	1287.24	764.57	0.096	0.4608	14.82	0.0001
ELISA - and Culture +	486.78	673.64	0.472	0.4231	14.32	0.0001
ELISA + or Culture +	753.28	593.84	0.206	0.4455	23.09	0.0001
ELISA +	961.03	601.58	0.112	0.4487	23.38	0.0001
Culture +	178.28	573.69	0.756	0.4402	22.62	0.0001

Table 6.6. Model Summaries of the relationship between *M. paratuberculosis* Test Status and **305 ME Fat** from multivariable linear regression models including days in milk and parity, with herd included as a fixed effect from prospective cohort study of the effect of *M. Paratuberculosis* test status on milk production and reproductive outcomes in paratuberculosis positive dairy herds monitored from May 1996 to November 1997 in Michigan, USA.

Case Definition	Parameter Estimate	Std. Error	P	Adjusted Model R^2	Model F	Model P
ELISA + and Culture +	24.57	68.58	0.721	0.2409	4.97	.0003
ELISA + and Culture -	17.70	44.06	0.688	0.3315	9.02	.0001
ELISA - and Culture +	60.71	42.48	0.156	0.2367	6.64	.0001
ELISA + or Culture +	32.34	33.74	0.339	0.3330	14.73	.0001
ELISA +	-18.54	34.34	0.590	0.3303	14.57	.0001
Culture +	47.90	32.30	0.140	0.3382	15.06	.0001

Table 6.7. Model Summaries of the relationship between *M. paratuberculosis* Test Status and **305 ME Protein** from multivariable linear regression models including days in milk and parity, with herd included as a fixed effect from prospective cohort study of the effect of *M. Paratuberculosis* test status on milk production and reproductive outcomes in paratuberculosis positive dairy herds monitored from May 1996 to November 1997 in Michigan, USA.

Case Definition	Parameter Estimate	Std. Error	P	Adjusted Model R^2	Model F	Model P
ELISA + and Culture +	45.53	34.26	0.1882	0.5090	13.96	0.0001
ELISA + and Culture -	37.61	22.66	0.1003	0.5145	18.13	0.0001
ELISA - and Culture +	12.98	20.50	0.5279	0.5001	19.18	0.0001
ELISA + or Culture +	21.56	18.06	0.2345	.4963	28.10	0.0001
ELISA +	35.24	19.62	0.0746	.5109	25.54	0.0001
Culture +	1.82	18.41	0.9214	.4992	24.43	0.0001

Table 6.8. Model Summaries of the relationship between *M. paratuberculosis* Test Status and **Days Open** from multivariable linear regression models including days in milk and parity, with herd included as a fixed effect from prospective cohort study of the effect of *M. Paratuberculosis* test status on milk production and reproductive outcomes in paratuberculosis positive dairy herds monitored from May 1996 to November 1997 in Michigan, USA.

Case Definition	Parameter Estimate	Std. Error	P	Adjusted Model R^2	Model F	Model P
ELISA + and Culture +	27.42	23.91	0.2561	0.3602	6.91	0.0001
ELISA + and Culture -	22.13	12.85	0.0896	0.4819	12.78	0.0001
ELISA - and Culture +	-7.56	11.15	0.4998	0.3559	9.75	0.0001
ELISA + or Culture +	3.93	11.08	0.7237	0.4051	16.89	0.0001
ELISA +[a]	27.94	11.37	0.0152	0.4303	18.62	0.0001
Culture +	-11.94	10.73	0.2680	0.4100	17.22	0.0001

a- Statistically significant at $p \leq 0.05$.

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SUMMARY, CONCLUSIONS, AND FUTURE RECOMMENDATIONS

Summary

This dissertation has investigated the epidemiology and economic impact of subclinical *M. paratuberculosis* infection in Michigan dairy herds. Herd-level and individual animal level approaches were used to determine the impact of paratuberculosis on productivity and risk factors associated with the distribution, transmission, and maintenance of *M. paratuberculosis* among Michigan dairy farms.

At the herd-level, a cross-sectional study was conducted that focussed on the distribution of *M. paratuberculosis* infected herds throughout the state. This study evaluated various management and soil associated risk factors, and sought to determine the economic impact of subclinical paratuberculosis on herd productivity.

At the level of the individual animal within the *M. paratuberculosis* infected herd, this dissertation has assessed the impact of subclinical *M. paratuberculosis* infection on the quantity and quality of milk produced and on reproductive outcomes. Efforts to reduce misclassification of infected cows as control cows were undertaken by incorporating a parallel testing strategy into the study

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design. However, even in parallel, misclassification of cases and controls continues to be a problem. In high prevalence herds false positive results from fecal culture reduces the reliability of these test results. The ELISA may be a better indicator of infection status, particularly when attempting to estimate changes in productivity for infected animals in high paratuberculosis prevalence herds.

Conclusions

The prevalence of *M. paratuberculosis* infected herds in the state of Michigan was greater than expected. It was anticipated that the prevalence of positive herds in Michigan would be similar to the 34% prevalence reported in Wisconsin (Collins *et al* 1993). In this stratified random sample 64% of the herds had one or more animals that tested positive for antibodies to *M. paratuberculosis*. Even after elimination of those herds in which only one animals tested positive, 55% of the sample had two or more positive animals. The actual number of positive animals was 7% (177 / 2,376) and within the range that would be expected based upon the 9% sero-prevalence rate reported in a previous, abattoir-based Michigan study (Kaneene *et al* 1992). Thus, while widely distributed among herds, the prevalence of positive animals within infected herds was not unexpectedly great.

The multivariable Poisson regression analysis of soil-related risk factors that controlled for management and soil texture/drainage class identified increased soil iron content and decreased soil pH as significant risk factors for *M.*

paratuberculosis infection. Controlling for herd hygiene, management, and productivity, application of lime to pasture areas (O.R. = 0.10, C.I. = 0.02 - 0.56) was associated with a reduced risk of positive herd paratuberculosis status.

Paratuberculosis positive herds experienced a 3% increase in cow and heifer mortality rate (p= 0.044). For each 10% increase in the prevalence of paratuberculosis positive animals there was a 74 pound decreases in the average culling weight (p= 0.047).

At the level of the individual animal, no significant differences were found in mature equivalent milk, fat, or protein production between infected cows and their negative herdmates. ELISA positive cows had a 28 day increase in the number of days open when compared to ELISA negative herdmates (p = 0.015).

The validity of the results from the cross-sectional study were limited by the potential for volunteer or information biases. Evaluation of the economic outcomes in particular was limited by a poor response rate for many questionnaire items resulting in inadequate statistical power. The prospective analysis of individual animal productivity may have been impeded by misclassification of cases and controls. This problem may be particularly severe in herds with very high prevalence rates.

Despite its limitations this study has made an important contribution to the body of knowledge regarding bovine paratuberculosis. This is the first population-based study in which acidic soil pH was associated with an increased prevalence of paratuberculosis-positive cows. Not only was soil pH identified as

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a risk factor for paratuberculosis, but soil iron availability was discerned as the probable mechanism by which soil pH influences the epidemiology of the disease. Increased soil iron content was associated both with an increase in the number of infected herds and the number of infected animals. Lastly, use of lime on pasture areas was identified as a management measure associated with a reduction in paratuberculosis positive herds and animals. This last piece of information provides a practical application for the information contributed by this study. Elevation of soil pH by application of lime reduces iron availability thus perhaps limiting the viability of *M. paratuberculosis* in the external environment.

Future Recommendations

The possibility that soil characteristics and soil management practices such as application of lime to pasture areas are important in the transmission and maintenance of *M. paratuberculosis* is an important finding. Further study of this issue is warranted. A statistically valid soil sampling scheme and the application of both cross-sectional and prospective study designs are needed.

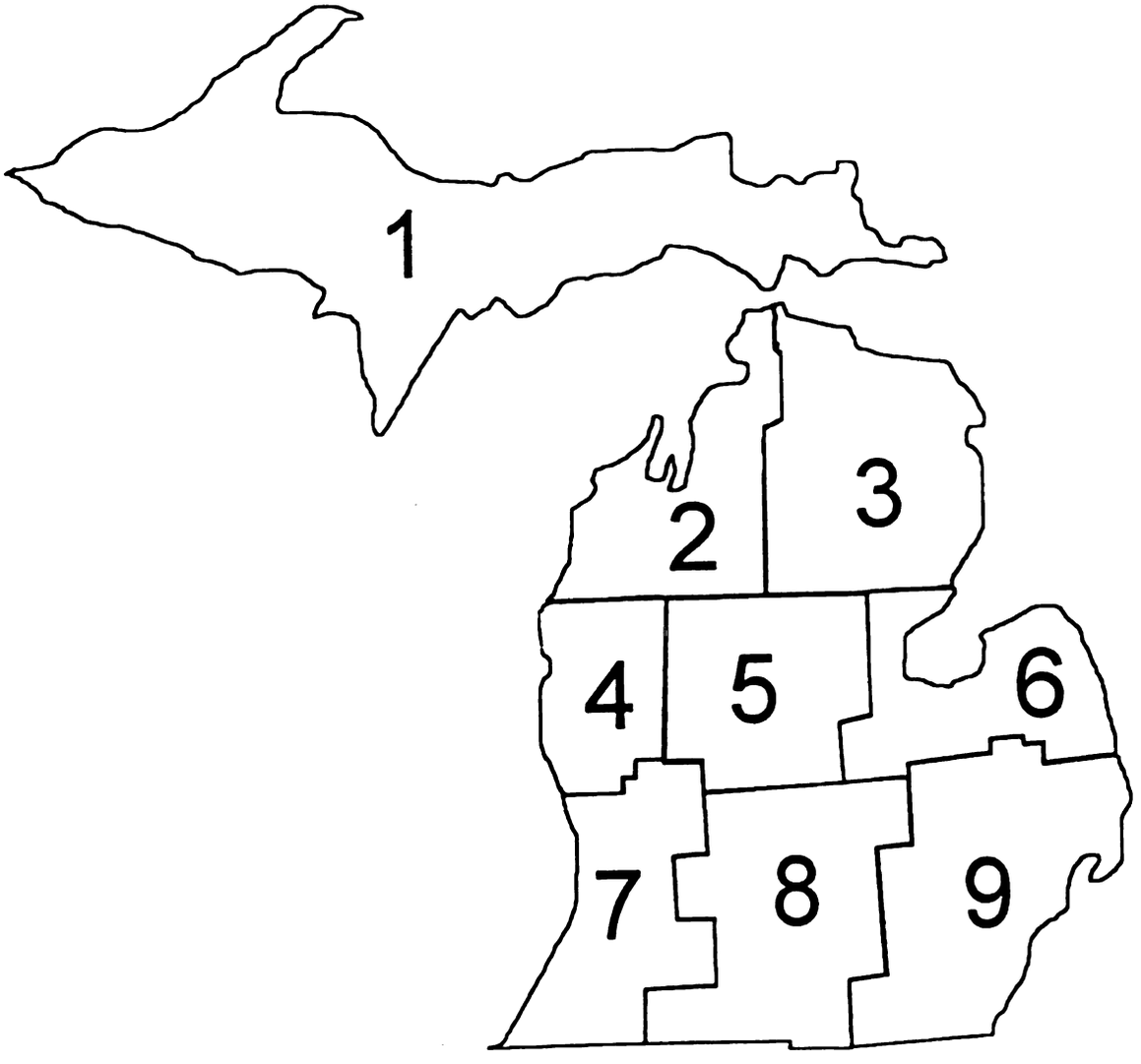
Further evaluation of the economic impact of subclinical paratuberculosis is needed at both the herd and individual animal levels. The results of this study are consistent with previous reports in the literature finding inconsistent associations between milk production and subclinical paratuberculosis depending upon the parity, stage of lactation, and stage of *M. paratuberculosis* infection. The possibility that high potential cows are at an increased risk of

paratuberculosis and premature culling secondary to complications from paratuberculosis may complicate efforts to quantify the economic losses attributable to subclinical paratuberculosis. While these high potential animals may produce more than negative herdmates in the early stages of disease, the subsequent decline in production may represent an even greater loss than currently estimated. The concurrent loss in the genetic potential of these animals also becomes a vital issue if this theory proves to be true.

All of the previous discussion regarding the impact of subclinical paratuberculosis, however, is contingent upon paratuberculosis continuing to be considered only pathogenic to ruminants. Recent indications that *M. paratuberculosis* may somehow be associated with Crohn's disease in humans may drastically change the estimation of the economic impact of paratuberculosis. Should *M. paratuberculosis* prove to be an etiologic agent or a significant risk factor in human disease, there will be major changes in the food safety and public health policies with respect to infected animals and infected herds. The cost of the disease to producers will then increase dramatically. Research in the epidemiology of Crohn's disease is also an important area for future research because ultimately these findings have the potential to affect the cattle industry too.

APPENDIX A

Appendix A. Map of Michigan Agricultural Districts



APPENDIX B

Appendix B. Questionnaire Part I FARM ID # _____

HERD PREVALENCE AND ASSOCIATED RISK FACTORS FOR SUBCLINICAL JOHNE'S DISEASE IN MICHIGAN DAIRY CATTLE

QUESTIONNAIRE - PART 1

Dr. John Kaneene
Dr. Yvette Johnson-Ifearulundu

Population Medicine Center
Michigan State University
A-109 Veterinary Medical Center
East Lansing, Michigan 48824-1314

(517) 353-5941

THANK YOU FOR PARTICIPATING!

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Appendix B (cont'd)

INSTRUCTIONS

Please complete the following questionnaire in ink.
Do not leave any items blank. When appropriate, fill in 0, not applicable, don't know, or decline to answer.

The following documents may be helpful for completing this questionnaire:

1) 1995 IRS form 1040F
2) Weigh slips for dairy cattle culled during the past three months.

Please consult farm records for additional values or give your best estimate if records are unavailable.

All responses will be kept confidential. If you have any questions feel free to contact Dr. Yvette Johnson or ask your data collector when (s)he arrives to collect specimens from your farm.

The following definitions may be helpful in completing the questionnaire:

Average Market Value/Head	Your best estimate of the fair market value (the price you would receive if you sold them today).
Dairy herd	Cows, first lactation heifers, heifer calves, bull calves, and bulls raised primarily for the production and sale of milk or as a result milk production. This does NOT include steers, veal calves, feeders, or finishers being raised for beef markets.
Cows	Mature female cattle (second lactation or older), both milking and dry.
First Lactation Heifers	Cows that are in their first lactation (first calf heifers).
Heifer Calves	Female cattle from one day of age until freshening
Bull Calves	Male cattle less than one year of age
Bulls	Mature male cattle (greater than one year of age)
Dairy Replacement Heifers	Heifers raised or purchased to add to your dairy herd
Dairy Replacement Bulls	Bulls raised or purchased to add to your dairy herd
Cull Cows	Mature dairy cows sold for slaughter or to a dairy market
Beef herd	Beef and/or dairy breeds of cattle raised primarily for sale to a slaughter market includes veal calves, feeders, and steers produced by the dairy herd.
Beef Replacement Heifers	Heifers raised or purchased to add to your beef herd
Beef Replacement Bulls	Bulls raised or purchased to add to your beef herd
Unpaid family labor	Members of the main family or families that own and manage the farm. Example: If your child works for you but only earns money out of what the family withdraws from the farm, he/she is considered unpaid. However, if the child draws a wage periodically he/she is considered paid labor.
Paid labor	Any labor used on the farm that was compensated (including bartered or traded).
Farm Inventory	Include: all stored feeds such as dry hay, silage, grains and supplements, and other feeds; all stored bedding such as sand, sawdust, shavings, straw, and other bedding; and all supplies on hand such as semen, antibiotics, other drugs, towels, teat dip, and other supplies.

Appendix B (cont'd)

FARM ID #_____

HERD PREVALENCE AND ASSOCIATED RISK FACTORS FOR
SUBCLINICAL JOHNE'S DISEASE IN MICHIGAN DAIRY CATTLE

QUESTIONNAIRE - PART 1

Please complete the following information about your DAIRY HERD

1) Your DAIRY HERD INVENTORY as of January 1, 1996

	Number of Head	Average Market Value/Head
Cows and First Lactation Heifers		
Heifer Calves (birth to first freshening)		
Bull Calves (less than 1 yr of age)		
Bulls		

2) Your DAIRY HERD INVENTORY as of January 1, 1995

	Number of Head	Average Market Value/Head
Cows and First Lactation Heifers		
Heifer Calves (birth to first freshening)		
Bull Calves (less than 1 yr of age)		
Bulls		

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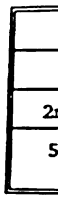

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FARM ID#_____

Appendix B (cont'd)

3) Do any of your dairy cows receive bovine Somatotropin (bST) treatments?

Circle one: YES NO

4) If YES, please fill in the following information about use of bST in your dairy herd.

	Number that will receive bST this lactation
1st Lactation Heifers	
2nd - 4th Lactation Cows	
5th or greater Lactation Cows	

5) Did any of the cattle in your DAIRY herd DIE (due to illness, accident, injury, or euthanasia) during the past year?

YES NO

6) If YES, please fill in the information for those DAIRY CATTLE that DIED between January 1, 1995 and January 1, 1996.

	Number of Head
Cows and First Lactation Heifers	
Heifer Calves (birth to first freshening)	
Bull Calves (less than 1 yr of age)	
Bulls	

7) DAIRY CATTLE Purchases

	1995		1994		1993	
	# Head	Total $	# Head	Total $	# Head	Total $
Replacement Heifers						
Replacement Bulls						
Other (specify)						

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FARM ID# _____

Appendix B (cont'd)

8) Clinical signs of Johne's disease in cattle include chronic diarrhea and weight loss that doesn't respond to treatment despite a normal appetite. In the last 12 months, how many of the cull cows from the **dairy herd** showed these signs?

_____head

9) **Please complete the following information about the Dairy Cows that were culled (either for slaughter or to a dairy market) in the last 90 days. Attach additional sheet(s) if needed.**

Date Culled	Live weight	Price/lb or head	Reason Culled

Date Culled	Live Weight	Price/lb or head	Reason Culled

10)

11)

12)

13)

Appendix B (cont'd)

FARM ID#_____

10) In the last 90 days, how many of the **culled dairy cows** were in their:

A) first lactation _____head

B) second lactation _____head

C) third lactation _____head

D) fourth or more lactation _____head

11) In the last 90 days, of those **culled dairy cows** that had a previous lactation, how many had peak milk production in that previous lactation that was:

A) Above herd average (top 1/3) _____head

B) Near herd average (middle 1/3) _____head

C) Below herd average (bottom 1/3) _____head

12) In the last 90 days, how many of the **culled dairy cows** were in their:

A) first 100 days of lactation _____head

B) second 100 days of lactation _____head

C) third 100 days of lactation _____head

D) dry _____head

13) In the last 90 days how many of the **culled dairy cows** were pregnant?

_____head

Appendix B (cont'd)

FARM ID#_____

14) Do you raise beef cattle (either beef or dairy breeds)?

Circle one: YES NO

If YES, please complete questions 15 and 16 about your BEEF HERD.

If NO, go to question #17.

15) **Please fill in the information for your BEEF HERD as of January 1, 1996.**

	Number of Head	Average Market value/head
Cows		
Youngstock (calves, heifers and steers)		
Bulls		

16) **BEEF CATTLE Purchases**

	1995		1994		1993	
	# Head	Total $	# Head	Total $	# Head	Total $
Replacement Heifers						
Replacement Bulls						
Other (specify)						

FARM ID#_____

Appendix B (cont'd)

17) **Farm Sales for the year 1995.**

	Pounds	Total Value
Milk		
Dairy Cattle:	**Number of Head**	**Total Value**
Cull Cattle		
Calves		
Breeding Cattle (heifers)		
Breeding Cattle (bulls)		

18) **Total farm product sales for 1995 from IRS form 1040F line 11.**
(All dairy, cash crop, beef, and other sales)

$_____

19) **Total farm cash expenses for 1995 from IRS form 1040F line 35.**
(Purchased feeds, fuels, paid labor, veterinary expenses, interest paid, taxes paid, etc.)

$_____

20) **Total farm depreciation expenses for 1995 from IRS form 1040F line 16.**

$_____

21) **Estimate the total number of hours of unpaid family labor in 1995.**

_____hours

22) **Estimate the total number of hours of paid labor in 1995.**

_____hours

23) **Estimate the total value of the farm inventory (feeds, supplies, bedding, semen, etc.) on January 1, 1996?**

$_____

24) **Estimate the total value of the farm inventory (feeds, supplies, bedding, semen, etc.) on January 1, 1995?**

$_____

APPENDIX C

Appendix C. Questionnaire Part II

HERD PREVALENCE AND ASSOCIATED RISK FACTORS FOR SUBCLINICAL JOHNE'S DISEASE IN MICHIGAN DAIRY CATTLE

QUESTIONNAIRE - PART 2

Dr. John Kaneene
Dr. Yvette Johnson-Ifearulundu

Population Medicine Center
Michigan State University
A-109 Veterinary Medical Center
East Lansing, Michigan 48824-1314

(517) 353-5941

THANK YOU FOR PARTICIPATING!

Appendix C (cont'd)

INSTRUCTIONS

Please complete the following questionnaire in ink.
Do not leave any items blank. When appropriate, fill in 0, not applicable, don't know, or decline to answer
All responses will be kept confidential.

The following definitions may be helpful in completing this questionnaire:

Permanent Pasture	A field that is always planted in grass and used for grazing, usually land that is not suitable for crop production.
Rotational Pasture	A fallow field that is used for grazing as a part of a crop rotational pattern.
Exercise Lot	A fenced area outside the barn for cow exercise, may be paved or unpaved (dirtlot).
Fresh Chopped Forage	Forage is green cut and delivered to cows at the confinement unit.
Slurry	A mixture of feces and urine often also with water, food residues and bedding material in a liquid or semi-liquid state.
Slurry Cellar/Pit	A manure storage facility beneath a slotted floor.
Lagoon	A structure for containing liquid wastes. An earth-banked structure partly above ground level.
Manure Pack	Manure and bedding material allowed to collect for 6 months to one year.
Composting	Treatment of manure through controlled microbial action, primarily that of bacteria, actinomycetes, and fungi. The heat generated from the process kills most weed seeds and plant pathogens.
Surface Application	Use of irrigation equipment to deliver diluted slurry to fields (organic irrigation).
Soil Injection	Application of diluted liquid slurry beneath the soil surface.
Solid Spreader	Application of solid wastes to a field using a manure spreader.
Gutter	An area located behind the stalls in a stanchion or tie stall barn 11 to 16 inches deep and 16 to 18 inches wide for the collection of manure. It may have a metal grate over it. It is cleaned by scraping or flushing with water.
Alley	A paved or concrete passageway behind stalls that separates rows of adjoining stalls. It is cleaned scraping (with a tractor and blades or a mechanical scraper) or periodical flushing with water.
Maternity Pen	A stall that is located away from the milking herd in the same barn or in different quarters.
Calf Hutch	An individual shelter for a calf.
Dam	The female parent (mother).
Johne's Disease	Infection with Mycobacterium paratuberculosis, clinical signs: chronic diarrhea and weight loss that doesn't respond to treatment despite a normal appetite.

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Appendix C (cont'd)

HERD PREVALENCE AND ASSOCIATED RISK FACTORS FOR
SUBCLINICAL JOHNE'S DISEASE IN MICHIGAN DAIRY CATTLE

QUESTIONNAIRE - PART 2

Please complete the following information about your management practices now and three years ago for your adult cows (milking and dry) and heifers (at or above 6 months of age to first freshening).

	NOW			3 YEARS AGO		
	MILKING COWS	DRY COWS	HEIFERS	MILKING COWS	DRY COWS	HEIFERS
25) Do you have pasture for the dairy cattle?	Y N	Y N	Y N	Y N	Y N	Y N
If answer to #25 is NO, go to #30						
26) Is the pasture: Permanent or Rotational?	P R	P R	P R	P R	P R	P R
27) How many acres of pasture are in use for each cattle group?	____ acres	____ acres	____ acres	____ acres	____ acres	____ acres
28) How many pasture paddocks are in use for each cattle group?	____ paddocks	____ paddocks	____ paddocks	____ paddocks	____ paddocks	____ paddocks
29) How often do you rotate the cattle in the paddocks? Every:	____ days	____ days	____ days	____ days	____ days	____ days
30) How often do you scrape the barns for each cattle group? Every:	____ days	____ days	____ days	____ days	____ days	____ days
If answer to #30 is NO, go to #34						
31) Do you have exercise lots for the cattle?	Y N	Y N	Y N	Y N	Y N	Y N
32) Are the exercise lots paved?	Y N	Y N	Y N	Y N	Y N	Y N
33) How often do you scrape the exercise lots? Every:	____ days	____ days	____ days	____ days	____ days	____ days

Appendix C (cont'd)

34) Circle the waste-storage or treatment system(s) in use and indicate which group (if any) has access/exposure to the system:	NOW						3 YEARS AGO					
	MILKING COWS		DRY COWS		HEIFERS		MILKING COWS		DRY COWS		HEIFERS	
A) Slurry Cellar/Pit	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
B) Lagoon	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
C) Manure pack (inside barn)	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
D) Outside solid manure storage not in drylot or pen	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
E) Outside solid manure storage in drylot or pen	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
F) Indoor solid manure storage with no cattle access	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
G) Composting	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
H) Other (specify)	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N

	NOW		3 YEARS AGO	
35) What method(s) of cattle waste disposal are used on this operation?				
A) Surface Application of Dilute Slurry	Y	N	Y	N
B) Soil Injection of Dilute Slurry	Y	N	Y	N
C) Solid Spreader	Y	N	Y	N
D) Hauling off the farm	Y	N	Y	N
E) Other (specify)	Y	N	Y	N
36) Do you use any of the following to remove manure from cow housing areas?				
A) Gutter cleaner	Y	N	Y	N
B) Alley scraper (mechanical or tractor)	Y	N	Y	N
C) Alley flushed with water	Y	N	Y	N
D) Other (specify)	Y	N	Y	N
37) If #36 C is Yes - Is water used for flushing manure from cow housing areas recycled for multiple flushings?	Y	N	Y	N

Appendix C (cont'd)

	NOW			3 YEARS AGO		
38) Does this operation incorporate manure into the soil within 24 hours after application? (Always Sometimes Never)	A	S	N	A	S	N
39) Does this operation routinely use any of the following on fields that are used as pasture?						
A) Inorganic fertilizer	Y		N	Y		N
B) Pesticides/Herbicides	Y		N	Y		N
C) Limestone	Y		N	Y		N
40) What is the distance between manure storage area and nearest drinking site (pond, trough, etc.) for cattle?	_____ feet / miles (Circle one)			_____ feet / miles (Circle one)		
41) Do you use maternity pens for the cows that are about to freshen? (Always Sometimes Never)	A	S	N	A	S	N
If the answer to #41 is NEVER, go to #44						
42) Do you clean the maternity pens after EACH use?	Y		N	Y		N
43) Are the maternity pens also used as a hospital area for sick cows? (Always Sometimes Never)	A	S	N	A	S	N
44) How long does the calf stay with the dam after birth (in hours)?	_____ hours			_____ hours		
45) Are the dams' teats washed before colostrum is collected or calf nurses? (Always Sometimes Never)	A	S	N	A	S	N
46) Are the dams' udders washed before colostrum is collected or calf nurses? (Always Sometimes Never)	A	S	N	A	S	N
47) Are the heifer calves housed individually?	A	S	N	A	S	N
If answer to #47 is Never go to #51						
48) Can the heifer calves make nose-to-nose contact with each other?	Y		N	Y		N
49) Are the hutches moved or the pens cleaned after EACH calf?	Y		N	Y		N

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Appendix C (cont'd)

	NOW	3 YEARS AGO
50) How old are the heifer calves when they are removed from individual housing?	_____ weeks	_____ weeks
51) After removal from the dam, at what age do heifers first have contact with adult cows in the herd?	_____ months	_____ months
52) Is manure handling equipment also used to handle feed given to heifer calves below 6 months of age? (i.e. skid loader)	A S N	A S N
53) Do heifer calves below less than 6 months of age share common feed and/or water sources with adult cattle? (Always Sometimes Never)	A S N	A S N
54) Do heifer calves below 6 months of age eat feed grown where manure was spread during the growing season? (Always Sometimes Never)	A S N	A S N
If answer to #54 was Never, go to #57		
55) Circle the type of feed grown for heifers where manure was spread:	pasture hay fresh chop	pasture hay fresh chop
56) How many days do you wait after applying manure before heifers are allowed to graze the pasture or eat the fresh chopped forage?	_____ days	_____ days
57) Do adult cows eat feed where manure was spread during the growing season? (Always Sometimes Never)	A S N	A S N
If answer to #57 was Never, go to #60		
58) Circle the type of feed grown for cows where manure was spread:	pasture hay fresh chop	pasture hay fresh chop
59) How many days do you wait after applying manure before adult cows are allowed to graze the pasture or eat the fresh chopped forage?	_____ days	_____ days
	NOW	**3 YEARS AGO**
60) Before bringing cattle (either dairy or beef) onto the farm, does this operation require tests for Johne's Disease?	A S N	A S N
61) Does this operation vaccinate ANY of the dairy cattle against Johne's Disease?	Y N	Y N

Appendix C (cont'd)

	Within the past 3 years		More than 3 years ago	
62) Have ANY cattle from this operation left for fairs or shows and returned to the premises?	Y	N	Y	N
63) Have ANY cattle on this farm exhibited clinical signs of Johne's Disease?	Y	N	Y	N
64) Have ANY cattle on this farm tested positive for Johne's Disease?	Y	N	Y	N

REMARKS:

THANK YOU POR PARTICIPATING!

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