SEROPREVALENCE, PRODUCTION IMPACTS, ECONOMICS AND RISK FACTORS OF *Mycobacterium avium* subspecies *paratuberculosis* IN CANADIAN DAIRY CATTLE

A Thesis

Submitted to the Graduate Faculty

in Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

in the Department of Health Management

Atlantic Veterinary College

University of Prince Edward Island

Dr. Ashwani Tiwari

Charlottetown, P. E. I.

September, 2005

Your file Votre référence
ISBN: 978-0-494-22842-5
Our file Notre référence
ISBN: 978-0-494-22842-5

CONDITION OF USE

The author has agreed that the Library, University of Prince Edward Island, may make this thesis freely available for inspection. Moreover, the author has agreed that permission for extensive copying of this thesis for scholarly purposes may be granted by the professor or professors who supervised the thesis work recorded herein or, in their absence, by the Chairman of the Department or the Dean of the Faculty in which the thesis work was done. It is understood that due recognition will be given to the author of this thesis and to the University of Prince Edward Island in any use of the material in this thesis. Copying or publication or any other use of the thesis for financial gain without approval by the University of Prince Edward Island and the author's written permission is prohibited.

Requests for permission to copy or to make any other use of material in this thesis in whole or in part should be addressed to:

Chairman of the Department of Health Management

Atlantic Veterinary College

University of Prince Edward Island

Charlottetown, P. E. I.

Canada C1A 4P3

SIGNATURE

PAGE(S)

REMOVED

SIGNATURE

PAGE(S)

iv

REMOVED

Abstract

The purpose of this thesis was to determine the seroprevalence, production impacts, economics and risk factors associated with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in Canadian dairy herds. Seroprevalence of bovine leukemia virus (BLV) and bovine viral diarrhoea virus (BVDV), and production impacts of BLV, BVDV and *Neospora caninum* (NC) were also estimated. Within 373 randomly selected Canadian herds (from New Brunswick, Nova Scotia, Prince Edward Island, Quebec, Ontario, Manitoba, Saskatchewan and Alberta), a serum sample was obtained from approximately 30 randomly selected lactating animals for ELISA testing for exposure to BLV, MAP and NC, while 5 unvaccinated cattle over 6 months of age were selected for virus neutralization testing for exposure to BVDV.

Seroprevalence at the cow level was 30.3% and 3.1% for BLV and MAP, respectively. At the herd level, 76.6% had at least one seropositive cow for BLV, 31.2% had at least one animal with a titer $\geq$1:64 for BVDV, and 18.9% had at least two seropositive cows for MAP. The Global Moran's I statistic for BLV and MAP indicated significant positive spatial autocorrelation of 0.16 and 0.15. The K-function for BVDV did not indicate any significant spatial autocorrelation. Using the spatial scan statistic, 4 and 1 significant clusters were detected for BLV and MAP-seroprevalence, respectively. No significant clusters were detected for BVD.

Regarding 305 day milk production (305 d milk), MAP-seropositivity was significantly associated with a lower 305 d milk of 212 kg in 4-plus lactation cows. NC-seropositivity was associated with a lower 305 d milk of 158 kg in primiparous cows compared to NC-seronegative primiparous cows. Cows in BVDV-seropositive herds (at least one unvaccinated animal with a titer $\geq$ 1:64) had significantly lower 305 d milk (by 368 kg) compared to cows in BVDV-seronegative herds. Among 1st lactation animals, NC-seropositivity was associated with a significant reduction of 5.5 kg and 3.3 kg of 305 d fat and 305 d protein yields, respectively. However, there were no statistically significant effects of MAP-seropositivity on 305 d fat or 305 d protein yields. BVDV-seropositivity (at the herd level) was associated with reductions in 305 d fat and 305 d protein yields of 10.20 kg/cow and 9.46 kg/cow, respectively. Regarding somatic cell counts, NC- (4+ lactation) and MAP-seropositivity were associated with a 0.094 decrease and 0.098 increase in the lactational mean of the $\log_{10}$ somatic cell count. The cows in BVDV-seropositive herds had a significant increase in the mean $\log_{10}$ somatic cell count of 0.096. BLV-seropositivity was not associated with 305 d milk, 305 d fat, 305 d protein or mean $\log_{10}$ somatic cell count. There was no significant effect modification of any of the outcomes examined from interaction between seropositivity for any of the pathogens.

For all reasons of culling, MAP-seropositive cows had a 1.38 (95% C.I., 1.05 – 1.81) times increased hazard of culling compared to MAP-seronegative cows. Seropositivity for the other pathogens was not associated with an increased risk of overall culling. Among cows that were culled because of either decreased reproductive efficiency or decreased milk production or mastitis, MAP-seropositive cows were associated with 1.55 (95% C.I., 1.12 – 2.15) times increased hazard compared to MAP-seronegative

cows. Among cows that were culled because of reproductive inefficiency, NC-seropositive cows had a 1.43 (95% C.I., 1.15 – 1.79) times greater hazard than NC-seronegative cows. Among cows that were culled because of decreased milk production, cows in BVDV-seropositive herds had a 1.86 (95% C.I., 1.28 – 2.70) times increased hazard compared to cows in BVDV-seronegative herds. BLV-seropositive cows did not have an increased risk of reason-specific culling as compared to BLV-seronegative cows. No significant interaction on culling among seropositivity for the pathogens was detected, but only a limited number of cows tested seropositive for multiple pathogens. Results from our research will help in better understanding the economic impacts of these pathogens and justification for their control.

For the MAP-seropositive Canadian dairy herds, the mean loss per 61-cow herd was CD $2,992 (95% C.I., $143 – $9741) annually, or CD $49 per cow per year. Herd additional culling losses were responsible for 46% (CD $1374) of the total losses from MAP. Decreased milk production, mortality and reproductive losses accounted for 9% (CD $254), 16% (CD $488) and 29% (CD $875) of the losses, respectively.

The following factors were significantly associated with the number of MAP-seropositive cows: "more than one cow in the maternity pen", "group housing in pre-weaned calves in winter", "purchasing open heifers", "direct contact with beef cattle", "BVDV-seropositive herds" and "not having proper BVD vaccination in calves (i.e. animals were not boostered 2-4 weeks after their first killed inoculation)" with count ratios of 1.69, 2.03, 2.34, 1.27, 1.41 and 1.77, respectively. The variable "herds having BVDV modified live vaccination in calves" was associated with 0.44 times fewer MAP-seropositive cows. There were significant regional differences in the number of positive cows per herd that were not explained by the management factors included in the final multivariable model. In the logistic portion of the final Zero Inflated Negative Binomial model, a one unit increase in the mean lactation number of cows tested in a herd was associated with an increase of 1.44 times the odds of being herds having at least one MAP-seropositive cow. Herds having "feeding total mixed ration" and "bedding that was not added frequently to calving areas (for each calving)" were associated with 3.1 and 2.7 times greater odds of being MAP-seropositive herds, respectively. The variables "NC-seropositivity" and "area of pasture (more than 200 acres)" were marginally significant ($P < 0.010$), while "BLV-seropositivity" was not significantly associated ($P > 0.10$) with MAP-seropositivity.

The results from this study will improve the knowledge of seroprevalence, production impacts, economical losses and risk factors of MAP in Canadian dairy industry and will assist management recommendations in national Johne's control programs.

ACKNOWLEDGEMENTS

Just as no man is an island, it would have been impossible for me to complete my PhD without the help of such a supportive network. My appreciation must be extended foremost to my co-supervisors, Dr. John VanLeeuwen and Dr. Ian Dohoo. John provided the attentive and patient guidance necessary to navigate the turbulent waters of daily project issues and communication with the provincial representatives. Ian, through sharing his wealth of epidemiological knowledge, gave me the foresight to conduct a high level of research. Together, both were inspirational through their dedication to student based teaching, continual learning of new epidemiological methods and most of all, for their faith in my capabilities.

Extending from these two masts are the sails of the ship, the other members of my supervisory committee: Drs. Greg Keefe, Rob Tremblay, and Fred Markham. All provided invaluable comments stemming from their particular areas of expertise. In addition, Dr. Henryk Stryhn's extensive knowledge helped sound the statistical depths of this study.

This journey should not have been complete without the comradeship of other graduate students and the dedicated hard work of the technicians. I would like to express my thanks to my fellow graduate students: Shawn Mckenna, Carol McClure, Javier Sanchez, Pipat Arunvipas, Aboubakar Mounchili, Joao Paulo Haddad, Fortune Sithole, Carolyn Sanford, Neil LeBlanc, Jillian Westcott, Wendela Wapenaar,Vicky Benson,

Andre Drapeau, Fabienne Uehlinger, Elmabrok Masaound, Pascale Nerette and Richard Olde Riekerink, whose continuing, successful journeys have been very encouraging. I also gratefully acknowledge those whose efforts helped chart the waters: Ricky Milton, Theresa Andrews, and Lloyd Dalziel.

Sincere thanks must also be given to the following agencies for having the vision to fund this study: Dairy Farmers of Canada, Canadian Food Inspection Agency, and Production Limiting Diseases Committee, Atlantic Veterinary College, Dairy Farmers of PEI, PEI Agricultural Research Investment Fund, Dairy Farmers of Ontario, Manitoba Agriculture, Dairy Farmers of Manitoba, Saskatchewan Agriculture, Western Economic Partnership Agreement, and the Food Safety Division of Alberta Agriculture, Food and Rural Development. Without their financial support this study would have been dead in the water!

My endless gratitude goes to my family, Sunita, Abhay, Brahm Tiwari, Sudesh, Mool Raj Dogra and to Donya, for their boundless patience and sustained concern for ship maintenance. I would also like to thank my grandmother, Durga Devi, whose dearest wish it was that I should continue in the footsteps of her son, Dr. Sneh Dogra, and complete a PhD in Canada.

Finally, I dedicate this thesis to all those whose efforts and lives have affected me and helped, in their own way, to steer this course.

Dedicated To:

To my family and professors

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

BLV – Bovine leukemia virus

BVDV - Bovine viral diarrhoea virus

MAP – *Mycobacterium avium* subspecies *paratuberculosis*

NC - *Neospora caninum*

LAM – Lipoarabinomanan

ELISA – Enzyme Linked ImmunoSorbent Assay

WTO – World Trade Organisation

NAHMS – National Animal Health Monitoring System

LISA – Local indicators of spatial autocorrelation

PLDC – Production limiting disease committee

ZINB – Zero inflated negative binomial model

PEI – Prince Edward Island

NS - Nova Scotia

NB - New Brunswick

QUE - Quebec

ONT - Ontario

MAN - Manitoba

SASK - Saskatchewan

ALTA – Alberta

Se – Sensitivity

Sp - Specificity

LIST OF TABLES

LIST OF FIGURES

LIST OF MAPS

CHAPTER 1: Introduction

1.1 Background to the initiation of this research project

In 2003, the dairy industry in Canada generated CD $4.5 billion in total farm cash receipts. Sales from dairy processors was CD $11 billion (http://www.cdc-ccl.gc.ca/cdc/main_e.asp?catid=87). Infectious diseases negatively affect the net return to farmers through lower production levels (e.g. milk production), low reproductive efficiency, higher additional culling, loss of market opportunities (eg. international and/or local sales), and reduced demand for its products when there is relationship (real or perceived) with human diseases (eg. Crohn's disease). In 1997, all of these concerns led to the formation of the Production Limiting Disease Committee (PLDC) in Canada. The main objectives of this committee were to determine the prevalence, impacts, risk factors and economic losses associated with infectious diseases in Canadian cattle, beginning with four diseases: bovine leukosis, bovine viral diarrhea (BVD), Johne' disease (JD), and neosporosis.

These four diseases were selected because they were thought to cost the industry substantially, and were insidious in nature, in that they could exist and spread within farms through the presence of persistently infected animals that could only be detected through laboratory tests. Furthermore, there has been very little research on how the agents that cause these diseases might interact if co-infecting the same farms and the same animals. This thesis will concentrate on JD, although the effects of co-infections will also be addressed.

1.2. Introduction to Johne's disease

1.2.1 The Organism

Paratuberculosis, or Johne's disease (JD), is a chronic infectious enteritis found in domestic and wild ruminants. It is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), a slow growing, Gram-positive and acid-fast bacterium (Barrow and Feltham (ed), 1993; Krieg, 1986). Johne and Frothingham (1895) first described this fecal-orally transmitted disease as an atypical case of bovine tuberculosis in 1895, although the causative agent was not isolated until 1912 by Twort and Ingram (1912). MAP belongs to the *M. avium* complex in the genus *Mycobacterium*, which is the only genus in the family *Mycobacteriaceae*. The original name given to the causative bacteria was *Mycobacterium enteritidis chronicae pseudotuberculosis bovis* Johne. The name was then shortened to *Mycobacterium paratuberculosis,* but further studies found that it was closely related to *Mycobacterium avium*. The two bacteria have a DNA homology of more than 99% (van der Giessen et al., 1992), and therefore are currently regarded as one species and given the names *Mycobacterium avium* subspecies *paratuberculosis* and *Mycobacterium avium* subspecies *avium*. MAP can be differentiated phenotypically from *M. avium* subspecies *avium* by its dependence on mycobactin (Thorel et al., 1990) and genotypically by the presence of multiple copies of an insertion element, IS900 (Collins and de Lisle, 1986; Green et al., 1989).

The natural hosts for MAP are wild and domesticated ruminants, including: dairy and beef cattle, sheep, goats, red deer, cervids, and camelids (Kennedy and Benedictus, 2001). MAP is considered more tolerant than most other pathogenic bacteria to temperature, pH variations and exposure to ultraviolet light (Larsen et al., 1956; Sung and Collins, 1998). The bacteria are obligate pathogens of animals and cannot multiply outside the animal host (Thorel et al., 1990).

1.2.2 Johne's disease and Crohn's disease

In the last few years, there has been increased concern about paratuberculosis in the dairy industry because of mounting evidence about the possible role of MAP in Crohn's disease in humans (Baksh et al., 2004; Romero et al., 2005). The scientific committee on Animal Health and Animal Welfare (from European Union member states) concluded that the currently available evidence is insufficient to confirm or to disprove that MAP is a causative agent of at least some cases of Crohn's disease in humans. However, the perception that these two diseases are linked may deter consumer confidence in milk products.

1.2.3 Prevalence

Consumer confidence is also a function of the likelihood of the bacteria being found in consumer products, which is related to the prevalence of infection in the dairy industry.

While the bacteria is found world-wide, there is a wide variation in the herd-level prevalence of MAP between countries, from 7% in Austria (Gasteiner et al., 1999) to 54% in the Netherlands (Muskens et al., 2000). These differences may represent real differences in the distribution of the organism, due to variations in the distribution of risk factors for transmission, or due to differences in testing protocols and the challenges of detecting infected cattle with the variety of tests available.

In Canada, seroprevalence at the animal level in dairy cattle ranged from 1.3% (Prince Edward Island) (Vanleeuwen et al., 2001) to 7.0% (Alberta) (Sorensen et al., 2003). At the herd level, 9.8% (Ontario) (Vanleeuwen et al., 2000) to 40.0% (Alberta) (Sorensen et al., 2003) of herds had at least 2 seropositive cows. These animal level estimates are substantially lower than the 16.1% of tissue culture-positive cows found in culled dairy cows in the Maritime provinces in 2001 (McKenna et al., 2004), or the 15.2% of culled dairy cows testing seropositive in Ontario in 1986 (McNab et al., 1988). The differences between these estimates may be partly due to different sampling strategies, location of animals, and tests utilized, and therefore it is not clear whether there are provincial differences in MAP prevalence. It is clear that there are a substantial number of cows and herds with MAP-infection in Canada.

1.2.4 Impact on Productivity

Another reason for the recently increasing concern over paratuberculosis in the dairy industry is the impact that infection has on productivity and culling. Clinical effects of

paratuberculosis are well documented (Benedictus et al., 1987). These include thickened intestinal mucosa resulting in malabsorptive diarrhea, and severe negative energy balance leading to emaciation and reduced milk production. Paratuberculosis has no known cure (Chiodini et al., 1984).

Losses associated with subclinical infection with MAP have been more difficult to quantify because of the difficulty of accurately detecting subclinically infected cattle with the currently available diagnostic tests (McKenna et al., 2005; Sockett et al., 1992). Dairy cattle subclinically infected with MAP (either seropositive or fecal culture positive for MAP) have been associated with premature culling (Benedictus et al., 1987; Goodle et al., 2000), decreased milk production (Abbas et al., 1983; Benedictus et al., 1987), increased mortality (Kreeger, 1991), and decreased reproductive efficiency (Abbas et al., 1983; Johnson-Ifearulundu et al., 2000). However, there is still a need to do further research to better quantify these impacts in order to determine better estimates of the economic costs of paratuberculosis to dairy farms with the infection, and to the industry as a whole. Accurate estimates will better demonstrate the need for vigilant control efforts.

1.2.5 Economic Impact

Based on an Atlantic Canadian MAP-seroprevalence survey and literature estimates of costs associated with MAP infection, average annual direct farm costs were $2472 per infected herd using an average herd size of 50 cows and an average within-herd MAP-

seroprevalence of 7% (Chi et al., 2002). While the costs due to clinical paratuberculosis (US$ 401 to 959 per infected cow per year) (Ott et al., 1999) are significant and can be observed by the farmer, the costs due to subclinical paratuberculosis (US$123 to 696 per infected cows per year) (Ott et al., 1999) can be more devastating due to the effects occurring on a larger proportion of cattle.

1.2.6 Risk factors

Most often, MAP-infections arise through fecal-oral transmission, due to the ingestion of feed or water contaminated with infected fecal matter. Level of exposure (dose of organisms) and age at the time of exposure are major factors in determining whether an animal eventually becomes infected and how severe that infection becomes (Chiodini, 1996; Hagan, 1938). There have been a number of studies investigating important risk factors for the transmission of MAP between and within farms (Collins et al., 1994; Jakobsen et al., 2000; Obasanjo et al., 1997; Wells and Wagner, 2000). Putative risk factors include: breed, herd size, region, purchasing cattle, nutrition, calving and calf management. However, it is unclear how relevant these risk factors are to the Canadian dairy industry.

1.3. Thesis objectives

The specific objectives of this thesis were:

a) to determine the seroprevalence and spatial distribution of MAP at the animal, herd, regional and national level in Canada;

b) to determine associations between MAP-seroprevalence and risk of culling, 305 day milk, fat and protein production, and somatic cell count in Canadian dairy cattle (associations with reproductive parameters were examined by a co-researcher);

c) to determine the economic losses associated with MAP-seroprevalence in Canadian dairy cattle, based on national estimates of MAP-seroprevalence and impacts associated with MAP-seroprevalence (objectives a and b); and

d) to determine the management factors associated with MAP-seroprevalence in Canadian dairy herds.

The results from this thesis will help in refining the Canadian national Johne's control program and help Canadian farmers in making decisions related to paratuberculosis.

1.4. Outline of the thesis

In order to address the listed objectives, the thesis has been divided into the following chapters. Chapter 2 critically examines the literature regarding the stages of infection, the advantages and limitations of commonly used diagnostic tests, and the regional distribution of MAP prevalence in dairy cattle, focusing on literature relevant to Canadian dairy practitioners and producers. This paper is the first of a two-part literature review of Johnes Disease (JD) in dairy cattle in Canada. The second paper examines the impacts, economics, risk factors and control of MAP. The two papers were a collaborative effort, and therefore Dr. Shawn McKenna was listed as the first author of this second paper (included in the appendices).

Chapter 3 presents the seroprevalence and spatial distribution of MAP in 373 herds from 8 of 10 provinces in Canada. A choropleth map was created to demonstrate the proportion of herds within each region of each province having at least two MAP-seropositive cows. In order to identify whether there was significant spatial clustering of seroprevalence estimates, the Moran's I statistic was calculated. Local indicators of spatial autocorrelation were determined to identify specific locations of spatial clustering.

In Chapter 4, for the sampled herds on monthly milk recording (the majority of tested farms), associations between MAP-seropositivity and 305 day milk, 305 day protein, 305 day fat and somatic cell count were determined. These analyses adjusted for herd clustering and controlled for the effects of seropositivity for three other pathogens (BLV,

BVDV and NC) because most participating farms were tested for exposure to all 4 pathogens.

In Chapter 5, a subset of the sampled dairy cattle was followed from their test dates to their culling dates in four provinces where laboratory testing was conducted early in the project. Using these data, associations between MAP-seropositivity and risk of culling were determined, while controlling for the possible effects of seropositivity for the other three pathogens and adjusting for herd clustering.

In Chapter 6, the economic losses associated with subclinical MAP-infection were estimated, incorporating estimated productivity, mortality, reproductive and culling impacts from earlier chapters of this thesis, along with estimates from the scientific literature. The losses were calculated by utilizing a stochastic partial budget model.

Chapter 7 determines the management factors associated with the number of MAP-seropositive cows in the study herds where a management questionnaire was completed. Those factors associated with a herd having at least two MAP-seropositive cows were also determined. The second analysis was intended to determine risk factors associated with a farm having JD, regardless of the prevalence, utilizing a conservative definition of an infected farm to minimize misclassification of infected and non-infected farms.

Chapter 8, a general discussion chapter, summarizes the main findings of the thesis in the context of the scientific literature, discusses how the results can be applied to the Canadian dairy industry, and identifies potential future areas of research.

1.5 Reference List

Abbas, B., Riemann, H.P., Hird, D.W., 1983a. Diagnosis of Johne's disease (paratuberculosis) in Northern California cattle and a note of it's economic significance. Calif. Vet. 37, 20-24.

Baksh, F.K., Finkelstein, S.D., Ariyanayagam-Baksh, S.M., Swalsky, P.A., Klein, E.C., Dunn, J.C., 2004. Absence of *Mycobacterium avium* subsp. *paratuberculosis* in the microdissected granulomas of Crohn's disease. Mod. Pathol. 17, 1289-1294.

Barrow and Feltham (ed), 1993. Cowan and Steel's manual for the identification of medical bacteria. In: Cambridge University Press., pp. 92-93.

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

Bergey's manual of systemic bacteriology. Vol 2.1986. Williams and Wilkins. Baltimore.

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

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

Chiodini, R.J., Van Kruiningen, H.J., Merkal, R.S., 1984. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. Cornell Vet 74, 218-262.

Collins, D.M., de Lisle, G.W., 1986. Restriction endonuclease analysis of various strains of *Mycobacterium paratuberculosis* isolated from cattle. Am. J Vet Res. 47, 2226-2229.

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

Gasteiner, J., Wenzl, H., Fuchs, K., Jark, U., Baumgartner, W., 1999. Serological cross-sectional study of paratuberculosis in cattle in Austria. Zentralbl. Veterinarmed. B 46, 457-466.

Goodle, G.M., Hirst, H., Garry, F., Dinsmore, P., 2000. Comparison of cull rates and milk production of cilinially normal dairy cows grouped by ELISA *Mycobacterium avium parauberculosis* serum antibody results. Proc. Intern Symp Vet Epidemiol Econ, Breckenridge, CO, USA.

Green, E.P., Tizard, M.L., Moss, M.T., et al, 1989. Sequence and characteristics of IS900, an insertion element identified in a human Crohn's disease isolate of Mycobacterium paratuberculosis. Nucleic Acids Res. 17, 9063-9073.

Hagan, W.A., 1938. Age as a factor in susceptibility to Johne's disease. Cornell Vet. 28, 34-40.

Jakobsen, M.B., Alban, L., Nielsen, S.S., 2000. A cross-sectional study of paratuberculosis in 1155 Danish dairy cows. Prev. Vet Med. 46, 15-27.

Johne, H.A., Frothingham, L., 1895. Ein eigenthumlicher Fall von Tuberculose beim Rind. Deutsch Zeitschr Teirmed Vergl Path. 21, 438-454.

Johnson-Ifearulundu, Y.J., Kaneene, J.B., Sprecher, D.J., Gardiner, J.C., Lloyd, J.W., 2000. The effect of subclinical *Mycobacterium paratuberculosis* infection on days open in Michigan, USA, dairy cows. Prev. Vet Med. 46, 171-181.

Kennedy, D.J., Benedictus, G., 2001. Control of *Mycobacterium avium* subsp. *paratuberculosis* infection in agricultural species. Rev. Sci. Tech. 20, 151-179.

Kreeger, J.M., 1991. Ruminant paratuberculosis--a century of progress and frustration. J Vet Diagn. Invest 3, 373-382.

Larsen, A.B., Merkal, R.S., Vardaman, T.H., 1956. Survival time of *Mycobacterium paratuberculosis*. Am. J Vet Res. 17, 549-551.

McKenna, S.L., Keefe, G.P., Barkema, H.W., McClure, J., Vanleeuwen, J.A., Hanna, P., Sockett, D.C., 2004. Cow-level prevalence of paratuberculosis in culled dairy cows in Atlantic Canada and Maine. J. Dairy. Sci. 87, 3770-3777.

McKenna, S.L.B., Keefe, G.P., Barkema, H.W., Sockett, D.C., 2005. Evaluation of three ELISAs for *Mycobacterium avium* subsp. *paratuberculosis* using tissue and fecal culture as comparison standards. Accepted in Vet. Microbiology.

McNab, W.B., Meek, A.H., Martin, S.W., 1988. An epidemiological study of paratuberculosis in Ontario dairy cattle: study design and lipoarabinomannan antigen ELISA test results. Acta Vet Scand. Suppl 84, 206-208.

Muskens, J., Barkema, H.W., Russchen, E., Van Maanen, K., Schukken, Y.H., Bakker, D., 2000. Prevalence and regional distribution of paratuberculosis in dairy herds in The Netherlands. Vet Microbiol. 77, 253-261.

Obasanjo, I.O., Grohn, Y.T., Mohammed, H.O., 1997. Farm factors associated with the presence of *Mycobacterium paratuberculosis* infection in dairy herds on the New York State Paratuberculosis Control Program. Prev. Vet Med. 32, 243-251.

Ott, S.L., Wells, S.J., Wagner, B.A., 1999. Herd-level economic losses associated with Johne's disease on US dairy operations. Prev. Vet. Med. 40, 179-192.

Romero, C., Hamdi, A., Valentine, J.F., Naser, S.A., 2005. Evaluation of Surgical Tissue From Patients with Crohn's Disease for the Presence of *Mycobacterium avium* Subspecies *paratuberculosis* DNA by In Situ Hybridization and Nested Polymerase Chain Reaction. Inflamm. Bowel. Dis. 11, 116-125.

Sockett, D.C., Conrad, T.A., Thomas, C.B., Collins, M.T., 1992. Evaluation of four serological tests for bovine paratuberculosis. J Clin. Microbiol. 30, 1134-1139.

Sorensen, O., Rawluk, S., Wu, J., Manninen, K., Ollis, G., 2003. *Mycobacterium paratuberculosis* in dairy herds in Alberta. Can Vet J 44, 221-226.

Sung, N., Collins, M.T., 1998. Thermal tolerance of *Mycobacterium paratuberculosis*. Appl. Environ. Microbiol. 64, 999-1005.

Thorel, M.F., Krichevsky, M., Levy-Frebault, V.V., 1990a. Numerical taxonomy of mycobactin-dependent mycobacteria, emended description of *Mycobacterium avium*, and description of *Mycobacterium avium* subsp. *avium* subsp. *nov.*, *Mycobacterium avium* subsp. *paratuberculosis* subsp. *nov.*, and *Mycobacterium avium* subsp. *silvaticum* subsp. *nov.* Int. J Syst. Bacteriol. 40, 254-260.

Twort, F.C., Ingram, G.L.Y., 1912. A method of isolating and cultivating the *Mycobacterium enteritides chronicae pseudotuberculosae bovis* Johne and some experiments on the preparation of a diagnostic vaccine for pseudotuberculous enteritis of bovines. Vet J 68, 353-365.

van der Giessen, J.W., Eger, A., Haagsma, J., Haring, R.M., Gaastra, W., van der Zeijst, B.A., 1992. Amplification of 16S rRNA sequences to detect *Mycobacterium paratuberculosis*. J Med. Microbiol. 36, 255-263.

Vanleeuwen, J.A., Tremblay, R., Keefe, G.P., Wichtel, J., 2000. Seroprevalence of Production Limiting Diseases in Ontario and Maritime Dairy Cattle. Proceedings of the Canadian Veterinary Medical Association Annual Convention, St. John, Canada.

Vanleeuwen, J.A., Keefe, G.P., Tremblay, R., Power, C., Wichtel, J.J., 2001. Seroprevalence of infection with Mycobacterium avium subspecies paratuberculosis, bovine leukemia virus, and bovine viral diarrhea virus in maritime Canada dairy cattle. Can Vet J 42, 193-198.

1 Wells, S.J., Wagner, B.A., 2000. Herd-level risk factors for infection with
2 *Mycobacterium paratuberculosis* in US dairies and association between
3 familiarity of the herd manager with the disease or prior diagnosis of the disease
4 in that herd and use of preventive measures. J Am. Vet Med. Assoc. 216, 1450-
5 1457.
6

CHAPTER 2: Johne's disease in Canada Part I: Clinical symptoms, pathophysiology, diagnosis and prevalence in dairy herds

(Review Article)

Ashwani Tiwari, John A. VanLeeuwen, Shawn L.B. McKenna,

Greg P. Keefe, Herman W. Barkema

Department of Health Management

Atlantic Veterinary College, Prince Edward Island, 550 University Avenue,

Charlottetown, Prince Edward Island, C1A 4P3 Canada

2.1 Abstract

Recent international developments in the area of infectious disease control and nontariff trade barriers, along with possible zoonotic concerns, have provoked a revival of interest in Johne's disease in Canada and elsewhere. The bacterium causing Johne's disease, *Mycobacterium avium* subspecies *paratuberculosis*, is distributed worldwide and causes chronic granulomatous enteritis, also known as paratuberculosis, in domestic and exotic ruminants, including cattle. The subclinical form of this disease results in progressive weight loss, reduced milk production, lower slaughter value, and premature culling, with possible impacts on fertility and udder health. Eventually, infection can lead to the clinical form that manifests as chronic diarrhea, emaciation, debilitation, and eventual death. Currently, available tests to detect infected animals produce many false-negative results and some false-positives, particularly in subclinically infected animals, thus making their interpretation and utilization challenging in control programs.

The objective of this two-part review is to critically review the literature about Johne's disease in dairy cattle for bovine practitioners in Canada. Part I covers the clinical stages, pathophysiology, diagnosis, and prevalence of infection in Canada, while Part II discusses impacts, risk factors, and control programs relevant to Canadian dairy farms. By reviewing the scientific literature about Johne's disease, control of the disease could be pursued through informed implementation of rational biosecurity efforts and the strategic use of testing and culling.

2.2 Introduction

Paratuberculosis, or Johne's disease (JD), is a chronic infectious enteritis of domestic and wild ruminants. It is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), a hardy, slow growing, gram-positive, and acid-fast bacterium (1,2). Despite having 99% DNA homology (3), MAP can be differentiated phenotypically from *M. avium* subspecies *avium* and *M. avium* subspecies *sylvaticum* by its dependence on mycobactin (4), and genotypically by the presence of multiple copies of an insertion element, IS900 (5,6).

Restriction endonuclease analysis has identified variations in 2 principal types of MAP, a cattle type (C) and a sheep type (S), that were first identified by Collins et al. (7). Other variations have also been identified, although their importance is unclear (8-10). Paratuberculosis in cattle, goats, deer, and camelids is caused mainly by type C, whereas sheep are usually infected by type S. However, the cattle type can infect sheep and vice versa (11).

The natural hosts for MAP are wild and domesticated ruminants, including dairy and beef cattle, sheep, goats, red deer, cervids, and camelids (12). However, other nonruminant wildlife, such as the fox, weasel, crow, rat, wood mouse, rabbit, hare and badger, have also been found to harbor MAP (13). Calves inoculated with MAP from a free living rabbit developed typical histological lesions consistent with Johne's disease, demonstrating that wild animals other than ruminants may also contribute to the spread of the disease (14). However, calves are more likely to be exposed to manure from other mature cattle than from wildlife; therefore, the major sources of infection on most farms are likely to be infected domesticated ruminants that shed the bacterium in their feces.

1 The route of infection is usually through ingestion, be it contaminated water, milk, or
2 feed. The purpose of this first part of a two-part series of review articles is to critically
3 review the literature on clinical stages of JD, pathophysiology, diagnostic and screening
4 tests, and prevalence estimates of infection in Canada to enable bovine practitioners in
5 Canada to successfully implement control strategies.

2.3 Methods

Due to substantial differences in management and production between dairy and beef cattle, and the extensive literature on JD for both dairy and beef cattle, this paper focuses on dairy cattle. Also, because the intended audience for this paper is primarily veterinarians in Canada, we have emphasized Canadian references as much as possible.

Medline (accessed via PubMed from 1950 to present), The Commonwealth Animal Bureaux (CAB) (accessed via VetCD and ParasiteCD from 1973 to present), and Agricola, produced by the National Agricultural Library of the U.S. Department of Agriculture (accessed via the National Agricultural Library from 1970 to present) were used to collect the majority of the references that were used in this paper. The keywords used in the search of the databases were Johne's disease or paratuberculosis, Canada or Canadian, dairy and cattle. In addition, a number of papers were included from the reference lists of other papers, or personal knowledge of reports or conference proceedings, where the literature search did not identify papers with salient information for this review.

All relevant material collected from the above process was included in the review, provided that it was pertinent to the methods of production within the Canadian dairy industry. Exclusion of material was only done if information was redundant or outdated and had been directly refuted. Otherwise, all available information was included.

2.4 Pathophysiology

Ingested MAP bacteria enter the intestinal wall through the small intestinal mucosa, primarily in the region of the ileum, via M cells (specialized absorptive mucosal cells) residing in the Peyer's patches (15). Where they are resistant to intracellular degradation. They are eventually phagocytosed by sub-epithelial macrophages (16). While the bacteria are in the mucosal tissue and submucosal macrophages, there is little or no detectable reaction to the infection. This delayed detectable humoral immune response is one reason for the poor Se of serological diagnostic tests for MAP, as explained in detail later.

Eventually, the infected macrophages migrate into local lymphatics (17,18), spreading the infection to regional lymph nodes. In the regional lymph nodes, the organisms are capable of stimulating inflammatory and immunological responses (19). The immune response towards MAP resembles that of other mycobacterial infections. Most animals mount a cellular immune response involving a variety of cells, most importantly T lymphocytes (20). Cytokines produced by T helper cells also contribute to the protective response against mycobacterial infections, especially the cytokine gamma interferon (IFN-γ). Production of IFN- γ has been recognized as a key step in resistance against mycobacterial diseases in general, and it may provide a means to help monitor early infection in some animals (21). In some cows, the cellular immune response has been shown to be able to control the infection, with the cows never developing clinical signs, but remaining subclinically infected for life (22). In those animals in which the cellular immunity is unable to control the disease, a detectable humoral immune response will develop, along with increased shedding of bacteria (22).

Typically, the organism proliferates slowly in the ileal mucosa and regional lymph nodes. However, poor nutrition, stress related to transport, lactation, parturition, and immunosuppression by agents like bovine viral diarrhea virus have been proposed as accelerating or precipitating the onset of the clinical phase of infection (23).

The physiological mechanism for development of diarrhea in clinically affected animals is thought to be related to antigen-antibody reactions in infected tissue, with subsequent release of histamine (24-26). However, the effect on villi of the mucosal epithelium of the ileum is the major component of the pathogenesis resulting in a malabsorption syndrome. Macroscopic lesions, if present, are seen primarily in the intestine and it's draining mesenteric lymph nodes, more specifically in the region of the ileum, although they can occur throughout the whole length of the intestinal tract. The intestinal wall is thickened and edematous, and the mucosa has exaggerated transverse folds, mimicking the appearance of corrugated cardboard. The serosal and mesenteric lymphatic vessels can be dilated and thickened. Subsequent muscle atrophy, emaciation, alopecia, renal infarcts, anemia, and leukopenia are thought to be mediated by cytokines (23,27). There is no evidence to suggest that the pathophysiology or progression of disease differ between infected cattle in Canada and elsewhere.

2.5 Clinical effects and stages of paratuberculosis

Infection with MAP can be divided into 4 stages as described by Whitlock & Buergelt (28), depending on the severity of clinical signs, potential for shedding organisms into the environment, and the ease with which the disease may be detected by using current laboratory methods.

2.5.1 Silent infection

This stage generally includes young stock up to 2 y of age; it is called "silent" because 1) there are no clinical signs of infection 2) there are no measurable subclinical effects of infection, and 3) there are no cost-effective diagnostic tests that can detect infection. The only means of detecting infected cattle at this early stage is by demonstration of the established bacteria in the intestinal tract, either by culture or by histologic demonstration of microgranulomas in the intestine or regional lymph nodes, a cost-prohibitive procedure if multiple animals require testing. Other diagnostic tests, such as johnin (sterile solution of growth products of Johne's bacillus) skin testing and gamma-interferon tests, that utilize the cell mediated response (CMI), have also been used to detect this stage of the disease. However, there are common antigens between MAP and other environmental *Mycobacteria* spp., resulting in low Sp for these tests (29,30), making them ineffective as a routine screening test. Infected animals in this stage may shed infectious organisms into the farm environment at levels below the threshold of detection (31).

2.5.2 Sub-clinical infection

Animals with subclinical MAP infection do not yet have clinical signs of infection, but may be detected as infected by using cost-effective diagnostic tests and may begin to have measurable effects of infection (as discussed in part 2) (32,33). Some of these infected cattle may be detected by fecal culture and subsequently removed from the herd. However, focal lesions, variable rates of disease progression and shedding, and dilution of organisms in large volumes of intestinal content result in intermittent detection of fecal shedding (34). Therefore, these infected animals test negative by using current fecal culture techniques, yet they may be shedding low numbers of organisms in the manure, which contaminate the environment and pose a threat to other animals on the farm. Some animals may have detectable antibodies to MAP, an altered cellular immune response, or both, particularly if they are getting close to entering the next stage of the disease (clinical phase) (12). However, the MAP fecal shedding usually occurs before a detectable antibody response (35).

2.5.3 Clinical infection

Initial clinical signs follow a prolonged incubation period of 2 to 10 y, depending on the exposure level and the capacity of an animal to fight the infection (36,37). The first apparent sign is gradual weight loss, despite a normal or, occasionally, an increased appetite. During a period of 3-6 mo, concurrent with the weight loss, the manure consistency becomes more fluid. The diarrhea may be persistent, or intermittent, at first,

with periods of normal manure consistency. Thirst is usually increased and milk production is decreased. However, appetite and vital signs (heart rate, respiratory rate, and temperature) remain normal (28).

Most animals at this stage have a positive fecal culture and have increased serum antibody levels detectable by the commercial enzyme linked immunosorbant assay (ELISA) and agar gel immunodiffusion (AGID) test. It is estimated that only 10 to 15% of infected animals survive to this stage of infection, because they are often culled due to reduced productivity earlier in the subclinical stage (38). One study showed that out of 113 herds that did not have a clinical case of JD in the previous 5 y, only 32 herds were completely test-negative during 9 pooled fecal-culture tests done every 6 mo (39).

2.5.4 Advanced clinical infection

Clinically affected animals, if not culled, become increasingly lethargic, weak, and emaciated. "Water-hose"or "pipestream" diarrhea, hypoproteinemia, and intermandibular edema (bottle jaw) characterize the advanced stage of the disease. In the last stage of JD, cows become cachectic, anemic and too weak to rise (40). Most animals are culled from the herd before this time due to the chronic or intermittent diarrhea, decreased milk production and or weight loss in the earlier stages of disease (28).

2.6 Diagnosis

The diagnostic tests to detect infection with MAP can be categorized into those that identify the organism, and those that identify an immunological reaction to the organism. Evaluation of the performance of diagnostic tests is typically done by comparing estimates of sensitivity (Se – ability of the test to detect infected cattle) and specificity (Sp – ability of the test to identify healthy cattle) based on a "gold standard" that has identified animals as truly infected and truly noninfected. However, comparisons of the Se and Sp of diagnostic tests for MAP should be interpreted with great caution, because there are a number of factors that have a major impact on these estimates, including 1) the type of gold standard used 2) the stage of infection of the study animals and 3) the type of farms utilized to source animals for testing. Each of these will be discussed in turn.

Due to delays of 2 to 10 y between time of infection and development of measurable immune system reactions and shedding, various "gold standards" have been utilized for MAP-infection status in the past. Tissue culture of MAP is considered the ideal gold standard test, because, even before fecal shedding or an immune response is present, it can detect growth of MAP in multiple organs, including the intestinal mucosa and submucosa, and regional lymph nodes (41). However, fecal culture has been used as the gold standard in many studies (42-48) in the past, due to the high cost and logistical difficulties of sampling for tissue culture. The methods for fecal and tissue culture are identical and are described below.

The delays in immune response or shedding also mean that tests performed on animals with clinical JD will have a better Se than when they are used on animals for

subclinical JD (43,49), because the clinically affected animals are much more likely to be shedding bacteria or have developed a detectable immune response (19). False-negative test results are common, particularly in calves, heifers, and even 1st lactation cows (cattle in silent and subclinical stages) (50). Test results from animals with subclinical paratuberculosis can be a challenge to interpret, because clinical signs are not present to assist in their interpretation (42,49,51).

Regarding farm type, on farms with a known history of clinical JD, there will likely be more bacteria in the environment and higher exposure to MAP of the youngstock than on farms without history of clinical JD. If youngstock undergo higher exposure to MAP bacteria on a particular farm, they are more likely to develop a detectable immunological reaction or begin shedding bacteria earlier in life (24,25,52), leading to higher detectable prevalence of infection on that farm and increased shedding of bacteria in those infected cattle on those farms compared with farms where there is lower exposure to MAP. Therefore, evaluation of test performance using cattle on these farms will lead to higher estimates of Se and Sp than on farms without history of clinical JD.

As a result, in Table 2.1, the test Se of the most widely used diagnostic tests for MAP infection are categorized with respect to testing for clinical versus subclinical infection and, for subclinical infections, testing in farms with high prevalence (≥25%) of MAP infection versus those with low prevalence (<25%).

2.6.1 Identification methods

2.6.1.1. Culture on tissue or feces (individual and pooled samples)

A number of different media have been used to culture MAP. The standard culture procedure utilized in Canada is Herrold's egg yolk medium (HEYM); however, culture time is 16 wk before observable growth is seen for this slow growing bacterium (28). A radiometric system has been developed that reduces the culture time by half, because detection of growth is not visual but through the detection of metabolized radioisotopes in the media (53). However, because the system requires expensive safety equipment to handle the radioisotopes, at the time of publication, only laboratories in British Columbia (Animal Health Monitoring Laboratory, Abbotsford) and Ontario (Animal Health Laboratory, Guelph) are offering this system on a commercial basis, with the Manitoba provincial laboratory (Veterinary Services Branch, Winnipeg) conducting research and development with the system. Recently, a specialized broth media system has been developed that has reduced the detection time to 6 wk, without loss in test Se, through the detection of alterations in oxygen, CO_2, or pressure within a sealed bottle (54). At the time of publication, the only Canadian laboratory currently offering broth culture testing is the Atlantic Veterinary College in Charlottetown, Prince Edward Island (PEI). The Agri-Food Laboratories Branch laboratory in Edmonton, Alberta, does MAP cultures, using the standard HEYM method, but only for research purposes and for some of the samples submitted under the Alberta Johne's Control Program.

If bacterial growth is detected, the bacterium is isolated and its identity is confirmed through the morphologic characteristics and mycobactin dependency of the bacterial colonies, acid fast staining, and, sometimes, through the detection of the insertion sequence IS900 by polymerase chain reaction (PCR) (see description below).

With identification of MAP, the animal is considered infected. However, laboratory error can occasionally lead to cross-contamination and false-positive test results (55). Also, the phenomenon of "pass-through" of bacteria through the gastrointestinal tract could lead to other false-positives, but this remains a hypothesis and has not been fully substantiated. Therefore, Sp of fecal culture is described as being virtually 100% (49) and fecal culture is considered an excellent confirmatory diagnostic test of paratuberculosis for animals that test positive to immunological tests (Table 1).

Due to the lengthy duration of testing and the specialized equipment and media required for culturing MAP, the cost per sample tested is high (CD$35 to $60/sample). Therefore, pooling fecal samples has been utilized to test large numbers of animals for less cost per animal (60), while still maintaining reasonable Se to detect infected animals (61). In comparing conventional culture to pooled culture on a herd level basis, 94% of pooled samples with moderate to high numbers of MAP yielded positive culture results (62). Pooled fecal culturing has been shown to have a herd level Se of 73% (60), meaning that 73% of infected herds were detected with a single set of strategically (by age cohorts) pooled samples. However, the maximum number of negative animals that can be mixed in with a sample from a positive animal (while still getting a positive pooled test) needs to be determined, and this number will need to be appropriate to infection prevalence, severities, and shedding levels seen in Canada. Based on initial results from outside Canada, 3 to 5 fecal samples in a pool may be the optimal number (60,62,63). Any Canadian laboratory that is equipped to conduct MAP cultures should also be able to conduct pooled fecal cultures.

2.6.2.2 Polymerase chain reaction on feces

Since the discovery of the IS900 insertion sequence, attempts have been made to perform PCR techniques directly on clinical samples (64,65). Through amplification of this piece of genetic material, the PCR is able to provide a much faster result compared with culture techniques, with a turnaround time of a 4 d for most laboratories (55). However, PCR is less sensitive than culture due to the presence of inhibitory substances in fecal specimens (66). Another concern is that IS900 may not be as specific for MAP as once believed. The IS900 element has been detected in other mycobacterial strains isolated from the feces of ruminants (67). At the time of publication, PCR testing is being offered commercially in Canada only at BIOVET Inc. in St. Hyacinthe, Québec, and at the Faculté de Médecine Vétérinaire in Montréal, Québec. It is being used as a confirmation of positive cultures where culturing is being done. If PCR were to be done on a large scale, laboratory error could lead to false-positive test results if very strict adherence to preventing even minute cross-contamination was not implemented.

2.6.2 Immunological methods

2.6.2.1. Enzyme linked immunosorbant assays on serum and milk

The main type of immunological test that is widely available and commonly used is the enzyme linked immunosorbant assay (ELISA), which detects an optical density in serum (68) or milk (69,70) that correlates with an antibody response to MAP. The ELISAs have been desirable tests to use because of their ease of sample collection (blood or milk), rapid test results (within a week), and relatively low cost (approximately $10 per sample).

However, for several reasons, results from ELISAs need to be interpreted with caution. Due to the long delay between infection and presentation of bacteria to the immune system in sufficient numbers to develop a detectable immune response, the reported Se of the serum ELISAs for detecting subclinically infected cattle are much lower than the Se of fecal cultures, leading to many false-negative results (42,45,46,69). In fact, many studies have estimated the Se of the serum ELISA on the basis of the proportion of fecal shedders that were seropositive, with these estimates ranging from 15% to 75% (42,43,49).

Milk ELISA testing has recently been introduced as another immunological test for detecting subclinically infected cattle, with the obvious practical advantage of ease of sample collection. However, independent, peer-reviewed evaluation of the operating characteristics of the ELISA of milk is still limited. A recent study in Ontario (58) reported only moderate agreement between serum and milk ELISAs, and the milk ELISA detected 12% fewer infected cows than the serum ELISA. These findings make biological sense considering that antibody concentrations in milk depend not only on levels in serum, but also on milk production (58), parity, and days in milk (71). The added variability in antibody levels in milk (72) makes interpretation of milk ELISA results even more challenging than that of serum ELISA results at the animal level, which have inherent laboratory variability (73). Further research may identify a role for the milk ELISA as a practical method of monitoring MAP infection at the herd level or instigating interest in controlling JD.

Another caution regarding the use of ELISAs in low prevalence herds is that false-positive test results can also be a problem when a large number of cattle are tested

with a test that has a Sp that is not very close to 100% (42). While some studies have reported Sp estimates of 99% (43,49,74), another study (42) likely provides a more realistic estimate of Sp (96.8%), because it utilized multiple sources of samples (more representative of the North American dairy cattle industry as a whole), providing more possibility of cross reactivity with other micro-organisms to give false-positive test results. Therefore if an ELISA was used on 100 uninfected cattle, it would likely produce 1 (Sp of 99%) to 3 (Sp = 97%) false-positive test results, which could erroneously categorize an uninfected herd as infected if confirmatory tests were not utilized on the ELISA-positive cattle. In a low prevalence herd of 100 cows with 5 truly infected animals, the low sensitivity of ELISAs would lead to only 1 of the 5 infected animals likely testing seropositive and 1 to 3 false-positive test results. Therefore, with only 1 of 2 to 4 test positives being truly infected, the predictive values of a positive test result would vary between 25% and 50%, making it difficult to know how to interpret and act on positive test results. Therefore, apparently healthy cows that are ELISA-positive should be fecal cultured to confirm infection status, particularly in herds suspected of having a low prevalence of infection. If the fecal culture is negative, these ELISA-positive cows should be retested in 6-12 mo because the owner does not know if these nonshedding ELISA-positive cows are truly uninfected or just not shedding in detectable numbers at the time of sampling.

One additional caution regarding the interpretation of ELISA results relates to the form in which they are reported. Interpretation of results has generally been made on a single cut-off value that allows for dichotomous test results, positive or negative. Although this would appear to make results easier to interpret and allow for Se and Sp

calculations, valuable information is lost, because the likelihood of true infection is much higher in cattle with a high optical density, particularly in herds that have a history of clinical JD infection (57). As a result, some laboratories are appropriately utilizing a 3-level result system - negative, suspect, and positive (75), or 4-level result system – negative, suspect, weak positive, and strong positive, based on categorizations of likelihood ratios generated from the optical densities (55).

At the time of publication, there are only 2 serum ELISAs currently offered on a commercial basis in Canada, with different provinces using different ELISAs. It is unlikely that there is a large and significant difference in the test performance of the 2 ELISAs; both tests have difficulty detecting, subclinically infected cattle.

2.6.2.2 Agar gel immunodiffusion

There is one other immunological test that is available in Canada, the agar gel immunodiffusion (AGID) test. It was developed as a quick test for animals that were showing clinical signs of JD. Some reports estimate that when AGID results are positive, there is a 95% chance of actual MAP infection in a clinically affected cow (28). However, the Se of the AGID for subclinical cows is poor, with one report of an Se of 18.9% (76). Therefore, use of the AGID is restricted to animals showing clinical signs of JD. The ELISAs are equally sensitive at detecting MAP in clinically affected cattle; and therefore, it is unlikely that the AGID offers any advantage over the ELISA.

2.7 Prevalence

The results of seroprevalence studies done recently in dairy cattle in Canada are shown in table 2.2. Seroprevalence at the animal level in dairy cattle ranged from 1.3% (PEI) (77) to 7.0% (Alberta) (81). At the herd level, 9.8% (Ontario) to 40.0% (Alberta) (81) of herds had at least 2 seropositive cows. The provincial differences in seroprevalence may represent real differences in the distribution of the organism due to variations in management or other risk factors for transmission. Conversely, these seroprevalence differences may be due to variations in sampling and testing protocols. Normally, estimated true prevalences of infection can be calculated to adjust for differences in testing protocols; however, for JD, there is little consensus on the adjustments test Se and Sp required to calculate true prevalence estimates. Therefore, even comparisons of estimated true prevalences should be interpreted with caution.

All of the above provincial studies were conducted by using the same number of animals per herd and the same IDEXX ELISA test (except for Alberta), with the same cut-off value for interpretation of test-positives. However, comparisons between these seroprevalence estimates should be interpreted with caution for a number of reasons. Sera from Manitoba were tested at a separate laboratory from the other provinces, possibly impacting on the survey results. Even the sera that were tested at the same laboratory were tested at different times with different lots of test kits, also possibly impacting on survey results. Furthermore, the Ontario study consisted of herds that were purposely chosen to monitor disease (mastitis) incidence, and likely an underestimate of the true prevalence of paratuberculosis in dairy herds in Ontario. A 1986-89 survey of 14,932 cows in 304 dairy herds in Ontario, using a lipoarabinomannan antigen enzyme-linked

immunosorbent assay (LAM-ELISA), found 15.2% of the animals tested seropositive (82). All of the differences between these 2 estimates are unlikely to be due to differences in test performance, because of the similarities in the two tests used (IDEXX-ELISA and LAM-ELISA). A control program for JD did not exist in Ontario during the time between the 2 studies, so that the prevalence of MAP infection is unlikely to have declined between 1989 and 1998.

While it is possible to compare seroprevalences between provinces with the above studies, they are likely underestimate the true prevalences of infection at the animal level due to the low sensitivities of ELISAs for MAP. Very few Canadian studies have been conducted to determine the prevalence of MAP infection in dairy cattle based on fecal culture. In the study in Alberta (81), fecal samples were collected from cows in 50 dairy herds and cultured in pools of 3 samples. Three point four percent of pools were found to be culture-positive, meaning that from 3.4% to 10.2% of cattle were test-positive (individual cattle results were not reported), leading to the conclusion that the estimated true herd-level prevalence ranged from 28% to 57%, depending on whether 1, 2, or all 3 individual fecal samples in the positive fecal pools were culture-positive.

While fecal culture testing has a better Se than ELISAs for MAP, it still produces many false-negatives, particularly in young infected cattle that have not yet started to shed MAP bacterium. A recent prevalence estimate based on tissue culture testing of ileocecal lymph nodes and ileum from culled dairy cows at a slaughterhouse in New Brunswick was that 16.1% of dairy cows were culture-positive for MAP (41). This prevalence estimate is likely to be a close approximation of the true infection prevalence, because culturing the ileum and ileocecal lymph node of the selected animals is better

than fecal culturing at detecting cows that are infected but not yet shedding bacteria in their feces.

Many other prevalence surveys have been carried out around the world, A brief summary of recent international seroprevalence estimates from representative samples is provided in Table 2.3. While comparisons between countries should be conducted with caution, there does appear to be a large variation in the reported seroprevalences between countries and even within countries. Seroprevalences at the animal level in Canada are similar to those in other countries, ranging from 0.8% in Belgium (89) to 17.1% in Florida, USA (83). At the herd level, the proportion of herds with 2 or more seropositive cows in Canada (77) was also similar to that in other studies, ranging from 17% for the 20 tested states in the USA (90) to 44% in Michigan, USA (85). Some Scandinavian countries have very low seroprevalence for MAP, leading those countries to seriously consider eradication efforts (91,92).

The significant advances in the quality of the diagnostic tests used to detect MAP make it difficult to determine if the prevalence of MAP infection is increasing. There are very few, if any, data from random samples of the same area over time, using similar diagnostic tests. Before the evolution of ELISAs in the late 80s, most of the initial reports of JD prevalence were limited to slaughterhouse data. Perhaps future studies will address this paucity of data and clarify whether MAP is becoming more prevalent or not.

2.8 References

1. Krieg NR ed. Bergey's Manual of Systemic Bacteriology. Vol 2. London: Williams and Wilkins, 1986.

2. Barrow and Feltham (ed). Cowan and Steel's Manual for the Identification of Medical bacteria. Cambridge University Press., 1993: 92-93.

3. van der Giessen JW, Eger A, Haagsma J, Haring RM, Gaastra W, van der Zeijst BA. Amplification of 16S rRNA sequences to detect *Mycobacterium paratuberculosis*. J Med Microbiol 1992;36:255-263.

4. Thorel MF, Krichevsky M, Levy-Frebault VV. Numerical taxonomy of mycobactin-dependent mycobacteria, emended description of *Mycobacterium avium*, and description of *Mycobacterium avium* subsp. *avium* subsp. *nov.*, *Mycobacterium avium* subsp. *paratuberculosis* subsp. *nov.*, and *Mycobacterium avium* subsp. *silvaticum* subsp. *nov.* Int J Syst Bacteriol 1990;40:254-260.

5. Collins DM, de Lisle GW. Restriction endonuclease analysis of various strains of *Mycobacterium paratuberculosis* isolated from cattle. Am J Vet Res 1986;47:2226-2229.

6. Green EP, Tizard ML, Moss MT, et al. Sequence and characteristics of IS900, an insertion element identified in a human Crohn's disease isolate of *Mycobacterium paratuberculosis*. Nucleic Acids Res 1989;17:9063-9073.

7. Collins DM, Gabric DM, de Lisle GW. Identification of two groups of *Mycobacterium paratuberculosis* strains by restriction endonuclease analysis and DNA hybridization. J Clin Microbiol 1990;28:1591-1596.

8. Cousins DV, Williams SN, Hope A, Eamens GJ. DNA fingerprinting of Australian isolates of *Mycobacterium avium* subsp *paratuberculosis* using IS900 RFLP. Aust Vet J 2000;78:184-190.

9. Moreira AR, Paolicchi F, Morsella C et al. Distribution of IS900 restriction fragment length polymorphism types among animal *Mycobacterium avium* subsp. *paratuberculosis* isolates from Argentina and Europe. Vet Microbiol 1999;70:251-259.

10. Pavlik I, Bejckova L, Pavlas M, Rozsypalova Z, Koskova S. Characterization by restriction endonuclease analysis and DNA hybridization using IS900 of bovine, ovine, caprine and human dependent strains of *Mycobacterium paratuberculosis* isolated in various localities. Vet Microbiol 1995;45:311-318.

11. Ris DR, Hamel KL, Ayling JM. Can sheep become infected by grazing pasture contaminated by cattle with Johne's disease? N Z Vet J 1987;35:137.

12. Kennedy DJ, Benedictus G. Control of *Mycobacterium avium* subsp. *paratuberculosis* infection in agricultural species. Rev Sci Tech 2001;20:151-179.

13. Beard PM, Daniels MJ, Henderson D, et al. Paratuberculosis infection of nonruminant wildlife in Scotland. J Clin Microbiol 2001;39:1517-1521.

14. Beard PM, Rhind SM, Buxton D, et al. Natural paratuberculosis infection in rabbits in Scotland. J Comp Pathol 2001;124:290-299.

15. Momotani E, Whipple DL, Thiermann AB, Cheville NF. Role of M cells and macrophages in the entrance of *Mycobacterium paratuberculosis* into domes of ileal Peyer's patches in calves. Vet Pathol 1988;25:131-137.

16. Tessema MZ, Koets AP, Rutten VP, Gruys E. How does *Mycobacterium avium* subsp. *paratuberculosis* resist intracellular degradation? Vet Q 2001;23:153-162.

17. Kim JC, Sanger VL, Whitemark DL. Ultrastructural studies of bovine paratuberculosis. Agri Pract 1976;12:78.

18. Jorgensen JB. Pathogenicity and immunogenicity of atypical mycobacteria for calves: A short summary. Rev Infect Dis 1981;3:979.

19. Chiodini RJ, Van Kruiningen HJ, Merkal RS. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. Cornell Vet 1984;74:218-262.

20. Stabel JR. Transitions in immune responses to *Mycobacterium paratuberculosis*. Vet Microbiol 2000;77:465-473.

21. Wood PR, Corner LA, Rothel JS, et al. Field comparison of the interferon-gamma assay and the intradermal tuberculin test for the diagnosis of bovine tuberculosis. Aust Vet J 1991;68:286-290.

22. Zurbrick BG, Czuprynski CJ. Ingestion and intracellular growth of *Mycobacterium paratuberculosis* within bovine blood monocytes and monocyte-derived macrophages. Infect Immun 1987;55:1588-1593.

23. Rice CE, Annau E, Duhamel L. Studies of Johne's disease in Canada. VI. An attempt to passively transfer sensitivity to johnin by means of plasma fractions. Can J Comp Med 1959;23:59-64.

24. Bendixen PH. Immunological reactions caused by infection with *Mycobacterium paratuberculosis*. A review. Nord Vet Med 1978;30:163-168.

25. Merkal RS, Richard JL, Thurston JR, Ness RD. Effect of methotrexate on rabbits infected with *Mycobacterium paratuberculosis* or *Dermatophilus congolensis*. Am J Vet Res 1972;33:401-407.

26. Merkal RS, Kopecky KE, Larsen AB. Immunologic mechanisms in bovine paratuberculosis. Am J Vet Res 1970;31:475-485.

27. Merkal RS, Witzel DA. Passive transfer of johnin hypersensitivity with reactivity resembling clinical paratuberculosis in calves. Infect Immun 1973;7:196-198.

28. Whitlock RH, Buergelt C. Preclinical and clinical manifestations of paratuberculosis (including pathology). Vet Clin North Am Food Anim Pract 1996;12:345-356.

29. Kalis CH, Collins MT, Hesselink JW, Barkema HW. Specificity of two tests for the early diagnosis of bovine paratuberculosis based on cell-mediated immunity: the Johnin skin test and the gamma interferon assay. Vet Microbiol 2003;97:73-86.

30. McDonald WL, Ridge SE, Hope AF, Condron RJ. Evaluation of diagnostic tests for Johne's disease in young cattle. Aust Vet J 1999;77:113-119.

31. de Lisle GW, Duncan JR. Bovine paratuberculosis III. An evaluation of a whole blood lymphocyte transformation test. Can J Comp Med 1981;45:304-309.

32. Tiwari A, VanLeeuwen JA, Dohoo IR, Stryhn H, Keefe GP, Haddad JP. Effects of seropositivity for bovine leukemia virus, bovine viral diarrhoea virus, Mycobacterium avium subspecies paratuberculosis, and Neospora caninum on culling in dairy cattle in four Canadian provinces. Vet Microbiol 2005;109:147-158.

33. Tiwari A, Vanleeuwen JA, Dohoo IR, Stryhn H, Keefe GP. Effects of seropositivity for bovine Leukemia virus, *Mycobacterium avium* subspecies *paratuberculosis,* and *Neospora caninum* on calving to conception interval in maritime Canadian dairy cattle. Proc Soc Vet Epidemiol Prev Med, Warwick, England 2003: 243-252.

34. Merkal RS, Thurston JR. Comparison of *Mycobacterium paratuberculosis* and other mycobacteria, using standard cytochemical tests. Am J Vet Res 1966;27:519-521.

35. Lepper AW, Wilks CR, Kotiw M, Whitehead JT, Swart KS. Sequential bacteriological observations in relation to cell-mediated and humoral antibody responses of cattle infected with *Mycobacterium paratuberculosis* and maintained on normal or high iron intake. Aust Vet J 1989;66:50-55.

36. Kopecky KE, Larsen AB. Intravenous johnin and tuberculin tests in cattle vaccinated with *Mycobacterium paratuberculosis* cells and subsequently inoculated with *Mycobacterium* bovis. Am J Vet Res 1975;36:1727-1729.

37. Mathews HT. On Johne's disease. Vet Rec 1947;59:397-401.

38. Abbas B, Riemann HP, Hird DW. Diagnosis of Johne's disease (paratuberculosis) in Northern California cattle and a note of it's economic significance. Calif Vet 1983;37:20-24.

39. Kalis CH, Collins MT, Barkema HW, Hesselink JW. Certification of herds as free of *Mycobacterium paratuberculosis* infection: actual pooled faecal results versus certification model predictions. Prev Vet Med 2004;65:189-204.

40. Manning EJ, Collins MT. *Mycobacterium avium* subsp. *paratuberculosis*: pathogen, pathogenesis and diagnosis. Rev Sci Tech 2001;20:133-150.

41. McKenna SL, Keefe GP, Barkema HW, McClure J, Vanleeuwen JA, Hanna P et al. Cow-level prevalence of paratuberculosis in culled dairy cows in Atlantic Canada and Maine. J Dairy Sci 2004;87:3770-3777.

42. Dargatz DA, Byrum BA, Barber LK, Sweeney RW, Whitlock RH, Shulaw WP et al. Evaluation of a commercial ELISA for diagnosis of paratuberculosis in cattle. J Am Vet Med Assoc 2001;218:1163-1166.

43. Sweeney RW, Whitlock RH, Buckley CL, Spencer PA. Evaluation of a commercial enzyme-linked immunosorbent assay for the diagnosis of paratuberculosis in dairy cattle. J Vet Diagn Invest 1995;7:488-493.

44. Collins MT, Sockett DC. Accuracy and economics of the USDA-licensed enzyme-linked immunosorbent assay for bovine paratuberculosis. J Am Vet Med Assoc 1993;203:1456-1463.

45. Sockett DC, Conrad TA, Thomas CB, Collins MT. Evaluation of four serological tests for bovine paratuberculosis. J Clin Microbiol 1992;30:1134-1139.

46. Collins MT, Sockett DC, Ridge S, Cox JC. Evaluation of a commercial enzyme-linked immunosorbent assay for Johne's disease. J Clin Microbiol 1991;29:272-276.

47. Cox JC, Drane DP, Jones SL, Ridge S, Milner AR. Development and evaluation of a rapid absorbed enzyme immunoassay test for the diagnosis of Johne's disease in cattle. Aust Vet J 1991;68:157-160.

48. Milner AR, Mack WN, Coates KJ, Hill J, Gill I, Sheldrick P. The sensitivity and specificity of a modified ELISA for the diagnosis of Johne's disease from a field trial in cattle. Vet Microbiol 1990;25:193-198.

49. Whitlock RH, Wells SJ, Sweeney RW, Van Tiem J. ELISA and fecal culture for paratuberculosis (Johne's disease): sensitivity and specificity of each method. Vet Microbiol 2000;77:387-398.

50. Sweeney RW. Transmission of paratuberculosis. Vet Clin North Am Food Anim Pract 1996;12:305-312.

51. Bech-Nielsen S, Jorgensen JB, Ahrens P, Feld NC. Diagnostic accuracy of a *Mycobacterium* phlei-absorbed serum enzyme-linked immunosorbent assay for diagnosis of bovine paratuberculosis in dairy cows. J Clin Microbiol 1992;30:613-618.

52 Nielsen SS, Gronbaek C, Agger JF, Houe H. Maximum-likelihood estimation of sensitivity and specificity of ELISAs and faecal culture for diagnosis of paratuberculosis. Prev Vet Med 2002;53:191-204.

53. Collins MT, Kenefick KB, Sockett DC, Lambrecht RS, McDonald J, Jorgensen JB. Enhanced radiometric detection of *Mycobacterium paratuberculosis* by using filter-concentrated bovine fecal specimens. J Clin Microbiol 1990;28:2514-2519.

54. Stich RW, Byrum B, Love B, Theus N, Barber L, Shulaw WP. Evaluation of an automated system for non-radiometric detection of *Mycobacterium avium paratuberculosis* in bovine feces. J Microbiol Methods 2004;56:267-275.

55. Collins MT. Diagnosis of paratuberculosis. Vet Clin North Am Food Anim Pract 1996;12:357-371.

56. Sockett DC, Carr DJ, Collins MT. Evaluation of conventional and radiometric fecal culture and a commercial DNA probe for diagnosis of *Mycobacterium paratuberculosis* infections in cattle. Can J Vet Res 1992;56:148-153.

57. Kalis CH, Barkema HW, Hesselink JW, van Maanen C, Collins MT. Evaluation of two absorbed enzyme-linked immunosorbent assays and a complement fixation test as replacements for fecal culture in the detection of cows shedding *Mycobacterium avium* subspecies *paratuberculosis*. J Vet Diagn Invest 2002;14:219-224.

58. Hendrick SH, Duffield TE, Kelton DE, Leslie KE, Lissemore KD, Archambault M. Evaluation of enzyme-linked immunosorbent assays performed on milk and serum samples for detection of paratuberculosis in lactating dairy cows. J Am Vet Med Assoc 2005;226:424-428.

59. Klausen J, Huda A, Ekeroth L, Ahrens P. Evaluation of serum and milk ELISAs for paratuberculosis in Danish dairy cattle. Prev Vet Med 2003;58:171-178.

60. Kalis CH, Hesselink JW, Barkema HW, Collins MT. Culture of strategically pooled bovine fecal samples as a method to screen herds for paratuberculosis. J Vet Diagn Invest 2000;12:547-551.

61. Wells SJ, Godden SM, Lindeman CJ, Collins JE. Evaluation of bacteriologic culture of individual and pooled fecal samples for detection of *Mycobacterium paratuberculosis* in dairy cattle herds. J Am Vet Med Assoc 2003;223:1022-1025.

62. Wells SJ, Whitlock RH, Lindeman CJ, Fyock T. Evaluation of bacteriologic culture of pooled fecal samples for detection of *Mycobacterium paratuberculosis*. Am J Vet Res 2002;63:1207-1211.

63. van Schaik G, Stehman SM, Schukken YH, Rossiter CR, Shin SJ. Pooled fecal culture sampling for *Mycobacterium avium* subsp. *paratuberculosis* at different herd sizes and prevalence. J Vet Diagn Invest 2003;15:233-241.

64. Collins DM, Stephens DM, de Lisle GW. Comparison of polymerase chain reaction tests and faecal culture for detecting *Mycobacterium paratuberculosis* in bovine faeces. Vet Microbiol 1993;36:289-299.

65. Gwozdz JM, Reichel MP, Murray A, Manktelow W, West DM, Thompson KG. Detection of *Mycobacterium avium* subsp. *paratuberculosis* in ovine tissues and blood by the polymerase chain reaction. Vet Microbiol 1997;57:233-244.

66. Englund S, Bolske G, Johansson KE. An IS900-like sequence found in a *Mycobacterium* sp. other than *Mycobacterium avium* subsp. *paratuberculosis*. FEMS Microbiol Lett 2002;209:267-271.

67. Cousins DV, Whittington R, Marsh I, Masters A, Evans RJ, Kluver P. Mycobacteria distenct from *Mycobacterium avium* subsp. *paratuberculosis* isolated from the faeces of ruminants possess IS900-like sequences detectable IS900 polymerase chain reaction: implications for diagnosis. Mol Cell Probes 1999;13:431-442.

68. Yokomizo Y, Merkal RS, Lyle PA. Enzyme-linked immunosorbent assay for detection of bovine immunoglobulin G1 antibody to a protoplasmic antigen of *Mycobacterium paratuberculosis*. Am J Vet Res 1983;44:2205-2207.

69. Hardin LE, Thorne JG. Comparison of milk with serum ELISA for the detection of paratuberculosis in dairy cows. J Am Vet Med Assoc 1996;209:120-122.

70. Sweeney RW, Whitlock RH, Buckley CL, Spencer P, Rosenberger AE, Hutchinson LJ. Diagnosis of paratuberculosis in dairy cattle, using enzyme-linked immunosorbent assay for detection of antibodies against *Mycobacterium paratuberculosis* in milk. Am J Vet Res 1994;55:905-909.

71. Nielsen SS, Enevoldsen C, Grohn YT. The *Mycobacterium avium* subsp. *paratuberculosis* ELISA response by parity and stage of lactation. Prev Vet Med 2002;54:1-10.

72 Kudahl A, Nielsen SS, Sorensen JT. Relationship between antibodies against Mycobacterium avium subsp. paratuberculosis in milk and shape of lactation curves. Prev Vet Med 2004;62:119-134.

73 Nielsen SS. Variance components of an enzyme-linked immunosorbent assay for detection of IgG antibodies in milk samples to Mycobacterium avium subspecies paratuberculosis in dairy cattle. J Vet Med B Infect Dis Vet Public Health 2002;49:384-387.

74. Collins MT, Sockett DC, Ridge S, Cox JC. Evaluation of a commercial enzyme-linked immunosorbent assay for Johne's disease. J Clin Microbiol 1991;29:272-276.

75. Adaska JM, Munoz-Zanzi CA, Hietala SK. Evaluation of result variability with a commercial Johne's disease enzyme-linked immunosorbent assay kit and repeat testing of samples. J Vet Diagn Invest 2002;14:423-426.

76. Sherman DM, Gay JM, Bouley DS, Nelson GH. Comparison of the complement-fixation and agar gel immunodiffusion tests for diagnosis of subclinical bovine paratuberculosis. Am J Vet Res 1990;51:461-465.

77. VanLeeuwen JA, Keefe GP, Tremblay R, Power C, Wichtel JJ. Seroprevalence of infection with *Mycobacterium avium* subspecies *paratuberculosis*, bovine leukemia virus, and bovine viral diarrhea virus in maritime Canada dairy cattle. Can Vet J 2001;42:193-198.

78. VanLeeuwen JA, Tremblay R, Keefe GP, Wichtel J. Seroprevalence of production limiting diseases in Ontario and Maritime dairy cattle. Proc 52nd Annu Conv Can Vet Med Assoc, St John, 2000.

79. VanLeeuwen JA, Whiting T, Tiwari A. Seroprevalence of antibodies against bovine leukemia virus, bovine viral diarrhea virus, *Mycobacterium avium* subspecies *paratuberculosis* and *Neospora caninum* in Manitoba dairy and beef cattle. Submitted to Can Vet J 2005.

80. VanLeeuwen JA, Forsythe L, Tiwari A, Chartier R. Seroprevalence of antibodies against bovine leukemia virus, bovine viral diarrhea virus, *Mycobacterium avium* subspecies *paratuberculosis*, and Neospora caninum in dairy cattle in Saskatchewan. Can Vet J 2005;46:56-58.

81. Sorensen O, Rawluk S, Wu J, Manninen K, Ollis G. *Mycobacterium paratuberculosis* in dairy herds in Alberta. Can Vet J 2003;44:221-226.

82. NcNab WB, Meek AH, Duncan JR, Martin SW, Van Dreumel AA. An epidemiological study of paratuberculosis in dairy cattle in Ontario: study design and prevalence estimates. Can J Vet Res 1991;55:246-251.

83. Braun RK, Buergelt CD, Littell RC, Linda SB, Simpson JR. Use of an enzyme-linked immunosorbent assay to estimate prevalence of paratuberculosis in cattle of Florida. J Am Vet Med Assoc 1990;196:1251-1254.

84. Collins MT, Sockett DC, Goodger WJ, Conrad TA, Thomas CB, Carr DJ. Herd prevalence and geographic distribution of, and risk factors for, bovine paratuberculosis in Wisconsin. J Am Vet Med Assoc 1994;204:636-641.

85. Johnson-Ifearulundu Y, Kaneene JB. Distribution and environmental risk factors for paratuberculosis in dairy cattle herds in Michigan. Am J Vet Res 1999;60:589-596.

86. US Department of Agriculture, Animal and Plant Health Inspection Service. Johnes's Disease on US Dairy Operations, NAHMS Dairy '96, Center for Animal Health Monitoring, Report N245, 1997.

87. Muskens J, Barkema HW, Russchen E, Van Maanen K, Schukken YH, Bakker D. Prevalence and regional distribution of paratuberculosis in dairy herds in The Netherlands. Vet Microbiol 2000;77:253-261.

88. Gasteiner J, Wenzl H, Fuchs K, Jark U, Baumgartner W. Serological cross-sectional study of paratuberculosis in cattle in Austria. Zentralbl Veterinarmed B 1999;46:457-466.

89. Boelaert F, Walravens K, Biront P, Vermeersch JP, Berkvens D, Godfroid J. Prevalence of paratuberculosis (Johne's disease) in the Belgian cattle population. Vet Microbiol 2000;77:269-281.

90. Wells SJ, Wagner BA. Herd-level risk factors for infection with *Mycobacterium paratuberculosis* in US dairies and association between familiarity of the herd manager with the disease or prior diagnosis of the disease in that herd and use of preventive measures. J Am Vet Med Assoc 2000;216:1450-1457.

91. Paisley LG, Tharaldsen J, Jarp J. A simulated surveillance program for bovine paratuberculosis in dairy herds in Norway. Prev Vet Med 2000;44:141-151.

92. Bolske G, Sternberg S, Viske D, Larsson B. Use of culture and serology in tracing and screening for paratuberculosis in Sweden. Proc 6th Int Coll Paratuberculosis 1999;349-352.

Table 2.1. Characteristics of currently available diagnostic tests for Johne's disease in Canada

		Sensitivity			Specificity
		Sub-clinical cow		Clinical cow	
		Low prevalence herds and/or low shedders[a]	High prevalence herds and/or high shedders[b]		
Fecal culture	Estimate	19%[c]	53%[d]	>90%[e]	Approaching 100%[e]
	Reference	(56)	(56)	(49)	(49)
Serum ELISA[n]	Estimate	12%[f] - 15%[f]	40%[g] - 75%[g] - 95%[h]	87%[i] - 88%[i]	96.8%[j] - 99%[i]
	Reference	(57) - (42,43)	(57) - (42,43) - (58)	(43) - (42)	(42) - (56)
Cow milk ELISA[n]	Estimate	-[k]	51%[l] - 84%[h]	-[k]	92%[i] - 96%[i]
	Reference		(58) - (58)		(59) - (59)

[a]Low prevalence herds (<25%) and/or low shedders (<10CFU[m])

[b]High prevalence herds (≥25%) and/or high shedders (≥10CFU)

[c]Tissue culture was gold standard in low prevalence herds

[d]Tissue culture was gold standard in high prevalence herds

[e]Tissue culture was gold standard

[f]Fecal culture was gold standard in low shedders

[g]Fecal culture was gold standard in high shedders

[h]Fecal culture was gold standard in high shedders in high prevalence herds

[i]Fecal culture was gold standard

[j]Tissue culture or fecal culture or history of herd was gold standard

[k]No published reports found

[l]Fecal culture was gold standard in low shedders in high prevalence herds

[m]Colony forming unit

[n]Enzyme linked immunosorbant assays

18 Table 2.2 Seroprevalence estimates of bovine paratuberculosis at the animal and herd level in Canadian dairy herds (from East to West)

Province (Reference #)	Number of herds	Number of animals	Animals test +ve[a] (%)	Herds with 1 test +ve[b] (%)	Herds with 2 test +ve[c] (%)	Animals test + in herds with 1 test +ve[d] (%)	Animals test + in herds with 2 test +ve[e] (%)
Nova Scotia (77)	30	814	3.3	53.3	16.7	5.3	8.1
Prince Edward Island (77)	30	816	1.3	33.3	16.7	5.0	6.3
New Brunswick (77)	30	804	2.9	43.3	16.7	6.4	11.0
Ontario (78)	60	-	2.2	37.0	9.8	4.6	8.4
Manitoba (79)	40	1204	4.5	68.4	43.1	6.6	8.7
Saskatchewan (80)	51	1530	2.7	43.6	24.2	6.3	8.6
Alberta (81)	50	1500	7.0	74.0	40.0	- [f]	- [f]

[a]Animals testing positive

[b]Herds with at least 1 animal testing positive

[c]Herds with at least 2 animals testing positive

[d]Animals testing positive in herds with at least 1 animal testing positive

[e]Animals testing positive in herds with at least 2 animals testing positive

[f]Published report did not include these figures

Table 2.3. Seroprevalence estimates of bovine paratuberculosis in dairy herds from major dairy producing countries and states outside of Canada (from high prevalence to low prevalence at the animal level)

Country (Reference #)	Areas	Number of Herds	Number of animals	Animals test +ve[a] (%)	Herds with 1 test +ve[b] (%)	Herds with 2 test +ve[c] (%)	Animals test + in herds with 1 test +ve[d] (%)	Animals test + in herds with 2 test +ve[e] (%)
U.S.A. (83)	Florida	452	4491	17.1	-	-	-	-
U.S.A. (84)	Wisconsin	158	4990	7.3	50	-	20	
U.S.A. (85)	Michigan	121	3886	6.9	66	44	8	12
U.S.A. (86)	20 states	967	31,745	2.5	41	17		
Netherlands (87)	National	378	15,822	2.5	54	28	-	-
Austria (88)	National	2757	11,028	1.9	7	-	-	-
Sweden (12)	National	-	4000	1.2	-	-	-	-
Belgium (89)	National	556	13,317	0.8	18	-	3	-

[a]Animals testing positive

[b]Herds with at least 1 animal testing positive

[c]Herds with at least 2 animals testing positive

[d]Animals testing positive in herds with at least 1 animal testing positive

[e]Animals testing positive in herds with at least 2 animals testing positive

CHAPTER 3: Seroprevalence and spatial distribution of bovine leukemia virus, bovine viral diarrhea virus and Mycobacterium avium subspecies paratuberculosis in Canadian dairy cattle

3.1 Abstract

The purpose of this research was to determine the seroprevalence and spatial distribution of exposure to bovine leukemia virus (BLV), bovine viral diarrhea virus (BVDV) and Mycobacterium avium subspecies paratuberculosis (MAP) in Canadian dairy herds. Within each randomly selected herd, a serum sample was obtained from approximately 30 randomly selected lactating animals for BLV and MAP ELISA testing, while 5 unvaccinated cattle over 6 months of age were selected for BVDV virus neutralization testing. After visual exploration using choropleth maps, the spatial-scan statistic and Moran's I were utilized for detection of significant clusters and spatial autocorrelation. Seroprevalence at the cow level was 30.3 and 3.1% for BLV and MAP, respectively. At the herd level, 76.6% had at least one seropositive cow for BLV, 31.2% had at least one animal with a titer $\geq$1:64 for BVDV, and 18.9% had at least two seropositive cows for MAP. For the spatial scan statistic, 4 and 1 significant clusters were detected for BLV and MAP, respectively. No significant clusters were detected for BVDV. The Global Moran's I statistic for BLV and MAP indicated significant positive spatial autocorrelation of 0.16 and 0.15. The K-function for BVDV did not indicate any significant spatial autocorrelation. Exposure to BLV is high in Canadian dairy cattle, with significant clustering in certain parts of the country, while exposure to MAP is lower and

somewhat spatially clustered. There is evidence that a third of Canadian dairy farms have been exposed to BVDV in the recent past, without any significant regional differences. Given the prevalence and the potential undetected spread of these 3 pathogens, dairy farmers and veterinarians should consider utilizing herd screening tests and then control programs for those herds testing positive.

3.2 Introduction

Infections with bovine leukemia virus (BLV), bovine viral diarrhea virus (BVDV) and *Mycobacterium avium* subspecies *paratuberculosis* (MAP), the causative agents of Enzootic Bovine Leukosis, Bovine Viral Diarrhea and Johne's disease, respectively, have significant health and economic impacts on the cattle industry. These effects may include the loss of international market opportunities, lower domestic productivity and production efficiency, and the potential for reduced consumer confidence in dairy products (1). The World Trade Organisation's (WTO) new trading rules state that health certification standards for imported cattle, semen and embryos cannot exceed those required under domestic regulatory programs (WTO, Act 1994). As a result, there is renewed interest in these diseases in many countries, including Canada. There have been historical or isolated studies of the seroprevalence of exposure to these pathogens in parts of Canada in the past, as described below, but whether these reflects the representativeness for the entire country has been questionable.

BLV is a retrovirus that can cause a fatal malignant lymphosarcoma in up to 5% of infected cattle. In addition to tumour development, other signs of lymphosarcoma can include decreased milk production, weight loss, fever and loss of appetite (2). However, most BLV infections are asymptomatic. There is no curative treatment for BLV-infection. In a recent survey (1995) done by the National Animal Health Monitoring System (NAHMS), 79% of herds in the United States had at least one BLV-seropositive cow (3). This estimate was similar to some recent surveys done in the Canadian Maritime provinces and in the province of Saskatchewan where 70% and 89% of herds had at least

one BLV-seropositive cow, respectively (1,11). A national survey of BLV was conducted in Canada in 1980, reporting animal and herd level seroprevalence levels of 3.6% and 19.7%, respectively. However, substantial changes in seroprevalence could have occurred between since then (4).

BVDV is a pestivirus that has a wide range of clinical manifestations, including pneumonia, abortion, birth defects and immunosuppression, and mucosal disease in persistently infected cattle that were originally infected with a non-cytopathogenic strain of BVDV between 45 and 125 days of gestation (5). BVDV prevalence can be reported in two different ways: proportion of cattle transiently infected with BVDV (i.e. having antibodies indicating previous exposure to BVDV); or proportion of persistently infected cattle (that typically do not produce antibodies). The prevalence of transiently and persistently infected cattle can range from 0% to 89% and 0% to 2% (5). In recent surveys of dairy cattle in the Canadian Maritime Provinces and in Saskatchewan, the proportion of randomly sampled herds testing positive for recent exposure to BVDV (having at least one tested animal with a titer $\geq$ 1:64) was reported to be 46.1% and 29.2%, respectively (1,11).

MAP is distributed worldwide but there appears to be a wide variation in the prevalence between countries and even within countries. MAP prevalence estimates using the culture of ileocecal lymph nodes and ileum of apparently healthy dairy cattle at slaughter range from 2.9% (United States) (6) to 16.1% (Maritime provinces, Canada) (7). Seroprevalence estimates among on-farm dairy cattle range from 17.1% (Florida, US) (8) to 0.8% (Belgium) (9) at the cow level, and 74% (Missouri, US) (10) to 18% (Belgium) (9) at the herd level. In recent surveys of dairy cattle in Saskatchewan and the

maritime provinces of Canada, the animal level seroprevalences were 2.7% (11) and 2.6% (1), respectively, and herd level seroprevalences ($\geq$ 2 seropositive cattle) were 24.2% (11) and 16.7% (1), respectively. However in 1991, using a LAM-ELISA, a survey of 14,932 cows in 304 dairy herds in Ontario found 15.2% of the animals tested seropositive (12).

To assist efforts toward control of pathogens, knowing the spatial distribution of the seroprevalence estimates can focus where control efforts could be concentrated. Spatial visualisation methods (such as choropleth maps) can provide powerful tools for understanding the epidemiology of the disease process. Statistical tests have been developed to describe and quantitatively interpret apparent disease clusters. Global methods (such as the Moran's I statistic) (13) determine the presence of spatial clustering in a study population, while local methods (such as the Scan statistic) (14) point out the location of these clusters.

In 1997, individuals and organizations involved in the cattle industry (veterinarians, livestock genetics companies, livestock exporters and national dairy breed associations) formed the Production Limiting Diseases Committee (PLDC). The committee is interested in maintaining and enhancing the ability of Canadian cattle producers to sell products domestically and internationally in the future. To achieve this mission, the PLDC initiated research to estimate the prevalence, spatial distribution, risk factors and economic impact of BLV, BVDV, MAP and Neospora caninum (NC). Estimated seroprevalence levels for antibodies against these four pathogens were determined and published for the Maritime provinces and Saskatchewan (1; 11). However, since the majority of dairy farms in Canada are located in other provinces, and with possible

regional differences in management and ecological conditions between the tested and untested provinces, there remained a need to determine the seroprevalence of these pathogens in other provinces of Canada. The purpose of this study was to determine the seroprevalence levels and spatial distribution for exposure to BLV, BVDV and MAP in a nationally representative sample of Canadian dairy cattle. Seroprevalence and spatial distribution of exposure to NC is reported elsewhere (15). Results of these studies will also be used to determine the effects of these diseases and the risk factors for their spread.

3.3 Materials and methods

3.3.1 Serum sample collection

A stratified two-stage random sampling procedure was employed. During the summer of 1998 in Atlantic Canada, participating Dairy Herd Improvement herds were randomly selected (using computer generated random numbers) until ninety herds were recruited, thirty each from Prince Edward Island (PEI), New Brunswick (NB), and Nova Scotia (NS). Similar recruitment procedures (that included non Dairy Herd Improvement herds) were utilized to recruit one hundred four, forty, fifty-one, and seventy-nine herds from the provinces of Quebec (QUE), Manitoba (MAN), Saskatchewan (SASK), and Alberta (ALTA) in 2002, 2002, 2001, and 2002/03, respectively. Sampling of herds in ALTA was first stratified by veterinary practices servicing dairy herds in the provinces, and then within these practices, herds were randomly selected. For the province of Ontario (ONT), a different sampling protocol was utilized to select herds with extensive records on clinical disease occurrences in order to determine relationships between seroprevalence for these 4 agents and recorded clinical disease (not reported here).

Using computer generated random numbers: 1) approximately 30 (on all cows if the total number of cows in herd was less than 30 cows) lactating animals were randomly selected for blood collection in each participating herd. For BVDV, five unvaccinated (for BVDV) cattle >6 months old were selected for blood collection. In unvaccinated herds, five animals of the 30 cows tested for the other three diseases were selected. In vaccinated herds, five unvaccinated heifers >6 months old were selected. The sampling technique was based upon Houe's study (16). Within 24 hours, the blood samples were

centrifuged, and the serum was harvested and stored at -20°C until all the samples were collected and ready for testing.

The sample size formula used to determine the number of required herds for the original prevalence surveys assumed a herd level seroprevalence of 10%, an allowable error of 10%, and a confidence level of 95%. The lowest expected seroprevalence of any of the investigated diseases was 10% (17); therefore this seroprevalence was used to calculate herd sample size. This calculation lead to a total of approximately 30 herds being needed to be tested in each province. The sample size formula used to determine the number of required cows in each herd for the original prevalence surveys assumed an average herd size of 45 cows, an average within-herd prevalence estimate of 10%, confidence of 90%, and sensitivity of the enzyme linked immunosorbent assay (ELISA) test for MAP of 43.0%, the test with the a lowest sensitivity among the four diseases (18). This calculation lead to a total of approximately 30 cattle being needed to be tested in each herd to detect at least 1 infected animal in a herd.

3.3.2 Laboratory analysis

The test utilized by all provinces for BLV antibodies was an Enzyme Linked Immunosorbant Assay (ELISA)[1] (19). A cow was considered to be infected with BLV if the serum-to-positive ratio on the ELISA was ≥ 0.50, as recommended by the manufacturer of the test kit. The BLV ELISA test kit also requires a confirmation of positive tests, using a sample-to-negative host-cell ratio of ≥ 1.8. The BLV testing for NS, NB, PEI, ONT, MAN, SASK, and ALTA was conducted at the national BLV testing

[1] IDEXX ELISA - IDEXX Corporation - Idexx Laboratories, Westbrook, Maine, USA

laboratory in Prince Edward Island (now in Quebec), which is certified to conduct BLV testing for international trade purposes. BIOVET Inc. laboratory in Quebec was utilized for the dairy farms in QUE (table 3.1).

The test utilized by all provinces for MAP antibodies was also an ELISA, with ALTA farms being tested with one brand of ELISA, according to the wishes of the directors of the study in that province[2] (18). The farms in the rest of the participating provinces were tested with a different ELISA[3] (20). For the first ELISA, an animal was considered to be seropositive for MAP if the optical density value was greater than the mean of the negative control plus 0.100 for bovine sera, as recommended by the manufacturer of the test kit. For the second ELISA, an animal was considered to be seropositive for MAP if the serum-to-positive ratio on the ELISA was $\geq$0.25, as recommended by the manufacturer of the test kit. The MAP-testing for NS, NB, PEI, ONT, and SASK was conducted at Prairie Diagnostic Services in SASK, which is accredited for MAP-ELISA testing by the United States Dept of Agriculture. The BIOVET Inc. laboratory in QUE, the Manitoba Agricultural Laboratory in MAN, and the Alberta Agriculture, Food and Rural Development Food Safety Division Laboratory in ALTA were utilized for their respective provincial testing. The serum samples were tested in duplicate at all locations (table 3.1).

For all provinces, 5 serum samples per farm were tested for antibody against type 1 genotype BVDV, using virus neutralization to the cytopathic Singer strain (21). A herd was considered to be infected with BVDV if at least one of the animals tested had a titer of $\geq$1:64 for BVDV. Testing for BVDV was conducted at Animal Diseases Research

[2] BIOCOR-CSL ELISA - BIOCOR Animal Health, Inc., Omaha, Nebraska, USA
[3] IDEXX ELISA - IDEXX Corporation - Idexx Laboratories, Westbrook, Maine, USA

Institute in Alberta for dairy herds in NS, NB, PEI, ONT, MAN, SASK, ALTA, and at the Armand Frappier Laboratory in Quebec for dairy herds in QUE (table 3.1).

3.3.3 Statistical analysis

For BLV and MAP, the seroprevalence estimates and 95% confidence intervals were determined for the proportion of cattle and herds that were seropositive by utilizing survey commands in STATA (Statistical package, v.8; Stata Press, College Station, Texas, USA) which adjusted for within herd clustering, sampling weights and stratification at the provincial level. Herd level seroprevalence was calculated using 2 definitions: 1) positive herds had at least one test positive animal; and 2) positive herds had at least two test positive animals. Furthermore, estimated true prevalences at the animal and herd levels were calculated (17), correcting for test sensitivity and specificity (22).

Herd level estimates of BVDV prevalence were calculated using 2 definitions: 1) a positive herd had at least one animal with antibodies against BVDV, and 2) a positive herd had at least one animal with a titer of $\geq$ 1:64 for BVDV. The latter definition is based on Houe's study (23), where titers in this range in unvaccinated cattle over six months of age were likely to represent recent exposure to BVDV, either from the presence of a persistently infected animal on the farm, or an acutely infected animal recently introduced to the farm. An analysis of the distribution of titers in the current study showed that most herds with cattle having titers of $\geq$1:64 also had cattle with titers of 1:256 (the highest dilution tested). Conversely, most herds with cattle having titers of

1:32 did not have cattle with titers greater than 1:32. Therefore, a titer of ≥1:64 was determined to be a natural cut point for this population.

3.3.4 Spatial analysis

For confidentiality reasons, regions within provinces were identified for geographical representation of seroprevalence data at a subprovincial level without revealing the specific location of farms. The regions were aggregations of contiguous census divisions that were large enough to contain at least four study herds, ensuring confidentiality of results and reasonable stability and representative of region estimates. An exception to this aggregation process was in northern, sparsely inhibited, parts of provinces where regions without study herds were identified and indicated as such.

A choropleth map for herd level BVDV seroprevalence was created, showing the percentage of farms in each region that had at least 1 seropositive (≥1:64 titer) animal. For MAP, due to the low animal level seroprevalence, a choropleth map for herd level seroprevalence was also created, showing the percentage of farms in each region that had at least two seropositive animals. Because more than 75% of farms had at least one test positive animal for BLV, and there was a wide, right-skewed, distribution in animal level seroprevalences on positive farms (3-100%), a choropleth map showing simple herd level seroprevalence would be less informative than a map that somehow combines both animal and herd level seroprevalence information. Therefore, in preparation for the BLV map, the median within herd animal level seroprevalence for all farms was first determined. It was then determined whether each farm had a seroprevalence that was above or below this median. Finally, a choropleth map for BLV exposure was created

showing, by region, the percentage of farms with higher "within herd seroprevalence" than the median seroprevalence. All mapping utilized the software package ArcView GIS (version 3.3).

In order to identify whether there was significant spatial clustering of seroprevalence levels, the Moran's I statistic for BLV- and MAP-seroprevalence and the K-function statistic for BVDV-seropositivity were calculated using GeODa 0.9.5[4] and R[5]. Local indicators of spatial autocorrelation (LISA) were determined using SaTScan[6] to identify specific locations of spatial clustering. The distribution of BLV and MAP cases was assumed to be Poisson and the distribution of herds having at least one seropositive ($\geq$1:64 titer) animal was assumed to be binomial. Scanning windows of up to 50% of the study population were used to identify clusters and a likelihood-ratio test statistic was calculated for each cluster identified. The position of these significant clusters (>100 km radius) are shown in choropleth maps (3.1-3.3).

[4] Luc Anselin and The Regents of the University of Illinois
[5] R Development Core Team (2003). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-00-3, URL http://www.R-project.org.
[6] M Kulldorff and Information Management Services Inc. SaTScan version 3.03 (http://srab.cancer.gov/satscan/)

3.4 Results

3.4.1 Descriptive statistics

The final dataset for BLV and MAP contained 398 and 373 herds with 10768 and 10578 cows. Logistical problems (eg. errors in animal or vial identifications on the farms or at the different labs) lead to the small differences in the number of cows tested. Of the 398 herds, BVDV testing was done for 323 herds because 75 herds had aggressive BVDV vaccination protocols, and therefore did not have unvaccinated cattle > 6 months of age.

Overall, the production records were obtained from 346 herds. Comparison of the sampled cows and herds to national industry averages for farms utilizing monthly milk testing (Table 3.2) did not identify any significant differences. Therefore, the random selection of study herds and cows produced a sample population that was representative of the Canadian dairy industry as a whole.

In total, 30.3% and 3.1% of the cattle were positive for the antibodies to BLV and MAP, respectively. At the herd level, 76.6% of herds had at least one seropositive cow for BLV, 31.2% had at least one animal with a titer $\geq$1:64 for BVDV, and 18.9% had at least two seropositive cows for MAP. Tables 3.3-3.5 provide detailed results of BLV, BVDV and MAP seroprevalences, by province, and overall.

Table 3.3 shows the proportion (and 95% confidence interval) of cows seropositive for BLV, the proportion of herds with at least 1 BLV-seropositive cow, and the average within-herd BLV-seroprevalence (in herds having at least 1 BLV-seropositive cow). The dairy herds in MAN had a significantly higher proportion of BLV-seropositive cows and

seropositive herds compared to dairy herds in all other provinces, with only a few exceptions; the proportion of herds having at least one BLV-seropositive cow in MAN was not significantly different from ONT and SASK ($P > 0.05$), and the average within herd seroprevalence for BLV in MAN was not significantly different from herds in NB. The estimated true prevalence of exposure to BLV, adjusting for test sensitivity and specificity, were determined to be 30.7% and 75.9% at the animal and herd levels, respectively.

Table 3.4 shows the proportion (and 95% CI) of BVDV-seropositive cattle, the proportion of cattle with a titer $\geq$ 1:64, the proportion of BVDV-seropositive herds, and proportion of herds with at least one animal with a BVDV titer $\geq$ 1:64. In PEI, more cattle were seropositive and had significantly more number of cattle with titers $\geq$ 1:64 compared other provinces. However, at the herd level, no significant differences between provinces were observed when comparing the proportions of herds with at least one animal with a titer $\geq$ 1:64. In ALTA, there were significantly fewer herds with at least one animal with a BVDV titer compared to herds in PEI and NB. The estimated true prevalence of exposure to BVDV, adjusting for test sensitivity and specificity, was determined to be 28.17% at the herd level.

Table 3.5 shows the proportion (and 95% confidence interval) of cows seropositive for MAP, the proportion of herds with at least 1 and 2 MAP-seropositive cows, and the within herd MAP-seroprevalence in herds having at least 1 and 2 MAP-seropositive cows. The dairy herds in ALTA had significantly more MAP-seropositive cows and a significantly higher within-herd seroprevalence (in herds with at least 1 seropositive cow) compared to dairy herds in other provinces. ALTA also had more herds with at least 2

seropositive cows than all other provinces except the MAN. Among herds with at least 2 seropositive cows, the average within-herd seroprevalence among herds in ALTA was significantly higher than PEI, MAN and SASK. There were significantly fewer seropositive dairy herds (herds with at least 1 seropositive cow) in PEI compared to MAN and ALTA. The estimated true prevalence of exposure to MAP, adjusting for test sensitivity and specificity, were determined to be 21% and 40% at the animal and herd levels, respectively.

3.4.2 Spatial analyses

For BLV, Map 3.1 shows the percentage of farms in each region with higher "within herd prevalence" than the median seroprevalence (22%) at the animal level. The regions in MAN show higher percentage of farms having BLV than other parts of Canada. For the BVDV, Map 3.2 shows the percentage of farms in each region that had at least one seropositive ($\geq$1:64 titer) animal. The choropleth map did not identify any areas with high percentage of farms having recent BVDV infection. For MAP, Map 3.3 shows the percentage of farms in each region that had at least 1 seropositive animal. Certain regions in the western Canadian provinces of ALTA and MAN showed higher percentages of farms with high seroprevalence of MAP as compared to regions in the eastern provinces.

The Global Moran's I statistic for BLV and MAP indicated significant ($P < 0.05$) positive spatial autocorrelation of 0.16 and 0.15 (see Maps 3.2 and 3.3). The K-function for BVDV did not find significant spatial autocorrelation. The SaTScan statistical analyses detected 4 and 1 significant clusters for BLV and MAP, respectively. The radius of the clusters were 303, 77, 35 and 104 km for BLV, and 165 km for MAP. No

1 significant clusters were detected for BVDV. The position and size of the clusters (radius
2 > 100 km) for BLV and MAP are shown in Maps 3.1-3.3.

3.5 Discussion

This study was part of a national project designed to determine the seroprevalence, spatial distribution, effects and risk factors of BLV-, BVDV-, MAP- and NC-seropositivity in Canadian dairy cattle. Farmers in eight out of ten Canadian provinces (which comprised 95% of dairy herds in Canada)[7] participated in this study. Similar random selection procedures at the herd (except in ONT) and cow level were utilized to ensure that the study population was representative of the target population. Summary statistics (see Table 3.1) from the study herds were compared to national parameters and indicated good representativeness. The summary statistics from ONT herds compared well to the other provinces, and the herd level residual normal probability plots in the model diagnostic tests indicated that the representative assumption was not violated for the ONT herds (data not shown).

Survey statistics were utilized to estimate the apparent animal and herd level seroprevalence, which allowed prevalence estimates to be adjusted for probability weights of each sampled cow and herd, and stratification at the provincial level. Spatial data analyses included two steps: 1) visualization (descriptive interpretation); and 2) exploration (identification of pathogen exposure clusters through global and local statistic). The Satscan statistic was particularly useful for the identification of the presence and location of clusters because it utilized the full cow-level prevalence data rather than just herd-level seroprevalence of the randomly selected herds. The global statistics (eg. Moran's-I) for BLV and MAP-seropositivity were significantly correlated with the Satscan statistic, providing corroboration of the findings of the Satscan statistic.

[7] Canadian Diary Herd Improvement, Dairy Section, AAFC.

BLV, BVDV and MAP are mostly transmitted between regions and herds through introduction of infected cattle. Clusters of BLV- and MAP-seropositivity may indicate where some herds share common risk factors (management practices like grazing, vaccination, testing or buying/exchanging cattle with neighbours). Herd and cow level risk factors have been determined and described elsewhere (24,25). Areas having clusters can be targeted in the initial phase of control programs to prevent the spread of disease to less affected areas.

In the past (1980, 1986, 1989), surveys done in Canada, ONT and PEI reported that 19.7%, 47% and 49.2% of herds had at least one BLV-seropositive cow (BLV-seropositive herd) and 3.6%, 24.2% and 5.5% of the cattle were BLV-seropositive (4,26,27). The lack of a widely adopted control program for BLV is likely partially responsible for the apparent increase in herd and cow level prevalence since the 1970s and 1980s. A recent survey (1996) in the United States reported herd and animal level seroprevalence estimates of 89% and 43% (3). The European Economic Community has been able to reduce the animal level seroprevalence for BLV to 0.5% - 1.5% by utilizing a "test and cull" control program. However, with higher herd and animal level BLV-seroprevalence in North American dairy herds, testing and implementation of corrective management may be a better option, especially in herds with a high BLV-seroprevalence. Reasons for clusters of elevated seroprevalences in some parts of Canada will be examined and reported elsewhere.

Houe et al. (1992) (23) showed that when 5 cattle (unvaccinated for BVDV and at least 6 month old) were sampled per herd, the probability of finding at least 1 seropositive animal was 98% if there was a Persistently Infected animal in the herd, and

the probability of having at least 1 seropositive animal if there was no PI in the herd was 5%(16). However, introduction of a transiently infected and shedding animal into a herd could also produce antibody titers in unvaccinated animals. Therefore, our results indicate that 31% of herds were recently exposed to either a persistently or transiently infected animal with BVDV. There were an additional 14% of tested herds which had BVDV-seropositive animals but without any titers $\geq$ 1:64, and some of these herds could be harbouring a persistently infected animal that has not had recent exposure to the 5 tested cattle.

Similar sampling strategies have been utilized efficiently in detecting herds with carriers in European BVDV eradication programs. Our study identifies a similar Canadian estimate of BVDV exposure to Denmark before that country embarked on its eradication program (28) and lower than in Sweden (29). Persistently infected cattle are important sources of infection to other cattle and their identification and elimination is an important feature of an effective BVDV control program, along with a proper vaccination protocol.

MAP-seroprevalence is difficult to estimate because of the chronic nature of the infection and the lack of accurate diagnostic tests for subclinically infected cattle (20). The estimated true prevalence, adjusting for test sensitivity and specificity, is particularly important to examine for MAP due to the poor sensitivity and specificity of ELISA tests for MAP exposure.

Although all of the provincial studies were conducted on the same number of animals per herd with the same IDEXX ELISA test for MAP exposure, using the same cut-off value for interpretation of test positives, interprovincial comparisons between these

seroprevalence estimates should be interpreted with caution for a number of reasons. First, the Ontario study was not a randomly selected population of herds and therefore, may have produced biased prevalence estimates. The study in Ontario primarily consisted of herds that were purposely chosen to monitor disease incidence through accurate record keeping, and therefore could have lead to an underestimate of the true prevalence in Ontario (this may also be applicable to the BVDV- and BLV-seroprevalence estimates). Support of this likely underestimation comes from an earlier large MAP study in Ontario conducted in 1986-89. This survey of 14,932 cows in 304 dairy herds in Ontario found 15.2% of the animals tested seropositive using a LAM-ELISA (12), much higher than the current study of 2.4% test positive cows. It is unlikely that the difference in these estimates is entirely due to differences in test performance of these two ELISA tests. A control program for JD did not exist in Ontario during the time between the two studies, therefore it is highly unlikely that the MAP-seroprevalence would have declined between 1989 and 1998.

Furthermore, sensitivity estimates for MAP-ELISAs differ widely with stage of disease, and therefore differences in age distribution and other factors related to stage of disease would also be important in seroprevalence interpretations. Adjustments to the apparent seroprevalences to calculate estimated true prevalences are unlikely to completely clarify prevalence comparisons between provinces or within a province over time.

Finally, while the vast majority of the testing was done in the same laboratory, directors of sampling for some provinces elected to have laboratory testing done at a laboratory in their own province, occasionally even utilizing a different test. Funding was

not available to test a subsample of sera at all participating laboratories to confirm agreement of results between laboratories. Even when the same test was utilized at the same laboratory, differences in technical staff or protocols over time may have also affected some of the results due to the study being conducted over a period of 5 years. Funding for the project was obtained incrementally, with directors from each province in charge of obtaining the funding necessary for the dairy herds in that province to be tested. The large size and scope of this project, with multiple jurisdictions and coordinators made avoidance of these possible biases impossible.

In conclusion, exposure to BLV is high in Canadian dairy cattle, with significant clustering in certain parts of the country, while exposure to MAP is lower and somewhat spatially clustered. There is evidence that a third of Canadian dairy farms have been exposed to BVDV in the recent past, without any significant regional differences. Given these prevalences and the potential undetected spread of these pathogens, dairy farmers and veterinarians should consider herd screening tests and control programs for herds testing positive.

3.6 Acknowledgements

The authors wish to acknowledge Dr. LeeAnn Forsythe and Ms. Renee Chartier of the Saskatchewan Department of Agriculture (Food and Rural Revitalization, Inspection and Regulatory Management Branch, Animal Health Unit), Karen Kemp of Manitoba Agriculture, the accredited Johne's veterinarians of Alberta, and laboratory scientists and staff of Alberta Agriculture, Food and Rural Development for their technical and logistical support in the collection of samples and data from the participating farmers in Saskatchewan, Manitoba and Alberta, respectively.

The authors wish to acknowledge the following funding sources. Dairy Farmers of Canada, Canadian Food Inspection Agency, and Production Limiting Diseases Committee, Atlantic Veterinary College, Dairy Farmers of PEI, PEI Agricultural Research Investment Fund, Dairy Farmers of Ontario, Manitoba Agriculture, Dairy farmers of Manitoba, Saskatchewan Agriculture, Western Economic Partnership Agreement, and Food Safety Division of Alberta Agriculture, Food and Rural Development.

3.7 References

1. Vanleeuwen JA, Keefe GP, Tremblay R, Power C, Wichtel JJ. Seroprevalence of infection with Mycobacterium avium subspecies paratuberculosis, bovine leukemia virus, and bovine viral diarrhea virus in maritime Canada dairy cattle. Can Vet J 2001;42(3):193-198.

2. Pelzer KD. Economics of bovine leukemia virus infection. Vet Clin North Am Food Anim Pract 1997;13(1):129-141.

3. Ott SL, Johnson R, Wells SJ. Association between bovine-leukosis virus seroprevalence and herd-level productivity on US dairy farms. Prev Vet Med 2003;61(4):249-262.

4. Samagh BS, Kellar JA. Seroepidemiological survey of bovine leukaemia virus infection in canadian cattle. Fourth International Symposium on Bovine Leukosis 1982.

5. Houe H. Epidemiology of bovine viral diarrhea virus. Vet Clin North Am Food Anim Pract 1995;11(3):521-547.

6. Merkal RS, Whipple DL, Sacks JM, Snyder GR. Prevalence of Mycobacterium paratuberculosis in ileocecal lymph nodes of cattle culled in the United States. J Am Vet Med Assoc 1987;190(6):676-680.

7. McKenna SL, Keefe GP, Barkema HW, McClure J, Vanleeuwen JA, Hanna P et al. Cow-level prevalence of paratuberculosis in culled dairy cows in Atlantic Canada and Maine. J Dairy Sci 2004;87(11):3770-3777.

8. Braun RK, Buergelt CD, Littell RC, Linda SB, Simpson JR. Use of an enzyme-linked immunosorbent assay to estimate prevalence of paratuberculosis in cattle of Florida. J Am Vet Med Assoc 1990;196(8):1251-1254.

9. Boelaert F, Walravens K, Biront P, Vermeersch JP, Berkvens D, Godfroid J. Prevalence of paratuberculosis (Johne's disease) in the Belgian cattle population. Vet Microbiol 2000;77(3-4):269-281.

10. Thorne JG, Hardin LE. Estimated prevalence of paratuberculosis in Missouri, USA cattle. Prev Vet Med 1997;31(1-2):51-57.

11. VanLeeuwen JA, Forsythe L, Tiwari A, Chartier R. Seroprevalence of antibodies against bovine leukemia virus, bovine viral diarrhea virus, Mycobacterium avium subspecies paratuberculosis, and Neospora caninum in dairy cattle in Saskatchewan. Can Vet J 2005;46(1):56-58.

12. McNab WB, Meek AH, Martin SW, Duncan JR. Associations between dairy production indices and lipoarabinomannan enzyme-immunoassay results for paratuberculosis. Can J Vet Res 1991;55(4):356-361.

13. Moran PAP. Notes on continuous stochastic phenomena. Biometrika 1950;37:17-23.

14. Kulldorff M, Nagarwalla N. Spatial disease clusters: detection and inference. Stat Med 1995;14(8):799-810.

15. Haddad JP, Dohoo IR, VanLeeuwen JA, Keefe GP, Tiwari A. Seroprevalence and spatial distribution of neospora caninum in canadian dairy herds. Planned to be submiited to Can Vet Journal 2005.

16. Houe H. Serological analysis of a small herd sample to predict presence or absence of animals persistently infected with bovine viral diarrhoea virus (BVDV) in dairy herds. Res Vet Sci 1992;53(3):320-323.

17. Martin SW, Meek AH, Willeberg P. Veterinary Epidemiology - Principles and Methods. Ames, Iowa: Iowa State Univ Pr, 1987.

18. Sockett DC, Conrad TA, Thomas CB, Collins MT. Evaluation of four serological tests for bovine paratuberculosis. J Clin Microbiol 1992;30(5):1134-1139.

19. Johnson RJ, Kaneene JB. Bovine Leukemia Virus. Part I. Descriptive Epidemiology, Clinical Manifestations, and Diagnostic tests. Compendium on Continuing Education 1991;13:328.

20. Dargatz DA, Byrum BA, Barber LK, Sweeney RW, Whitlock RH, Shulaw WP et al. Evaluation of a commercial ELISA for diagnosis of paratuberculosis in cattle. J Am Vet Med Assoc 2001;218(7):1163-1166.

21. Deregt D, Smithson S, Kozub GC. A short incubation serum neutralization test for bovine viral diarrhea virus. Can J Vet Res 1992;56(2):161-164.

22. McKenna SLB, Keefe GP, Barkema HW, Sockett DC. Evaluation of three ELISAs for *Mycobacterium avium* subsp. *paratuberculosis* using tissue and fecal culture as comparison standards. Accepted in Veterinary Microbiology 2005.

23. Houe H. Serological analysis of a small herd sample to predict the presence of animals persistently infected with bovine viral diarrhoea virus (BVDV) in dairy herds. Res Vet Sci 1992;53:320-323.

24. Tiwari A, VanLeeuwen JA, Dohoo IR, Stryhn H, Haddad JP, Keefe GP. Prevalence, spatial distribution and management factors associated with seroprevalence for *Mycobacterium avium* subspecies *paratuberculosis* in Canadian dairy cattle. Proceedings of tenth International Symposium of Veterinary Epidemiology and Economics 2003.

25. Tiwari A, VanLeeuwen JA, Dohoo IR, Haddad JP, Keefe GP. Prevalence, spatial distribution and management factors associated with seroprevalence for Bovine leukemia virus in Canadian dairy cattle. Proceedings of tenth International Symposium of Veterinary Epidemiology and Economics 2003.

26. Herald MTS, Waltner_Toes D, Jacobs RM. The prevalence of anti-bovine leukemia virus antibodies in dairy cows and association with farm management practices, production and culling in Ontario. Prev Vet Med 1992;14:45-55.

27. Richardson GF, MacAulay EB. The prevalence of bovine leukemia virus infection in dairy cows on Prince Edward Island. Can Vet J 1992;33:188-189.

28. Houe H, Baker JC, Maes RK, Lloyd JW, Enevoldsen C. Comparison of the prevalence and incidence of infection with bovine virus diarrhoea virus (BVDV) in Denmark and Michigan and association with possible risk factors. Acta Vet Scand 1995;36(4):521-531.

29. Niskanen R. Relationship between the levels of antibodies to bovine viral diarrhoea virus in bulk tank milk and the prevalence of cows exposed to the virus. Vet Rec 1993;133(14):341-344.

Table 3.1: Year of sample collection, test and laboratory utilized for BLV, BVDV and MAP testing in a seroprevalence survey of eight Canadian provinces.

Province	Year	BLV		BVDV		MAP	
		Diag test	Diag lab	Diag test	Diag lab	Diag test	Diag lab
ALTA	2002-03	IDEXX-E	Nat BLV lab[a]	VN - Singer	ADR Insti	BIOCOR-E	Alta Agri Lab
MAN	2002	IDEXX-E	Nat BLV lab[a]	VN - Singer	ADR Insti	IDEXX-E	Man Agri Lab
NB	1998	IDEXX-E	Nat BLV lab[a]	VN - Singer	ADR Insti	IDEXX-E	Prairie Diag
NS	1998	IDEXX-E	Nat BLV lab[a]	VN - Singer	ADR Insti	IDEXX-E	Prairie Diag
ONT	1998	IDEXX-E	Nat BLV lab[a]	VN - Singer	ADR Insti	IDEXX-E	Prairie Diag
PEI	1998	IDEXX-E	Nat BLV lab[a]	VN - Singer	ADR Insti	IDEXX-E	Prairie Diag
QUE	2002	IDEXX-E	BIOVET Inc[b]	VN - Singer	ARM FRAPP	IDEXX-E	BIOVET Inc[b]
SASK	2001	IDEXX-E	Nat BLV lab[a]	VN - Singer	ADR Insti	IDEXX-E	Prairie Diag

ALTA = Alberta, MAN = Manitoba, NB = New Brunswick, NS = Nova Scotia, ONT = Ontario, PEI = Prince Edward Island, QUE = Quebec and SASK = Saskatchewan, Year- Year of sample collection, Diag test- Diagnostic test, Diag lab- Diagnostic laboratory, E-ELISA, a- National BLV testing laboratory in Prince Edward Island (now in Quebec), b- BIOVET Inc. laboratory in Quebec, VN - Virus Neutralizing antibody test, ADR Insti- Animal Diseases Research Institute in Alberta, ARM FRAPP – Armand Frappier Institute in Quebec; SEMI-COLONS BETWEEN ALL; Prairie Diag- Prairie Diagnostic Services in Saskatchewan, Man Agri Lab- Manitoba Agricultural Laboratory in Manitoba, Alta Agri lab- Alberta Agriculture, Food and Rural Development Food Safety Division Laboratory.

Table 3.2 Demographies and productivity of 10768 sample cows from 398 Canadian dairy herds versus national industry averages.

	Study Population estimates (95% Confidence interval)	Canadian Dairy Industry estimates (from 1999-2003)[8]
Mean 305 d milk	8637-9363 kg	8960-9519 kg
Mean 305 d fat	317-337 kg	331-351 kg
Mean 305 d protein	276-300 kg	290-306 kg
Mean herd size of milking cows	58-76	50-62

[8]http://www.dairyinfo.gc.ca/pdf_files/statsbook2005.pdf.

Table 3.3 Animal and herd level seroprevalence results for Bovine Leukemia Virus for 10768 dairy cows from 398 Canadian farms.

	Cows +[a] p (95% CI)	Herds 1+[b] p (95% CI)	+ in Herds 1+[c] p (95% CI)
Canada	30.3 (25-36)	76.6 (69-84)	40.5 (35-46)
ALTA	29.2 (23-35)	85.0 (76-93)	34.3 (28-41)
MAN	59.8 (51-69)	97.4 (93-100)	61.4 (52-70)
NB	28.7 (18-41)	76.7 (61-93)	39.0 (25-53)
NS	16.1 (10-25)	70.0 (52-87)	21.7 (13-30)
ONT	35.1 (25-45)	85.3 (73-98)	41.0 (31-51)
PEI	16.6 (9-29)	63.3 (45-82)	27.3 (12-42)
QUE	[f]	[f]	[f]
SASK	37.4 (29-46)	89.1 (81-97)	42.0 (33-51)

[a]Cows testing positive

[b]Herds with at least 1 cow testing positive

[c]Cows testing positive in herds with at least 1 cow testing positive

[f]Results for Quebec have not been publicly released at the time of publication

p = Proportion

CI = Confidence Interval

ALTA = Alberta, MAN = Manitoba, NB = New Brunswick, NS = Nova Scotia, ONT = Ontario, PEI = Prince Edward Island, QUE = Quebec and SASK = Saskatchewan.

Table 3.4 Animal and herd level seroprevalence results for Bovine Viral Diarrhea Virus for 323 Canadian dairy farms.

	Cows +[a]	Cows ≥1:64[b]	Herds +[c]	Herds ≥1:64[d]
	p (95% CI)	p (95% CI)	p (95% CI)	p (95% CI)
Canada	19.9 (17-23)	12.3 (10-15)	44.7 (41-48)	31.2 (28-34)
ALTA	17.6 (7-28)	17.1 (7-27)	27.6 (13-42)	27.6 (13-42)
MAN	16.4 (4-29)	15.6 (3-28)	32.0 (12-51)	28.1 (9-47)
NB	37.5 (30-45)	23.7 (17-30)	66.7 (49-85)	46.7 (28-66)
NS	22.0 (15-29)	16.0 (10-22)	51.7 (32-71)	37.9 (19-57)
ONT	22.0 (10-34)	18.7 (7-30)	43.3 (24-62)	36.7 (18-55)
PEI	53.4 (45-61)	44.6 (36-53)	80.0 (65-95)	53.3 (34-72)
QUE	[e]	[e]	[e]	[e]
SASK	28.1 (16-40)	16.8 (6-27)	48.7 (31-66)	29.2 (13-45)

[a]Cows testing positive with BVDV titers ≥1:2

[b]Cows testing positive with BVDV titers ≥ 1:64

[c]Herds with at least 1 cow testing positive with BVDV titers ≥1:2

[d]Herds with at least 1 cow with a BVDV titer ≥ 1:64

[e]Results for Quebec have not been publicly released at the time of publication

p = Proportion

CI = Confidence Interval

ALTA = Alberta, MAN = Manitoba, NB = New Brunswick, NS = Nova Scotia, ONT = Ontario, PEI = Prince Edward Island, QUE = Quebec and SASK = Saskatchewan.

Table 3.5: Animal and herd level seroprevalence results for Mycobacterium avium subspecies paratuberculosis for 10578 dairy cows from 373 Canadian farms.

	Cows +[a]	Herds 1+[b]	Herds 2+[c]	+ in Herds 1+[d]	+ in Herds 2+[e]
	p (95% CI)	p (95% CI)	p (95% CI)	p (95% CI)	p (95% CI)
Canada	3.1 (2.3-3.8)	39.1 (30-48)	18.9 (13-25)	7.8 (6.7-8.8)	11.8 (10.1-13.5)
ALTA	9.1 (6.3-12)	73.2 (59-87)	56.4 (42-71)	12.4 (9.2-16)	15.1 (11.3-19)
MAN	4.5 (2.8-6.2)	68.4 (52-84)	43.1 (25-61)	6.6 (4.6-8.6)	8.7 (6.7-10.6)
NB	2.9 (1.6-5.2)	43.3 (24-62)	16.7 (2-31)	6.4 (3.8-9.1)	11.0 (6.3-15.8)
NS	3.3 (1.7-6.0)	53.3 (34-72)	16.7 (2-31)	5.3 (3.7-7.0)	8.1 (4.5-11.7)
ONT	2.4 (1.3-3.4)	52.9 (35-71)	11.8 (1-23)	4.4 (3.2-5.7)	8.3 (3.0-13.6)
PEI	1.3 (0.7-2.6)	33.3 (15-51)	16.7 (2-31)	5.0 (3.8-6.2)	6.3 (4.7-7.9)
QUE	[f]	[f]	[f]	[f]	[f]
SASK	2.7 (1.6-3.9)	43.3 (27-59)	24.3 (10-39)	6.3 (4.8-7.7)	8.6 (6.7-10.6)

[a]Cows testing positive

[b]Herds with at least 1 cow testing positive

[c]Herds with at least 2 cows testing positive

[d]Cows testing positive in herds with at least 1 cow testing positive

[e]Cows testing positive in herds with at least 2 cows testing positive

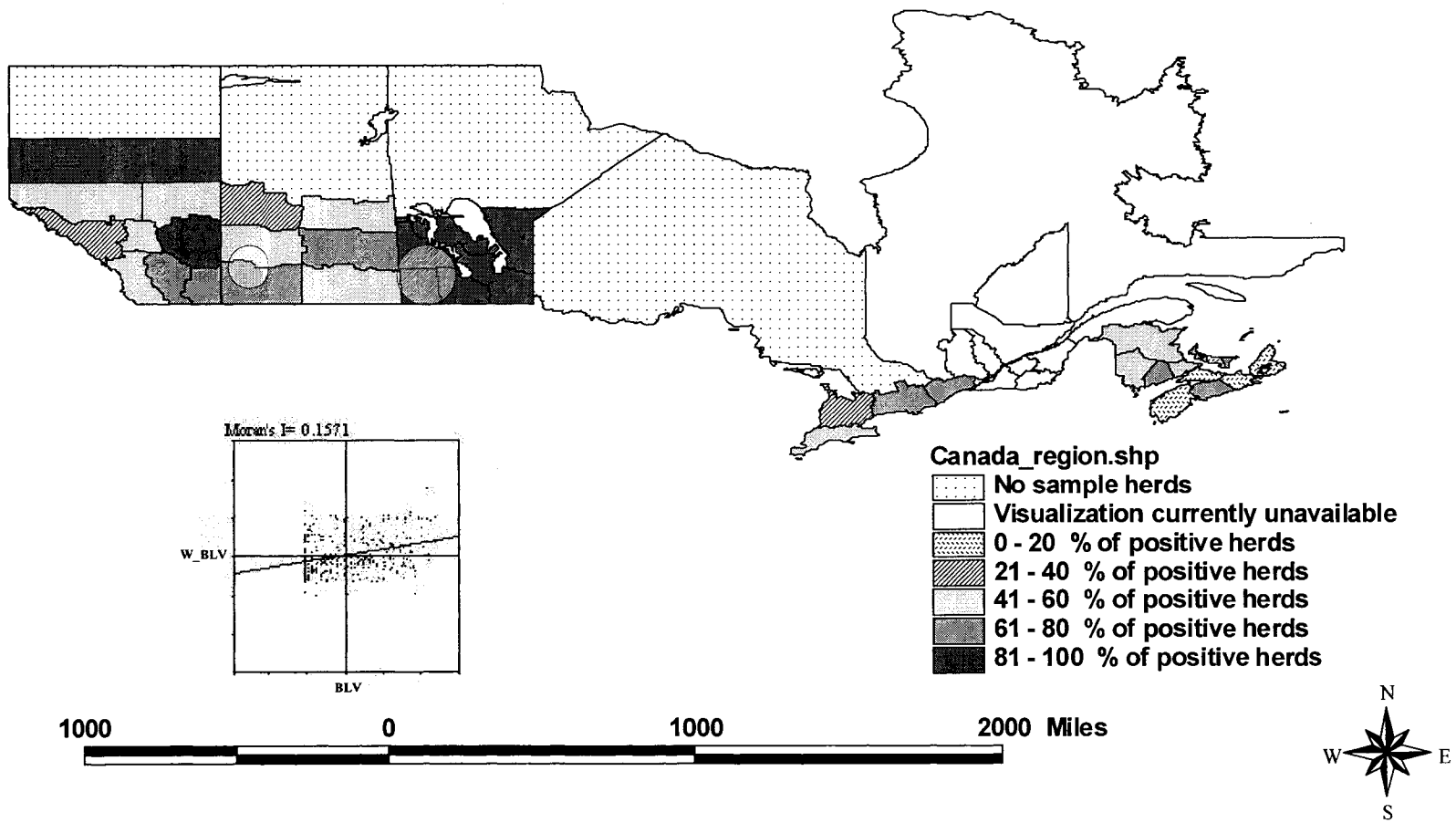
[f]Results for Quebec have not been publicly released at the time of publication

p = Proportion

CI = Confidence Interval

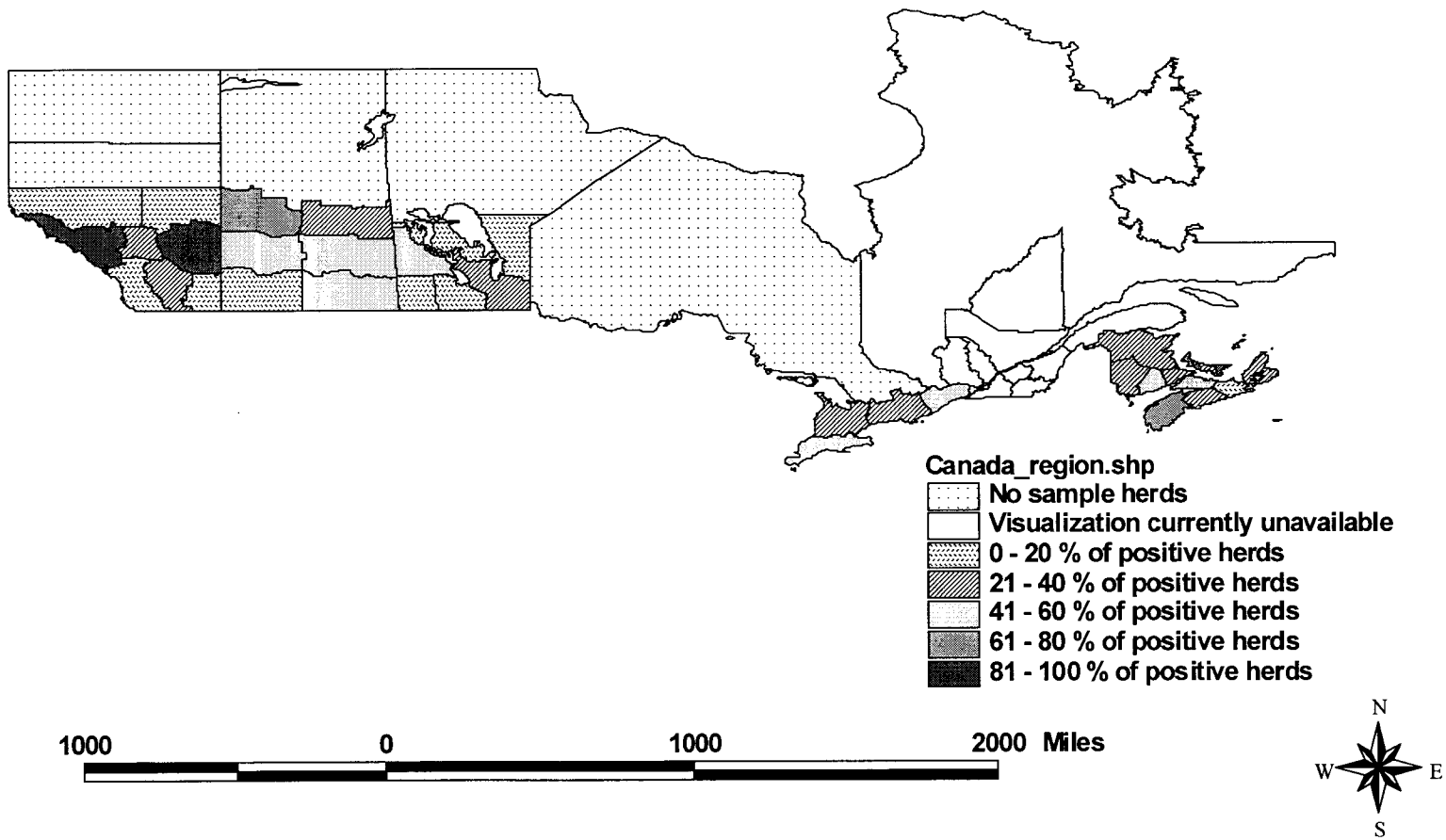
ALTA = Alberta, MAN = Manitoba, NB = New Brunswick, NS = Nova Scotia, ONT = Ontario, PEI = Prince Edward Island, QUE = Quebec and SASK = Saskatchewan.

Map 3.1: Choropleth map showing the percentage of dairy farms with higher "within herd BLV-seroprevalence" than the Canadian median, by region, based on 398 tested farms. Embedded Graph: Moran's I statistic results of spatial autocorrelation of seroprevalence for BLV in Canadian dairy farms[9]. Embedded circles: show the position and size of significant clusters.



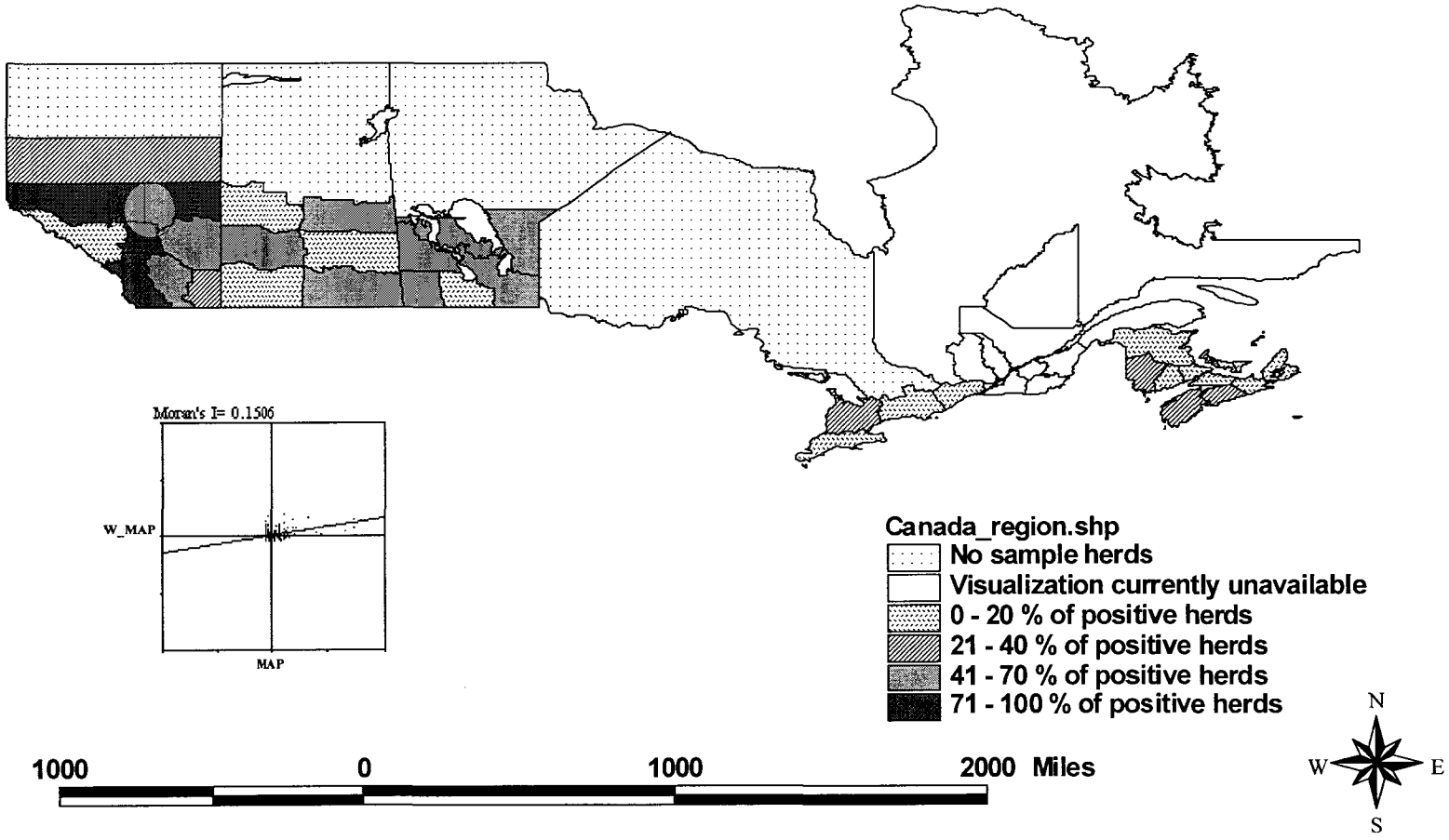
[9] Due to the fact that the results from the province of Qubec are not publicly released at the time of publication, the seroprevalence, and existence and location of clusters in Quebec cannot be reported

Map 3.2: Choropleth map showing the percentage of dairy farms containing unvaccinated cattle ≥ 6 mo of age with BVDV titers ≥ 1:64, by region, based on 323 tested Canadian farms[10].



[10] Due to the fact that the results from the province of Qubec are not publicly released at the time of publication, the seroprevalence, and existence and location of clusters in Quebec cannot be reported

Map 3.3: Choropleth map showing the percentage of farms in each region that had at least 1 seropositive animal, by region, based on 373 tested farms. Embedded Graph: Moran's I statistic results of spatial autocorrelation of seroprevalence for MAP in Canadian dairy farms[11]. Embedded circle: shows the position and size of the significant cluster.



[11] Due to the fact that the results from the province of Qubec are not publicly released at the time of publication, the seroprevalence, and existence and location of clusters in Quebec cannot be reported

CHAPTER 4: Effects of bovine leukemia virus, bovine viral diarrhea virus, *Mycobacterium avium* subspecies *paratuberculosis*, and *Neospora caninum* on milk, fat and protein production and somatic cell count

4.1 Abstract

The purpose of this research was to determine associations among seropositivity for bovine leukemia virus (BLV), bovine viral diarrhea virus (BVDV), *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and *Neospora caninum* (NC) and each of 4 outcome variables (305 day milk, fat and protein production, and somatic cell count) in Canadian dairy cattle. Serum samples from approximately 30 randomly selected cows from each of 342 herds were tested for antibodies against BLV, MAP and NC using commercially available ELISA test kits, while 5 unvaccinated cattle over 6 months of age from each herd were tested for virus neutralizing antibodies to BVDV. Linear mixed models with province, herd and cow as random variables were fitted with restricted maximum likelihood estimates of outcome effects being obtained, while controlling for potential confounding variables.

Regarding 305 day milk production (305 d milk), NC-seropositivity was associated with a 158 kg decrease in 305 d milk in primiparous cows compared to NC-seronegative primiparous cows. MAP-seropositivity was significantly associated with a 212 kg decrease in 305 d milk in 4-plus lactation cows. Cows in BVDV-seropositive herds (at least one unvaccinated animal with a titer $\geq$ 1:64) had significantly lower 305 d milk (by 368 kg) compared to cows in BVDV-seronegative herds.

Among 1st lactation animals, NC-seropositivity was associated with a significant reduction of 5.54 kg and 3.3 kg of 305 d fat and 305 d protein yields, respectively. There were no statistically significant effects of MAP-seropositivity on 305 d fat or 305 d protein yields. BVDV-seropositivity (at the herd level) was associated with reductions in 305 d fat and 305 d protein yields of 10.20 kg/cow and 9.46 kg/cow, respectively. Regarding somatic cell counts, NC- (4+ lactation) and MAP-seropositivity were associated with a 0.094 decrease and 0.098 increase in the lactation mean of the $\log_{10}$ somatic cell count. Cows in BVDV-seropositive herds had a significant increase in the mean $\log_{10}$ somatic cell count of 0.096. BLV-seropositivity was not associated with 305 d milk, 305 d fat, 305 d protein or mean $\log_{10}$ somatic cell count. There was no significant effect modification of any of the outcomes examined from interaction between seropositivity for any of the pathogens.

Approximately, 34.16%, 21.58% and 44.26% of average variations in 305 d milk volume, 305 d fat and protein volumes were at the herd, cow and lactation levels. However, 12.27%, 32.25% and 55.48% of the variation in mean log10 somatic cell count was at the herd, cow and lactation levels. Results from our research will assist better understanding of the economic impacts of these pathogens and justification for their control.

(**Key words:** Enzootic bovine leukosis; bovine viral diarrhea; paratuberculosis; neosporosis; milk production; fat production; protein production; somatic cell count; multilevel modeling.)

Abbreviation key: BLV = bovine leukemia virus, **BVDV** = bovine viral diarrhea virus, **MAP** = *Mycobacterium avium* subspecies *paratuberculosis,* **NC** = *Neospora caninum.*

4.2 Introduction

Infectious disease agents, such as bovine leukemia virus (BLV), bovine viral diarrhea virus (BVDV), *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and *Neospora caninum* (NC), that can be harbored in apparently healthy animals, are undergoing increased scrutiny and research as a result of new World Trade Organization (WTO) regulations concerning animal health and animal movement between countries (WTO, Act 1994). In addition to the trade concerns, each of these organisms can lead to substantial economic losses on infected farms. Based on an Maritime Canada (New Brunswick - NB, Nova Scotia – NS, and Prince Edward Island - PEI) prevalence survey of the above four pathogens, and literature estimates of associated costs, average direct farm costs associated with infection with these organisms were estimated for infected Atlantic Canadian dairy herds (Chi et al., 2002). The annual costs for BLV, BVDV, MAP and NC were reported to be $806, $2421, $2472 and $2304 per infected herd, respectively, assuming an average herd size of 50 cows and an average within herd seroprevalence of BLV, MAP and NC in infected herds of 31%, 7% and 24%, respectively (Keefe and VanLeeuwen, 2000; Vanleeuwen et al., 2001). However, there were limited and/or inconsistent reports regarding the effects of subclinical infection with these pathogens on milk production parameters and somatic cell counts that were applicable to Canadian dairy herds and therefore, very conservative estimates of these effects were included in these cost estimates (Chi et al., 2002). Salient published reports of the effects on production, by pathogen, are summarized below.

The estimated effects of BLV infection on milk volume vary considerably in the published literature, from a negative relationship (Brenner et al., 1989; D'Angelino et al., 1998; Ott et al., 2003; Sargeant et al., 1997) to either no relationship or even a positive relationship (Huber et al., 1981; Pollari et al., 1992; Wu et al., 1989). Seropositive cows produced 3.5% less milk in a case-control study of 102 seropositive and seronegative cattle pairs, matched on age and herd (Brenner et al., 1989). In another study, BLV-seropositive cows produced more milk volume, protein and somatic cell count compared to BLV-seronegative cows, but no significant associations were present after adjusting for possible confounding variables such as age (Jacobs et al., 1991). Milk volume in BLV-infected herds has been reported to be 3% less than BLV-negative herds (Ott et al., 2003). However, these herd differences in milk volume could simply be due to difference in management practices or herd characteristics (e.g. distribution of parity), factors that were not controlled in the analyses in this study. There are no published reports of effects of BLV-seropositivity on milk fat or somatic cell count.

David et al., (1994) reported 23% lower milk volume in affected cows among herds having BVDV outbreaks in the United Kingdom over a 2-week period. Another study conducted in 13,971 Norwegian dairy herds, reported a 7% increase in the incidence of clinical mastitis in the year of apparent BVDV exposure (Waage, 2000). While it is understandable that herds with clinically affected animals would likely have a low milk volume, fat, protein and increased incidence of clinical mastitis, it is still unclear whether herds with primarily subclinically infected animals have lower milk, fat or protein volume, or increased somatic cell count compared to uninfected herds.

For MAP, Benedictus et al., (1987) reported a 6% reduction in milk volume in the second to last lactation and a 16% reduction in the final lactation prior to culling in histopathologically positive, subclinically infected cows compared to culled cows without histopathological evidence of MAP infection. Similarly, a 15% (835 kg) reduction in mean annual milk yield in fecal culture positive subclinically infected cows has been reported, compared to fecal culture negative cows (Abbas et al., 1983). A 4% (376 kg) reduction in mature equivalent milk production in ELISA-positive cows has been reported, compared to ELISA-negative cows (Nordlund et al., 1996). In contrast, others have reported no significant decrease in milk volume in culled, asymptomatic, fecal culture-positive or histopathologically positive cows compared to test-negative culled cows (Buergelt and Duncan, 1978), or ELISA seropositive and fecal culture positive cows (Johnson et al., 2001), compared to test-negative cows. Some of these studies (Johnson et al., 2001; Wilson et al., 1993) have suggested that the observed reduction in milk volume may not occur across all lactations. However, there is still a wide variation in the estimates of reduced milk volume between studies, which may be due to differences in study design, diagnostic tests used, biological differences between the study populations or productivity differences over time.

The effects of MAP-seropositivity on 305 d fat, 305 d protein production and somatic cell count are less well documented and less consistent in the published literature than effects on 305 d milk production. The relationship between MAP-seropositivity and 305 d fat and 305 d protein production was not significant in one study (Johnson et al., 2001). However, in another study, the daily milk fat and protein were significantly lower for fecal culture positive cows compared to test-negative cows (Merkal et al., 1975; Sweeney

et al., 1994). McNab et al., (1991) reported a significant increase in somatic cell count in LAM-ELISA seropositive cows compared to seronegative cows and a higher risk of culling due to mastitis in culture positive cows compared to culture negative cows (Merkal et al., 1975). However, deLisle and Milestone, (1989) and Wilson et al., (1993) failed to find an increased risk of mastitis in cows testing positive for MAP compared to test-negative cows, based on results from serum ELISA and fecal culture tests, respectively.

Published reports on the effects of NC-seropositivity on milk volume are not consistent. NC-seropositive first lactation heifers (Thurmond and Hietala, 1997b) and multiparous cows (Hernandez et al., 2001) produced 384 kg and 385 kg less milk than NC-seronegative first lactation heifers and multiparous cows in 2000 and 700 cow herds, respectively. In contrast, NC-seropositivity in a 600 cow dairy herd was associated with an increase of 0.4 litres/cow/day of milk when compared to NC-seronegative cows (Pfeiffer et al., 2002). In a case-control study including dairy herds in Ontario, NC-seropositive cows in herds with abortion attributable to NC produced significantly less 305 d milk (276 kg) compared to NC-seronegative cows (Hobson et al., 2002). However, in the same project, the NC-seropositive cows from 57 non-randomly selected herds did not have a decrease in milk volume when compared to NC-seronegative cows (Hobson et al., 2002). Therefore, it is still unclear whether NC-seropositivity has an impact on milk production, and whether this putative impact depends upon factors such as age of the cow or whether the herd is experiencing abortion problems.

There is only one published study investigating the relationship between NC-seropositivity and milk protein production. In this study, NC-seropositivity was

associated with higher milk protein production in a 600-cow New Zealand dairy herd compared to NC-seronegative cows (Pfeiffer et al., 2002). Some studies suggest an increased (Hassig and Gottstein, 2002) or decreased (Ould-Amrouche et al., 1999; Peregrine et al., 2004) risk of mastitis with NC-seropositivity in herds with and without abortion problem due to NC. However, whether this relationship is valid in randomly selected herds in other countries is unclear. There are no published studies reporting on the effects of NC-seropositivity on milk fat.

The results from the above studies on associations between these milk production parameters and seropositivity for the four pathogens might not be representative of the true state of nature in the dairy industry in general (or Canadian dairy herds in particular) due to one or more of the following reasons. 1) Many studies used a single large herd, which may not be representative of other large herds or small herds due to differences in management or seroprevalence. 2) If multiple herds were used, these herds were often not randomly selected, leading to possible herd selection bias. 3) Confounding variables, such as parity, were often not controlled in the design or analyses. 4) Associations between the milk production parameters and seropositivity for one pathogen may vary with co-infection with other pathogens (e.g. BLV and BVDV impair cellular immunity) and the effects of these co-infections have been rarely studied in past.

The primary objective of this study was to determine the relationships among seropositivity for BLV, BVDV, MAP and NC and each of 4 outcome variables (305 d milk, 305 d fat, and 305 d protein production and milk somatic cell count) in Canadian dairy cattle while controlling for possible confounding variables, such as lactation number and seropositivity for the other pathogens, and adjusting for clustering at the

lactation, cow, herd and provincial levels. A secondary objective was to determine the contribution of each level of the hierarchical structure (province, herd, cow, lactation) to the total variation in the various outcome variables.

While most investigations in this area of research have been conducted with testing for exposure to 1 of the 4 agents, this investigation is unique in that testing for exposure to all 4 of the agents was conducted on the sampled farms at the same time, allowing for the control of other agent exposures when determining the association between one agent and each outcome variable. Separate analyses were conducted for each of the 4 outcome variables, and therefore the results and tables are presented according to the significant variables associated with each of the 4 outcome variables.

4.3 Materials and methods

The data utilized for this study were from recent prevalence surveys of Canadian dairy herds in Prince Edward Island (PEI), New Brunswick (NB), and Nova Scotia (NS) (Keefe and VanLeeuwen, 2000; Vanleeuwen et al., 2001), Saskatchewan (Vanleeuwen et al., 2005), Ontario (Vanleeuwen et al., 2000), Quebec (CQIASA, 2003), Manitoba (VanLeeuwen et al., 2003) and Alberta (Scott et al., 2005). Briefly summarized, nationally, 30.3, 3.1, and 11.4% of cows were seropositive for exposure to BLV, MAP, and NC (Haddad et al., 2005; Tiwari et al., 2005a), respectively. At the herd level, 76.6% of herds had at least one cow seropositive for BLV and, 18.9% and 64.8% of herds had at least two cows seropositive for MAP and NC, respectively. For BVDV, 31.5% of the herds had at least one unvaccinated heifer/cow with a BVDV titer $\geq$1:64. The sampling for these surveys is briefly described below.

4.3.1 Serum sample collection

A stratified two-stage random sampling procedure was employed. The following criteria were utilized to select participating herds; willingness to participate and provide cows and calves for blood sampling; willingness to allow the blood samples to be tested for antibodies to the 4 pathogens, and willingness to release the results of those blood tests to the research team. In MAN, PEI, NS and NB, eligible herds were also required to be on a monthly milk testing program through their local dairy herd improvement (DHI) organization. The directors from the remaining provinces preferred to include herds not on DHI for their provincial seroprevalence estimates, and therefore, of all the sampled

herds in QUE, ONT, SASK and ALTA, only the herds that were on DHI were eligible for this study.

During the summer of 1998 in Atlantic Canada, dairy herds were randomly selected (using computer generated random numbers) from eligible farms until ninety herds were recruited, thirty from PEI, NB, and NS. Similar recruitment procedures were utilized to select seventy-five, twenty-seven, forty, forty-four, and sixty-six herds from the provinces of Quebec (QUE), Ontario (ONT) Manitoba (MAN), Saskatchewan (SASK), and Alberta (ALTA) in 2002, 1998, 2002, 2001, and 2002/03, respectively. Sampling in ALTA first involved recruiting veterinary practices who serviced dairy herds in the province, and then within these practices, herds were randomly selected. For the ONT, a different sampling protocol was utilized to select 60 herds with extensive records on clinical disease occurrences in order to determine relationships between seroprevalence for these four agents and recorded clinical disease (not reported here). Of these herds, 27 herds met the inclusion criteria and were included in the sample population for this study.

Approximately 30 (less if the total number of cows in the herd was less than 30 cows) lactating animals were randomly selected for blood collection and BLV, MAP and NC testing in each participating herd. For BVDV testing, five unvaccinated (for BVDV) cattle >6 months old were selected for blood collection. In unvaccinated herds, five animals of the 30 cows tested for the other three pathogens were selected. In vaccinated herds, five unvaccinated heifers >6 months old were selected, if available. The BVDV sampling technique was based upon Houe's study (Houe, 1992). Within 24 hours after collection, the blood samples were centrifuged, and the sera were harvested and stored at -20°C until all the samples were collected and ready for testing.

Sample size calculations to determine significant differences in milk production parameters or SCC between seropositive and seronegative cows were not conducted because the primary purpose of the original study was to obtain a reasonably accurate seroprevalence estimate for each province at a reasonable cost. The sample size calculations used to determine the number of required herds for the original prevalence surveys assumed a herd level seroprevalence of 10%, an allowable error of 10%, and a confidence level of 95%. The lowest expected herd seroprevalence of any of the investigated diseases was 10%; therefore this value was used to calculate herd sample size. This calculation lead to a minimum of 30 herds being needed to be tested in each province. The sample size formula used to determine the number of required cows in each herd for the original prevalence surveys assumed an average herd size of 45 lactating cows, an average within-herd prevalence estimate of 10%, confidence of 90%, and sensitivity of the enzyme linked immunosorbent assay (ELISA) test for MAP of 43.0%, the test with the a lowest sensitivity among the four diseases (Sockett et al., 1992). This calculation lead to a total of 30 cattle being needed to be tested in each herd to detect at least 1 infected animal in a herd.

4.3.2 Laboratory analysis

The test utilized by all provinces for BLV antibodies was an enzyme linked immunosorbant assay (ELISA)[1] (sensitivity 98.5%, specificity 99.9%) (Johnson and Kaneene, 1991). A cow was considered to be seropositive for BLV if the serum-to-positive ratio on the ELISA was ≥ 0.50, as recommended by the manufacturer of the test

[1] IDEXX ELISA - IDEXX Corporation, Westbrook, Maine, USA

kit. The BLV ELISA test kit also required a confirmation of positive tests, using a sample-to-negative host-cell ratio of ≥1.8. The BLV testing for all provinces except QUE was conducted at the national BLV testing laboratory of the Canadian Food Inspection Agency in PEI (now in QUE), which is certified to conduct BLV testing for international trade purposes. The BIOVET Inc. laboratory in QUE was utilized for the dairy farms in QUE.

The test utilized by all provinces for MAP antibodies was also an ELISA, with ALTA farms being tested with one brand of ELISA, according to the wishes of the directors of the study in that province[2] (Sockett et al., 1992). The farms in the rest of the participating provinces were tested with a different ELISA[1] (Dargatz et al., 2001). For the first ELISA, an animal was considered to be seropositive for MAP if the optical density value was greater than the mean of the negative control plus 0.100 for bovine sera, as recommended by the manufacturer of the test kit. For the second ELISA, an animal was considered to be seropositive for MAP if the serum-to-positive ratio on the ELISA was ≥0.25, as recommended by the manufacturer of the test kit. The MAP testing for NS, NB, PEI, ONT, and SASK was conducted at Prairie Diagnostic Services in SASK, which is accredited for MAP-ELISA testing by the United States Dept of Agriculture. The BIOVET Inc. laboratory in QUE, the Manitoba Agricultural Laboratory in MAN, and the Alberta Agriculture, Food and Rural Development Food Safety Division Laboratory in ALTA were utilized for their respective provincial testing. The serum samples were tested in duplicate at all locations.

[2] BIOCOR-CSL ELISA - BIOCOR Animal Health, Inc., Omaha, Nebraska, USA

The test utilized by all provinces for NC antibodies was also an ELISA, with ALTA farms being tested with one brand of ELISA, again according to the wishes of the directors of the study in that province[1] (sensitivity 97.6%, specificity 99.5%) (Wu et al., 2002). The farms in the rest of the participating provinces were tested with a different ELISA[3] (sensitivity 99.0%, specificity 98.4%) (Bergeron et al., 2000). A cow was considered to be seropositive for NC if the serum-to-positive ratio for the first and second ELISAs were $\geq$0.60 and $\geq$0.40, respectively. The NC testing was conducted at the BIOVET Inc. laboratory in Quebec for dairy farms from NS, NB, PEI, ONT, MAN, SASK, and the Food Safety Division Laboratory of Alberta Agriculture, Food and Rural Development in ALTA for dairy farms in ALTA. The serum samples were tested in duplicate at both locations. Serum samples from dairy farms in QUE were not tested for antibodies to NC according to the wishes of the directors of the study in that province.

For all provinces, up to five serum samples per farm were tested for antibody against type 1 genotype BVD virus, using virus neutralization to the cytopathic Singer strain (sensitivity 99.6% and specificity 100%;) (Deregt et al., 1992). A herd was considered to be seropositive for BVDV if at least one of the animals tested had a titer above $\geq$1:64 for BVD virus. Testing for BVDV was conducted at the Animal Diseases Research Institute in ALTA for dairy herds in NS, NB, PEI, ONT, MAN, SASK, ALTA, and at the Armand Frappier Laboratory in QUE for dairy herds in QUE.

[3] BIOVET ELISA - BIOVET Inc. - St. Hyacinthe, Quebec, Canada

1

2 4.3.3 Data collection

3 For each tested animal, demographic data, the actual and predicted (if more than 200
4 days in milk) 305-day milk, fat, and protein volumes, somatic cell count data and culling
5 data from January 1, 1999 to December 31st, 2003 were gathered electronically from a
6 central milk-recording database. Somatic cell count was transformed using a logarithm to
7 the base 10 in order to give the variable a normal distribution (base 2 did not produce a
8 normal distribution). Then, the mean was calculated for all of the $\log_{10}$ somatic cell
9 counts for every lactation of each cow. With this transformation and calculation, there
10 was one record for each lactation of each cow during this time period, with each record
11 containing four continuous outcome variables: 305 d milk, 305 d fat, 305 d protein and
12 mean $\log_{10}$ somatic cell count ($ML_{10}SCC$). Cow records with lactation numbers greater
13 than 4 were reclassified as lactation 4 (allowing cows in lactation 3 to be analysed
14 separately from older cows) due to the small number of cows in lactations of 4 or more.
15

16 4.3.4 Statistical analysis

17 Standard descriptive statistics were calculated, and unconditional associations between
18 the independent variables and each of the four outcome variables were computed in Stata,
19 Release 8[4]. Data for independent variables with unconditional associations significant at

[4] Stata - Statistical package, Release.8; Stata Press, College Station, Texas, USA

the $P < 0.15$ were transferred to MLwiN version 1.2[5] to fit the multilevel multivariable models.

In MLwiN, initially, restricted maximum likelihood estimates of parameters were obtained for each of the 4 outcome variables for a simple random intercept model containing only a constant (i.e. no other independent variables) to determine the distribution of variation among the 4 levels (province, herd, animal, lactation) of data and guide the model-building process. Due to non-significant variation at the province level, only 3 levels were utilized with the addition of province as fixed effect. Study design, statistical significance, and biological knowledge of the four pathogens were also utilized to construct the hierarchical causal diagrams and models for each outcome variable.

A three-step approach was utilized for modelling the effects of seropositivity for BLV, BVDV, MAP and NC on each of the 4 outcome variables. First, the effect of seropositivity for BLV, BVDV, MAP and NC on 305 d milk, 305 d fat, 305 d protein and $ML_{10}SCC$ count was estimated while controlling for possible confounding variables, such as lactation number, province and seropositivity for the other three pathogens, and adjusting for clustering at the lactation, cow and herd levels. All independent variables previously identified as having unconditional associations with the dependent variable were added to the models as fixed effects. Variables that were not significant at $P \leq 0.05$ were removed from each outcome model, starting with the variable with the highest P-value.

Second, effect modification among model variables on 305 d milk, 305 d fat, 305 d protein and $ML_{10}SCC$, were examined by creating first-order interaction variables among the model variables. In particular, first-order interaction terms between seropositivity for

[5] MLwiN - Centre for Multilevel Modelling, Institute of Education, London, UK

each of the 4 pathogens and lactation number (1, 2, 3 and 4plus) were examined to determine if the production effects may be occurring within specific lactations. The interaction term between BVDV-seropositivity and lactation number was a mixed level term, with BVDV-seropositivity being a herd level variable while the lactation number was a cow level variable. Also, in order to examine the possible effect of some tests being conducted in different laboratories, first-order interaction terms between the different laboratories and BLV-, BVDV-, MAP- and NC-serostatus were also added to the final models. Again, interaction variables that were not significant at $P \leq 0.05$ were removed from the final models, starting with the variable with the highest P-value.

Finally, the effects of seropositivity for BLV, BVDV, MAP and NC on 305 d milk, 305 d fat, 305 d protein and $ML_{10}SCC$ were allowed to vary at the herd level by using random slope models. If there was significant variation in the effects across herds, the random slopes were retained in the final model.

The diagnostics of the models were evaluated by examining normal probability plots of residuals and plots of residuals vs. predicted values to check on the assumptions of normality and homogeneity of variance.

4.4 Results

4.4.1 Descriptive statistics

The final dataset contained 342 herds with 9,834 cows and 22,665 lactations. There were, on average, 2.3 (maximum 4) lactations per cow. Of the 342 herds, 70 herds had aggressive BVDV vaccination protocols and therefore did not have BVDV results due to the unavailability of unvaccinated heifers > 6 months of age. Comparison of production data from the sampled cows to national industry averages (Table 4.1) did not identify any substantial differences. Therefore, the random selection of herds and cows appeared to have produced a sample population that was representative of the Canadian dairy industry as a whole.

4.4.2 Analytical statistics

There was no significant effect modification of seropositivity for any of the pathogens by other pathogens in any of the 305 d milk, fat, protein and ML10SCC models. Similarly, the first-order interaction terms between the different laboratories and BLV-, BVDV-, MAP- and NC-seropositvity were not significant at the p-value of 0.10 for any of the outcome variables. Therefore, none of these interaction variables was included in the following final models.

4.4.3 305 d milk volume modeling

The results from multilevel linear regression analyses for BLV-, BVDV-, MAP- and NC-seropositivity (by lactation) on 305 d milk volume are shown in Table 4.2. When all lactations were pooled together, the cows in BVDV-seropositive herds had a lower 305 d milk volume of 368 kg/cow ($P = 0.014$) compared to cows in BVDV-seronegative herds, and this decrease in 305 d milk was not substantially different among lactations. MAP-seropositivity in lactation 4+ was associated with a lower 305 d milk volume of 212 kg ($P = 0.048$). NC-seropositivity in first lactation heifers was significantly associated with a lower 305 d milk volume of 158 kg ($P = 0.012$). BLV-seropositive, 4+ lactation cows had a lower 305 d milk volume of 21 kg (data not shown) but this association was not statistically significant ($P > 0.10$).

4.4.4 305 d fat volume modeling

The results from multilevel linear regression analyses for BLV-, BVDV-, MAP- and NC-seropositivity (by lactation) on 305 d fat are shown in Table 4.3. Cows in BVDV-seropositive herds had a marginally lower 305 d fat volume of 10.2 kg ($P = 0.052$) compared to cows in BVDV-seronegative herds. NC-seropositivity in first lactation heifers was significantly associated with a lower 305 d fat of 5.54 kg ($P = 0.027$). There was no significant relationship between BLV- and MAP-seropositivity and 305 d fat volume.

4.4.5 305 d protein volume modeling

The results from multilevel linear regression analyses for BLV-, BVDV-, MAP- and NC-seropositivity (by lactation) on 305 d protein are shown in Table 4.3. The cows in BVDV-seropositive herds had a significantly lower 305 d protein of 9.46 kg (P = 0.036) compared to cows in BVDV-seronegative herds. NC-seropositivity in first lactation heifers was associated with a marginally lower 305 d protein of 3.30 kg (P = 0.080). BLV- and MAP-seropositive cows were not significantly associated with 305 d protein volume.

4.4.6 Mean log10 somatic cell count modeling

The results from multilevel linear regression analyses for BLV-, BVDV-, MAP- and NC-seropositivity (by lactation) on $ML_{10}SCC$ are shown in Table 4.3. As expected, mean log10 somatic cell count increased as lactation number increased. The cows in BVDV-seropositive herds had a significantly higher $ML_{10}SCC$ of 0.096 (P = 0.032) compared to cows in BVDV-seronegative herds. MAP-seropositivity was significantly associated with increased $ML_{10}SCC$ of 0.098 (P = 0.022) compared to MAP-seronegative cows. NC-seropositivity in lactation 4+ heifers was associated with a significantly lower $ML_{10}SCC$ of 0.094 (P = 0.018). BLV-seropositivity was not significantly associated with the $ML_{10}SCC$.

The normal probability plots of residuals, and plots of residuals vs. predicted values for all of the final models indicated that the basic assumptions of normality and homogeneity of variance were not violated for any of the models fit.

4.4.7 Fit of the final models, and proportion of variance

With no fixed effects in the models, the proportions of variance for 305 d milk, 305 d fat, 305 d protein and somatic cell count explained at the province, herd, cow and lactation levels are shown in Table 4.4. Approximately, 34.16%, 21.58% and 44.26% of average variations in 305 d milk volume, 305 d fat and protein volumes were at the herd, cow and lactation levels. However, 12.27%, 32.25% and 55.48% of the variation in mean log10 somatic cell count was at the herd, cow and lactation levels. When the significant fixed effects were in the final models (305 d milk, 305 d fat, 305 d protein and $ML_{10}SCC$), the proportion of variance estimates at the cow and herd levels were increased, while the proportion of variance estimates at the lactation level were decreased.

4.5 Discussion

This study is the first published report of the relationships among seropositivity for four pathogens (BLV, BVDV, MAP and NC) and each of four outcome variables (305 d milk, 305 d fat, and 305 d protein production and milk somatic cell count) in dairy cattle, while controlling for possible confounding variables, such as lactation number and seropositivity for the other pathogens. This study was part of a national study designed to determine the seroprevalence of BLV, BVDV, MAP and NC in Canadian dairy cattle. Eight out of ten Canadian provinces (which comprised 95% of dairy herds in Canada) (DFC, 2002) participated in this study. Similar random selection procedures at the herd (except in ONT) and cow level were utilized to ensure that the study population was representative of the target population. The summary statistics from ONT herds compared to the other provinces, and the herd level residual normal probability plots in the model diagnostic tests indicated that the assumption of representativeness was not violated for the ONT herds (data not shown).

There was no significant effect modification on 305 d milk, fat, protein and ML_{10}SCCs from interactions between seropositivity for any of the pathogens. While it is possible that there truly are no interactive effects from seropositivity for more than one of the pathogens tested in our study, there are other possible explanations for not detecting any interactions in our study. First, there were limited numbers of cattle that were seropositive for multiple pathogens in our study, providing somewhat limited power to detect interactions between seropositivity (Tiwari et al., 2005a). Due to the low sensitivity of ELISAs to detect MAP-infected cattle (Dargatz et al., 2001), the power to

detect interactions was particularly problematic. For BVDV, only five unvaccinated cattle over 6 mo of age were tested per herd, which were calves on farms where cows were vaccinated for BVDV (70 farms). Therefore, the serostatus of cows for BVDV exposure was unknown on 19.3% of farms in our study.

Another possible reason for no observed interactions on milk production or SCC between seropositivity for the four pathogens tested in this study could be that the timing of the effects of infection did not coincide frequently enough to be detected. It is known that the clinical manifestations of infections with BLV and NC generally occur at differing times relative to the time of infection. Cattle infected with BLV usually do not develop lymphosarcoma until later in life (Detilleux et al., 1991). Conversely, abortion due to NC often occurs during the pregnancy immediately after infection. For animals infected through vertical transmission, abortion often occurs during their first pregnancy as a heifer, or second pregnancy as a first-calf heifer (Pfeiffer et al., 2002; Thurmond and Hietala, 1997a). For animals infected through horizontal transmission, abortion is often during the pregnancy at or immediately after the time of infection (Dubey et al., 1992). If clinical manifestations do not frequently coincide in time, then it is reasonable to believe that any subclinical impacts on milk production or SCC may not frequently coincide in time either. For BVDV, any cows that were seropositive due to exposure to circulating virus on the farm were likely to be only transiently infected, and at some unknown point in time prior to blood sampling. Impacts on production and SCC may no longer be occurring in these cows at the time of blood sampling, and these impacts also may not be coinciding with impacts from infection of the other pathogens. Furthermore, depending

on the protection afforded by BVDV vaccination of the cows on a farm, transiently infected cows may not undergo any detectable impacts on production or SCC.

4.5.1 Multilevel models

The use of multilevel models has grown recently due to an increase in computational power and the recognition of biased results produced by regular regression analyses when they are applied to clustered animal study populations. Clustered study populations violate the assumption of independence of observations in ordinary regression models. Multilevel models involve two or more levels of relationships among variables that are arranged in a hierarchy (e.g. lactations in a cow, cows in a herd, etc.). Restricted maximum likelihood estimates were utilized because they are less prone to bias than the full maximum-likelihood estimates (Kreft and De Leeuw, 1998). Although lactations within cows are repeated measures, the correlations among all lactations within a cow in our study were assumed to be equal due to the small number of lactations within each cow (mean = 2.3). The multilevel models were also utilized to identify the level, which accounts for the greatest amount of variability in the dependent variable, so that interventions can be targeted at this level in order to have the greatest impact on the variable.

In our study, data from all available lactations for the sampled cattle were included in the final dataset for 2 reasons. Most cattle infected with BVDV (Houe, 1995), MAP (Hagan, 1938; Larsen et al., 1975) and NC (Davison et al., 1999a; Pare et al., 1996) are likely infected in utero or early in life. Furthermore, utilizing all lactations would provide more statistical power to detect significant differences between seropositive and

seronegative cows and herds. However, a subset analysis was conducted utilizing only the lactations in which the blood testing occurred, producing very similar but less statistically significant results (results not shown). Therefore, all lactations were utilized.

4.5.2 BLV

Relationships between BLV-seropositivity and milk volume, fat and mastitis have been reported (Emanuelson et al., 1992; Jacobs et al., 1991; Pollari et al., 1993; Wu et al., 1989), but these studies were either in small numbers of herds with high seroprevalence, or had inappropriate control of confounding variables or adjustments for clustering. After controlling for herd size and age, no significant relationships between BLV-seropositivity and milk volume and mastitis were found (Jacobs et al., 1991). In a study involving 150 herds (Jacobs et al., 1995), BLV-seropositive cows older than 5 yrs had higher somatic cell counts than BLV-seronegative cows. However, this association was not adjusted for within-herd clustering, and for effects of other infectious agents, such as BVDV. In our study of randomly selected cows and herds with a wide variety of seroprevalences, after controlling for age and other infective agents effects, seropositivity for BLV was not associated with 305 d milk, 305 d fat, 305 d protein or $ML_{10}SCC$. However, there was a trend seen, where BLV-seropositive cows in 4-plus lactations had a slightly lower milk volume than BLV-seronegative cows in 4-plus lactations.

BLV can cause alterations in cell-mediated immunity; with up to 30% of BLV-seropositive cattle eventually developing persistent lymphocytosis, while less than 5% of BLV-seropositive cows develop lymphosarcoma during their normal lifetime in commercial dairy herds (Detilleux et al., 1991). Lymphosarcoma usually develops in

cattle between 4 and 8 years of age (Muller et al., 1987), perhaps explaining the trend toward reduced 305 d milk volume seen in BLV-seropositive, 4-plus lactation cows in our study. However, with a low proportion of seropositive cows surviving normal culling pressures and developing lymphosarcoma in higher parities, significant associations between BLV-seropositivity and milk volume, fat and protein or mastitis are unlikely to be detected, except perhaps in high prevalence herds where the herd burden of virus is large, potentially leading to a higher likelihood of negative impacts. In our study the BLV-seroprevalence was 31.7%. It remains unclear whether persistent lymphocytosis leads to low milk volume and high somatic cell count either directly or through another ailment.

4.5.3 BVDV

In our randomly selected population of cattle and herds, cows in BVDV-seropositive herds were significantly associated with a lower milk volume, fat, protein and increased somatic cell count compared to cows in BVDV-seronegative herds. The pathogenesis underlying this relationship is unclear, but a number of hypotheses are possible, including clinical disease related to pneumonia, mastitis, abortion or immunosuppression. Each is discussed in turn.

Another study (Waage, 2000), based upon acute clinical BVDV infection, have found impaired milk production parameters among the affected cattle relative to non-affected cattle. It is unknown whether the BVDV-seropositive herds in our study were undergoing clinical manifestations of BVDV infection, leading to the observed associations. In a related study, we found that cows in BVDV-seropositive herds had a 1.86 and 1.43 times

higher hazard of culling for low milk volume and mastitis, respectively, than cows in BVDV-seronegative herds (Tiwari et al., 2005b).

David et al., (1994) reported a 23% loss in milk following an abortion. The risk of abortion among susceptible cattle that become infected with BVDV has been reported to vary from 4.8% in Denmark (Meyling et al., 1990) to 26% in England (Murray, 1990). Therefore abortions due to BVDV-seropositivity may have also contributed to a loss of milk volume, fat and protein.

During BVDV infection, neutrophil (Roth et al., 1981) and lymphocyte (Lamontagne et al., 1989) suppression result in impaired resistance and act as an important predisposing factor for intra-mammary or other infections in the cow (Cai et al., 1994; Houe et al., 1995). However, in our study, there was no significant effect modification on 305 d milk, fat, protein and ML_{10}SCCs from interactions between seropositivity for any of the pathogens.

4.5.4 MAP

In our study, after adjusting for province, herd and cow clustering and controlling for possible confounding variables, MAP-seropositivity in 4+ lactation cows was significantly associated with a lower 305 d milk of 219 kg ($P = 0.028$). These results support conclusions made in previous studies (Johnson et al., 2001; Kudahl et al., 2004; Sockett et al., 1992; Wilson et al., 1993) that the effect of MAP-seropositivity on milk volume largely depends upon the parity of the cow. This effect modification of lactation assists in understanding the results from previous studies, which range from no effect

(Johnson et al., 2001) to 19.5% lower milk production in fecal culture positive cows compared to fecal culture negative cows (Benedictus et al., 1987).

Our results for 305 d fat and 305 d protein volumes support results from previous studies by Johnson et al., (2001). However, the opposite result was found by Sweeney et al., (1994) and Collins and Nordlund, (1991). These differences could be due to either study design (non random selection of 14, 23 and 7 herds in Sweeney et al., 1994; Collins et al., 1991 and Johnson et al., 2001, respectively), diagnostic test utilized (fecal culture in Sweeney et al., 1994; serum ELISA in Collins et al., 1991 and fecal culture and ELISA in Johnson et al., 2001), or statistical analyses (effect of MAP-seropositivity in each lactation was not estimated due to small sample sizes).

McNab et al., (1991) found an increased somatic cell count in LAM-ELISA-positive cows compared to negative cows, which was confirmed by our study results. However, it is unclear whether there is also an increased risk of clinical mastitis in MAP-seropositive cows compared to seronegative cows, as this would require data that were not collected in our study.

The results of our study likely represent very conservative estimates of the MAP-seropositivity on 305 d milk, 305 d fat, 305 d protein and increased $ML_{10}SCC$ because of the misclassification bias associated with ELISA test results for MAP (Whitlock et al., 2000). Due to the very poor sensitivity of ELISA tests for MAP exposure, ranging from 8% to 80% (McKenna et al., 2004; Sockett et al., 1992), depending on the stage of infection of the test population, numerous infected animals likely gave false negative test results. Specificity of ELISAs for MAP exposure are also not ideal, ranging from 90-99% (Dargatz et al., 2001), particularly when utilized on a large number of animals in a low

prevalence population, leading to numerous false positive test results (low positive predictive values). The impact of these misclassifications, assuming they are not differential in nature (other than with respect to age), would bias these model estimates toward the null.

It is certainly biologically plausible for MAP infection to lead to reduced milk volume in higher parities. MAP infection typically occurs early in life and then causes changes in cell-mediated immunity and progressive, localized granulomatous lesions in lymph nodes and the lamina propria of the terminal ileum. Granulomatous lesions can also be disseminated throughout the entire gastrointestinal (GI) tract, leading to reduced nutrient absorption (Chiodini et al., 1984). However the bacteria are very slow growing, typically leading to clinical gastroenteritis in higher parity cows. Reduced GI absorption might worsen a negative energy balance in early lactation, while alterations in cell-mediated immunity may decrease the resistance of cows towards other infectious diseases, possibly resulting in a reduced milk volume (Kudahl et al., 2004; Wilson et al., 1993) and increased somatic cell count (McNab et al., 1991).

4.5.5 NC

After adjusting for clustering effects and controlling for potential confounding variables, NC-seropositive first lactation heifers had a 161 kg lower 305 d milk volume compared to NC-seronegative first lactation heifers. This finding confirms that this relationship is applicable to a variety of herd sizes and management levels of dairy farms and not just on a single 2000 cow Holstein herd in California with a history of abortion problems associated with NC (Thurmond and Hietala, 1997b). Similarly, in a case-

control study involving 83 dairy herds in Ontario (with 28 herds having abortions attributable to NC), NC-seropositivity was significantly associated with a decrease of 276 kg of 305 d milk volume ($P \leq 0.05$). Much of this difference was due to the discovery that there was a trend toward first lactation NC-seropositive heifers having 295 kg lower 305 d milk volume ($P = 0.11$) (Hobson et al., 2002). Our lower estimate of impact on 305 d milk production may be due to the mixing of NC-infected herds with and without active abortion problems (data on abortions were not available).

We also found that seropositivity for NC in first lactation heifers was associated with lower 305 d milk fat and protein volumes compared to NC-seronegative first lactation heifers. Conversely, in one New Zealand herd, NC-seropositivity was associated with higher milk protein (Pfeiffer et al., 2002). However, the effect of NC on different lactations was not determined in that study. We found that, over all lactations, NC-seropositivity was not significantly associated with milk fat and protein. NC-seropositive cows that are high milk producers are less likely to be culled from a herd due to their superior milk production compared to NC-seropositive low milk producers, which may explain the lack of association when all lactations were considered.

The biological rationale of reduced milk volume due to NC-seropositivity in first lactation heifers is still not clear but may be related to reduced milk volume after an abortion in first lactation heifers. The major clinical effect of NC in cattle is abortion and the percentage of abortions among dairy cattle attributed to NC has been estimated to be from 12.5% (Davison et al., 1999b) to 38.7% (Mainar-Jaime et al., 1999), making it one of the most common causes of abortion in cattle. In one abortion epidemic due to NC in New Zealand, where 33% of the breeding herd aborted, abortions were most common in

cows 4 yrs old or younger (Thornton et al., 1994). In another study, conducted in one 600 cows herd in New Zealand, the abortion risk was highest in heifers and decreased with the age of the cow (Pfeiffer et al., 2002). This increased abortion risk in young cattle may be due to transplacental transmission of NC from cow to calf being the major route of infection in cattle. On the basis of pre-colostral antibodies in calves, this transmission varies from 95% (Pare et al., 1996) to 81% (Davison et al., 1999a), and perhaps even lower, depending on the age-structure of the herd, with older cows being less likely to abort than first lactation cows (Dijkstra et al., 2003) showed that cattle produce less milk following an abortion, perhaps related to abortion sequel, such as retained placenta after birth and metritis.

In 57 Ontario herds without abortion problem (Peregrine et al., 2004), the NC-seropositive cows were less likely to have high linear score of somatic cell count compared to NC-seronegative cows. To a certain extent, this study corroborates our results that suggest that high lactation NC-seropositive cows were associated with improved udder health (low $ML_{10}SCC$). In the study in Ontario, however, the interaction between lactation and NC-positivity was not examined. The reason that mature NC-seropositive cows had improved udder health is not clear but may because the younger NC-seropositive cattle were in an active phase of immunological response to the NC infection, while older NC-seropositive cattle were no longer actively responding to the NC infection, allowing the older cattle to more effectively fight other udder pathogens. This speculation is based on the premise that the majority of NC infection are vertically transmitted (Davison et al., 1999a; Pare et al., 1996).

Our results suggested that the 44.65%, 21.95% and 33.40% of average variation in 305 d milk volume, 305 d fat and protein volumes was at the lactation, cow and herd levels. We also found that the 55.62%, 32.51% and 11.87% of the variation in $ML_{10}SCC$ was at the lactation, cow and herd levels, showing that even more of the variation for SCC is at the lactation level compared to the milk production parameters. Conversely, in calving to conception interval, 86% of the variation resided at the lactation level with only 7%, 6% and 2% at the cow, herd and regional level (Dohoo et al., 2001).

A higher percentage of variation in milk production indices at the herd level as compare to inter-calving interval shows the importance of herd level factors (like cattle feed) in targeting interventions to have desired impact. MAP, BLV and NC were tested and interpretable at the cow level, while BVDV was tested at the animal level, but interpreted at the herd level. Significant associations were detected for MAP- and NC-seropositivity, but only for certain lactations. Therefore, efforts to reduce transmission of MAP and NC should be made at the animal and herd levels, but efforts to reduce impacts on milk production parameters and SCC should be focused at the lactation level. For BVDV, there was not a substantial difference in the associations between milk production and BVDV-seropositivity at the herd level, and therefore efforts to reduce impacts of BVD infection on milk production should not differ among lactations.

4.6 Conclusions

In contrast to the abstract and body of this paper, this conclusion will summarize the results by pathogen rather than outcome. NC-seropositivity in first lactation heifers was significantly associated with a lower 305 d milk and 305 d fat volume of 158 kg and 5.54 kg. However, NC-seropositivity in 4+ lactation cows were significantly associated with a lower $ML_{10}SCC$ of 0.094. MAP-seropositivity in 4+ lactation cows was significantly associated with a lower 305 d milk of 212 kg. When all lactations were pooled together, the MAP-seropositivity was also significantly associated with increased $ML_{10}SCC$ of 0.098. When all lactations were pooled together, the cows in BVDV-seropositive herds had a lower 305 d milk, 305 d fat and 305 d protein volume of 368 kg, 10.2 kg and 9.46 kg, respectively. The cows in BVDV-seropositive herds had an increased $ML_{10}SCC$ of 0.096. The BLV-seropositivity was not associated with volume of 305 d milk, fat, or protein or $ML_{10}SCC$. There were no interactions between seropositivity for the 4 pathogens with respect to the 4 outcome variables investigated. Based on the final models, approximately, 34.16%, 21.58% and 44.26% of average variations in 305 d milk volume, 305 d fat and protein volumes were at the herd, cow and lactation levels. However, 12.27%, 32.25% and 55.48% of the variation in mean log10 somatic cell count was at the herd, cow and lactation levels. Results from our research will help in better understanding the economic impacts of these pathogens and justification for their control.

4.7 Acknowledgement

The authors wish to acknowledge Dr. LeeAnn Forsythe and Ms. Renee Chartier of the Saskatchewan Department of Agriculture, Food and Rural Revitalization (Inspection and Regulatory Management Branch, Animal Health Unit), Karen Kemp of Manitoba Agriculture and Food, the accredited Johne's veterinarians of Alberta, and laboratory scientists and staff of Alberta Agriculture, Food and Rural Development for their technical and logistical support in the collection of samples and data from the participating farmers in Saskatchewan, Manitoba and Alberta, respectively.

The authors also wish to acknowledge the following funding sources. Dairy Farmers of Canada, Dairy Herd Improvement companies, Canadian Food Inspection Agency, and Production Limiting Diseases Committee, Atlantic Veterinary College, Dairy Farmers of PEI, PEI Agricultural Research Investment Fund, Dairy Farmers of Ontario, Manitoba Agriculture, Saskatchewan Agriculture, Western Economic Partnership Agreement, and Food Safety Division of Alberta Agriculture, Food and Rural Development.

4.8 Reference List

Abbas, B., Riemann, H.P., Hird, D.W., 1983. Diagnosis of Johne's disease (paratuberculosis) in Northern California cattle and a note of it's economic significance. Calif. Vet. 37, 20-24.

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

Bergeron, N., Fecteau, G., Pare, J., Martineau, R., Villeneuve, A., 2000. Vertical and horizontal transmission of Neospora caninum in dairy herds in Quebec. Can. Vet J 41, 464-467.

Brenner, J., Van Haam, M., Savir, D., Trainin, Z., 1989. The implication of BLV infection in the productivity, reproductive capacity and survival rate of a dairy cow. Vet Immunol. Immunopathol. 22, 299-305.

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

Cai, T.Q., Weston, P.G., Lund, L.A., Brodie, B., McKenna, D.J., Wagner, W.C., 1994. Association between neutrophil functions and periparturient disorders in cows. Am. J Vet Res 55, 934-943.

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

Chiodini, R.J., Van Kruiningen, H.J., Merkal, R.S., 1984. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. Cornell Vet 74, 218-262.

Collins, M.T., Nordlund, K., 1991. Milk production levels in cow ELISA positive for serum antibodies to M. paratuberculosis. Proceedings of the Third International Colloquim on Paratuberculosis. Orlando, Florida, USA. 401-409.

CQIASA 2003. Centre Québécois d'inspection des aliments et de santé animale. Rapport final: Enquête de prévalence de la paratuberculose, de la leucose bovine enzootique et des immunotolérants à la diarrhée virale bovine dans les troupeaux laitiers du Québec.

D'Angelino, J.L., Garcia, M., Birgel, E.H., 1998. Productive and reproductive performance in cattle infected with bovine leukosis virus. J Dairy Res 65, 693-695.

Dargatz, D.A., Byrum, B.A., Barber, L.K., Sweeney, R.W., Whitlock, R.H., Shulaw, W.P., Jacobson, R.H., Stabel, J.R., 2001. Evaluation of a commercial ELISA for diagnosis of paratuberculosis in cattle. J Am. Vet Med. Assoc. 218, 1163-1166.

David, G.P., Crawshaw, T.R., Gunning, R.F., Hibberd, R.C., Lloyd, G.M., Marsh, P.R., 1994. Severe disease in adult dairy cattle in three UK dairy herds associated with BVD virus infection. Vet Rec 134, 468-472.

Davison, H.C., Otter, A., Trees, A.J., 1999a. Estimation of vertical and horizontal transmission parameters of Neospora caninum infections in dairy cattle. Int. J Parasitol. 29, 1683-1689.

Davison, H.C., Otter, A., Trees, A.J., 1999b. Significance of Neospora caninum in British dairy cattle determined by estimation of seroprevalence in normally calving cattle and aborting cattle. Int. J Parasitol. 29, 1189-1194.

deLisle,G.W., B.A.Milestone, 1989. The economic impact of johne's disease in New Zealand. In: Milner,A., Wood,P. (Eds) Johne's disease. East Melbourne, CSIRO, pp. 41-45.

Deregt, D., Smithson, S., Kozub, G.C., 1992. A short incubation serum neutralization test for bovine viral diarrhea virus. Can J Vet Res 56, 161-164.

Detilleux, J.C., Freeman, A.E., Miller, L.D., 1991. Comparison of natural transmission of bovine leukemia virus in Holstein cows of two genetic lines selected for high and average milk production. Am. J Vet Res 52, 1551-1555.

Dijkstra, T., Barkema, H.W., Eysker, M., Beiboer, M.L., Wouda, W., 2003. Evaluation of a single serological screening of dairy herds for Neospora caninum antibodies. Vet Parasitol. 110, 161-169.

Dohoo, I.R., Tillard, E., Stryhn, H., Faye, B., 2001. The use of multilevel models to evaluate sources of variation in reproductive performance in dairy cattle in Reunion Island. Prev. Vet Med. 50, 127-144.

Dubey, J.P., Lindsay, D.S., Anderson, M.L., Davis, S.W., Shen, S.K., 1992. Induced transplacental transmission of Neospora caninum in cattle. J Am. Vet Med. Assoc. 201, 709-713.

Emanuelson, U., Scherling, K., Pettersson, H., 1992. Relationships Between herd bovin leukemia virus infection status and reproduction, disease incidence, and productivity in Swedish dairy herds. Prev. Vet. Med. 12, 121-131.

Haddad, J.P., Dohoo, I.R., VanLeeuwen , J.A., Tiwari, A., Keefe, G.P., Scott, M., Whiting, T., 2005. Seroprevalence and spatial distribution of Neospora caninum in Canadian dairy cattle. Submitted to Can Vet J .

Hagan, W.A., 1938. Age as a factor in susceptibility to Johne's disease. Cornell Veterinarian 28, 34-40.

Hassig, M., Gottstein, B., 2002. Epidemiological investigations of abortions due to Neospora caninum on Swiss dairy farms. Vet Rec. 150, 538-542.

Hernandez, J., Risco, C., Donovan, A., 2001. Association between exposure to Neospora caninum and milk production in dairy cows. J Am. Vet Med. Assoc. 219, 632-635.

Hobson, J.C., Duffield, T.F., Kelton, D., Lissemore, K., Hietala, S.K., Leslie, K.E., McEwen, B., Cramer, G., Peregrine, A.S., 2002. Neospora caninum serostatus and milk production of Holstein cattle. J Am. Vet Med. Assoc. 221, 1160-1164.

Houe, H., 1992. Serological analysis of a small herd sample to predict presence or absence of animals persistently infected with bovine viral diarrhoea virus (BVDV) in dairy herds. Res Vet Sci 53, 320-323.

Houe, H., 1995. Epidemiology of bovine viral diarrhea virus. Vet Clin. North Am. Food Anim Pract. 11, 521-547.

Houe, H., Baker, J.C., Maes, R.K., Lloyd, J.W., Enevoldsen, C., 1995. Comparison of the prevalence and incidence of infection with bovine virus diarrhoea virus (BVDV) in Denmark and Michigan and association with possible risk factors. Acta Vet Scand. 36, 521-531.

Huber, N.L., DiGiacomo, R.F., Evermann, J.F., Studer, E., 1981. Bovine leukemia virus infection in a large Holstein herd: prospective comparison of production and reproductive performance in antibody-negative and antibody-positive cows. Am. J Vet Res 42, 1477-1481.

Jacobs, R.M., Heeney, J.L., Godkin, M.A., Leslie, K.E., Taylor, J.A., Davies, C., Valli, V.E., 1991. Production and related variables in bovine leukaemia virus-infected cows. Vet Res Commun. 15, 463-474.

Jacobs, R.M., Pollari, F.L., McNab, W.B., Jefferson, B., 1995. A serological survey of bovine syncytial virus in Ontario: associations with bovine leukemia and immunodeficiency-like viruses, production records, and management practices. Can J Vet Res 59, 271-278.

Johnson, R.J., Kaneene, J.B., 1991. Bovine Leukemia Virus. Part I. Descriptive Epidemiology, Clinical Manifestations, and Diagnostic tests. Compendium on Continuing Education 13, 328.

Johnson, Y.J., Kaneene, J.B., Gardiner, J.C., Lloyd, J.W., Sprecher, D.J., Coe, P.H., 2001. The effect of subclinical Mycobacterium paratuberculosis infection on milk production in Michigan dairy cows. J Dairy Sci 84, 2188-2194.

Keefe, G.P., VanLeeuwen, J.A., 2000. Neospora then and now: prevalence of Neospora caninum in Maritime Canada in 1979, 1989, and 1998. Can. Vet J 41, 864-866.

Introducing Multilevel Modeling1998, Sage, London.

Kudahl, A., Nielsen, S.S., Sorensen, J.T., 2004. Relationship between antibodies against Mycobacterium avium subsp. paratuberculosis in milk and shape of lactation curves. Prev. Vet Med. 62, 119-134.

Lamontagne, L., Lafortune, P., Fournel, M., 1989. Modulation of the cellular immune responses to T-cell-dependent and T cell-independent antigens in lambs with induced bovine viral diarrhea virus infection. Am. J Vet Res 50, 1604-1608.

Larsen, A.B., Merkal, R.S., Cutlip, R.C., 1975. Age of cattle as related to resistance to infection with Mycobacterium paratuberculosis. Am. J Vet Res 36, 255-257.

Mainar-Jaime, R.C., Thurmond, M.C., Berzal-Herranz, B., Hietala, S.K., 1999. Seroprevalence of Neospora caninum and abortion in dairy cows in northern Spain. Vet Rec. 145, 72-75.

McKenna, S.L., Keefe, G.P., Barkema, H.W., McClure, J., Vanleeuwen, J.A., Hanna, P., Sockett, D.C., 2004. Cow-level prevalence of paratuberculosis in culled dairy cows in Atlantic Canada and Maine. J Dairy Sci 87, 3770-3777.

McNab, W.B., Meek, A.H., Martin, S.W., Duncan, J.R., 1991. Associations between dairy production indices and lipoarabinomannan enzyme-immunoassay results for paratuberculosis. Can J Vet Res 55, 356-361.

Merkal, R.S., Larsen, A.B., Booth, G.D., 1975. Analysis of the effects of inapparent bovine paratuberculosis. Am. J Dig. Dis. 20, 597-598.

Meyling, A., Houe, H., Jensen, A.M., 1990. Epidemiology of bovine virus diarrhoea virus. Rev. Sci Tech. 9, 75-93.

Muller, M., Wittmann, W., Liebermann, H., 1987. [The effect of the selection of calves having antibodies against the bovine leukosis virus at the time of birth--progress and results of leukosis prevention in young cattle breeding facilities]. Arch. Exp. Veterinarmed. 41, 738-742.

Murray, R.D., 1990. A field investigation of causes of abortion in dairy cattle. Vet Rec 127, 543-547.

Nordlund, K.V., Goodger, W.J., Pelletier, J., Collins, M.T., 1996. Associations between subclinical paratuberculosis and milk production, milk components, and somatic cell counts in dairy herds. J Am. Vet Med. Assoc. 208, 1872-1876.

Ott, S.L., Johnson, R., Wells, S.J., 2003. Association between bovine-leukosis virus seroprevalence and herd-level productivity on US dairy farms. Prev. Vet Med. 61, 249-262.

Ould-Amrouche, A., Klein, F., Osdoit, C., Mohammed, H.O., Touratier, A., Sanaa, M., Mialot, J.P., 1999. Estimation of Neospora caninum seroprevalence in dairy cattle from Normandy, France. Vet Res. 30, 531-538.

Pare, J., Thurmond, M.C., Hietala, S.K., 1996. Congenital Neospora caninum infection in dairy cattle and associated calfhood mortality. Can. J Vet Res. 60, 133-139.

Peregrine, A.S., Duffield, T.F., Wideman, G., Kelton, D., Hobson, J., Cramer, G., Hietala, S.K., 2004. Udder health in dairy cattle infected with Neospora caninum. Prev. Vet Med. 64, 101-112.

Pfeiffer, D.U., Williamson, N.B., Reichel, M.P., Wichtel, J.J., Teague, W.R., 2002. A longitudinal study of Neospora caninum infection on a dairy farm in New Zealand. Prev. Vet Med. 54, 11-24.

Pollari, F.L., DiGiacomo, R.F., Evermann, J.F., 1993. Use of survival analysis to compare cull rates between bovine leukemia virus seropositive and seronegative dairy cows. Am. J Vet Res 54, 1400-1403.

Pollari, F.L., Wangsuphachart, V.L., DiGiacomo, R.F., Evermann, J.F., 1992. Effects of bovine leukemia virus infection on production and reproduction in dairy cattle. Can J Vet Res 56, 289-295.

Roth, J.A., Kaeberle, M.L., Griffith, R.W., 1981. Effects of bovine viral diarrhea virus infection on bovine polymorphonuclear leukocyte function. Am. J Vet Res 42, 244-250.

Sargeant, J.M., Kelton, D.F., Martin, S.W., Mann, E.D., 1997. Associations between farm management practices, productivity, and bovine leukemia virus infection in Ontario dairy herds. Prev. Vet Med. 31, 211-221.

Scott, H.M., Sorenson, O., Chow, E., Manninen, K., VanLeeuwen, J.A., 2005. Seroprevalence of Mycobacterium avium subspecies paratuberculosis, Neospora caninum, bovine leukemia virus, and bovine viral diarrhea virus infection in Alberta dairy cattle and agro-ecological risk factors associated with seropositivity. Submitted to Can Vet J .

Sockett, D.C., Conrad, T.A., Thomas, C.B., Collins, M.T., 1992. Evaluation of four serological tests for bovine paratuberculosis. J Clin. Microbiol. 30, 1134-1139.

Sweeney, R.W., Whitlock, R.H., Buckley, C.L., Spencer, P., Rosenberger, A.E., Hutchinson, L.J., 1994. Diagnosis of paratuberculosis in dairy cattle, using enzyme-linked immunosorbent assay for detection of antibodies against Mycobacterium paratuberculosis in milk. Am. J Vet Res 55, 905-909.

Sweeney, R.W., Hutchinson, L.J., Whitlock, R.H., 1994. Effects of Mycobacterium paratuberculosis infection on milk production in dairy cattle. Proceedings of the Fourth International Colloquim on Paratuberculosis. 133-139.

Thornton, R.N., Gajadhar, A., Evans, J., 1994. Neospora abortion epidemic in a dairy herd. NZ Vet. J. 42, 190-191.

Thurmond, M.C., Hietala, S.K., 1997a. Effect of congenitally acquired Neospora caninum infection on risk of abortion and subsequent abortions in dairy cattle. Am. J Vet Res. 58, 1381-1385.

Thurmond, M.C., Hietala, S.K., 1997b. Effect of Neospora caninum infection on milk production in first-lactation dairy cows. J Am. Vet Med. Assoc. 210, 672-674.

Tiwari, A., VanLeeuwen , J.A., Dohoo, I.R., Keefe, G.P., Haddad, J.P., 2005a. Seroprevalence and spatial distribution of bovine leukemia virus, bovine viral diarrhea virus and Mycobacterium avium subspecies paratuberculosis in Canadian dairy cattle. Submitted to Can Vet J .

Tiwari, A., VanLeeuwen , J.A., Dohoo, I.R., Stryhn, H., Keefe, G.P., Haddad, J.P., 2005b. Effects of seropositivity for bovine leukemia virus, bovine viral diarrhoea virus, Mycobacterium avium subspecies paratuberculosis, and Neospora caninum on culling in Canadian dairy cattle. Accepted in J. Vet. Micro.

Vanleeuwen, J.A., Keefe, G.P., Tremblay, R., Power, C., Wichtel, J.J., 2001. Seroprevalence of infection with Mycobacterium avium subspecies paratuberculosis, bovine leukemia virus, and bovine viral diarrhea virus in maritime Canada dairy cattle. Can Vet J 42, 193-198.

VanLeeuwen, J.A., Tiwari, A., Dohoo, I.R., Keefe, G.P., 2003. Prevalence of Johne's Disease Infection in Canada - National Production Limiting Diseases Study. Proc. Annual Meeting of Atlantic Bovine Practitioners' Association, Moncton, Canada.

Vanleeuwen, J.A., Tiwari, A., Forsythe, L., Chartier, R., 2005. Seroprevalence of antibodies against bovine leukemia virus, bovine viral diarrhea virus, *Mycobacterium avium* subspecies *paratuberculosis* and *Neospora caninum* in Saskatchewan dairy cattle. Can Vet J .

Vanleeuwen, J.A., Tremblay, R., Keefe, G.P., Wichtel, J., 2000. Seroprevalence of Production Limiting Diseases in Ontario and Maritime Dairy Cattle. Proceedings of the Canadian Veterinary Medical Association Annual Convention, St. John, Canada.

Waage, S., 2000. Influence of new infection with bovine virus diarrhoea virus on udder health in Norwegian dairy cows. Prev. Vet Med. 43, 123-135.

Whitlock, R.H., Wells, S.J., Sweeney, R.W., Van Tiem, J., 2000. ELISA and fecal culture for paratuberculosis (Johne's disease): sensitivity and specificity of each method. Vet Microbiol. 77, 387-398.

Wilson, D.J., Rossiter, C., Han, H.R., Sears, P.M., 1993. Association of Mycobacterium paratuberculosis infection with reduced mastitis, but with decreased milk production and increased cull rate in clinically normal dairy cows. Am. J Vet Res 54, 1851-1857.

Wu, J.T., Dreger, S., Chow, E.Y., Bowlby, E.E., 2002. Validation of 2 commercial Neospora caninum antibody enzyme linked immunosorbent assays. Can. J Vet Res. 66, 264-271.

Wu, M.C., Shanks, R.D., Lewin, H.A., 1989. Milk and fat production in dairy cattle influenced by advanced subclinical bovine leukemia virus infection. Proc Natl. Acad. Sci U. S. A 86, 993-996.

World Trade Organisation. (1994) Final Act of the 1986-1994 Uruguay Round of trade negotiations.http://www.wto.org/english/docs_e/legal_e/ursum_e.html

Table 4.1 Comparison of the study 9,834 cows (342 herds) with national industry averages.

	Study Population estimates (95% Confidence interval)	Canadian Dairy estimates[6] (From 1999-2003)
Mean 305 d milk	8906-9174 kg	8960-9519 kg
Mean 305 d fat	324-333 kg	331-351 kg
Mean 305 d protein	286-294 kg	290-306 kg
Mean herd size of milking cows	58-76	50-62

[6] Canadian Dairy Herd Improvement, Dairy Section, AAFC. (http://www.dairyinfo.gc.ca/pdf_files/statsbook2005.pdf)

Table 4.2 The final model of associations between 305 d milk volume (kilograms) and BLV-, BVDV-, MAP- and NC-seropositivity (by lactation number) from multilevel linear regression analyses of 22665 lactations in 9,834 dairy cows in 342 Canadian dairy herds.

Variables	305 d milk		
	Variance	S.E.[7]	P-value[7]
Random Effects			
Herd	1357807	108252	0.000
Cow	986178	23682	0.000
Lactation	1189563	14674	0.000
Fixed Effects			
	Estimate	S.E.	P-value
Constant	7033.07	233.16	0.000
BLV	36.07	34.79	0.300
BVDV			0.000
- Lac 1	-348.05	155.01	
- Lac 2	-427.61	154.71	
- Lac 3	-410.24	155.88	
- Lac 4plus	-302.28	155.60	
BVDV not tested[8]			
- Lac 1	155.30	193.76	
- Lac 2	201.99	193.98	
- Lac 3	76.86	195.48	
- Lac 4plus	101.62	195.98	
MAP			0.004
- Lac 1	-125.57	99.59	
- Lac 2	45.81	98.09	
- Lac 3	-135.79	102.88	
- Lac 4plus	-212.12	107.36	
NC			0.000
- Lac 1	-158.13	63.11	
- Lac 2	-58.52	61.91	
- Lac 3	91.01	65.24	
- Lac 4plus	45.61	64.39	
Lactation			0.000
1	1	-	-
2	1306.19	29.75	
3	1845.01	33.80	
≥4 plus	2000.78	35.68	
Province			0.000
PE	1	-	-
NB	-255.47	308.90	
NS	741.18	307.96	
QUE	707.75	259.27	
ONT	1438.08	317.89	
MAN	1423.26	298.81	
SASK	1058.60	290.50	
ALTA	1327.57	282.56	

[7]Standard error and P-value of variance component.

[8]These herds did not had the BVDV unvaccinated calves less than 6 months.

Table 4.3. The final models of associations between 305 d fat (kilograms), 305 d protein (kilograms) and mean Log_{10} Somatic Cell Count outcomes, and BLV-, BVDV-, MAP- and NC-seropositivity and lactation number from multilevel linear regression analyses of 22,665 lactations in 9,834 dairy cows in 342 Canadian dairy herds.

	305 d fat			305 d protein			Mean Log_{10} SCC		
Random Effects									
	Variance	S.E.[7]	P-value[7]	Variance	S.E.[7]	P-value[7]	Variance	S.E.[7]	P-value[7]
Herd	1592.61	129.06	0.000	1297.36	102.99	0.000	0.102	0.010	0.000
Cow	1478.51	36.76	0.000	836.04	20.84	0.000	0.318	0.009	0.000
Lactation	1945.19	23.95	0.000	1109.80	13.68	0.000	0.549	0.007	0.000
Fixed Effects									
	Estimate	S.E.	P-value	Estimate	S.E.	P-value	Estimate	S.E.	P-value
Constant	258.35	8.03	0.000	227.84	7.18	0.000	4.146	0.070	0.000
BLV	-0.04	1.36	0.972	1.02	1.03	0.322	0.016	0.021	0.446
BVDV			0.002			0.002			0.003
-BVDV neg	1	-	-	1	-	-	1	-	-
-BVDV pos	-10.20	5.21		-9.46	4.67		0.096	0.045	
-BVDV nt[8]	6.85	6.50		5.49	5.90		-0.032	0.056	
MAP	-3.81	2.79	0.154	-2.06	2.11	0.328	0.098	0.043	0.022
NC			0.005			0.038			0.024
- Lac 1	-5.54	2.51		-3.30	1.89		0.009	0.041	
- Lac 2	-3.07	2.46		-1.46	1.86		0.010	0.040	
- Lac 3	1.27	2.60		0.86	1.96		-0.041	0.042	
- Lac 4plus	0.71	2.55		0.76	1.93		-0.094	0.040	
Lactation			0.000			0.000			0.000
- Lac 1	1	-	-	1	-	-	1	-	-
- Lac 2	45.08	0.92		40.48	0.70		0.229	0.016	
- Lac 3	65.70	1.04		53.67	0.80		0.485	0.018	
- Lac 4plus	72.83	1.10		56.93	0.83		0.732	0.018	
Province			0.000			0.000			0.001
PE	1	-	-	1	-	-	1	-	-
NB	-8.11	10.65		-8.41	9.53		0.028	0.092	
NS	25.13	10.62		20.50	9.50		-0.044	0.092	
QUE	37.05	8.94		24.20	7.99		-0.092	0.077	
ONT	45.13	10.96		45.10	9.81		0.076	0.094	
MAN	43.61	10.31		49.69	9.22		-0.038	0.089	
SASK	27.57	10.01		34.39	8.96		0.031	0.086	
ALTA	38.05	9.74		40.24	8.72		-0.243	0.084	

[7]Standard error of estimate of variance component. [8]These herds did not have the BVDV unvaccinated calves less than 6 months.

Table 4.4. Summary of the percent of the variance explained at each level of the hierarchy for 305 d milk, 305 d fat, 305 d protein and Mean Log10 SCC (no fixed effects in model) in 342 dairy herds with 9,834 cows from 8 provinces in Canada.

Source of variation	Proportion of variance			
	305 d milk	305 d fat	305 d protein	Mean Log10 SCC
Herd	35.83	28.72	37.93	12.27
Cow	21.33	24.10	19.32	32.25
Lactation	42.84	47.18	42.75	55.48

CHAPTER 5: Effects of seropositivity for bovine leukemia virus, bovine viral diarrhoea virus, Mycobacterium avium subspecies paratuberculosis, and Neospora caninum on culling in dairy cattle in four Canadian provinces

5.1 Abstract

The purpose of this research was to determine the effects of seropositivity for exposure to bovine leukemia virus (BLV), bovine viral diarrhoea virus (BVDV), Mycobacterium avium subspecies paratuberculosis (MAP) and Neospora caninum (NC) on overall and reason-specific culling in Canadian dairy cattle. Serum samples from approximately 30 randomly selected cows from 134 herds were tested for antibodies against BLV, MAP and NC using commercially available ELISA test kits, while 5 unvaccinated cattle over 6 months of age were tested for antibodies to bovine viral diarrhoea virus (BVDV). For analyzing the time (in days) to culling of cows after the blood testing, a two-step approach was utilized, non-parametric (Kaplan-Meier survival graphs) visualization and then semi-parametric survival modelling (Cox proportional hazards model), while controlling for confounding variables and adjusting for within herd clustering.

For all reasons of culling, MAP-seropositive cows had a 1.38 (1.05 – 1.81, 95% C.I.) times increased hazard of culling compared to MAP-seronegative cows. Seropositivity for the other pathogens was not associated with an increased risk of overall culling. Among cows that were culled because of either decreased reproductive efficiency or decreased milk production or mastitis, MAP-seropositive cows were associated with 1.55

(1.12 – 2.15, 95% C.I.) times increased hazard compared to MAP-seronegative cows. Among cows that were culled because of reproductive inefficiency, NC-seropositive cows had a 1.43 (95% C.I., 1.15 – 1.79) times greater hazard than NC-seronegative cows. Among cows that were culled because of decreased milk production, cows in BVDV-seropositive herds had a 1.86 (1.28 – 2.70 95% C.I.) times increased hazard compared to cows in BVDV-seronegative herds. BLV-seropositive cows did not have an increased risk of reason-specific culling as compared to BLV-seronegative cows. No significant interaction on culling among seropositivity for the pathogens was detected, but only a limited number of cows tested seropositive for multiple pathogens. Results from our research will help in better understanding the economic impacts of these pathogens and justification for their control.

Keywords: Enzootic bovine leukosis; bovine viral diarrhoea; Johne's disease; Neosporosis; culling; survival analysis.

5.2. Introduction

Infectious disease agents that can be harboured in apparently healthy animals, such as bovine leukemia virus (BLV), bovine viral diarrhoea virus (BVDV), Mycobacterium avium subspecies paratuberculosis (MAP) and Neospora caninum (NC), are undergoing increasing scrutiny and research as a result of new World Trade Organization (WTO) regulations concerning animal health and animal movement between countries (WTO, Act 1994). These regulations state that health certification standards for imported cattle, semen and embryos cannot exceed those required under domestic regulatory programs. Development and implementation of such programs would require research on the current prevalence of these diseases and the feasibility of implementing domestic health assurance programs for them, based on the costs of import/export restrictions and losses in productivity. While these organisms lead to clinical diseases and their associated costs, there are still questions regarding the impacts of subclinical infections with these agents.

Based on an Atlantic Canadian (New Brunswick -NB, Nova Scotia – NS, and Prince Edward Island - PEI) prevalence survey of the above four pathogens, and literature estimates of associated costs, average direct farm costs associated with infection with these organisms were estimated for infected Atlantic Canadian dairy herds. The annual costs for BLV, BVDV, MAP and NC, respectively, were reported to be $806, $2421, $2472 and $2304 per infected herd using an average herd size of 50 cows (Chi et al., 2002). However, there were limited and/or inconsistent reports regarding the effects of

the diseases on premature culling costs applicable to Canadian dairy herds and consequently these were not included in the economic impact assessment.

The published effects of BLV on culling rates are very inconsistent. In a single large herd in Maryland demonstrated no effect of BLV infection status on culling was observed (Rhodes et al., 2003). No effect was also found in an Ontario study, (Herald et al., 1992). Conversely, greater culling rates were seen in BLV-seropositive versus seronegative cows in one large, high prevalence dairy farm in Washington State, USA (Pollari et al., 1993). Similarly, culling rates for all reasons were reported to be higher for BLV infected herds compared to cows in BLV non-infected herds in Sweden (Emanuelson et al., 1992). However, other unmeasured herd factors correlated with BLV status may be responsible for this difference in culling.

The effect of seropositivity for NC on culling rates also varies, from no effect in 56 dairy herds in Ontario (Cramer et al., 2002) to higher culling rates (1.7 times) in NC-seropositive versus seronegative cows (Thurmond and Hietala, 1996). However, this increased culling rate was estimated from an unconditional comparison of percentage of seropositive and seronegative cows culled after 3 years of follow up in a single dairy herd of 442 cows with abortion problems due to NC (Thurmond and Hietala, 1996). There was no information on abortions in the Ontario study and therefore, it is unclear whether NC infection may have an impact on overall culling or culling due to reproductive inefficiency.

David et al. (1994) found that 11% of cows were culled prematurely (due to chronic illness) because of BVDV in three dairy herds in England. Similarly, another study (Pritchard et al., 1989) reported that 15 (8%) cows died and 20 (11%) were culled (mainly because of acute infection) because of BVDV in a 183 cow dairy herd in Norfolk, England. In endemically infected herds, a 2% increased culling risk associated with BVDV infection has been reported (Meyling et al., 1990). While it is understandable that herds with outbreaks of clinically affected animals would likely undergo increased involuntary culling, it is still unclear whether endemic herds with subclinically infected animals have increased culling compared to uninfected herds.

For MAP, a higher cull rate has been reported in MAP culture-positive cows as compared to MAP negative cows in a 210-cow Holstein herd in New York (Wilson et al., 1993). In another 900-cow Guernsey herd with subclinically infected cows, 22.6% of fecal culture positive cows and 3.6% of fecal culture negative cows were reported culled due to mastitis, and 68.8% versus 60.2% were culled due to infertility, respectively (Merkal et al., 1975). Similarly, in a study in Colorado dairy herd 48.9% of seropositive cows (based on ELISA test) were culled over one year as compared to 33% of seronegative cows (Goodell et al., 2000), although misclassification of serology results may have affected these results. MAP is the only one of the above four diseases for which there appear to be consistent evidence of increased culling risk associated with animals that test positive. However, there is still a wide variation in the estimates of culling risk between studies, which may be due to differences in study design and analyses, or biological differences between the study populations.

The results from the above studies on risk of culling associated with BLV, BVDV, MAP or NC-seropositivity might not be representative of the true state of nature in the dairy industry in general (or Canadian dairy herds in particular) due to one or more of the following reasons. 1) Many studies used a single large herd which may not be representative of other large herds or small herds due to differences in management or seroprevalence. 2) If multiple herds were used, these herds were not randomly selected, leading to possible herd selection bias. 3) Confounding variables were not controlled in the design or analyses. 4) Time to culling was not appropriately examined in the analyses. Furthermore, the culling impact of seropositivity for one pathogen may also vary with co-infection with other pathogens (e.g. BLV and BVDV impair cellular immunity).

Our study objective was to determine the effect of seropositivity for infection with bovine leukemia virus (BLV), bovine viral diarrhoea (BVDV), Mycobacterium avium subspecies paratuberculosis (MAP) and Neospora caninum (NC) on hazard ratios for culling (overall and reason-specific) in a large population of randomly selected dairy cows and herds, after controlling for possible confounding effects of other important predictor variables, and adjusting for herd clustering effects and time to culling.

5.3. Materials and Methods

The data utilized for this study were from recent prevalence surveys of Canadian dairy herds in NB, NS, PEI (Keefe and VanLeeuwen, 2000; VanLeeuwen et al., 2001) and Saskatchewan (VanLeeuwen et al., 2005). The methodologies of the two studies were very similar, making the merging of their datasets feasible. Briefly summarized, 89.1% and 70.0% of herds had at least one seropositive animal for BLV in Atlantic Canada and Saskatchewan (SASK), respectively. For BVDV, 46.1% and 16.8% of the herds had at least one unvaccinated heifer/cow with a BVDV titre above 1:32 in Atlantic Canada and Saskatchewan (SASK), respectively. For MAP, 16.7% and 24.1% of the herds had at least two seropositive animals for MAP in the two regions, respectively. For NC, 78.9% and 43.8% of herds had at least two seropositive animals in the two regions, respectively.

5.3.1. Serum sample collection

A stratified two-stage random sampling procedure was employed. During the summer of 1998 in Atlantic Canada, participating dairy herds were randomly selected (using computer generated random numbers) until ninety herds were recruited, thirty from Prince Edward Island (PEI), New Brunswick (NB), and Nova Scotia (NS). Only herds that were enrolled in a monthly, individual cow milk-testing program through the Atlantic Dairy Livestock Improvement Corporation (ADLIC) were eligible for participation. Approximately 60-70% of dairy herds in Atlantic Canada are enrolled with ADLIC. During the winter of 2000-01, similar herd level exclusion criteria and sampling

procedures were utilized to recruit forty-four herds from the province of Saskatchewan. The sample size formula used to determine the number of required herds as 30 per province for the original prevalence surveys assumed 300 herds per province on DHI, a herd level seroprevalence of 10%, an allowable error of 10%, and a confidence level of 95%.

Using computer generated random numbers, up to thirty lactating animals were randomly selected for blood collection in each herd. In the original prevalence survey (VanLeeuwen et al., 2001), with an average herd size of 45 cows, 30 cattle were needed to be tested in each herd to detect at least 1 infected animal in a herd, based on an average within-herd prevalence estimate of 10%, confidence of 90%, and sensitivity of the enzyme linked immunosorbent assay (ELISA) test for MAP of 43.0%, the test with the lowest sensitivity among the four diseases (Sockett et al., 1992).

For BVDV, five unvaccinated (for BVDV) cattle >6 months old were selected for blood collection. In unvaccinated herds, five animals of the 30 cows tested for the other three diseases were selected. In vaccinated herds, five unvaccinated heifers >6 months old were selected. The sampling technique was based upon Houe's study (Houe, 1992).

5.3.2. Laboratory analyses

Within 24 hours, the blood samples were centrifuged, and the serum was harvested and stored at -20°C until all the samples were collected. The serum samples were

subsequently assessed for antibody against: BLV using an ELISA[1] (sensitivity 98.5%, specificity 99.9%) (Johnson and Kaneene., 1991); MAP using an ELISA[1] (sensitivity 43.0%, specificity 99.0%) (Sockett et al., 1992), tested in duplicate; and NC using an ELISA[2] (sensitivity 99.0%, specificity 98.4%) (Bergeron et al., 2000), tested in duplicate. An animal was considered to be infected with BLV, MAP or NC if the serum-to-positive ratio on the ELISA was ≥ 0.50, ≥ 0.25, and ≥ 0.60, respectively, as recommended by the manufacturers of the various test kits. The BLV ELISA test kit requires a confirmation of positive tests, using a sample-to-negative host-cell ratio of ≥ 1.8.

Up to 5 serum samples per farm were tested for antibody against type 1 genotype BVD virus, using virus neutralization to the cytopathic Singer strain (Animal Diseases Research Institute, Lethbridge, Alberta – sensitivity 99.6% and specificity 100%; Deregt et al., 1992). A herd was considered to be infected with BVDV if at least one of the animals tested had a titer of $\geq$1:64 for BVD virus.

BLV testing was conducted at the national BLV testing laboratory in Prince Edward Island (now in Quebec), which is certified to conduct BLV testing for international trade purposes. Testing for BVDV was conducted at Animal Diseases Research Institute in Alberta. MAP testing was conducted at Prairie Diagnostic Services in Saskatchewan, which is accredited for MAP ELISA testing by the United States Dept of Agriculture. NC testing was conducted at the BIOVET Inc. laboratory in Quebec.

[1] IDEXX ELISA - IDEXX Corporation - Idexx Laboratories, Westbrook, Maine, USA
[2] BIOVET ELISA - BIOVET Inc. - St. Hyacinthe, Quebec, Canada

5.3.3. Culling data collection and statistical analyses

For each tested animal, the culling and production data were gathered electronically from a central milk recording database for the follow-up period that extended from June, 1998 to Feb, 2002 for Atlantic herds, and from April, 2001 to Sept, 2002 for Saskatchewan herds because Atlantic and Saskatchewan herds were sampled in June-August, 1998 and April-May, 2001, respectively.

Cows that left herds for dairy purposes (sold to another herd) were excluded from the analyses because they could not be considered to be culled for reasons biologically related to the infections. All other cows were either culled for non-dairy purposes during the follow-up period, or remained in the herd to the end date of the study (censored data). Days at risk for culling were calculated by subtracting the date of serum sampling from the date of culling or censoring.

For analyzing the time (days) to event (culling of cows), a two-step approach was utilized: non-parametric Kaplan-Meier survival graphs were evaluated visually; and then semi-parametric survival models (Cox proportional hazards model) were carried out. The non-parametric analyses produced graphical representations that assisted in interpretation of the data. Cox proportional hazards models were fit for overall culling, regardless of reason, and for reason-specific culling, based on owner reporting of culling reasons. Main effects of seropositivity for BLV, BVDV, MAP and NC on hazard ratios for culling were estimated after controlling for lactation number (at the time of blood sampling), province

and seropositivity for the other three infectious agents. In addition, first order interactions between all predictors except province were examined. Stratified proportional hazard (which allows for herd specific baseline hazards) and shared frailty models were utilized to adjust the estimates of BLV-, MAP- and NC-seropositivity for within herd clustering. The shared frailty model used multiplicative gamma-distributed random effects on the hazard scale, and estimation was based on maximizing the profile likelihood function (Therneau and Grambsch, 2000). This modelling was based on the assumption that the ratio of the hazards for the exposed and non-exposed individuals was an exponential function of a set of explanatory variables and that the ratio was constant over time (i.e. hazards were proportional).

Diagnostics for the Cox model were based on Cox-Snell and scaled Schoenfeld residuals as well as interaction terms between time and each of the predictor variables, as described in Therneau and Grambsch (2000). All of the above analyses were done in the software Stata, version 8[3]. Results were considered statistically significant at $p<0.05$.

[3] Stata (Statistical package, v.8; Stata Press, College Station, Texas, USA)

5.4. Results

Demographic examination of sampled herds compared to regional industry averages did not identify any significant differences, confirming that the random selection of herds and cows were representative of the dairy industry as a whole. The mean number of cows in participating herds was 55. The mean 305-day milk production and calving to conception interval were 8838.5 kg and 138.7 Days, respectively.

Overall, 26.9%, 2.4% and 12.7% of cattle were test-positive for exposure to BLV, MAP and NC and, 38.1% of herds were test-positive for exposure to BVDV, respectively.

The final dataset for culling analyses contained 134 herds with 3531 cows, which had serological results for exposure to all three agents and production and culling data. A total of 1981 (56.1%) cows were culled for all reasons out of 3531 tested cows. The follow-up period was 3 years and 7 months for the Atlantic herds and 1 year and 4 months for Saskatchewan herds, therefore, the average time at risk from testing until culling for cows in NB, NS, PEI and SASK were 837.4, 706.5, 728.1 and 376.9 days, respectively. Of the 134 herds, 17 herds had aggressive BVDV vaccination protocols and therefore did not have BVDV results due to unavailability of unvaccinated heifers > 6 months of age.

5.4.1. Overall risk of culling

Survival curves, which estimate the probability for a cow to survive (not be culled) up to any given day after testing, were generated for cows testing positive and negative for BLV, MAP and NC (Fig 5.1 – 5.3) and for cows in herds testing positive and negative for BVDV (Fig 5.4). In general, seropositive cows had lower survival than seronegative cows for each of the pathogens. However, the differences were small for BLV, BVDV and NC and the graphical estimation method does not determine if the differences are statistically significant. Furthermore, unlike the following results from the modelling, the graphical method does not account for confounding or clustering within herds, which could alter final interpretations of significant relationships between culling and seropositivity for the pathogens.

The results from semiparametric survival analyses (Cox proportional hazard model), with and without multiplicative gamma frailty and stratified baseline hazards to adjust for within herd clustering, are presented in Table 5.1.

Estimates from the survival analyses with multiplicative gamma frailty showed that the hazard of culling of MAP-seropositive cows was 1.38 (1.05 – 1.81, 95% C.I.) times that of MAP-seronegative cows. Higher lactation number cows were significantly associated with higher hazards of culling. The hazard of culling in Nova Scotia, Prince Edward Island and Saskatchewan were 1.53, 1.38 and 1.43 times that of New Brunswick. The significant frailty variance in the gamma shared frailty model suggested heterogeneity among culling decisions in different herds. The other models produced approximately similar fixed effect estimates. The stratified model could not produce BVDV and

province estimates because the stratification by herd eliminated the variation of these two variables.

Graphical assessment and non-significant P-values (P >0.05) for the inclusions of time-varying interaction covariates with BLV, BVDV, MAP, NC and lactation number indicated that the basic assumption of proportional hazards was not violated. Cox-Snell and scaled Schoenfeld residuals indicated a good overall fit of the final model. The estimated random herd effects showed a good fit to the gamma distribution.

5.4.2. Reason-specific risk of culling

Overall, 572, 179 and 373 cows were culled because of poor reproduction, low milk production and mastitis, respectively. Table 5.2 shows the hazard ratios for reason-specific culling associated with seropositivity for BLV, BVDV and NC from semi-parametric survival analyses with multiplicative gamma frailty. Among cows that were culled because of reproductive inefficiency, NC-seropositive cows had a 1.43 (95% C.I., 1.15 – 1.79) times greater hazard of culling than NC-seronegative cows. Among cows that were culled because of low milk production, cows in BVDV-seropositive herds had a 1.86 (95% C.I., 1.28 – 2.70) times greater hazard of culling than cows in BVDV-seronegative herds. For cows culled for poor reproduction or low milk production, higher lactation cows were significantly associated with greater hazards. However, for cows culled for mastitis, lactation number was not significantly associated with hazard of culling, although the hazard was highest in the two oldest age groups (lactations 5 and 6

plus). Significant frailty variance suggests heterogeneity among herds for specific reasons of culling, confirming the need for adjustment due to within herd clustering. As with the model for overall culling, model diagnostics did not identify any problems with the fit or structure of the models.

The effect of MAP-seropositivity could not be estimated for reason-specific models due to the small numbers of MAP-seropositive cows that were culled for each of the three main reasons (n = 41). However, in a model for the three reasons combined (results not shown in Table), MAP-seropositive cows had a 1.55 (95% C.I., 1.11 – 2.15,) times greater hazard of culling as compared to MAP-seronegative cows, while controlling for other confounders and adjusting for random within herd clustering.

5.5. Discussion

This study was part of a national study designed to determine the seroprevalence of BLV, BVDV, MAP and NC in Canadian dairy cattle. While other provinces have been included in the overall prevalence survey, only herds from these four provinces were included in these analyses for two reasons. They used similar random selection procedures at the cow and herd level to ensure that the study population was representative of the target population. Also, there was sufficient time between sampling and statistical analyses to generate a large sample of culled cows for analyses. Other provinces either used a different selection protocol or were sampled and tested later, leading to insufficient follow up time to conduct culling analyses.

With regard to the methodology used in this study, multivariable hazard models (risk of culling at a particular time) were chosen to estimate the risk of culling instead of a comparison of percentages of culled seropositive and seronegative cows because it takes into account the actual time at risk for culling and allows for controlling of possible confounding variables. The inclusion or exclusion of potential confounding variables in the final model did not significantly change the hazard estimates for pathogen seropositivity. For example, the inclusion of province in the final model minimized any bias that may have resulted from the differences in follow-up time or culling for dairy purposes between the Saskatchewan and Atlantic herds. The proportion of total culled cows that left the herd for dairy purposes was 16.6% and 16.4% in Saskatchewan and Atlantic herds, respectively.

In survival analysis, stratified and shared frailty models are two approaches to take into account significant correlations among observations derived from a hierarchical structure (in our data, multiple cows within a herd). Stratification by herd allows baseline hazards to vary between herds and thereby accounts for herd differences without explicitly modelling them. The approach is robust but has the disadvantage of precluding the inclusion of group level predictors in the model (such as BVDV herd-status), because stratification by herd eliminates the variation of these herd level variables. Shared frailty models assume that random herd effects follow a specific (in our case, gamma) distribution and that these effects act multiplicatively on the hazard. In shared frailty models, the variability in the data is split into individual variability in time to event and variability common to individuals in the group (herd). The magnitude of the latter is described by the frailty variance. This approach requires more explicit assumptions (eg. distribution of the random herd effects) but gives more information (eg. frailty variance) and allows for herd-level predictors. In frailty models, the hazard ratio represents the effect of the factor at time = 0, but over the time this effect will diminish in favour of the frailty effect (Stata ref manual 8., 2003).

The stratified and shared frailty models produced similar fixed effect estimates. The standard errors of the fixed effects were largest (marginally compared to shared frailty models) in the stratified models and smallest in the initial models that did not take into account the hierarchical structure. We choose the shared frailty models because they showed no sign of poor fit, were more efficient (by estimating less parameters than

stratified models) and did not have the disadvantage of precluding the inclusion of group level predictors in the model (such as BVDV herd-status). Significant frailty variance (as seen by the significant p value in tables 5.1 and 5.2) suggests clustering within herds and heterogeneity among herds for overall and specific reasons of culling, confirming the need for adjustment due to within herd clustering.

Without adjusting for herd effects, cows in BVDV-seropositive herds had a slightly (p-value < 0.10) increased hazard of overall culling compared to cows in BVDV-seronegative herds but this effect was not significant once herd effects were accounted for. Other studies (Meyling et al., 1990; Pritchard et al., 1989) found similar relationships but their studies included only three and one herd, respectively. We also found that among cows that were culled because of decreased milk production, cows in BVDV-seropositive herds were significantly associated with greater hazard of culling than cows in BVDV-seronegative herds. These results support the findings of a companion study in which we found that cows in BVDV-seropositive herds had a decrease of 393.7 (95% C.I., 106 – 681.4) kg in 305-day milk production (Tiwari et al., 2004). It is unclear whether this impact on milk production and its associated culling is due to direct physical effects of the virus on feed consumption and/or milk production, or due to the indirect effects of other pathogens that are allowed to impart their effects due to the immunosuppression caused by BVD virus. In our study, there were no interactive effects on culling between seropositivity for pathogens examined. However, studies in the past have suggested that BVDV can cause increased susceptibility to other diseases (Houe,

1995), although, there are no reports of concurrent infections exacerbating the effects of BVDV.

Among cows that were culled because of either decreased milk production or mastitis, cows in herds with no BVDV test results had a slightly increased risk of culling compared to cows in BVDV-seronegative herds. The unavailability of unvaccinated heifers in these herds may suggest a more vigorous approach to vaccinate heifers in response to health problems in the herd as a result of BVD virus, potentially leading to enhanced culling.

In our study, after adjusting for within herd clustering effects, seropositivity for MAP for all reasons for culling was significantly associated with an increased hazard of culling, confirming that this impact on culling is applicable to a variety of sizes and management levels of dairy farms, even after controlling for confounding variables. Wilson et al., (1993) found similar results utilizing fecal culture to identify MAP infected cows in one large herd in New York with high MAP prevalence. We also found that seropositivity for MAP was significantly associated with reason-specific (decreased reproductive efficiency, decreased milk production and/or mastitis) risk of culling, confirming that the culling was not simply due to the procedure being aware of the positive MAP ELISA test results.

It is biologically plausible for MAP to lead to increased hazard of culling. MAP infection typically causes localized granulomatous lesions in lymph nodes and the lamina propria

of the terminal ileum but granulomatous lesions can also be disseminated throughout the entire gastrointestinal (GI) tract (Chiodini et al., 1984). The gastroenteritis produces mucosal thickening that results in reduced absorption of nutrients from the intestine and alterations in cell-mediated immunity. Reduced GI absorption might cause or worsen a negative energy balance in early lactation, while alterations in cell-mediated immunity may decrease the resistance of cows towards other infectious diseases, possibly resulting in culling of MAP-seropositive cows due to decreased reproductive efficiency (Johnson et al., 2001) or decreased milk production (Goodell et al., 2000) or mastitis (McNab et al., 1991).

In our randomly selected population of cattle and herds, after adjusting for herd effects, seropositivity for NC for all reasons of culling was not significantly associated with an increased hazard of culling. These results agree with those from a previous study in which reason-specific culling was not examined (Cramer et al., 2002). However, we also found that NC-seropositive cows were significantly associated with an increased risk of culling for reproductive reasons, which supports a previous study looking at culling in a single large dairy herd in California with a history of abortion storms (Thurmond and Hietala, 1996). Cows infected with NC can have a two- to three-fold increase in risk of fetal loss (Thurmond and Hietala, 1996). However, NC-seropositivity does not necessarily lead to abortion (Anderson et al., 1997), which may explain the inability of some previous studies (Cramer et al., 2002) to find an increased risk of culling, if there were insufficient herds in the study with a recent history of abortion problems to lead to detectable increased culling risk.

Significant relationships between BLV-seropositivity and decreased reproductive efficiency, decreased milk production or mastitis have been reported (Pelzer, 1997; Pollari et al., 1993), often in large herds with high seroprevalence. Conversely, Herald et al. (1992) found no relationship among a group of herds of varying seroprevalence in Ontario, Canada, but these cows were selected from non-randomly selected herds. In our study of randomly selected cows and herds with a wide variety of seroprevalences, after adjusting for herd effects, seropositivity for BLV was not associated with an increased hazard of overall culling or culling for any specific reason.

BLV can cause alterations in cell-mediated immunity; with up to 30% of BLV-seropositive cattle eventually developing persistent lymphocytosis, while less than 5 % of BLV-seropositive cows develop lymphosarcoma (Bradford ., 2002).

With the low proportion of seropositive cows developing lymphosarcoma, significant associations between seropositivity and culling due to clinical disease is unlikely except perhaps in high prevalence herds where the herd burden of virus is large, potentially leading to a higher likelihood of negative impacts and subsequent culling. However, in our study with a seroprevalence of 26.9%, seropositivity for BLV for all reasons of culling was slightly associated (p-value<0.30) with an increased hazard of culling after adjusting for herd effects. If only 5% of these animals develop lymphosarcoma, only 1.4% or 27 culled animals in the study may have developed this abnormality, a limited number to be able to detect a significant association between culling and BLV-

seropositivity. It is unclear whether persistent lymphocytosis leads to increased culling either directly or through another ailment.

Maritime and Saskatchewan farmers received the diagnostic tests results for BLV, BVDV, MAP and NC after 16-18 and 6 months, respectively. The effect of knowing the test results on overall and reason-specific culling was estimated by two approaches. First, the data set was divided into two (before and after getting the test results), and then the hazards of culling for BLV, BVDV, MAP and NC seropositivity were compared. Second, the effects of variables were allowed to vary with time (as a time varying co-variates). There was no difference in the hazard of culling before and after getting the test results for any of the pathogens, and none of the time varying co-variates remains significant in the final model. It was therefore concluded that knowing the test results did not significantly affect the hazard of culling for BLV, BVDV, MAP and NC seropositivity.

Due to a small number of cows being seropositive for more than one pathogen, this study had limited power to detect a relationship between culling and co-infections, and a type II error is possible (stating that there is no relationship when in fact there is one). At the cow level, there were 18, 100 and 13 cows that were seropositive with BLV and MAP, BLV and NC, and MAP and NC, respectively, with 5 cows seropositive for all 3 pathogens. Misclassification of test results from the NC-ELISA (Bergeron et al., 2000) and MAP-ELISA (Sockett et al., 1992) would also have reduced the likelihood of detecting an interaction for co-infections. It can be concluded that a strong relationship between culling and seropositivity for more than one pathogen was not detected, although

future research with a larger number of co-infected (co-exposed) animals, preferably with tests with less misclassification than the ELISAs utilized in this study, are warranted.

5.6 Conclusions

In conclusion, for all reasons of culling, MAP-seropositive cows were associated with 1.38 (1.05 – 1.81, 95% C.I.) times increased hazard of culling compared to MAP-seronegative cows. Among cows that were culled because of either decreased reproductive efficiency or decreased milk production or mastitis, MAP-seropositive cows were associated with 1.55 (1.12 – 2.15, 95% C.I.) times increased hazard compared to MAP-seronegative cows. Among cows that were culled because of reproductive inefficiency, NC-seropositive cows were associated with 1.43 (95% C.I., 1.15 – 1.79) times greater hazard than NC-seronegative cows. Among cows that were culled because of decreased milk production, cows in BVDV-seropositive herds were associated with 1.86 (1.28 – 2.70 95% C.I.) times increased hazard compared to BVDV-seronegative herds. BLV-seropositive cows did not have an increased risk of culling as compared to BLV-seronegative cows. Results from our research will help in better understanding the economic impacts of these pathogens and justification for their control.

5.7 Acknowledgements

The authors wish to acknowledge Dr. LeeAnn Forsyth and Ms. Renee Chartier of the Saskatchewan Department of Agriculture (Food and Rural Revitalization, Inspection and Regulatory Management Branch, Animal Health Unit) for their technical and logistical support in the collection of samples and data from the participating farmers in Saskatchewan. Authors acknowledge the following funding sources: Dairy Farmers of Canada, Atlantic Veterinary College, Production Limiting Diseases Committee, and PEI Agricultural Research Investment Fund.

5.8 References

Anderson, M.L., Reynolds, J.P., Rowe, J.D., Sverlow, K.W., Packham, A.E., Barr, B.C., Conrad, P.A., 1997. Evidence of vertical transmission of Neospora sp infection in dairy cattle. J. Am. Vet. Med. Assoc. 210, 1169-1172.

Bergeron, N., Fecteau, G., Pare, J., Martineau, R., Villeneuve, A., 2000. Vertical and horizontal transmission of Neospora caninum in dairy herds in Quebec. Can. Vet. J. 41, 464-467.

Bradford, P.S., 2002. Large animal internal medicine. Third Edition ed. St. Louis, MO: Mosby.

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

Chiodini, R.J., Van Kruiningen, H.J., Merkal, R.S., 1984. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. Cornell Vet. 74, 218-262.

Cramer, G., Kelton, D., Duffield, T.F., Hobson, J.C., Lissemore, K., Hietala, S.K., Peregrine, A.S., 2002. Neospora caninum serostatus and culling of Holstein cattle. J. Am. Vet. Med. Assoc. 221, 1165-1168.

David, G.P., Crawshaw, T.R., Gunning, R.F., Hibberd, R.C., Lloyd, G.M., Marsh, P.R., 1994. Severe disease in adult dairy cattle in three UK dairy herds associated with BVD virus infection. Vet. Rec. 134, 468-472.

Deregt, D., Smithson, S., Kozub, G.C., 1992. A short incubation serum neutralization test for bovine viral diarrhoea virus. Can. J. Vet. Res. 56, 161-164.

Emanuelson, U., Scherling, K., Pettersson, H., 1992. Relationships Between herd bovin leukemia virus infection status and reproduction, disease incidence, and productivity in Swedish dairy herds. Prev. Vet. Med. 12, 121-131.

Goodell, G.M., Hirst, H., Garry, F., Dinsmore, P., 2000. Comparison of cull rates and milk production of cilinially normal dairy cows grouped by ELISA Mycobacterium avium paraubercuIosis serum antibody results. Proceedings of The International Symposium of Vetrinary Epidemiology and Economics, Breckenridge, CO, USA.

Herald, M.T.S., Waltner-Toews, D., Jacobs, R.M., 1992. The prevalence of anti-bovine leukemia virus antibodies in dairy cows and association with farm management practices, production and culling in Ontario. Prev. Vet. Med. 14, 45-55.

Houe, H., 1992. Serological analysis of a small herd sample to predict the presence of animals persistently infected with bovine viral diarrhoea virus (BVDV) in dairy herds. Res. Vet. Sci. 53, 320-323.

Houe, H., 1995. Epidemiology of bovine viral diarrhoea virus. Vet. Clin. North Am. Food Anim Pract. 11, 521-547.

Johnson, R.J., Kaneene, J.B., 1991. Bovine Leukemia Virus. Part I. Descriptive Epidemiology, Clinical Manifestations, and Diagnostic tests. Compendium on Continuing Education 13, 328.

Johnson, Y.J., Kaneene, J.B., Gardiner, J.C., Lloyd, J.W., Sprecher, D.J., Coe, P.H., 2001. The effect of subclinical Mycobacterium paratuberculosis infection on milk

production in Michigan dairy cows. J. Dairy Sci. 84, 2188-2194.

Keefe, G.P., VanLeeuwen, J.A., 2000. Neospora then and now: prevalence of Neospora caninum in Maritime Canada in 1979, 1989, and 1998. Can. Vet. J. 41, 864-866.

McNab, W.B., Meek, A.H., Martin, S.W., Duncan, J.R., 1991. Associations between dairy production indices and lipoarabinomannan enzyme-immunoassay results for paratuberculosis. Can. J. Vet. Res. 55, 356-361.

Merkal, R.S., Larsen, A.B., Booth, G.D., 1975. Analysis of the effect of inapparent bovine paratuberculosis. Am. J. Vet. Res. 36, 837-838.

Meyling, A., Houe, H., Jensen, A.M., 1990. Epidemiology of bovine virus diarrhoea virus. Rev. Sci. Tech. Off. Int. Epiz 9, 75-93.

Pelzer, K.D., 1997. Economics of bovine leukemia virus infection. Vet. Clin. North Am. Food Anim Pract. 13, 129-141.

Pollari, F.L., DiGiacomo, R.F., Evermann, J.F., 1993. Use of survival analysis to compare cull rates between bovine leukemia virus seropositive and seronegative dairy cows. Am. J. Vet. Res. 54, 1400-1403.

Pritchard, G.C., Borland, E.D., Wood, L., Pritchard, D.G., 1989. Severe disease in dairy herd associated with acute infecion with bovine viral diarrhoea virus, Leptospira hardjo and Coxiella burnetii. Vet. Rec. 124, 625-629.

Rhodes, J.K., Pelzer, K.D., Johnson, Y.J., Russek-Cohen, E., 2003. Comparison of culling rates among dairy cows grouped on the basis of serologic status for bovine leukemia virus. J Am. Vet Med. Assoc. 223, 229-231.

Sockett, D.C., Conrad, T.A., Thomas, C.B., Collins, M.T., 1992. Evaluation of four serological tests for bovine paratuberculosis. J. Clin. Microbiol. 30, 1134-1139.

Stata Reference Manual 8, 2003.Cox proportional hazards model. Stata Survival analysis and epidemiological tables. Stata Corporation, College Station, Texas, pp.108-155.

Therneau, T.M. and Grambsch, P.M., 2000. Modeling Survival Data, Extending the Cox Model, Springer: New York.

Thurmond, M.C., Hietala, S.K., 1996. Culling associated with Neospora caninum infection in dairy cows. Am. J. Vet. Res. 57, 1559-1562.

Tiwari, A., VanLeeuwen, J.A., Dohoo, I.R., Stryhn, H., Keefe, G., Haddad, J.P., 2004. Effects of seropositivity for bovine leukemia virus, bovine viral diarrhoea, Mycobacterium avium subspecies paratuberculosis, and Neospora caninum on milk production in Canadian dairy cattle. Accepted to World Buiatrics Congress, Quebec, Canada.

VanLeeuwen, J.A., Keefe, G.P., Tremblay, R., Power, C., Wichtel, J.J., 2001. Seroprevalence of infection with Mycobacterium avium subspecies paratuberculosis, bovine leukemia virus, and bovine viral diarrhoea virus in maritime Canada dairy cattle. Can. Vet. J. 42, 193-198.

VanLeeuwen, J.A., Forsythe, L., Tiwari, A., Chartier, R., 2005. Seroprevalence of antibodies against bovine leukemia virus, bovine viral diarrhea virus, Mycobacterium avium subspecies paratuberculosis, and Neospora caninum in dairy cattle in Saskatchewan. Can Vet J 46, 56-58.

Wilson, D.J., Rossiter, C., Han, H.R., Sears, P.M., 1993. Association of Mycobacterium paratuberculosis infection with reduced mastitis, but with decreased milk production and increased cull rate in clinically normal dairy cows.

Am. J. Vet. Res. 54, 1851-1857.

World Trade Organisation. (1994) Final Act of the 1986-1994 Uruguay Round of trade negotiations.http://www.wto.org/english/docs_e/legal_e/ursum_e.html

Table 5.1. Hazard ratios associated with BLV-, BVDV-, MAP- and NC-seropositivity, test lactation number and province from Cox- proportional hazard models for all reasons of culling in dairy cows in Canadian herds.

	Cox model (no herd effect)			Stratified Cox model			Shared frailty Cox model		
	Estimate	P-value	95% C.I.	Estimate	P-value	95% C.I.	Estimate	P-value	95% C.I.
Effects / Parameter									
BLV	1.060	0.272	0.955 – 1.176	1.021	0.768	0.890 – 1.170	1.032	0.615	0.913 – 1.166
BVDV									
herd negative	1	-	-				1	-	-
herd positive	1.140	0.007	1.035 – 1.254		-		1.171	0.076	0.983 – 1.394
herds having no test	1.104	0.224	0.941 – 1.294		-		1.141	0.356	0.861 – 1.511
MAP	1.354	0.024	1.040 – 1.761	1.442	0.010	1.092 – 1.904	1.382	0.019	1.054 – 1.811
NC	1.041	0.520	0.920 – 1.179	1.062	0.399	0.922 – 1.223	1.053	0.450	0.920 – 1.204
Lactation		0.000			0.000			0.000	
1	1	-	-	1	-	-	1	-	-
2	1.174	-	1.033 – 1.333	1.234	-	1.082 – 1.407	1.212	-	1.065 – 1.379
3	1.582	-	1.384 – 1.806	1.723	-	1.498 – 1.980	1.696	-	1.480 – 1.943
4	1.722	-	1.482 – 1.999	1.826	-	1.557 – 2.140	1.860	-	1.594 – 2.168
5	2.066	-	1.726 – 2.472	2.223	-	1.837 – 2.689	2.177	-	1.809 – 2.618
>6	2.550	-	21.43 – 3.032	3.207	-	2.653 – 3.876	3.097	-	2.582 – 3.714
Province		0.000						0.003	
New Brunswick	1	-	-		-		1	-	-
Nova Scotia	1.527	-	1.343 – 1.736		-		1.547	-	1.222 – 1.957
Prince Edward Island	1.381	-	1.210 – 1.575		-		1.365	-	1.076 – 1.729
Saskatchewan	1.426	-	1.219 – 1.668		-		1.462	-	1.131 – 1.888
Frailty variance		-			-		0.133	0.000	

Table 5.2: Hazard ratios associated with BLV-, BVDV- and NC-seropositivity, test lactation number and province from Cox-proportional hazard models for specific reasons and shared frailty at herd level in dairy cows in Canadian herds.

	Culling reason								
	Poor Reproduction			Low Milk			Mastitis		
	Estimate	P-value	95% C.I.	Estimate	P-value	95% C.I.	Estimate	P-value	95% C.I.
Effects / Parameter									
BLV	0.894	0.327	0.714 – 1.118	1.135	0.369	0.861 – 1.494	1.044	0.826	0.711 – 1.531
BVDV									
Herd negative	1	-	-	1	-	-	1	-	-
Herd positive	0.839	0.196	0.642 – 1.094	1.863	0.001	1.283 – 2.704	1.427	0.094	0.941 – 2.164
herds having no test	1.091	0.686	0.714 – 1.665	2.050	0.023	1.103 – 3.811	2.388	0.009	1.244 – 4.582
NC	1.435	0.001	1.148 – 1.793	0.811	0.225	0.579 – 1.137	1.005	0.981	0.656 – 1.538
Lactation		0.000			0.000			0.638	
1	1	-	-	1	-	-	1	-	-
2	1.461	-	1.153 – 1.852	1.138	-	0.834 – 1.553	0.886	-	0.594 – 1.320
3	1.718	-	1.329 – 2.219	2.079	-	1.533 – 2.817	1.099	-	0.714 – 1.691
4	1.996	-	1.499 – 2.655	2.350	-	1.669 – 3.308	0.906	-	0.523 – 1.569
5	2.137	-	1.512 – 3.018	2.093	-	1.345 – 3.254	1.327	-	0.720 – 2.446
>6	2.600	-	1.830 – 3.693	3.345	-	2.189 – 5.110	1.485	-	0.764 – 2.885
Province		0.281			0.134			0.014	
New Brunswick	1	-	-	1	-	-	1	-	-
Nova Scotia	1.290	-	0.901 – 1.846	1.749	-	1.064 – 2.874	2.016	-	1.176 – 3.454
Prince Edward Island	1.418	-	0.993 – 2.025	1.430	-	0.866 – 2.360	1.059	-	0.590 – 1.901
Saskatchewan	1.277	-	0.857 – 1.899	1.116	-	0.626 – 1.988	0.897	-	0.463 – 1.734
Frailty variance	0.235	0.000		0.551	0.000		0.406	0.000	

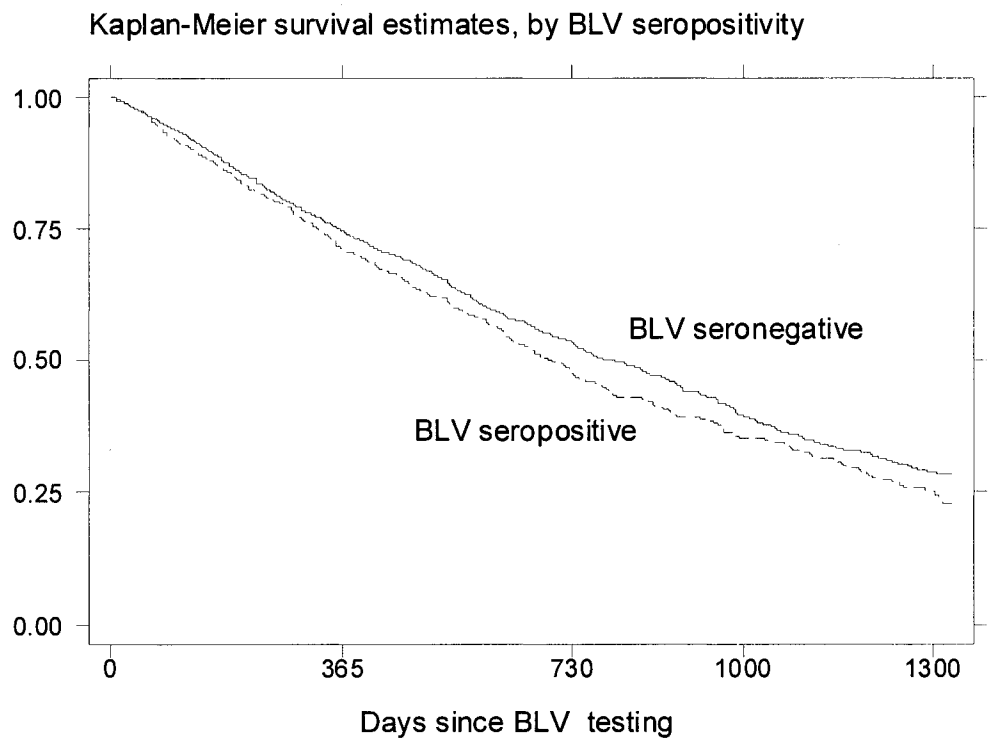


Fig. 5.1. Kaplan-Meier survival curves[1] for BLV-seropositive and seronegative cows in 3531 dairy cows in 134 Canadian herds.

BLV-seronegative cows _____

BLV-seropositive cows _ _ _ _ _ _

[1] Survival curves, which estimate the probability for a cow to survive (not be culled) up to any given day after testing (100% survival on day 0 - top-left corner of the curve).

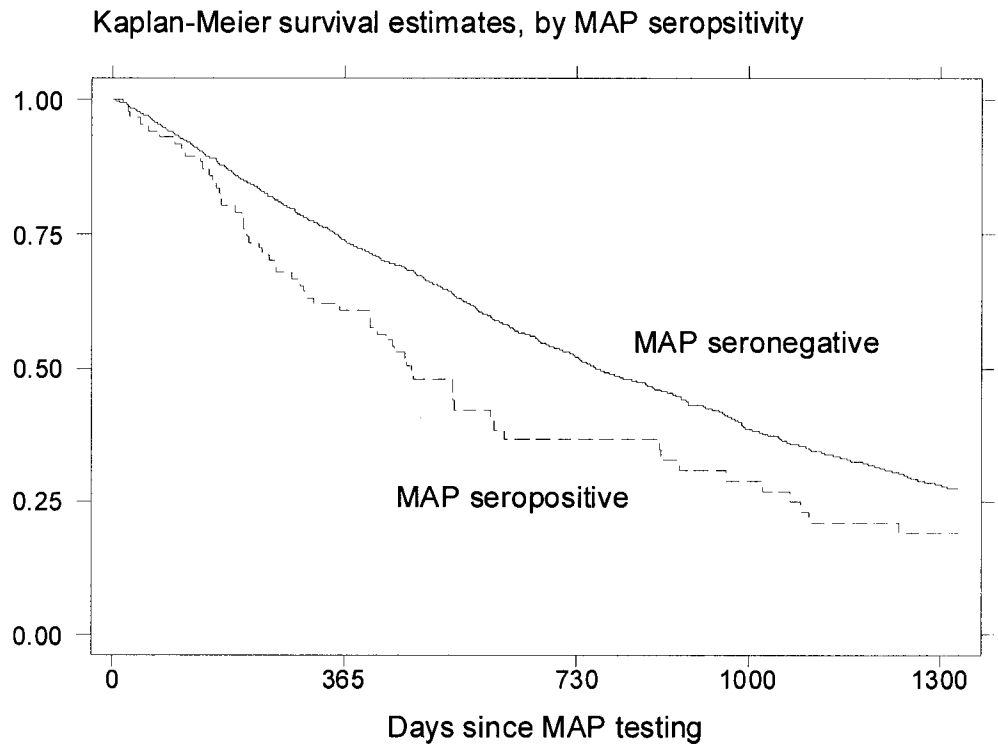


Fig. 5.2. Kaplan-Meier survival curves[1] for MAP-seropositive and seronegative cows in 3531 dairy cows in 134 Canadian herds.

MAP-seronegative cows _____

MAP-seropositive cows _ _ _ _ _ _

[1] Survival curves, which estimate the probability for a cow to survive (not be culled) up to any given day after testing (100% survival on day 0 - top-left corner of the curve).

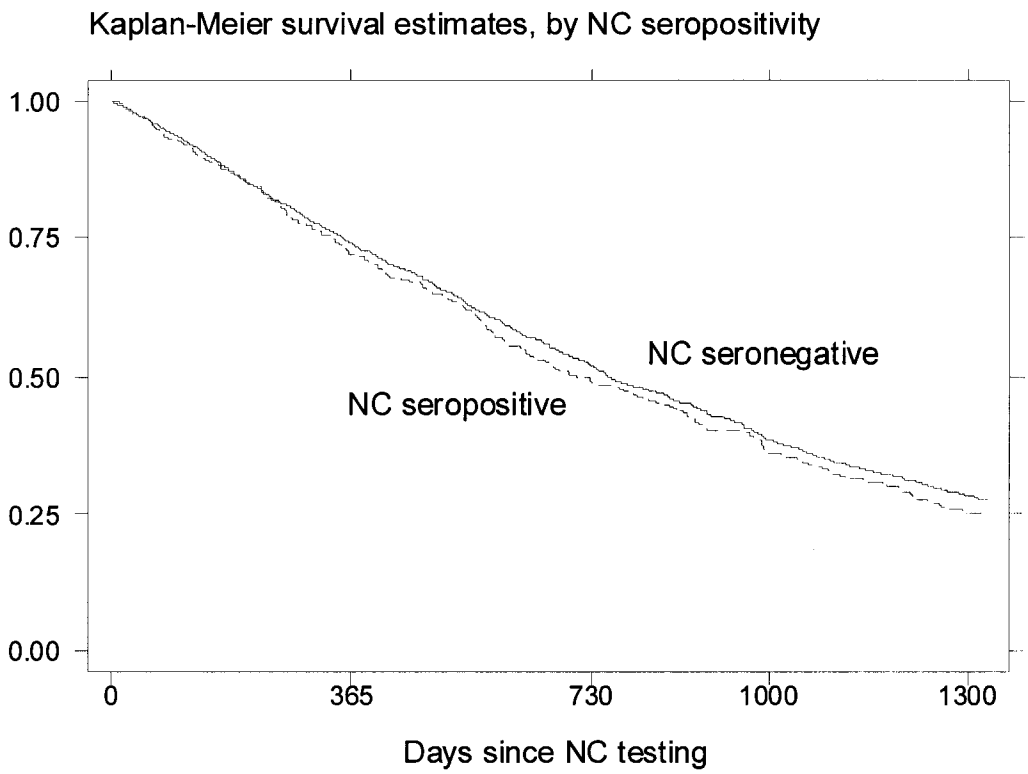


Fig. 5.3. Kaplan-Meier survival curves[1] for NC-seropositive and seronegative cows in 3531 dairy cows in 134 Canadian herds.

NC-seronegative cows _____

NC-seropositive cows _ _ _ _ _ _

[1]Survival curves, which estimate the probability for a cow to survive (not be culled) up to any given day after testing (100% survival on day 0 - top-left corner of the curve).

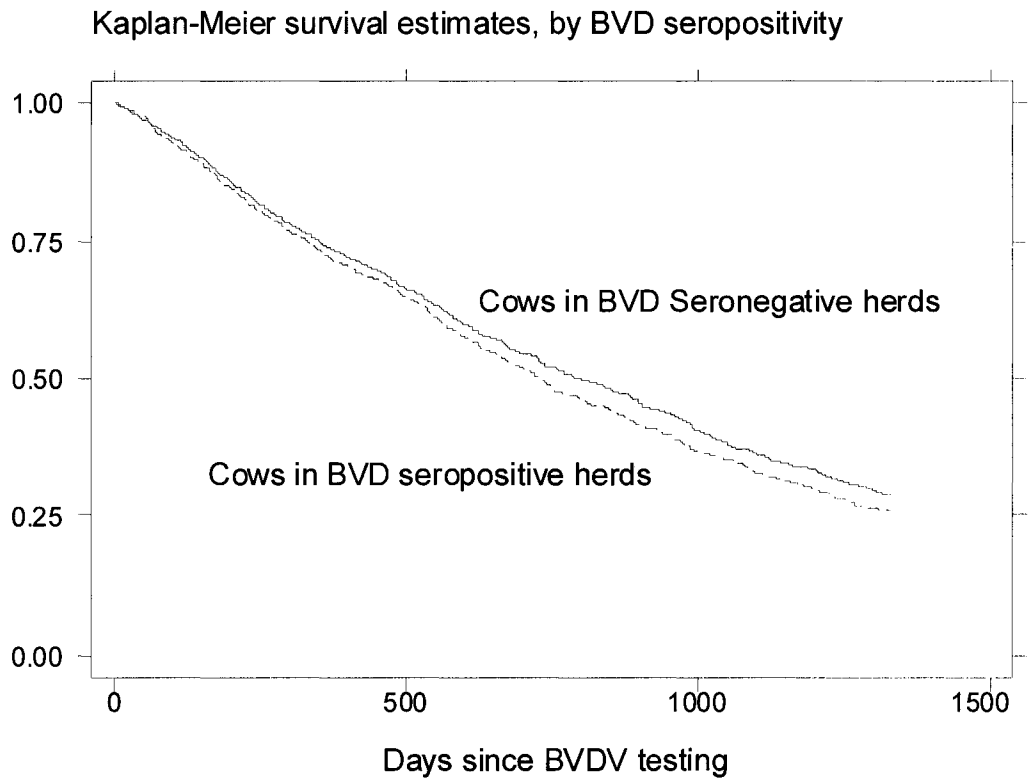


Fig. 5.4. Kaplan-Meier survival curves[1] for 3531 cows in BVDV-seropositive and seronegative Canadian dairy herds (n=134)

Cows in BVDV-seronegative herds _____

Cows in BVDV-seropositive herds _ _ _ _ _ _

[1]Survival curves, which estimate the probability for a cow to survive (not be culled) up to any given day after testing (100% survival on day 0 - top-left corner of the curve).

CHAPTER 6: Direct production losses from subclinical Mycobacterium avium subspecies paratuberculosis infection in Canadian dairy herds

6.1 Abstract

The objective of this study was to determine the estimate and range of annual direct production losses from Mycobacterium avium subspecies paratuberculosis (MAP) for an average MAP-seropositive Canadian dairy herd with 61 milking cows and a seroprevalence of 12%. A stochastic, partial budget, simulation model with four components of direct production losses (decreased milk production, premature voluntary culling, mortality and reproductive losses) was developed using @RISK software with Latin hypercube sampling and 10,000 iterations for each analysis. Input values were obtained primarily from a national study of 373 Canadian dairy farms in 8 of 10 provinces, with a few values coming from peer-reviewed literature. The model took into account the variability and uncertainty of the required input values and consequently produced probability distributions of the estimated losses.

For the MAP-seropositive Canadian dairy herds, the mean loss per 61 cow herd was CD $2,992 (95% C.I., $143 – $9741) annually, or CD $49 per cow per year. Herd additional culling losses were responsible for 46% (CD $1374) of the total losses from MAP. Decreased milk production, mortality and reproductive losses accounted for 9% (CD $254), 16% (CD $488) and 29% (CD $875) of the losses, respectively. MAP-seropositive

dairy farms sustain substantial, although somewhat variable, economic costs associated with these production losses, and dairy producers should use best management practices to reduce these annual losses.

6.2 Introduction

Recent international developments in the area of infectious disease control and non-tariff trade barriers, along with possible zoonotic concerns, have provoked a revival of interest in Johne's disease (JD) in Canada and elsewhere. The slow-growing, acid-fast bacterium causing Johne's disease (JD), Mycobacterium avium subspecies paratuberculosis (MAP), is distributed worldwide (Anon., 1997; Boelaert et al., 2000; Gasteiner et al., 1999; Muskens et al., 2000; Tiwari et al., 2005b) and causes chronic, progressive, granulomatous enteritis in domestic and exotic ruminants. Dairy cattle infected with MAP have been associated with premature culling (Benedictus et al., 1987; Tiwari et al., 2005e), decreased milk production (Abbas et al., 1983; Benedictus et al., 1987; Tiwari et al., 2005a), increased mortality (Kreeger, 1991), decreased reproductive efficiency (Abbas et al., 1983; Johnson-Ifearulundu et al., 2000) and possible increased susceptibility to other diseases (Tiwari et al., 2005d). Also known as paratuberculosis, JD has no known cure (Chiodini et al., 1984).

There have been studies done in the past to determine the economic losses from MAP, but the validity of these estimates in the Canadian dairy industry is questionable for the following reasons. 1) Some studies were based on research on a small number of herds that may not have been representative of the wide diversity of herds in the Canadian dairy industry and their differences in management or seroprevalence (Abbas et al., 1983; Benedictus et al., 1987; Chi et al., 2002). 2) Some studies utilized estimation methods, such as regression, or directly multiplied the estimated prevalence with costs of effects

associated with MAP (such as decreased milk yield) so that the interpretation of these estimates is limited to individual herds (Benedictus et al., 1987; Ott et al., 1999). 3) In a economic study done in Canadian maritime dairy herds (Chi et al., 2002), the utilized effects of MAP-seropositivity were estimated primarily from the scientific literature, in non-Canadian dairy herds. None of the studies done in the past utilized either the national Canadian MAP prevalence estimates or the effects of MAP on dairy cattle that have been estimated in Canadian dairy industry.

The objective of this study was to determine the economic losses from subclinical Mycobacterium avium subspecies paratuberculosis infection in Canadian dairy herds by utilizing Canadian estimates of MAP prevalence and impacts of subclinical MAP infection.

6.3. Materials and methods

6.3.1. Partial-budget model

A partial budget model is one which deals only with those aspects of an enterprise which are affected by a factor being investigated. The model used in this study was adapted from Bennett et al. (1999) and Chi et al. (2002), and included the impacts of MAP on milk yield, additional mortality, additional culling and reproductive losses .Considerations such as possible effects of MAP on human health, the ability of the farm to market livestock or other products, and other potential indirect costs were not included in the model.

6.3.2 Input parameters

Table 6.1 lists all of the input parameters used in the partial budget model, the distribution that was assumed to represent the range of possible values that each parameter might have, the characteristics which defined that distribution, and the source of the information about the parameter and its distribution. Each is discussed in more detail below.

6.3.2.1. Farm characteristics and prices

Two herd sizes were used in the analyses. To estimate the average losses for the Canadian dairy industry, a herd size of 100 cows was used and losses were expressed as being "per 100 cows" or "per cow". For estimating the range of possible losses at the individual infected herd level, the average size of a Canadian dairy herd, as reported by Dairy Farmers of Canada in 2002 (n=61 cows) was used. The average milk production

per cow per 305-day lactation (9,519 litres) was obtained from the Canadian Dairy Herd Improvement (DHI) (http://www.dairyinfo.gc.ca/pdf_files/statsbook2005.pdf). The average milk price ($0.59/liter) was obtained from the Canadian Dairy Information Centre web site (http://www.dairyinfo.gc.ca/pdf_files/pcan0304.pdf). Herd sizes, production levels and milk price were all treated as fixed values so that all estimates of MAP-associated losses were independent of differences in those parameters across herds.

Replacement cost of a cow (triangular distribution, min.= $1,500, max. = $2,500, most likely = $2,000 per head), average slaughter value (triangular distribution, min. = $300. max. = $700, most likely = $500 per head), and newborn calf value (triangular distribution, min. = $200, max. = $600, most likely = $400 per head) are representative values assigned by the authors following consultation with dairy clinicians familiar with the normal characteristics of these costs in Canada.

6.3.2.2. Seroprevalence of MAP

Data about the seroprevalence of MAP were obtained from a stratified two-stage random sample of 373 herds in eight Canadian Provinces (Tiwari et al., 2005b). During the summer of 1998 in Atlantic Canada, participating dairy herds were randomly selected (using computer generated random numbers) from all herds on monthly milk testing through the regional Dairy Herd Improvement (DHI) company until ninety herds were recruited, thirty from Prince Edward Island (PEI), New Brunswick (NB), and Nova Scotia (NS). Similar recruitment procedures were utilized to recruit thirty-one, seventy-nine, forty, fifty-one, and seventy-nine herds from the provinces of Ontario (ONT),

Quebec (QUE), Manitoba (MAN), Saskatchewan (SASK), and Alberta (ALTA) in 1998, 2002, 2002, 2001, and 2002/03, respectively. Within each herd, approximately 30 animals were randomly selected from the herd list and blood sampled. An ELISA test was utilized to determine whether cows were seropositive for MAP (details provided in Chapter 3). Overall 3.1% (2.3-3.8, 95% C.I.) of dairy cattle had positive tests for antibodies against MAP. For the model estimating overall losses for the Canadian dairy industry, the overall seroprevalence of MAP was assumed to fall within a normal distribution with a mean of 0.031 and S.D. of 0.004.

A much wider distribution for the within-herd seroprevalence was utilized for the estimate of the range of possible losses that individual infected Canadian herds might encounter. The mode of within-herd seroprevalence in herds having at least two MAP-seropositive cows was 6%, and 95% of the values were less than 33.3%. Consequently, a beta distribution (α=1.5488, β=9.5973) was utilized for estimating the range of infected herd losses.

6.3.2.3. Impact of MAP on milk yield.

For MAP, Benedictus et al., (1987) reported a 6% reduction in milk volume in the second to last lactation and a 16% reduction in the final lactation prior to culling in histopathologically positive, subclinically infected cows compared to culled cows without histopathological evidence of MAP-infection. Similar results have been reported elsewhere, with a 15% (835 kg) reduction in mean annual milk yield in fecal culture positive subclinically infected cows compared to fecal culture negative cows (Abbas et

al., 1983), and a 4% (376 kg) reduction in mature equivalent milk production in ELISA-positive cows compared to ELISA-negative cows (Nordlund et al., 1996) has been reported. In contrast, others have reported no significant decrease in milk volume in culled, asymptomatic, fecal culture-positive or histopathologically positive cows compared to test-negative culled cows (Buergelt and Duncan, 1978), or ELISA seropositive and fecal culture positive cows (Johnson et al., 2001), compared to test-negative cows. Some of these studies (Johnson et al., 2001; Wilson et al., 1993) have suggested that the observed reduction in milk volume may not occur across all lactations. In the largest evaluation of the effect of MAP-seroprevalence on milk production (22,665 lactations from 9,834 cows in 342 Canadian dairy herds), an interaction was found between MAP-seropositivity and lactation number (Table 6.2) (Tiwari et al., 2005a), with a statistically significant reduction in milk yield being observed only in 4th or higher lactation animals. This effect (loss of 212 kg (S.E. 106) in a 305 day lactation) would represent 2.34% of the average yield used in this study. The losses associated with 4 plus lactation animals was estimated by multiplying together the average milk yield (9519liters/per cow per year), milk price ($0.59/lt) and reduced milk yield (2.34%). Assuming a triangular distribution (minimum=0.20, most likely=0.25 and maximum=0.30) (Tiwari et al., 2005c) for the proportion of 4 plus lactation animals in a herd, the herd milk losses would be estimated by multiplying together the herd size, within herd seroprevalence of infection, losses associated with 4 plus lactation animals and the proportion of animals in this parity group.

6.3.2.4. Mortality.

Cow value at death was set equal to the cost of replacement because no carcass value was assumed for dead animals (Nix, 1996). There has been limited investigation of the mortality associated with MAP infection. In one study of 121 dairy herds in Michigan, USA (Johnson-Ifearulundu et al., 1999), mortality risk among herds positive for paratuberculosis was 3.15% higher than among negative herds. Consequently, this parameter was added to the model as a normal distribution with $\mu = 0.0315$ and $\sigma = 0.015$.

6.3.2.4. Premature culling and reduced slaughter value.

Internationally, a higher cull rate has been reported in MAP culture-positive cows as compared to MAP negative cows in a 210-cow Holstein herd in New York (Wilson et al., 1993). In another 900-cow Guernsey herd with subclinically infected cows, 22.6% of fecal culture positive cows and 3.6% of fecal culture negative cows were reported culled due to mastitis, and 68.8% versus 60.2% were culled due to infertility, respectively (Merkal et al., 1975). Similarly, in a study in a Colorado dairy herd, 48.9% of seropositive cows (based on an ELISA test) were culled over one year as compared to 33% of seronegative cows (Goodell et al., 2000).

Similar results were reported in 134 Canadian dairy herds (from NB, NS, PEI and SASK) with a follow-up period that ranged from 1.3 to 4 years. A total of 1981 of the 3531 cows were culled. The hazard of culling of MAP-seropositive cows was 1.38 (1.05 – 1.81, 95% C.I.) times that of MAP-seronegative cows. Interpreting the hazard ratio as a risk ratio,

assuming an average follow-up period of 2 years and using a within herd prevalence of MAP of 2.4%, the risk difference associated with MAP seropositivity was estimated to be 0.109 (SE = 0.04). Consequently, this parameter was added to the model as a normal distribution with $\mu = 0.109$ and $\sigma = 0.04$.

6.3.2.5. Reproductive losses.

In a sample of 533 animals from 7 dairy herds in Michigan, USA, ELISA-positive cows had a 28-day increase in calving conception interval compared to ELISA-negative cows (18). Kirk (1999) stated that a cost of increased days open is at least US$ 2.00-5.00 per day, which was converted to CD$ 2.5-6.25 per day by using an exchange rate of 1.25. Consequently, this cost of a day open was added to the model as a triangular distribution with minimum = 2.5, most likely = 4.375 and maximum = 6.25. The reproductive loss was added to the model as a normal distribution with $\mu = 27.9$ and $\sigma = 11.4$

6.3.2.6. Stochastic simulation of the partial budget model

A stochastic simulation model which combined the values presented in Table 6.1 into an overall estimate of the MAP-associated losses was developed using @RISK (2002) (Version 4.5.2, Palisade Corporation) with Latin hypercube sampling and 10,000 iterations for each analysis. The estimated distributions of total losses for the overall Canadian dairy industry and for infected individual dairy herds were determined and graphed. Mean and median values were determined along with the range of values that encompassed 95% of the estimates. The losses for each of the four main components of the model were determined. All calculations in the partial budget were carried out on an

annual basis except reproductive losses where it was estimated on a lactation basis. To convert this to an annual loss, an average calving interval of 13 months was assumed (based on consultation with dairy clinicians familiar with the normal calving interval in Canada) and the losses per lactation were multiplied by 0.923 (12/13).

A sensitivity analysis of key parameters was carried out as follows. For each of the following parameters, analyses were repeated, assuming expected values that were either 10% lower or 10% higher than estimated for: prevalence, reduction in milk yield, risk difference for mortality, risk difference for culling, and risk difference for infertility. For example, in the model evaluating overall effects in the Canadian dairy population, the increased culling was changed from 10.9% to 9.81% and 11.99% (the standard deviation of each of these distributions was left unchanged at 4%). The impacts of each of these 10% changes on the overall estimates of loss were compared to determine which factors were most influential in the model.

6.4 Results

For the Canadian dairy industry as a whole, the mean loss per 100 cows was $1196 annually ($385 per infected cow per year), regardless of their infection status, assuming a seroprevalence of 3.1% for this group of 100 cows. Additional culling costs, and reproductive, mortality and milk losses associated with seroprevalence for MAP were responsible for 45.8%, 29.2%, 16.2% and 8.8%, respectively (Table 6.2 and Figure 6.1). Figure 6.1 shows the potential variability around the estimate could be as low as $610 and as high as $1901 (not including the upper and lower 2.5% of estimates), based on the variability of the input estimates into the model and the stochastic nature of the model.

In MAP–seropositive dairy herds, the mean loss per 61 cows was $2992 annually, or $49 per cow per year, assuming a within herd seroprevalence of 12%. Additional culling losses were responsible for 46% (CD $1374) of the total herd losses from MAP. Decreased milk production, mortality and reproductive losses accounted for 9% (CD $254), 16% (CD $488) and 29% (CD $875) of the total herd losses, respectively (Table 6.3 and Figure 6.2). Figure 6.2 demonstrates that the mean loss could be as low as $143 and as high as $9741 (again, not including the upper and lower 2.5% of estimates), based on the variability of the input estimates into the model and the stochastic nature of the model.

Table 6.4 shows the results from the sensitivity analyses with the 10% changes in input estimates. The changes in MAP-seroprevalence lead to the largest difference (9.7%) in

the overall estimate of economic impact, from CD $1196 to CD $1076 and CD $1315 for minus and plus 10%, respectively, in the Canadian dairy industry, and from CD $2992 to CD $2691 and CD $3289, respectively, for a MAP-infected herd. A 10% change in additional culling, reproductive, mortality and milk losses resulted in a 4.6%, 2.9%, 1.6% and 1.2% change in annual losses due to MAP, respectively.

6.5 Discussion

Calculation of costs due to MAP-infection in the Canadian dairy industry requires estimating the MAP-prevalence and quantifying the losses that can be attributed to MAP infection. Clinical effects of paratuberculosis are well documented (Benedictus et al., 1987). However, due to the long incubation period of MAP, very few cattle show the signs of paratuberculosis before being culled (Merkal et al., 1975). Estimates suggest that for each animal with clinical signs of paratuberculosis in dairy herds, twenty-five subclinically infected cows are present (Whitlock et al., 1991). While the productivity costs due to clinical paratuberculosis are significant for that animal and can be observed by the farmer, the costs due to subclinical paratuberculosis can be more devastating due to the effects occurring on a larger number of cattle. Therefore, the costs due to subclinical paratuberculosis may be far more damaging at the herd and industry levels.

This paper updates a previous paper on the estimates of the effects of subclinical MAP-infection in maritime Canadian dairy herds, based on a spreadsheet model (Bennett et al., 1999) developed from data from the Maritime provinces of Canada (Chi et al., 2002). Major updates included in the current study were: national prevalence estimates; national estimates of milk losses and risk of culling associated with subclinical MAP infections; and making the model almost fully stochastic in nature. The prevalence estimates utilized in this study were based upon representative data of randomly sampled herds and cows from eight of the ten provinces in Canada. Actual milk losses and risk of culling associated with MAP infections in the Canadian dairy industry were included in this

economic study. Both of these aspects of the research make the economic results highly representative. Furthermore, the stochastic nature of the partial budget model allowed us to incorporate the variability of the input estimates into the model.

In MAP infected herds, our results suggested a range (95% C.I.) of CD$ 2 to 160 per cow per year (mean was CD$ 49 per cow per year). Ott et al., (1999) reported a similar range (90% C.I.) of US$ 2 to 120 per cow per year in MAP infected herds (in which less than 10% of culled cows were culled due to clinical signs similar to Johne's disease). These estimates (Ott et al., 1999) were considered similar to our results because more than 90% of positive herds in our study had herds where less than 10% of culled cows were culled due to clinical signs representative of Johne's disease.

Our results also suggested that the cost estimates due to subclinical MAP-infection ranged (95% C.I.) from CD$ 197 to 613 per infected cow (mean was CD$ 385 per infected cow per year). In a similar American study, by utilizing a same milk and replacement costs, MAP-infection costs ranged from US$ 123 to 696 per subclinically infected cow (Ott et al., 1999). Overlapping ranges of cost estimates suggest very similar economic losses due to MAP-infection in both the Canadian and American dairy industries.

The cost estimates due to subclinical MAP-infection from this study should be considered conservative estimates. The poor sensitivity (McKenna et al., 2005) of the current ELISA tests to correctly identify subclinically infected cattle (Dargatz et al., 2001; Sockett et al.,

1992) leads to substantial underestimation of the prevalence. However, the impacts of MAP-infection were also based upon the ELISA results and were considered appropriate because the sensitivity of ELISA is better (Sweeney et al., 1995) in later stages of MAP infection. The impact of MAP-infection in animals that were in early stages of MAP-infection (and ELISA negative) was assumed to be small or null. The effects of subclinical MAP-infection on the susceptibility of dairy cattle to other diseases such as BVDV and mastitis (which have large impacts on production) has not been determined but is suspected (Tiwari et al., 2005a).

Another reason why the cost estimates due to subclinical MAP-infection from this study should be considered conservative estimates is because the estimates do not include indirect costs. Additional indirect costs to society may be incurred due to the possible relationship between MAP in cattle and human Crohn's disease (Hermon-Taylor et al., 1998; Hermon-Taylor et al., 2000; Hermon-Taylor, 2000; Hermon-Taylor and Bull, 2002). However, due to the current absence of causal evidence, and the focus of this study being on direct costs to dairy producers and the dairy industry, this factor was not included in this study.

Mitigating this conservative estimate somewhat was the utilization of "normal" replacement cost and slaughter value of a healthy cow from the year before the discovery of Bovine Spongiform Encephalopathy in Canada. The period during which the border was closed to live cattle crossing the Canada-US border produced prices that were an aberration from normal prices.

While this study has numerous improvements over the economic study of MAP-infection in Maritime Canadian dairy herds, additional research is still needed to develop more precise estimates of the impacts of MAP-infection on dairy farms across Canada. For example, our estimates of milk production impact were conservative in nature for a number of reasons, including: inaccuracy of the ELISA leading to substantial misclassification bias, and the small number of test positive cows, leading to reduced power to detect impacts within different lactations. A study utilizing fecal cultures would likely produce less misclassification bias and therefore more accurate estimates of impacts on milk production relevant to the Canadian dairy industry. With regard to impacts on culling it would be desirable to analyse culling impacts of seropositivity for MAP, with this cohort of tested cows over a long time period, and the other pathogens. This additional analysis with a larger number of cows could provide more accurate and representative estimates of the impact of MAP-seropositivity on culling, particularly for specific reasons of culling. The current study had limited power to detect an association between MAP-seropositivity and specific reasons for culling due to the limited number of culled MAP-seropositive cows within each of the categories for reasons of culling. Misclassification bias of the ELISA may have also lead to a biasing of the result toward the null.

The mortality risk among herds positive for MAP-infection was assumed to be 3% higher than negative herds. Most of the MAP-infected cows are culled before they develop clinical signs (Tiwari et al., 2005e), therefore, the results from this study may be

overestimating the estimates of costs due to MAP-infection in our study. But, due to the lack of any other published estimate of impaction of MAP-infection on mortality risk, the author was not able to adjust for this possible overestimation.

With more detailed estimates of impacts on productivity and culling, more precise estimates of direct losses could be estimated. Furthermore, monitoring farms enrolled in a national Johne's disease control program could provide additional data on which to monitor prevalence estimates across Canada and estimates of impacts on productivity and culling, and improve the precision of the estimates of economic losses associated with MAP-infection in Canadian dairy herds.

6.6 Conclusions

For the MAP-seropositive Canadian dairy herds, the mean loss per 61 cow herd was CD $2,992 (95% C.I., $143 – $9741) annually, or CD $49 per cow per year. Herd additional culling losses were responsible for 46% (CD $1374) of the total losses from MAP. Decreased milk production, mortality and reproductive losses accounted for 9% (CD $254), 16% (CD $488) and 29% (CD $875) of the losses, respectively. MAP-seropositive dairy farms sustain substantial, although somewhat variable, economic costs associated with these production losses, and dairy producers should use best management practices to reduce these annual losses.

6.7 Reference List

1997. US Department of Agriculture, Animal and Plant Health Inspection Service, 1997. Johnes's Disease on US Dairy Operations, NAHMS Dairy '96, Center for Animal Health Monitoring, Report N245, 797.

Abbas, B., Riemann, H.P., Hird, D.W., 1983. Diagnosis of Johne's disease (paratuberculosis) in Northern California cattle and a note of it's economic significance. Calif. Vet. 37, 20-24.

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

Bennett, R., Christiansen, K., Clifton-Hadley, R., 1999. Preliminary estimates of the direct costs associated with endemic diseases of livestock in Great Britain. Prev. Vet Med. 39, 155-171.

Boelaert, F., Walravens, K., Bironf, P., Vermeersch, J.P., Berkvens, D., Godfroid, J., 2000. Prevalence of paratuberculosis (Johne's disease) in the Belgian cattle population. Vet Microbiol. 77, 269-281.

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

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

Chiodini, R.J., Van Kruiningen, H.J., Merkal, R.S., 1984. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. Cornell Vet 74, 218-262.

Dargatz, D.A., Byrum, B.A., Barber, L.K., Sweeney, R.W., Whitlock, R.H., Shulaw, W.P., Jacobson, R.H., Stabel, J.R., 2001. Evaluation of a commercial ELISA for diagnosis of paratuberculosis in cattle. J Am. Vet Med. Assoc. 218, 1163-1166.

Gasteiner, J., Wenzl, H., Fuchs, K., Jark, U., Baumgartner, W., 1999. Serological cross-sectional study of paratuberculosis in cattle in Austria. Zentralbl. Veterinarmed. B 46, 457-466.

Hermon-Taylor, J., 2000. Mycobacterium avium subspecies paratuberculosis in the causation of Crohn's disease. World J Gastroenterol. 6, 630-632.

Hermon-Taylor, J., Barnes, N., Clarke, C., Finlayson, C., 1998. Mycobacterium paratuberculosis cervical lymphadenitis, followed five years later by terminal ileitis similar to Crohn's disease. BMJ 316, 449-453.

Hermon-Taylor, J., Bull, T., 2002. Crohn's disease caused by Mycobacterium avium subspecies paratuberculosis: a public health tragedy whose resolution is long overdue. J Med. Microbiol. 51, 3-6.

Hermon-Taylor, J., Bull, T.J., Sheridan, J.M., Cheng, J., Stellakis, M.L., Sumar, N., 2000. Causation of Crohn's disease by Mycobacterium avium subspecies paratuberculosis. Can J Gastroenterol. 14, 521-539.

Johnson, Y.J., Kaneene, J.B., Gardiner, J.C., Lloyd, J.W., Sprecher, D.J., Coe, P.H., 2001. The effect of subclinical Mycobacterium paratuberculosis infection on milk production in Michigan dairy cows. J Dairy Sci 84, 2188-2194.

Johnson-Ifearulundu, Y., Kaneene, J.B., Lloyd, J.W., 1999. Herd-level economic analysis of the impact of paratuberculosis on dairy herds. J Am. Vet Med. Assoc. 214, 822-825.

Johnson-Ifearulundu, Y.J., Kaneene, J.B., Sprecher, D.J., Gardiner, J.C., Lloyd, J.W., 2000. The effect of subclinical Mycobacterium paratuberculosis infection on days open in Michigan, USA, dairy cows. Prev. Vet Med. 46, 171-181.

Kirk, J.H., 1999. Infectious abortions in dairy cows. On-Line Electronic Publications of the Veterinary Medical Extension, Veterinary Medicine Teaching and Research Center, University of California. Www. Vetmed. Ucdavis. Edu/Vetext/INF-DA_InfexAbortions. Html .

Kreeger, J.M., 1991. Ruminant paratuberculosis--a century of progress and frustration. J Vet Diagn. Invest 3, 373-382.

McKenna, S.L.B., Keefe, G.P., Barkema, H.W., Sockett, D.C., 2005. Evaluation of three ELISAs for *Mycobacterium avium* subsp. *paratuberculosis* using tissue and fecal culture as comparison standards. Accepted in Veterinary Microbiology .

Merkal, R.S., Larsen, A.B., Booth, G.D., 1975. Analysis of the effect of inapparent bovine paratuberculosis. Am. J Vet Res 36, 837-838.

Muskens, J., Barkema, H.W., Russchen, E., Van Maanen, K., Schukken, Y.H., Bakker, D., 2000. Prevalence and regional distribution of paratuberculosis in dairy herds in The Netherlands. Vet Microbiol. 77, 253-261.

Farm Management Pocketbook. 1996. Wye College, University of London, England.

Nordlund, K.V., Goodger, W.J., Pelletier, J., Collins, M.T., 1996. Associations between subclinical paratuberculosis and milk production, milk components, and somatic cell counts in dairy herds. J Am. Vet Med. Assoc. 208, 1872-1876.

Ott, S.L., Wells, S.J., Wagner, B.A., 1999. Herd-level economic losses associated with Johne's disease on US dairy operations. Prev. Vet Med. 40, 179-192.

Sockett, D.C., Carr, D.J., Collins, M.T., 1992. Evaluation of conventional and radiometric fecal culture and a commercial DNA probe for diagnosis of Mycobacterium paratuberculosis infections in cattle. Can J Vet Res 56, 148-153.

Sweeney, R.W., Whitlock, R.H., Buckley, C.L., Spencer, P.A., 1995. Evaluation of a commercial enzyme-linked immunosorbent assay for the diagnosis of paratuberculosis in dairy cattle. J Vet Diagn. Invest 7, 488-493.

Tiwari, A., VanLeeuwen , J.A., Dohoo, I.R., Keefe, G.P., Haddad, J.P., 2005a. Effects of seropositivity for bovine leukemia virus, bovine viral diarrhea virus, *Mycobacterium avium* subspecies *paratuberculosis,* and *Neospora caninum* and milk, fat and protein production and somatic cell count in Canadian dairy cattle. Submitted to J. Dairy Sci.

Tiwari, A., VanLeeuwen , J.A., Dohoo, I.R., Keefe, G.P., Haddad, J.P., 2005b. Seroprevalence and spatial distribution of bovine leukemia virus, bovine viral diarrhea virus and Mycobacterium avium subspecies paratuberculosis in Canadian dairy cattle. Submitted to Can Vet J .

Tiwari, A., VanLeeuwen, J.A., Dohoo, I.R., Keefe, G.P., Haddad, J.P., Tremblay, R., Scott, H.M., Whiting, T., 2005c. Effects of seropositivity for bovine leukemia virus, bovine viral diarrhoea, *Mycobacterium avium* subspecies *paratuberculosis,* and *Neospora caninum* on milk production in Canadian dairy cattle. Submitted to J Dairy Sci .

Tiwari, A., Vanleeuwen, J.A., Dohoo, I.R., Keefe, G.P., Haddad, J.P., Tremblay, R., Scott, H.M., Whiting, T., 2005d. Management risk factors associated with Mycobacterium avium subspecies paratuberculosis in Canadian dairy herds. Submitted to Prev. Vet. Med.

Tiwari, A., Vanleeuwen, J.A., Dohoo, I.R., Stryhn, H., Keefe, G.P., Haddad, J.P., 2005e. Effects of seropositivity for bovine leukemia virus, bovine viral diarrhoea virus, Mycobacterium avium subspecies paratuberculosis, and Neospora caninum on culling in dairy cattle in four Canadian provinces. Vet Microbiol. 109, 147-158.

Whitlock, R.H., Rosenberger, A.E., Siebert, M., Sweeny, R., 1991. Environmental survey of Mycobacterium paratuberculosis on dairy farms with a known history of Johne's disease. Proc. Annu. Meet. U. S. Anim. Hlth Assoc. 95, 280.

Wilson, D.J., Rossiter, C., Han, H.R., Sears, P.M., 1993. Association of Mycobacterium paratuberculosis infection with reduced mastitis, but with decreased milk production and increased cull rate in clinically normal dairy cows. Am. J Vet Res 54, 1851-1857.

1 Table 6.1 – Distribution, parameters and source of data for the model variables.

	Variable	Distribution/Formula	Parameters	Source of data
1	Average cattle population in herd	Fixed	61 or 100	Dairy Farmers of Canada (2002)
2	Seroprevalence of infection in the Canadian dairy population	Normal	$\mu = 0.031$, $\sigma = 0.004$	Tiwari et al., 2005
2	Seroprevalence of infection in seropositive dairy herds	Beta	$\alpha = 1.758$, $\beta = 21.317$, minimum=0.06	Tiwari et al., 2005
3	Milk yield (L per cow per year)	Fixed value	9519	CDHI
4	Milk price ($/L)	Fixed value	0.59	CDHI
5	Reduced milk yield	Normal distribution	$\mu = 2.34$, $\sigma = 1.18$	Tiwari et al., 2005
6	Losses associated with each low producing animal	**(3)*(4)*(5)**		
7	Percentage of infected animals affected	Triangular distribution	min=0.20, most likely=0.25, max=0.30	Assigned by author
ML	**Herd milk losses ($)**	**(1)*(2)*(6)*(7)**		
8	Replacement cost of cow ($ per head)	Triangular distribution	min=1500, most likely=2000, max=2500	Assigned by author
9	Percentage increased mortality risk in affected cattle	Normal distribution	$\mu = 0.0315$, $\sigma = 0.015$	Johnson-Ifearulundu et al., 1999
MO	**Herd mortality losses ($)**	**(1)*(2)*(8)*(9)**		

2

Table 1 continued

	Variable	Distribution	Parameters	Source of data
10	Slaughter value of healthy cattle ($ per head)	Triangular distribution	min=300, most likely=500, max=700	Assigned by author
11	Percentage of affected cattle with reduced slaughter value	Triangular distribution	min=0.2, most likely=0.25, max=0.3	Assigned by author
12	Losses associated with each culled animal ($)	**[(8)-{10-(11)*(10)}]**		
13	Excess culling risk for infected cattle	Normal distribution	μ= 0.109, σ= 0.04	Tiwari et al., 2005
CC	**Herd additional premature culling losses ($)**	**(1)*(2)*(12)*(13)**		
14	Increased calving interval (days)	Normal distribution	μ= 27.9, σ= 11.4	Johnson-Ifearulundu et al, 2000
15	Cost of increased calving interval ($/day)	Triangular distribution	Min=2.5, most likely=4.37, max=6.25	Kirk et al, 1999
RL	**Herd reproductive losses ($)**	**{(1)*(2)*(14)*(15)}*12/13**		
AL	**Herd annual Losses ($)**	**ML+MO+CC+RL**		

Figure 6.1 - Distribution of annual economic losses from MAP for the entire Canadian dairy industry, per 100 cows herd.

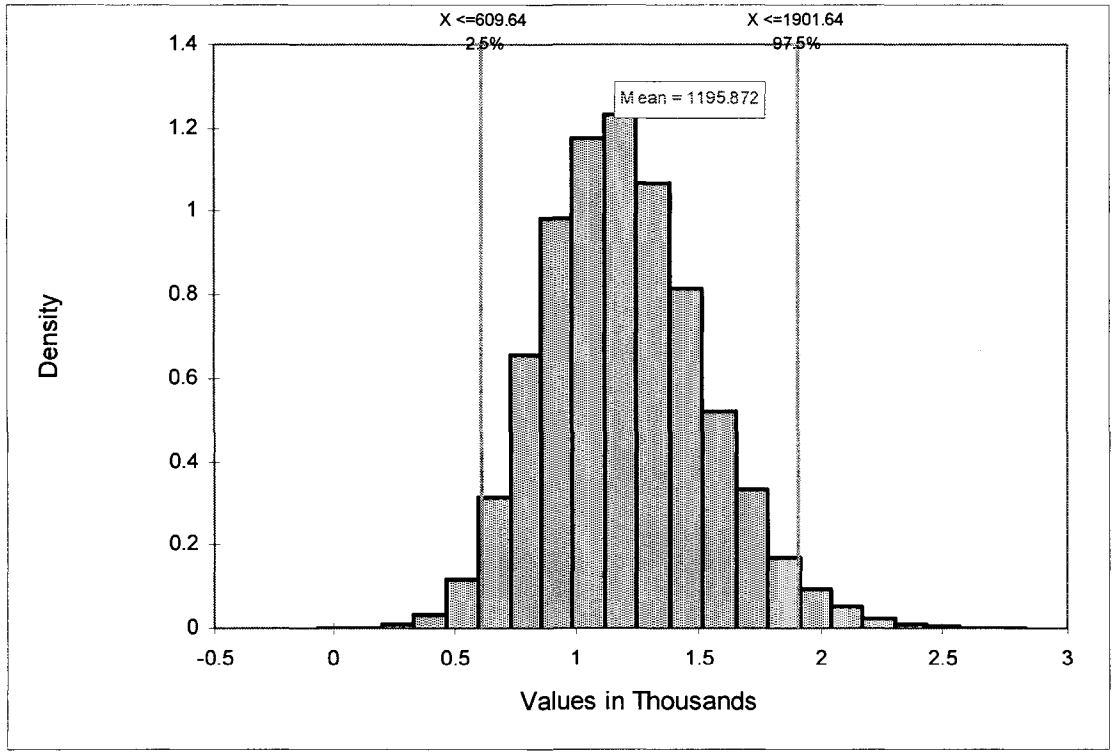
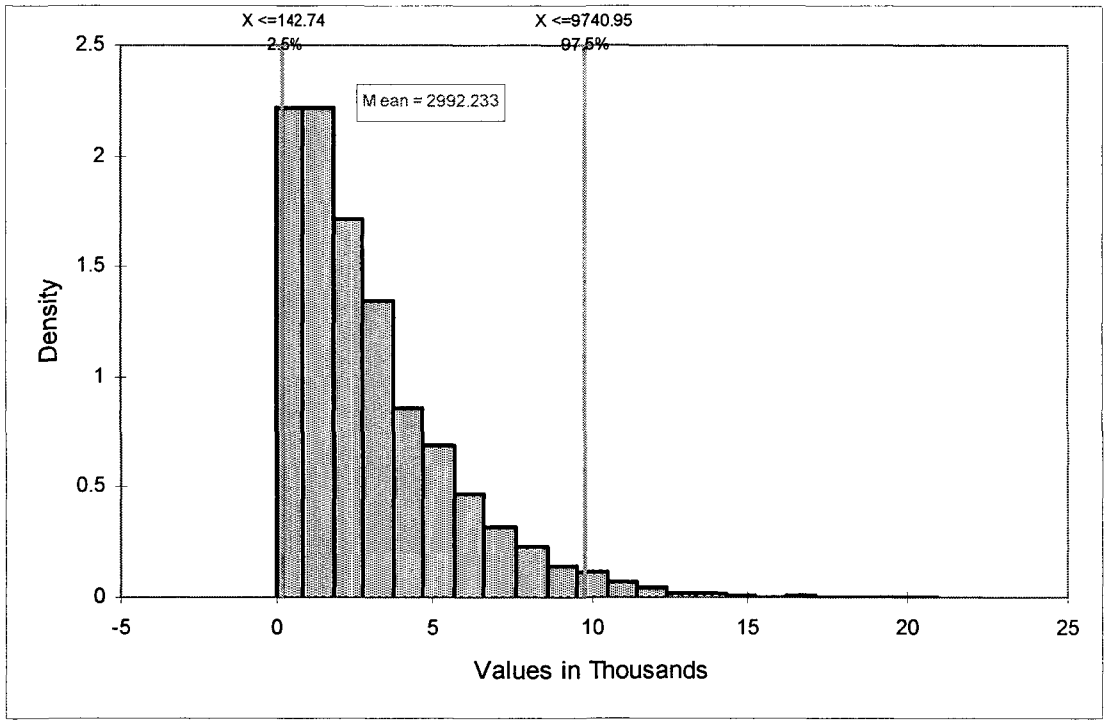


Figure 6.2 - Distribution of annual economic losses in a MAP-seropositive, 61 cow dairy herd in Canada.



15 Table 6.2. Economic losses with MAP for the entire Canadian dairy industry per 100 cow herd.

Var. #	Variable	Distribution	Mean	95% C.I.	
				Lower Limit	Upper Limit
1	Average cattle population in herd	Fixed	100		
2	Prevalence of infection in the Canadian dairy population	Normal	0.031	0.023	0.038
3	Milk yield (l per cow per year)	Fixed	9519	-	-
4	Milk price ($/l)	Fixed	0.59	-	-
5	Reduced milk yield	Normal	0.023	0.0012	0.0472
6	Losses associated with each low producing animal	**(3)*(4)*(5)**	132	1.5	262
7	Percentage of infected animals affected	Triangular	0.25	-	-
ML	**Herd milk losses ($)**	**(1)*(2)*(6)*(7)**	**102**	**1**	**216**
8	Replacement cost of cow ($ per head)	Triangular	2000	-	-
9	Increased mortality risk in affected cattle (%)/year	Normal	3.15	0.21	6.09
MO	**Herd mortality losses ($)**	**(1)*(2)*(8)*(9)**	**195**	**12**	**404**
10	Slaughter value of healthy cattle ($ per head)	Triangular	500	-	-
11	Percent of affected cattle with reduced slaughter value (%)	Triangular	25	-	-
12	Losses associated with each culled animal	**[(8)-{10-(11)*(10)}]**	1625	1215	2034
13	Excess culling risk for infected cattle (%)	Normal	10.9	3.1	18.7
CC	**Herd additional culling losses ($)**	**(1)*(2)*(12)*(13)**	**549**	**143**	**1035**
14	Increased calving interval (days)	Normal	28	6	50
15	Cost of increased calving interval ($/day)	Triangular	4	-	-
RL	**Herd reproductive losses**	**{(1)*(2)*(14)*(15)}*12/13**	**350**	**64**	**718**
AL	**Herd annual Losses**	**ML+MO+CC+RL**	**1196**	**610**	**1901**

16 Table 6.3. Economic losses in a MAP-seropositive, 61 cow dairy herd in Canada.

Var. #	Variable	Distribution	Mean	95% C.I.	
				Lower Limit	Upper Limit
1	Average cattle population in herd	Fixed	61	-	-
2	Prevalence of infection in individual dairy herds	Beta	12.7	0.65	38.47
3	Milk yield (l per cow per year)	Fixed	9519	-	-
4	Milk price ($/l)	Fixed	0.59	-	-
5	Reduced milk yield	Normal	0.023	0.0012	0.0472
6	Losses associated with each low producing animal	**(3)*(4)*(5)**	102	1	215
7	Percentage of infected animals affected	Triangular	0.25	-	-
ML	**Herd milk losses ($)**	**(1)*(2)*(6)*(7)**	**254**	**0.5**	**975**
8	Replacement cost of cow ($ per head)	Triangular	2000	-	-
9	Increased mortality risk in affected cattle (%)/year	Normal	3.15	0.21	6.09
MO	**Herd mortality losses ($)**	**(1)*(2)*(8)*(9)**	**488**	**5**	**1828**
10	Slaughter value of healthy cattle ($ per head)	Triangular	500	-	-
11	Percent of affected cattle with reduced slaughter value (%)	Triangular	25	-	-
12	Losses associated with each culled animal	**[(8)-{10-(11)*(10)}]**	1625	1215	2034
13	Excess culling risk for infected cattle (%)	Normal	10.9	3.1	18.7
CC	**Herd additional culling losses ($)**	**(1)*(2)*(12)*(13)**	**1374**	**46**	**4833**
14	Increased calving interval (days)	Normal	28	6	50
15	Cost of increased calving interval ($/day)	Triangular	4	-	-
RL	**Herd reproductive losses ($)**	**{(1)*(2)*(14)*(15)}*12/13**	**875**	**23**	**3259**
AL	**Herd annual Losses ($)**	**ML+MO+CC+RL**	**2992**	**143**	**9741**

17
18

Table 6.4. The possible range of annual losses due to JD, with a 10% change in seroprevalence, or losses associated with milk production, mortality, additional culling and reproductive losses.

Variables	Annual Losses with MAP			
	In entire Canadian Industry		In infected herds	
	Less 10%	More 10%	Less 10%	More 10%
Prevalence ($)	1076	1315	2691	3289
Milk loss ($)	1188	1209	2675	2722
Mortality loss ($)	1176	1215	2647	2735
Additional culling cost ($)	1141	1251	2568	2815
Reproductive losses ($)	1161	1231	2612	2770

CHAPTER 7: Management risk factors associated with Mycobacterium avium subspecies paratuberculosis infection in Canadian dairy herds

7.1 Abstract

The objective of this study was to determine the management factors associated with the seropresence and seroprevalence of Mycobacterium avium subspecies paratuberculosis in a large number of randomly selected Canadian dairy herds, controlling for important confounding variables and co-infections with bovine leukemia virus (BLV), bovine viral diarrhea virus (BVDV) and Neospora caninum (NC). Serum samples from approximately 30 randomly selected cows from 315 herds from 7 provinces were tested for antibodies against BLV, MAP and NC using commercially available ELISA test kits, while 5 unvaccinated cattle over 6 months of age were tested for antibodies to bovine viral diarrhoea virus (BVDV). A zero-inflated negative binomial (ZINB) model was utilized to simultaneously determine the management factors associated with the number of MAP-seropositive cows in a herd and the odds of herds having at least one MAP-seropositive cow as compared to having no MAP seropositive cows in a herd.

The following factors were significantly associated with the number of MAP-seropositive cows: "more than one cow in the maternity pen", "group housing in pre-weaned calves in winter", "purchasing open heifers", "direct contact with beef cattle", "BVDV-seropositive herds" and "not having proper BVD vaccination in calves (i.e. animals were not boostered 2-4 weeks after their first killed inoculation after 6 months of age)" with

count ratios of 1.69, 2.03, 2.34, 1.27, 1.41 and 1.77, respectively. The variable "herds having BVDV modified live vaccination in calves" was associated with 0.44 times fewer MAP-seropositive cows. There were also significant regional differences in the number of positive cows per herd. In the logistic portion of the final ZINB model, a one unit increase in the mean lactation number of cows tested in a herd was associated with 1.44 times greater odds of having at least one MAP-seropositive cow. The herds having "total mixed ration feeding" and "bedding that was not added frequently to calving areas (for each calving)" were associated with 3.1 and 2.7 times greater odds of being MAP-seropositive herds, respectively. The variables "NC-seropositivity" and "area of pasture (more than 200 acres)" were marginally significant ($P < 0.010$), while "BLV-seropositivity" was not significantly associated ($P > 0.10$) with MAP-seropositivity. The results from this study will assist management recommendations in national Johne's control programs.

7.2. Introduction

Paratuberculosis (Johne's disease) is caused by Mycobacterium avium subspecies paratuberculosis (MAP). It is a chronic infectious enteritis found in domestic and wild ruminants (Chiodini et al., 1984) and perhaps other species (Raizman et al., 2004). Reported productivity losses have included reduced milk production (Abbas et al., 1983; Nordlund et al., 1996; Tiwari et al., 2005a), increased culling (Benedictus et al., 1987; Tiwari et al., 2005c; Wilson et al., 1995), increased inter-calving period (Johnson-Ifearulundu et al., 2000; Tiwari et al., 2003) and mastitis (McNab et al., 1991; Merkal et al., 1975; Tiwari et al., 2005a). Costs due to reduced productivity have been estimated to be as high as US$200 per cow per year in herds where the prevalence of clinical cases among culled cows was ≥10% (Ott et al., 1999). In a Canadian study, the annual costs for MAP were estimated at CD$2472 per infected herd (having at least two seropositive cows) using an average herd size of 50 cows (Chi et al., 2002).

MAP is distributed worldwide but there does appear to be a wide variation in the prevalence between countries and even within countries. The seroprevalence estimates for dairy cattle at the animal level range from 17.1% (Florida, US) (Braun et al., 1990) to 0.8% (Belgium) (Boelaert et al., 2000), while herd level seroprevalences range from 74% in Missouri, US (Thorne and Hardin, 1997) to 18% in Belgium (Boelaert et al., 2000). A recent prevalence estimate from tissue culture testing of ileocecal lymph nodes and ileum from dairy cows at a slaughterhouse in New Brunswick found that 16.1% of dairy cows were culture-positive for MAP (McKenna et al., 2004). This prevalence estimate is likely

to be a close approximation of the true infection prevalence because culturing the ileum and ileocecal lymph node of the selected animals is a more sensitive test than fecal culturing for detecting cows that are infected but not yet shedding bacteria in their feces.

MAP is mainly transmitted from the feces of infected shedding cattle to susceptible youngstock through ingestion of fecally contaminated milk, water or feed. Other possible but less frequent routes of transmission are transplacental and colostral (Streeter et al., 1995; Sweeney et al., 1992a; Sweeney et al., 1992b). Johne's disease control programs have the following general objectives: 1) in non-infected herds, to prevent the entry of MAP into the herd; and 2) in infected herds, to detect and cull infected cattle, and to prevent the transmission of MAP to young livestock within the infected herd. In order to effectively accomplish these objectives, it is necessary to know what factors are associated with transmission of MAP within and between herds.

The following published factors have been associated with farm-to-farm transmission (presence of MAP infection on a farm):

a) regional differences, with certain regions having a higher prevalence (Wells and Wagner, 2000); (Collins et al., 1994);

b) herd size, with large herds being more likely to be infected with MAP (Collins et al., 1994; Wells and Wagner, 2000), perhaps related to large herds purchasing animals more frequently;

c) frequency of purchasing cattle, with percentage of cows born in other herds being related to the presence of MAP (Wells and Wagner, 2000); and

d) presence of farmed deer on the farm (Cetinkaya et al., 1997);

The following published factors have been associated with within-farm transmission (higher prevalence of MAP-infection):

a) higher lactation number (Jakobsen et al., 2000);

b) breed of cows in a herd, specifically, Channel Island breeds (Cetinkaya et al., 1997) (Jakobsen et al., 2000) have high prevalences;

c) housing management of preweaned calves, specifically, exposure of calves of ≤ 6 weeks of age to feces of adult cows (Obasanjo et al., 1997), and housing of pre-weaned calves and peri-parturient cows together for ≥ 24 hours (Wells and Wagner, 2000);

d) nutritional management, specifically, spreading of manure on fields from which forage is later harvested and fed to animals of any age group (Obasanjo et al., 1997), feeding whole milk containing antibiotic residues, or providing water for calves from birth (Ridge et al., 2005);

e) immunosuppression, due to poor nutrition or stress (with speculation of the latter being related to infections with pathogens such as bovine leukemia virus (BLV)) (Guilfoil et al., 1997);

f) use of exercise lot for lactating cows (Johnson-Ifearulundu and Kaneene, 1998);

Reported preventative factors reducing prevalence of infection include: application of lime to pasture areas, and cleaning of maternity pen after each use (Johnson-Ifearulundu and Kaneene, 1998), familiarity of farm manager with Johne's disease, and prior diagnosis of Johne's disease on farm (Wells and Wagner, 2000).

However, additional research into risk factors for MAP infection is needed for a number of reasons. 1) Some studies were based on a small number of herds that may not have been representative of the wide diversity of herds in the dairy industry and their differences in management or seroprevalence (Goodger et al., 1996; Ridge et al., 2005). 2) Herds were not randomly selected (were chosen either to assess the progress of control programs or to prove the absence of MAP) in many previous studies, leading to possible herd selection bias (Goodger et al., 1996; Obasanjo et al., 1997; Ridge et al., 2005) 3) Confounding variables (such as region or lactation number) were often not assessed and controlled in the design or analyses (Goodger et al., 1996; Johnson-Ifearulundu and Kaneene, 1998), an important consideration with certain regions and older cattle being known to have a higher risk of being seropositive. 4) Co-infections with other pathogens (e.g. bovine leukemia virus - BLV - and bovine viral diarrhea virus - BVDV – can impair cellular immunity) were usually not examined, co-infections which may be risk factors or which may interact with other risk factors. Furthermore, a national Johne's disease control program in Canada would also benefit greatly from a determination of important management risk factors for MAP in Canadian dairy cattle.

The objective of this study was to determine the management factors associated with the seropresence and seroprevalence of MAP in a large number of randomly selected Canadian dairy herds, controlling for important confounding variables and co-infections with BLV, BVDV and NC.

7.3. Materials and methods

The data utilized for this study were from recent prevalence surveys of Canadian dairy herds in Prince Edward Island (PEI), New Brunswick (NB), and Nova Scotia (NS) (Keefe and VanLeeuwen, 2000; VanLeeuwen et al., 2001), Saskatchewan (VanLeeuwen et al., 2005), Quebec (CQIASA, 2003), Manitoba (VanLeeuwen et al., 2003) and Alberta (Scott et al., 2005). The following sections provide a summary of the sampling protocol.

7.3.1 Serum Sample Collection

A stratified two-stage random sampling procedure was employed in all provinces. During the summer of 1998 in Atlantic Canada, participating dairy herds were randomly selected (using computer generated random numbers) from all herds on monthly milk testing through the regional Dairy Herd Improvement (DHI) company until ninety herds were recruited, thirty from Prince Edward Island (PEI), New Brunswick (NB), and Nova Scotia (NS). These herds met the herd level inclusion criteria which included willingness to: provide cattle for blood samplings; allow the blood to be tested for antibodies indicating exposure to the four pathogens (BLV, BVDV, MAP and NC); and release DHI data to the research team. Subsequently, similar (although not identical - see below) herd level inclusion criteria and sampling procedures were utilized to recruit seventy-five, forty, forty-four, and sixty-six herds from the provinces of Quebec (QUE), Manitoba (MAN), Saskatchewan (SASK), and Alberta (ALTA) in 2002, 2002, 2001, and 2002/03, respectively. Sampling of herds in ALTA was first stratified by veterinary practices

servicing dairy herds in the provinces, and then within these practices, herds were randomly selected.

Using computer generated random numbers; approximately 30 (less if the total number of cows in the herd was less than 30 cows) lactating animals were randomly selected for blood collection in each participating herd. Sample sizes for herd sampling within each province were based on calculations first determined and reported in detail for the Maritime provinces (VanLeeuwen et al., 2001), but adapted to the estimated herd prevalence for each province. The number of sampled herds per province was also dependent on available funds from the participating provinces. The sample size calculation for cow sampling was also based on calculations first determined and reported in detail for the Maritime provinces (VanLeeuwen et al., 2001). This sample size of 30 cows was adopted in the other provinces because a standard number of sampled cows per sampled herd was desirable for comparison purposes across provinces.

For BVDV, five unvaccinated (for BVDV) cattle > 6 months old were selected for blood collection. In unvaccinated herds, five animals of the 30 cows tested for the other three diseases were selected. In vaccinated herds, five unvaccinated heifers > 6 months old were selected to ensure that maternal antibodies would no longer be present. The BVDV sampling protocol was based upon Houe's study (Houe, 1992). Within 24 hours, the blood samples were centrifuged, and the serum was harvested and stored at -20°C until all the samples were collected and ready for testing for that province.

7.3.2 Laboratory Analysis

The test utilized by all provinces for BLV antibodies was an Enzyme Linked Immunosorbant Assay (ELISA) (sensitivity 98.5%, specificity 99.9%) (Johnson and Kaneene, 1991). A cow was considered to be infected with BLV if the serum-to-positive ratio on the ELISA was ≥0.50, as recommended by the manufacturer of the test kit. The BLV ELISA test kit also requires a confirmation of positive tests, using a sample-to-negative host-cell ratio of ≥1.8. The BLV testing for NS, NB, PEI, MAN, SASK, and ALTA was conducted at the national BLV testing laboratory in Prince Edward Island (now in Quebec), which is certified to conduct BLV testing for international trade purposes. The BIOVET Inc. laboratory in Quebec was utilized for the dairy farms in QUE for financial reasons.

The test utilized by all provinces for MAP antibodies was also an ELISA. Farms in all provinces except ALTA were tested with the same ELISA[1] (Dargatz et al., 2001). Alberta farms were tested with a different ELISA[2] (Sockett et al., 1992). For the first ELISA, an animal was considered to be seropositive for MAP if the serum-to-positive ratio on the ELISA was ≥ 0.25. For the second ELISA, if the optical density value was greater than the mean of the negative control for bovine sera plus 0.100, an animal was considered to be seropositive for MAP, as recommended by the manufacturer of the test kit. The MAP testing for NS, NB, PEI, and SASK was conducted at Prairie Diagnostic Services in SASK, which is accredited for MAP-ELISA testing by the United States Dept of

[1]IDEXX ELISA - IDEXX Corporation - Idexx Laboratories, Westbrook, Maine, USA
[2] BIOCOR-CSL ELISA –BIOCOR Animal Health, Inc., Omaha, Nebraska, USA

Agriculture. The BIOVET Inc. laboratory in QUE, the Manitoba Agricultural Laboratory in MAN, and the Alberta Agriculture, Food and Rural Development Food Safety Division Laboratory in ALTA were utilized for their respective provincial testing for financial reasons. The serum samples were tested in duplicate at all locations.

The test utilized by all provinces for NC antibodies was also an ELISA, with ALTA farms being tested with one brand of ELISA, again according to the wishes of the directors of the study in that province[1] (sensitivity 97.6%, specificity 99.5%) (Wu et al., 2002). The farms in the rest of the participating provinces were tested with a different ELISA[3] (sensitivity 99.0%, specificity 98.4%) (Bergeron et al., 2000). A cow was considered to be seropositive for NC if the serum-to-positive ratio for the first and second ELISAs were ≥ 0.60 and ≥ 0.40, respectively. The NC testing was conducted at the BIOVET Inc. laboratory in Quebec for dairy farms from NS, NB, PEI, ONT, MAN, SASK, and the Food Safety Division Laboratory of Alberta Agriculture, Food and Rural Development in ALTA for dairy farms in ALTA. The serum samples were tested in duplicate at both locations. Serum samples from dairy farms in QUE were not tested for antibodies to NC according to the wishes of the directors of the study in that province.

For all provinces, up to 5 serum samples per farm were tested for antibody against type 1 genotype BVD virus, using virus neutralization to the cytopathic Singer strain (sensitivity 99.6% and specificity 100%) (Deregt et al., 1992). A herd was considered to have been infected with BVDV if at least one of the animals tested had a titre of $\geq$ 1:64 for BVD virus, based on an observed natural division in the titres within farms, as reported

[3] BIOVET ELISA - BIOVET Inc. - St. Hyacinthe, Quebec, Canada

elsewhere (VanLeeuwen et al., 2001). Testing for BVDV was conducted at the Animal Diseases Research Institute in Alberta for dairy herds in NS, NB, PEI, MAN, SASK, ALTA, and at the Armand Frappier Laboratory in Quebec for dairy herds in QUE, for financial reasons.

7.3.3 Cow-level predictor data collection

Production and reproduction data of the sampled cows were obtained from the monthly milk test nearest the date of blood collection. The data for these cows were obtained electronically from Canadian Dairy Herd Management Services which processes DHI records for all of Canada. The cow-level predictors of interest included: lactation number (categorical – 1^{st}, 2^{nd}, 3plus), days in milk, 24-hour milk yield, 24-hour fat percent and 24-hour protein percent. These cow level variables were aggregated at the herd level to be utilized in the herd level statistical analyses described below.

The serostatus of NC and BLV were other cow-level predictors investigated. BLV and NC were defined as dichotomous variables (positive and negative based on the manufacturers' cutpoints of the diagnostic tests).

7.3.4 Herd-level predictor data collection

A number of herd-level predictors were determined through serological testing. A herd was considered positive for BVDV infection when at least one animal with a titer $\geq$ 1:64

1 was present. A herd was also considered positive for NC infection when at least two
2 animals were seropositive for NC. Also, a dichotomous variable was created from the
3 BLV within herd prevalence - whether or not the herd had a seroprevalence of $\geq$ 50%,
4 based on the maximum-likelihood of the cut-off value that dichotomized the herd-level
5 seroprevalence data best.
6
7 The majority of the herd-level predictors were obtained through personal interviews with
8 questionnaires administrated to managers at each farm visited. The questionnaire was
9 comprised of 11 sections with 21 pages and 423 variables, and was pre-tested on 5 farm
10 owners/employees for clarity and ease of administration. The questionnaires were
11 completed in an average of 45 minutes.
12
13 The questionnaire was divided into 11 sections. Section A covered the location and
14 identification of the farm and farmer. Section B covered the basic and demographic
15 information about the farm and farmer, including: age of the farmer, physical size of the
16 farm, cow breed, and number of employees. The herd population section (C) included the
17 number of dairy cattle currently present on the farm, and the number sold, culled, died
18 and purchased in the last 12-month period. These data were subdivided into the following
19 categories: pre-weaned calves, open heifers, bred heifers, milking cows, dry cows, and
20 bulls. The housing and pasture section (D) covered the type of housing utilized by each
21 category of animal in the winter and summer, and management practices for pasture.
22 Biosecurity was examined under various sections: purchase practices (E) and
23 requirements for the introduction of purchased animal; cattle contact (F) with domestic or

wild animals, either directly or through cattle water and food, and cattle contact with other people and their vehicles or equipment; transmission of disease through blood (G); and prophylactic use of medications and vaccines (H). The calving and calf management section covered colostrum and milk feeding of newborn calves, management of calves and calving area, and management of placentas and aborted fetuses (I). Another section (J) covered the type, origin and storage of food and water given to the dairy cattle, by age-sex categories of cattle, and general management of manure. The final section (K) looked at possible concurrent diseases, previous disease events and diagnostic tests carried out on the farm. The complete questionnaire is available from the corresponding author (Tiwari) on request.

7.3.5 Statistical analysis

The management factors were analysed in four steps using Stata, Release 8[4]. First, descriptive statistics (eg. means, proportions, correlations) were calculated for all cow and herd level predictors to assist in the subsequent modelling process. Zero-inflated negative binomial regression (ZINB) was utilized to determine the unconditional associations between the count of MAP-seropositive cattle on each farm, while adjusting for province, herd size and average lactation number in the herd as likely confounders. The number of cows tested in each farm was utilized as an offset. Those variables for which the P-value was ≤ 0.15 advanced to the second step – multivariable modeling. Guided by biological plausibility and statistical significance, a forward stepwise process was utilized to build the final multivariable model. Variables were considered significant with a P-value ≤ 0.05, and marginally significant with a P-value ≤ 0.10. BLV-, BVDV-

[4] Stata (Statistical package, Release.8; Stata Press, College Station, Texas, USA)

and NC–seropositivity, herd size, average lactation number in the herd, and province were forced in to all models as likely confounding factors. The third step was to identify significant interactions between significant main effects using a backward elimination process, allowing only significant (P-value ≤ 0.05) interaction variables to remain in the final model. Finally, regression diagnostics (including an evaluation of outliers) were carried out, and graphs of the observed verses predicted counts of MAP in a herd were generated. The likelihood ratio test was utilized for comparing poisson regression verses negative binomial regression and the Vuong test was utilized for comparing the ZINB model versus negative binomial regression model.

Logistic regression was also utilized to determine the cow and herd-level variables associated with herds having at least two MAP-seropositive cows compared to one or no MAP-seropositive cow in a herd, while adjusting for BLV-, BVDV- and NC-seropositivity province, average lactation number of cows in the herd, and herd size as possible confounders. A similar four-step approach to model building was utilized. The final model of the logistic regression was evaluated with the Hosmer-Lemeshow goodness-of-fit test, and plotting standardised residuals, leverage points and delta-beta values against observations.

7.3.6 Outcome variable

The number of cows seropositive for MAP was the outcome variable in the ZINB model, and a herd having at least 2 MAP seropositive cows was defined to be infected with MAP in the logistic model.

7.4. Results

7.4.1 Descriptive statistics

Of the 315 herds tested for exposure to the 4 pathogens, from 7 of the 10 Canadian provinces, questionnaires were completed for 285 farms. Due to missing data for certain questions in the questionnaire for some farms, only 257 herds within the 7 provinces were included in the dataset utilized for the final multivariable ZINB model. Comparison of the sampled cows and herds in the final dataset to national industry averages (Table 1) did not identify any significant differences (the range of industry averages are contained within the 95% confidence intervals of the sample population). Therefore, the random selection of herds and cows produced a sample population that was likely representative of the Canadian dairy industry as a whole.

Seroprevalence data are reported elsewhere. Briefly, for all Canadian dairy herds tested, 30.3% (Tiwari et al., 2005b), 3.1% (Tiwari et al., 2005b) and 11.4% (Haddad et al., 2005) of the cattle were positive for antibodies to BLV, MAP and NC, respeetively, while 31.2% (Tiwari et al., 2005b) of the herds had at least one cow with a titer of $\geq$ 1:64 for BVDV. The averages for these four agents in the sample population for this study were not significantly different from the national estimates, indicating minimal response bias with respect to BLV, BVDV, MAP and NC seroprevalences.

7.4.2 Management factor analyses

Table 2 reports the variables within each of the sections of the questionnaire that were associated ($P \leq 0.15$) with the count of MAP-seropositive cows in herds, after adjusting for province, herd size and average lactation number. These variables were eligible for subsequent steps of the modelling processes.

Table 3 reports the final model of the ZINB analyses after checking for interaction variables and model fit. In the negative binomial part of the model, the variables "more than one cow in maternity pen", "group housing in pre-weaned calves in winter", "purchasing open heifers", "direct contact with beef cattle", and "BVDV-seropositive herds" were associated with 1.69, 2.03, 2.34, 1.27, and 1.41 times more MAP-seropositive cows, respectively. "Purchasing bulls" was associated with 0.64 times fewer MAP-seropositive cows. Province was significantly associated with the number of MAP-seropositive cows, with the herds in ALTA being significantly associated with 5.82 times more MAP-seropositive cows compared to herds in PEI (referent category). There was also a trend towards herds in NB and MAN having 2.07 and 1.87 times more MAP-seropositive cows compared to herds in PEI, respectively.

BVDV vaccination in calves was associated with the number of MAP-seropositive cows. Specifically, "herds receiving modified live BVDV vaccination in calves" had 0.44 fewer MAP-seropositive cows, while "herds not having proper BVDV vaccination in calves" (after 6 months of age, calves not boostered 2-4 weeks after their first inoculation if a

killed vaccine was used) had 1.77 times more MAP-seropositive cows, compared to farms that did not use BVDV vaccine at all.

In the logistic part of the final ZINB model, a one unit increase in the mean lactation number of cows tested in the herd was associated with an increase of 1.44 times the odds of having at least one MAP-seropositive cow. "NC-seropositivity" and "area of pasture (more than 200 acres)" were marginally significant ($P < 0.010$), while "BLV-seropositivity" was not significantly associated ($P > 0.10$) with MAP-seropositivity. No interaction variables remained significant in the final model. The likelihood ratio test comparing poisson regression versus negative binomial regression was significant at the P-value of 0.001, indicating that the negative binomial distribution was preferred to the poisson distribution. The Vuong test was significant at the P-value of 0.005, indicating that the zero-inflated negative binomial model was preferred over the non-inflated model. The observed and predicted probabilities of each count (Graph 1) indicated that the model fit the data very well.

The following variables were significant in the final logistic regression model but not in the final ZINB model (whole model shown in table 4). Herds using a total mixed ration (TMR) were associated with 3.1 times greater odds of being MAP-seropositive (having at least 2 seropositive cows). Herds where bedding was not added to calving areas for each calving were associated with 2.7 times greater odds of being MAP-seropositive compared to herds where bedding was added to calving areas for each calving. The Hosmer-Lemeshow goodness-of-fit test was not significant, and plotting the standardised

residuals, and leverage and delta-beta values against the observations also indicated a good overall fit of the final model.

7.5. Discussion

This study was part of a national project designed to determine the seroprevalence, spatial distribution, effects and risk factors of BLV-, BVDV-, MAP- and NC-seropositivity in Canadian dairy cattle. Eight out of ten Canadian provinces (which comprises 94.95 % of dairy herds in Canada)[5] participated in the national study. Similar random selection procedures at the herd and cow level were utilized to ensure that the large study population was representative of the Canadian dairy industry.

The management questionnaire was completed on 285 farms (90%) out of the 315 farms in 7 provinces that participated in the study. Due to missing data for certain questions for some farms, responses from 28 farms were not included in the modelling process, leaving a final response rate for the final model of 82%, still indicating good representativeness. This response rate was comparable to the response rate reported in other studies (Johnson-Ifearulundu and Kaneene, 1998; Wells and Wagner, 2000). The summary statistics in Table 1 indicated that the farms in the 7 participating Canadian provinces were broadly representativeness of the national industry parameters.

One unique feature of this study was the investigation of seropositivity for other pathogens as risk factors for seropositivity for MAP. BVDV-seropositive herds were associated with MAP seroprevalence, and this may have been due to impaired immunity from the BVD virus. The immunosuppressive effects of BVDV include a decrease in percentage of circulating lymphocytes (Lamontagne et al., 1989) and an impairment of

[5] http://www.dairyinfo.gc.ca/pdf_files/statsbook2005.pdf

the normal protective function of neutrophils (Roth et al., 1981) and macrophages (Bolin et al., 1985; Reggiardo and Kaeberle, 1981). Infections with *Pasteurella multocida* or infectious bovine rhinotracheitis virus were severely aggravated by a concomitant acute BVDV infection (Potgieter et al., 1984a; Potgieter et al., 1984b). BVDV has also been associated with increased incidence of clinical mastitis (Waage, 2000) and somatic cell count (Tiwari et al., 2005a).

In this observational dataset, BVDV vaccination with modified live virus vaccine was also a significant protective factor for BVDV infection in a separate analysis (unpublished results), corroborating with results from clinical trials elsewhere (Cortese et al., 1998; Ellis et al., 2001). Therefore, it is biologically plausible that BVDV live virus vaccine would act as a preventive factor for the number of MAP-seropositive cows in a herd, as found in our results.

The marginal association between NC-seropositivity and MAP-seropositivity was unexpected and hard to explain. One explanation for this association could be a common unmeasured factor (confounder) in our study that was related to the introduction of both MAP and NC into the herds. Another explanation could be misclassification bias of the diagnostic tests for MAP and NC. The low-test sensitivity with the MAP ELISAs, and subsequent production of substantial numbers of false negatives in large populations has been documented (Dargatz et al., 2001; Sockett et al., 1992; Whitlock et al., 2000). Similarly, recent studies on various NC ELISAs have reported concerns regarding test sensitivity and specificity (Waldner et al., 2004). At the time of initiation of this study,

there was no study that independently evaluated test sensitivity and specificity of NC ELISAs. There have been studies since that time reporting on the accuracy of various NC ELISAs, but to date, no study has reported on the ELISA utilized in this study. Preliminary analyses by another researcher at UPEI have indicated that there are a substantial number of false positives with the ELISA utilized in this study, although recent changes in the ELISA appear to have eliminated most of these false positives (unpublished data). Seropositivity for BLV was not significantly associated with MAP-seropositivity but was forced into the final ZINB model in order to control for any potential confounding that it may have.

Herds purchasing open heifers were significantly associated with MAP-seroprevalence. Other studies have found that a similar risk factor "percentage of cows born on other dairy farms" was significantly associated with MAP-seropositivity (Wells and Wagner, 2000). In our study, the variable "herds purchasing any cattle" was also univariately associated with MAP-seroprevalence but that variable was highly collinear with purchasing open heifers. Therefore, only one of the two variables was allowed to remain as a significant variable in the final model. "Herds purchasing open heifers" was a stronger predictor for MAP-seropositivity than "herds purchasing any cattle", making the former variable a better fit for the final model.

"More than one cow in a maternity pen", "bedding not being added in calving areas between every calving", and "group housing for pre-weaned calves" were significantly associated with MAP-seropositivity. Similar factors and their directions were also

reported by Wells et al. (2000) and Johnson-Ifearulundu and Kaneene (1998). The effects of these risk factors depend upon the number of MAP organisms shed in feces and the organism's survival characteristics in the environment (Jorgensen, 1977). The primary method of MAP transmission is through a direct fecal-oral route, with calves being the most susceptible to infection (Chiodini et al., 1984). The specific number of organisms required for establishing infection for specific age groups has not been determined. Infection can occur in calves with a dose of $1.6x10^7$ organisms, which would easily be surpassed in a 2-gram sample of heavily infected feces (Sweeney et al., 1992b).

Farms with large areas of pasture (grazing) were marginally negatively associated with MAP-seropositivity. MAP is an obligate bacterium, and therefore, the survival of MAP on pasture is finite (Whittington et al., 2004). Larger pasture area provides the farmer the opportunity to increase the number of days between the spreading of feces on forage fields and the harvesting of those forages (Obasanjo et al., 1997).

Contact with beef cattle was significantly associated with number of MAP-seropositive cattle, suggesting that beef cattle could be a source of MAP to dairy cattle. MAP can also infect sheep, goats, farmed deer (Fawcett et al., 1995), alpacas (Cocito et al., 1994) and rabbits (Greig et al., 1997). The presence of farmed deer on dairy farms increased the risk of reporting paratuberculosis in 3772 dairy farmers in England and Wales (odds ratios ranged from 15.2 to 209) (Cetinkaya et al., 1997). Due to the low number of dairy farms which had other species of animals, our study was unable to confirm or reject the hypothesis that other species of animals pose as risk factors for MAP-seroprevalence.

Purchasing bulls was significantly associated with a lower number of MAP-seropositive cows, while other factors (such as use of artificial insemination or embryo collection) related to potential transmission of MAP through bull semen were not significantly associated with MAP-seroprevalence. None of the studies published in the scientific literature have reported a similar association. Inclusion or exclusion of this variable did not change the interpretation of other variables in the final model, and therefore it was left in the final model as a surrogate measure of some unmeasured preventive factor.

The association between an increase in mean lactation number of cows tested in a herd and an increase of 1.44 times the odds of having at least one MAP-seropositive cow can be explained by the chronic nature of the paratuberculosis (Chiodini et al., 1984). Many other studies have reported that older cattle are more likely to be test positive for MAP (Jakobsen et al., 2000). Most of the test-positive cattle had likely been infected in the first few months of their life but only become test positive later in life. Therefore, lactation number acts as a risk factor only because of the poor ability of the present diagnostic tests to detect young sub-clinically infected animals (Dargatz et al., 2001; Sockett et al., 1992; Whitlock et al., 2000).

Use of a total mixed ration (TMR) was associated with MAP-seropositivity in the logistic regression model. With herd size already forced in the model, the reason for TMR being in the model cannot be due to herd size. TMR is not being fed to calves and therefore is not likely to be a source of infection to calves. TMR may be a proxy for something else, such as a desire for saving on labour costs. The tractors /loaders that are used with TMRs

may also bring adult feces to calves. There is a need for future study on this possible association.

MAP-seropositivity was not significantly associated with herd size in either the ZINB or logistic model. However, herd size may act as a possible confounder, and therefore, was left in the final model. An American study found large herds (> 300 cows) having 4.6 higher odds of MAP infection (Wells and Wagner, 2000). The mean number of cows in herds in our study was 66, and 95% of the herds had less than 158 cows per herd. If herd size was split into 3 categories, < 50 cows, 50-299 cows, and ≥ 300 cows, the 95% confidence interval of the odds ratio for herds having ≥ 300 cows was statistically significant compared to herds having < 50 cows (referent category). However, the small number of herds having more than 300 cows in our study may explain the inability of our study to find a significant relationship between herd size and MAP-seropositivity.

Overall, region was significantly associated with MAP-seropositivity, an observation also reported in previous studies (Collins et al., 1994; Jakobsen et al., 2000; Wells and Wagner, 2000). However, "region" should be treated as a proxy for some other herd factors that may differ between these regions, with herd management practices within a region likely being similar as compared to herd management practices in different regions. Significant clusters of MAP-seropositivity in space were detected in a spatial analysis of these data, reported elsewhere (Tiwari et al., 2005b).

The zero-inflated negative binomial regression model (ZINB) was utilized to determine simultaneously the management factors associated with the number of MAP-seropositive cows in a herd, and odds of herds having no MAP-seropositive cows compared to having at least one MAP-seropositive cow in a herd (ie. included negative binomial and logistic components, respectively). The decision to utilize the ZINB model over a poisson regression model (PRM) or negative binomial regression model (NBRM) was based on the following two reasons. First, PRM and NBRM models assume that every herd in the study population has a positive probability of having MAP-seropositive cows (although it would not be necessary for all herds to have non-zero counts observed). However, some of the herds were likely to be truly negative, violating this assumption. In the ZINB model, the zero counts in the study population can be explained better by the logistic part of the model, while the non-zero counts can be explained by the negative binomial part of the model. Second, two statistical tests indicated that the ZINB model fit the data well. A likelihood ratio test for overdispersion showed that the negative binomial regression model (NBRM) was better than the poisson regression model (PRM). The Vuong test (Vuong, 1989) also showed that the ZINB regression model was better than the NBRM.

One limitation of this study has been the use of different test brands and different laboratories for some tests of pathogen exposure in some provinces. While these differences in laboratory testing may have an impact on comparisons between seroprevalences among provinces, they should have minimal impact on this study of risk factors for MAP-seropositive herds. The possible misclassification bias, if it exists, would likely be non-differential between positive and negative herds within provinces, biasing

toward the null, if at all. Therefore, the significant factors discovered to be associated with MAP-seropositivity were not likely to be due to differences in test brands or laboratories.

7.6. Conclusions

"More than one cow in the maternity pen", "group housing in pre-weaned calves in winter", "purchasing open heifers", "direct contact with beef cattle", "BVDV-seropositive herds" and "not having proper BVD vaccination in calves (i.e. animals were not boostered 2-4 weeks after their first shot after 6 months of age)" were associated with higher MAP-seroprevalence. The variable "herds having BVDV modified live vaccination in calves" was associated with lower MAP-seroprevalence. In the logistic part of the final ZINB model, an increase in the mean lactation number of cows tested in a herd was associated with an increased odds of having at least one MAP-seropositive cow. The variables "NC-seropositivity" and "area of pasture (more than 200 acres)" were marginally significant, while "BLV-seropositivity" was not significantly associated with MAP-seroprevalence. The herds having "total mixed ration feeding" and "bedding that was not added frequently to calving areas (for each calving)" were associated with greater odds of being MAP-seropositive herds. The results from this study will assist in the creation of management recommendations in national Johne's control programs.

7.7 Acknowledgements

The authors wish to acknowledge Dr. Lee Ann Forsythe and Ms. Renee Chartier of the Saskatchewan Department of Agriculture, Food and Rural Revitalization (Inspection and Regulatory Management Branch, Animal Health Unit), Terri Garner of the Department of Animal Science, University of Manitoba, the accredited Johne's veterinarians of Alberta, and laboratory scientists and staff of Alberta Agriculture, Food and Rural Development for their technical and logistical support in the collection of samples and data from the participating farmers in Saskatchewan, Manitoba and Alberta, respectively.

The authors also wish to acknowledge the following funding sources (monetary or in-kind contributions): the Food Safety Division of Alberta Agriculture, Food and Rural Development, Atlantic Veterinary College, Canadian Food Inspection Agency, Canadian Dairy Herd Management Services, Dairy Farmers of Canada, Dairy Farmers of PEI, Dairy Herd Improvement companies, Fédération des Producteurs de Lait du Québec, Dairy Farmers of Manitoba, Manitoba Agriculture and Food, PEI Agricultural Research Investment Fund, Production Limiting Diseases Committee, Saskatchewan Agriculture, Food and Rural Revitalization, and the Western Economic Partnership Agreement.

7.8 Reference List

Abbas, B., Riemann, H.P., Hird, D.W., 1983. Diagnosis of Johne's disease (paratuberculosis) in Northern California cattle and a note of it's economic significance. Calif. Vet. 37, 20-24.

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

Bergeron, N., Fecteau, G., Pare, J., Martineau, R., Villeneuve, A., 2000. Vertical and horizontal transmission of Neospora caninum in dairy herds in Quebec. Can. Vet J. 41, 464-467.

Boelaert, F., Walravens, K., Biront, P., Vermeersch, J.P., Berkvens, D., Godfroid, J., 2000. Prevalence of paratuberculosis (Johne's disease) in the Belgian cattle population. Vet. Microbiol. 77, 269-281.

Bolin, S.R., McClurkin, A.W., Cutlip, R.C., Coria, M.F., 1985. Severe clinical disease induced in cattle persistently infected with noncytopathic bovine viral diarrhea virus by superinfection with cytopathic bovine viral diarrhea virus. Am. J. Vet. Res. 46, 573-576.

Braun, R.K., Buergelt, C.D., Littell, R.C., Linda, S.B., Simpson, J.R., 1990. Use of an enzyme-linked immunosorbent assay to estimate prevalence of paratuberculosis in cattle of Florida. J. Am. Vet. Med. Assoc. 196, 1251-1254.

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

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

Chiodini, R.J., Van Kruiningen, H.J., Merkal, R.S., 1984. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. Cornell. Vet. 74, 218-262.

Cocito, C., Gilot, P., Coene, M., de Kesel, M., Poupart, P., Vannuffel, P., 1994. Paratuberculosis. Clin. Microbiol. Rev. 7, 328-345.

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

Cortese, V.S., Grooms, D.L., Ellis, J., Bolin, S.R., Ridpath, J.F., Brock, K.V., 1998. Protection of pregnant cattle and their fetuses against infection with bovine viral diarrhea virus type 1 by use of a modified-live virus vaccine. Am. J. Vet. Res. 59, 1409-1413.

CQIASA 2003. Centre Québécois d'inspection des aliments et de santé animale. Rapport final: Enquête de prévalence de la paratuberculose, de la leucose bovine enzootique et des immunotolérants à la diarrhée virale bovine dans les troupeaux laitiers du Québec.

Dargatz, D.A., Byrum, B.A., Barber, L.K., Sweeney, R.W., Whitlock, R.H., Shulaw, W.P., Jacobson, R.H., Stabel, J.R., 2001. Evaluation of a commercial ELISA for diagnosis of paratuberculosis in cattle. J. Am. Vet. Med. Assoc. 218, 1163-1166.

Deregt, D., Smithson, S., Kozub, G.C., 1992. A short incubation serum neutralization test for bovine viral diarrhea virus. Can. J. Vet. Res. 56, 161-164.

Dohoo, I.R., Martin, W., Stryhn, H., 2004. Veterinary Epidemiological Research. AVC Inc. Charlottetown, Canada .

Ellis, J., West, K., Cortese, V., Konoby, C., Weigel, D., 2001. Effect of maternal antibodies on induction and persistence of vaccine-induced immune responses against bovine viral diarrhea virus type II in young calves. J. Am. Vet. Med. Assoc. 219, 351-356.

Fawcett, A.R., Goddard, P.J., McKelvey, W.A., Buxton, D., Reid, H.W., Greig, A., Macdonald, A.J., 1995. Johne's disease in a herd of farmed red deer. Vet. Rec. 136, 165-169.

Goodger, W.J., Collins, M.T., Nordlund, K.V., Eisele, C., Pelletier, J., Thomas, C.B., Sockett, D.C., 1996. Epidemiologic study of on-farm management practices associated with prevalence of Mycobacterium paratuberculosis infections in dairy cattle. J. Am. Vet. Med. Assoc. 208, 1877-1881.

Greig, A., Stevenson, K., Perez, V., Pirie, A.A., Grant, J.M., Sharp, J.M., 1997. Paratuberculosis in wild rabbits (Oryctolagus cuniculus). Vet. Rec. 140, 141-143.

Guilfoil, V.Y., Roberts, N.J., Rodgers, S., McBridge, D., 1997. Regional and Country Seroprevalence of Bovine Leukosis Virus and Mycobacterium paratuberculosis (Johne's Disease) in Oklahoma Beef Cattle. Procc. Epidemiology and Economics Symposium, Fort Collins, Colorado.

Haddad, J.P., Dohoo, I.R., VanLeeuwen , J.A., Tiwari, A., Keefe, G.P., Scott, H.M., Whiting, T., 2005. Seroprevalence and spatial distribution of Neospora caninum in Canadian dairy cattle. Submitted to Can. Vet. J.

Houe, H., 1992. Serological analysis of a small herd sample to predict the presence of animals persistently infected with bovine viral diarrhoea virus (BVDV) in dairy

herds. Res. Vet. Sci. 53, 320-323.

Jakobsen, M.B., Alban, L., Nielsen, S.S., 2000. A cross-sectional study of paratuberculosis in 1155 Danish dairy cows. Prev. Vet. Med. 46, 15-27.

Johnson, R.J., Kaneene, J.B., 1991. Bovine Leukemia Virus. Part I. Descriptive Epidemiology, Clinical Manifestations, and Diagnostic tests. Compendium on Continuing Education 13, 328.

Johnson-Ifearulundu, Y.J., Kaneene, J.B., 1998. Management-related risk factors for M. paratuberculosis infection in Michigan, USA, dairy herds. Prev. Vet. Med. 37, 41-54.

Johnson-Ifearulundu, Y.J., Kaneene, J.B., Sprecher, D.J., Gardiner, J.C., Lloyd, J.W., 2000. The effect of subclinical Mycobacterium paratuberculosis infection on days open in Michigan, USA, dairy cows. Prev. Vet. Med. 46, 171-181.

Jorgensen, J.B., 1977. Survival of Mycobacterium paratuberculosis in slurry. Nord. Vet. Med. 29, 267-270.

Keefe, G.P., VanLeeuwen, J.A., 2000. Neospora then and now: prevalence of Neospora caninum in Maritime Canada in 1979, 1989, and 1998. Can. Vet. J. 41, 864-866.

Lamontagne, L., Lafortune, P., Fournel, M., 1989. Modulation of the cellular immune responses to T-cell-dependent and T cell-independent antigens in lambs with induced bovine viral diarrhea virus infection. Am. J. Vet. Res. 50, 1604-1608.

McKenna, S.L., Keefe, G.P., Barkema, H.W., McClure, J., VanLeeuwen, J.A., Hanna, P., Sockett, D.C., 2004. Cow-level prevalence of paratuberculosis in culled dairy cows in Atlantic Canada and Maine. J. Dairy. Sci. 87, 3770-3777.

McNab, W.B., Meek, A.H., Martin, S.W., Duncan, J.R., 1991. Associations between dairy production indices and lipoarabinomannan enzyme-immunoassay results for paratuberculosis. Can. J. Vet. Res. 55, 356-361.

Merkal, R.S., Larsen, A.B., Booth, G.D., 1975. Analysis of the effect of inapparent bovine paratuberculosis. Am. J. Vet. Res. 36, 837-838.

Nordlund, K.V., Goodger, W.J., Pelletier, J., Collins, M.T., 1996. Associations between subclinical paratuberculosis and milk production, milk components, and somatic cell counts in dairy herds. J. Am. Vet. Med. Assoc. 208, 1872-1876.

Obasanjo, I.O., Grohn, Y.T., Mohammed, H.O., 1997. Farm factors associated with the presence of Mycobacterium paratuberculosis infection in dairy herds on the New York State Paratuberculosis Control Program. Prev. Vet. Med. 32, 243-251.

Ott, S.L., Wells, S.J., Wagner, B.A., 1999. Herd-level economic losses associated with Johne's disease on US dairy operations. Prev. Vet. Med. 40, 179-192.

Potgieter, L.N., McCracken, M.D., Hopkins, F.M., Walker, R.D., 1984a. Effect of bovine viral diarrhea virus infection on the distribution of infectious bovine rhinotracheitis virus in calves. Am. J. Vet. Res. 45, 687-690.

Potgieter, L.N., McCracken, M.D., Hopkins, F.M., Walker, R.D., Guy, J.S., 1984b. Experimental production of bovine respiratory tract disease with bovine viral diarrhea virus. Am. J. Vet. Res. 45, 1582-1585.

Raizman, E.A., Wells, S.J., Godden, S.M., Bey, R.F., Oakes, M.J., Bentley, D.C., Olsen, K.E., 2004. The distribution of Mycobacterium avium sp. paratuberculosis in the environment surrounding Minnesota dairy farms. J. Dairy. Sci. 87, 2959-2966.

Reggiardo, C., Kaeberle, M.L., 1981. Detection of bacteremia in cattle inoculated with bovine viral diarrhea virus. Am. J. Vet. Res. 42, 218-221.

Ridge, S.E., Baker, I.M., Hannah, M., 2005. Effect of compliance with recommended calf-rearing practices on control of bovine Johne's disease. Aust. Vet. J. 83, 85-90.

Roth, J.A., Kaeberle, M.L., Griffith, R.W., 1981. Effects of bovine viral diarrhea virus infection on bovine polymorphonuclear leukocyte function. Am. J. Vet. Res. 42, 244-250.

Scott, H.M., Sorenson, O., Chow, E., Manninen, K., VanLeeuwen, J.A., 2005. Seroprevalence of Mycobacterium avium subspecies paratuberculosis, Neospora caninum, bovine leukemia virus, and bovine viral diarrhea virus infection in Alberta dairy cattle and agro-ecological risk factors associated with seropositivity. Submitted to Can. Vet. J.

Sockett, D.C., Conrad, T.A., Thomas, C.B., Collins, M.T., 1992. Evaluation of four serological tests for bovine paratuberculosis. J. Clin. Microbiol. 30, 1134-1139.

Streeter, R.N., Hoffsis, G.F., Bech-Nielsen, S., Shulaw, W.P., Rings, D.M., 1995. Isolation of Mycobacterium paratuberculosis from colostrum and milk of subclinically infected cows. Am. J. Vet. Res. 56, 1322-1324.

Sweeney, R.W., Whitlock, R.H., Rosenberger, A.E., 1992a. Mycobacterium paratuberculosis cultured from milk and supramammary lymph nodes of infected asymptomatic cows. J. Clin. Microbiol. 30, 166-171.

Sweeney, R.W., Whitlock, R.H., Rosenberger, A.E., 1992b. Mycobacterium paratuberculosis isolated from fetuses of infected cows not manifesting signs of the disease. Am. J. Vet. Res. 53, 477-480.

Thorne, J.G., Hardin, L.E., 1997. Estimated prevalence of paratuberculosis in Missouri, USA cattle. Prev. Vet. Med. 31, 51-57.

Tiwari, A., VanLeeuwen , J.A., Dohoo, I.R., Keefe, G.P., Haddad, J.P., 2005a. Effects of seropositivity for bovine leukemia virus, bovine viral diarrhea virus, Mycobacterium avium subspecies paratuberculosis, and Neospora caninum and milk, fat and protein production and somatic cell count in Canadian dairy cattle. Submitted to J. Dairy. Sci.

Tiwari, A., VanLeeuwen , J.A., Dohoo, I.R., Keefe, G.P., Haddad, J.P., 2005b. Seroprevalence and spatial distribution of bovine leukemia virus, bovine viral diarrhea virus and Mycobacterium avium subspecies paratuberculosis in Canadian dairy cattle. Submitted to Vet. Res.

Tiwari, A., Vanleeuwen, J.A., Dohoo, I.R., Stryhn, H., Keefe, G.P., Haddad, J.P., 2005. Effects of seropositivity for bovine leukemia virus, bovine viral diarrhoea virus, Mycobacterium avium subspecies paratuberculosis, and Neospora caninum on culling in dairy cattle in four Canadian provinces. Vet. Microbiol. 109, 147-158.

Tiwari, A., VanLeeuwen, J.A., Dohoo, I.R., Stryhn, H., Keefe, G.P., 2003. Effects of seropositivity for bovine Leukemia virus, Mycobacterium avium subspecies paratuberculosis, and Neospora caninum on calving to conception interval in maritime Canadian dairy cattle. Proceedings of SVEPM, Warwick, England. 243-252.

VanLeeuwen, J.A., Keefe, G.P., Tremblay, R., Power, C., Wichtel, J.J., 2001. Seroprevalence of infection with Mycobacterium avium subspecies paratuberculosis, bovine leukemia virus, and bovine viral diarrhea virus in maritime Canada dairy cattle. Can. Vet. J. 42, 193-198.

VanLeeuwen, J.A., Tiwari, A., Dohoo, I.R., Keefe, G.P., 2003. Prevalence of Johne's Disease Infection in Canada - National Production Limiting Diseases Study. Proc. Annual Meeting of Atlantic Bovine Practitioners' Association, Moncton, Canada.

VanLeeuwen, J.A., Tiwari, A., Forsythe, L., Chartier, R., 2005. Seroprevalence of antibodies against bovine leukemia virus, bovine viral diarrhea virus, Mycobacterium avium subspecies paratuberculosis and Neospora caninum in Saskatchewan dairy cattle. Can. Vet. J.

Vuong, Q.H., 1989. Likelihood ratio tests for model selection and non-nested hypotheses. Econometrica 57, 307-333.

Waage, S., 2000. Influence of new infection with bovine virus diarrhoea virus on udder health in Norwegian dairy cows. Prev. Vet. Med. 43, 123-135.

Waldner, C.L., Cunningham, G., Campbell, J.R., 2004. Agreement between three serological tests for Neospora caninum in beef cattle. J. Vet. Diagn. Invest. 16, 313-315.

Wells, S.J., Wagner, B.A., 2000. Herd-level risk factors for infection with Mycobacterium paratuberculosis in US dairies and association between familiarity of the herd manager with the disease or prior diagnosis of the disease in that herd and use of preventive measures. J. Am. Vet. Med. Assoc. 216, 1450-1457.

Whitlock, R.H., Wells, S.J., Sweeney, R.W., Van Tiem, J., 2000. ELISA and fecal culture for paratuberculosis (Johne's disease): sensitivity and specificity of each method. Vet. Microbiol. 77, 387-398.

Whittington, R.J., Marshall, D.J., Nicholls, P.J., Marsh, I.B., Reddacliff, L.A., 2004. Survival and dormancy of Mycobacterium avium subsp. paratuberculosis in the environment. Appl. Environ. Microbiol. 70, 2989-3004.

Wilson, D.J., Rossiter, C., han, H.R., Sears, P.M., 1995. Financial effects of Mycobacterium paratuberculosis on mastitis, milk production, and cull rate in clinically normal cows. Agri. Prac. 16, 12-18.

Wu, J.T., Dreger, S., Chow, E.Y., Bowlby, E.E., 2002. Validation of 2 commercial Neospora caninum antibody enzyme linked immunosorbent assays. Can. J. Vet. Res. 66, 264-271.

1 Table 7.1. Comparison of the sample of 8129 cows in 257 Canadian dairy herds to
2 national industry averages.

	Study Population estimates (95% Confidence interval)	Canadian Dairy estimates (From 1999-2003) (range of means)
Mean 305 day milk	8602-9390 kg	8960-9519 kg
Mean 305 day fat	315-337 kg	331-351 kg
Mean 305 day protein	275-301 kg	290-306 kg
Mean herd size of milking cows	59-73	50-62

3

Table 7.2. Variables associated ($P \leq 0.15$) with the count of MAP-seropositive cows in a herd, after adjusting for confounding variables province, herd size and mean lactation number in Canadian dairy herds.

Variables	Sec	Herds	C.R.	S.E.	P-value
Area of pasture	B				0.08
(<100 acres)		197	1	-	
(100-200 acres)		36	0.87	0.19	
(>200 acres)		21	0.52	0.15	
Percentage income from dairy (>50%)	B	208	1.65	0.43	0.05
Open heifers purchased	C	18	1.90	0.57	0.03
Group housed pre-weaned calves in winter	D	203	1.44	0.31	0.09
Tie stalls for lactating cows	D	120	1.32	0.24	0.13
Bulls access to a small field for exercise	D	23	1.57	0.46	0.13
Dry cow grazing	D	163	0.76	0.13	0.11
Bulls grazing	D	36	0.69	0.17	0.12
Bull purchased	E	39	0.55	0.12	0.01
Beef cattle direct contact with dairy cattle	F	65	1.81	0.49	0.03
Beef cattle in contact with dairy cattle water	F	62	1.78	0.47	0.03
Lending cows to other farms	F	32	0.64	0.16	0.07
Often see other dogs on farm	F	157	1.40	0.25	0.06
Often see skunk on farm	F	155	0.64	0.13	0.02
Farmers lending equipment to other farmers that could have manure contact	F	101	0.78	0.13	0.14
Use of cutting equipment for dehorning	G	170	1.87	0.52	0.02
Disinfect instruments for extra teat removal	G	118	0.67	0.13	0.05
More than one cow in maternity pen	I	69	1.45	0.26	0.04
Outdoor feed bunk or manger for dry cows	J	155	0.72	0.16	0.13
Heifers less than 12 months old share water trough with adult cattle	J	52	0.70	0.15	0.10
Distance from manure storage to farm well (>2000 feet)	J	33	0.59	0.15	0.03

Sec – Sections in Questionnaire
Herds - Number of herds in each category
C.R. - Count ratio
S.E. – Standard Error

Table 7.2. continued

Variables	Sec	Herds	C.R.	S.E.	P-value
BLV (> 50% within herd prevalence)	K	73	1.13	0.22	0.54
NC (herds having ≥ 2 NC seropositive)	K	150	1.20	0.24	0.35
BVDV	K				0.31
BVDV negative herd		122	1	-	
BVDV positive herd		70	1.37	0.28	0.12
BVDV not tested (vaccinated)		65	1.11	0.23	0.61
BVDV vaccine in calves (< 6 months old)	H				0.05
No vac		41	1	-	
No BVDV VAC		18	1.08	0.37	
BVDV VAC not done properly		37	1.44	0.40	
BVDV VAC killed done properly		22	1.48	0.43	
BVDV VAC M Live done properly		12	0.47	0.19	
BVDV vaccine in heifers	H				0.05
No vac		41	1	-	
No BVDV VAC		18	1.08	0.37	
BVDV VAC not done properly		45	1.41	0.36	
BVDV VAC killed done properly		62	1.03	0.25	
BVDV VAC M Live done properly		19	0.58	0.20	
BVDV vaccine in cows	H				0.15
No vac		41	1	-	
No BVDV VAC		18	1.08	0.38	
BVDV VAC not done properly		51	1.36	0.35	
BVDV VAC killed done properly		73	0.96	0.22	
BVDV VAC M Live done properly		14	0.58	0.23	

Sec – Sections in Questionnaire
Herds - Number of herds in each category
C.R. - Count ratio
S.E. – Standard Error
VAC – Vaccine
M Live – Modified live

Table 7.3: Final model of management and other risk factors associated with the number of MAP-seropositive cows in a herd, and odds of herds having no MAP-seropositive as compared to having at least one MAP-seropositive cow in a herd (n = 257 herds).

Variables	Herds	C.R.	S.E.	P-value
Negative binomial part of ZINB model				
Open heifer purchase	17	2.338	0.596	0.001
Bulls purchase	39	0.638	0.119	0.017
Beef cattle direct contact	64	1.938	0.458	0.005
No beef cattle	137	1.272	0.300	0.307
Area of pasture (100-200 acres)	36	0.787	0.154	0.224
(>200 acres)	21	0.611	0.157	0.056
More than one cow in maternity pen	66	1.692	0.271	0.001
Group housed pre-weaned calves in winter	189	2.032	0.380	<0.001
NC	146	1.355	0.238	0.084
BLV	64	0.916	0.150	0.598
BVDV				0.013
BVDV negative	106	1	-	-
BVDV positive	66	1.414	0.246	0.046
BVDV not tested	63	1.656	0.285	0.003
BVDV vaccine in calves				0.007
No VAC	40	1	-	-
No BVDV VAC	18	0.976	0.287	0.935
BVDV VAC not done properly	34	1.775	0.395	0.010
BVDV VAC (killed) done properly	21	1.107	0.249	0.649
BVDV VAC (M Live) done properly	11	0.440	0.166	0.030
Farmers not replied to VAC question	111	1.003	0.194	0.987
Province				<0.001
PEI	28	1	-	-
NS	24	1.641	0.653	0.213
NB	27	2.066	0.809	0.064
QUE	23	1.444	0.658	0.844
MAN	28	1.875	0.729	0.106
SASK	41	1.013	0.382	0.971
ALTA	64	5.821	1.935	<0.001
Logistic part of ZINB model	herds	O.R.	S.E.	P-value
Mean lactation number for herd	235	-1.442	0.613	0.019

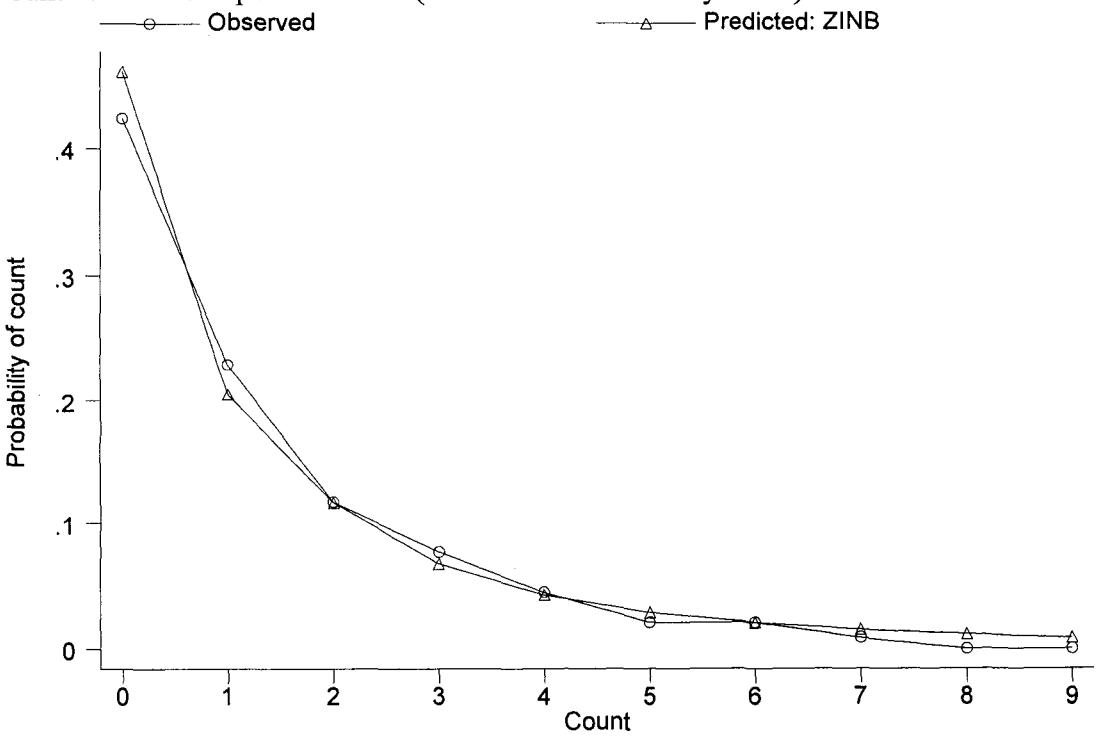
Herds - Number of herds in each category
C.R. - Count ratio
S.E. – Standard Error
VAC – Vaccine
M Live – Modified live

Table 7.4: Final logistic model of management and other risk factors associated with herds having at least 2 MAP-seropositive cows as compared to herds having zero or one MAP-seropositive cow (n = 257 herds).

Variables	Herds	O.R.	S.E.	P-value
TMR	136	3.096	1.220	0.004
				0.031
Bedding that was not added frequently to calving areas (for each calving)	53	2.726	1.151	0.018
Open heifers purchase	17	4.043	2.362	0.017
Mean lactation number for herd	243	1.401	0.463	0.308
Herd size	243	1.055	0.107	0.598
NC	146	2.904	1.229	0.012
BLV	64	1.191	0.464	0.654
BVDV				0.213
BVDV negative	106	1	-	-
BVDV positive	66	0.894	0.384	0.795
BVDV not tested	63	2.904	1.229	0.088
Province				0.020
PEI	28	1	-	-
NS	24	0.950	0.753	0.949
NB	27	0.783	0.618	0.758
QUE	23	0.340	0.434	0.398
MAN	28	1.790	1.438	0.468
SASK	41	0.680	0.929	0.778
ALTA	64	3.486	3.764	0.247

Herds - Number of herds in each category
O.R. - Odds ratio
S.E. – Standard Error

Figure 7.1: The observed and predicted (based on the final model) probabilities of each count of MAP-seropositive cows (n = 257 Canadian dairy herds).



CHAPTER 8: General discussion and conclusion

This final chapter summarizes the main findings of the thesis in the context of the scientific literature, discusses how the results can be applied to the Canadian dairy industry, and identifies potential future areas of research.

8.1 Prevalence of MAP infection

The first objective of this thesis was to determine the prevalence and spatial distribution of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection in Canadian dairy cattle and herds so that bovine practitioners and dairy farmers have a better understanding of how common is exposure to MAP bacteria in various parts of the Canadian dairy industry. The prevalence and spatial distribution of the other co-infections (bovine leukosis virus - BLV, bovine viral diarrhea virus - BVDV and *Neospora caninum* - NC) were also estimated to compare to MAP-seroprevalence.

A stratified two-stage random sampling procedure was employed. Herds on Dairy Herd Improvement (DHI) testing were randomly selected (sometimes non-DHI herds were included at the request of the provincial director) to recruit thirty, thirty, thirty, one hundred and four, forty, fifty-one, and seventy-nine herds from the provinces of Prince Edward Island (PEI), New Brunswick (NB), Nova Scotia (NS), Quebec (QUE), Manitoba (MAN), Saskatchewan (SASK), and Alberta (ALTA) in 1998, 1998, 1998, 2002, 2002, 2001, and 2002/03, respectively. From each selected herd, approximately 30 lactating

animals (all cows if the total number of cows in the herd was less than 30 cows) were randomly selected for blood collection. The serum samples were tested for MAP antibodies using an ELISA.

The final dataset of MAP test results contained 373 herds with 10,578 cows. Overall, 3.1% (2.3% - 3.8%, 95% C.I.) of the cattle were positive for antibodies to MAP, and 18.9% (13% - 25%, 95% C.I.) of herds had at least two seropositive cows for MAP. These seroprevalence estimates are similar to national seroprevalence estimates from the United States (2.5% cows were seropositive and 17% herds had at least 2 seropositive) (NAHMS, 1997) and the Netherlands (2.5% cows were seropositive and 28% herds had at least 2 seropositive cows) (Muskens et al., 2000).

A recent evaluation of the results of the ELISAs utilized in our study with respect to tissue culture indicated estimates of test sensitivity of 7% (McKenna et al., 2005) and test specificity of 98% (McKenna et al., 2005). Therefore, estimated true prevalence of exposure to MAP, adjusting for test sensitivity and specificity, were determined to be 21% (5.2 – 38.3, 95% C.I.) and 40% (27.3 – 52.1, 95% C.I.) at the animal and herd levels, respectively. Our estimate (21%) of true MAP prevalence at the animal level is similar to the estimate (16.1%) of a recent Canadian maritime study utilizing the culture of ileocecal lymph nodes and ileum of apparently healthy dairy cattle at slaughter (McKenna et al., 2004).

8.1.1. Spatial distribution

Regarding the spatial distribution of MAP infection, the Global Moran's I statistic resulted in a significant positive spatial autocorrelation of 0.15, indicating that certain

parts of Canada have a higher seroprevalence than others, higher than what was expected due to normal variation. Similar results have also been reported in the lone spatial study of MAP infection, conducted on results from dairy farms in the state of Indiana (Ward and Perez, 2004). In that study, significant spatial clustering of MAP-infection was identified in the north-eastern part of the state.

Visual exploration of the seroprevalence data in our study showed that there were significant differences between provinces, with high seroprevalence estimates at the herd and animal level found in Alberta (see Table 3.5), and this observation was confirmed by the presence of a statistically significant spatial cluster in an area of Alberta using the scan statistic. Furthermore, the province of Alberta was a significant predictor of MAP-seropositivity in the risk factor analysis.

Reasons for the apparent increased seroprevalence of MAP exposure in Alberta remain unclear, but are unlikely to be simply from the use of a different ELISA (IDEXX-ELISA and BIOCOR-ELISA produced similar sensitivity and specificity estimates (McKenna et al., 2005)) and its utilization at a laboratory in Alberta (which is also USDA accredited) rather than Saskatchewan where most of the other samples were tested. Clusters of MAP-seropositivity may arise when some herds share common risk factors (such as similar environmental conditions or housing/grazing management, or buying cattle from neighbours). Areas having MAP clusters can be targeted in the initial phase of Johne's disease control programs to prevent the further spread of infection to low prevalence areas or to other farms within the MAP cluster areas, and to reduce transmission and infection levels on infected farms.

8.1.2 Co-infection prevalence

In total, 30.3% and 11.9% of the cattle were positive for antibodies to BLV and NC (Haddad et al., 2005), respectively. At the herd level, 76.6% of herds had at least one seropositive cow for BLV, 31.2% had at least one animal with a titer $\geq$1:64 for BVDV, and 64.8% had at least two seropositive cows for NC. Tables (3.3-3.5) provide detailed results of BLV, BVDV and MAP-seroprevalences. With these results, the infection status for other pathogens were utilized as possible confounders to the potential impacts of MAP seropositivity on productivity, or as possible risk factors for MAP infection.

These seroprevalence results are believed to be representative of the Canadian dairy industry for a number of reasons. Samples were obtained from dairy herds in 8 of the 10 Canadian provinces. Study herds and animals were selected through similar random sampling procedures across all provinces to select a representative sample of Canadian dairy cattle and herds. Furthermore, there were no substantial differences between the production indices of sampled herds compared to indices obtained for all Canadian dairy herds on monthly milk recording (see table 3.2), suggesting that the sampled herds were representative of Canadian dairy industry.

8.2 Production impacts of MAP infection

A second objective of this thesis was to determine the production impacts (305 day milk, 305 day fat, 305 day protein and somatic cell count) of MAP infection in Canadian dairy cattle and herds so that bovine practitioners and dairy farmers have a better understanding of the detrimental effects that MAP infection has on production indices in the Canadian dairy industry.

A subset of the nationally sampled herds for the prevalence study was utilized for this research, those herds that were on monthly milk testing. Therefore, the sample population included 30, 30, 30, 75, 27, 40, 44, and 66 herds from the provinces of NB, NS, PEI, QUE, ONT, MAN, SASK, and ALTA, respectively, with their blood testing having been conducted in 1998, 1998, 1998, 2002, 1998, 2002, 2001, and 2002/03, respectively.

For each tested animal, demographic data, the actual and predicted (if more than 200 days in milk) 305-day milk, fat, and protein volumes, and somatic cell count (SCC) data from January 1, 1999 to December 31st, 2003 were gathered electronically from a central milk-recording database.

The final dataset contained 342 herds with 9,834 cows and 22,665 lactations. There were, on average, 2.3 (maximum 4) lactations per cow. MAP-seropositivity in lactation 4+ was significantly associated with a lower 305 day milk volume of 212 kg (2.34% lower milk production) compared to MAP-seronegative cows in lactation 4+. A significant reduction in milk production was not detected in younger cows, although a trend toward lower milk production was seen. However, MAP-seropositivity was not significantly associated with 305 day fat and 305 day protein volume.

These results support suggestions made in previous studies (Johnson et al., 2001; Kudahl et al., 2004; Sockett et al., 1992; Wilson et al., 1993) that the effect of MAP-seropositivity on milk volume largely depends upon the parity of the cow.

In our study, MAP-seropositivity was also significantly associated with a 0.098 increased lactational average of the log (to base 10) of SCC ($ML_{10}SCC$) compared to MAP-seronegative cows. McNab et al., (1991) also found an increased somatic cell count

in LAM-ELISA-positive cows compared to negative cows. However, it is unclear whether there is also an increased risk of clinical mastitis in MAP-seropositive cows compared to seronegative cows, as this would require data that were not collected in our study.

The results of our study likely represent very conservative estimates of the effect of MAP-seropositivity on 305 d milk, 305 d fat, 305 d protein and ML_{10}SCC because of the misclassification bias associated with ELISA test results for MAP (Whitlock et al., 2000). Due to the very poor sensitivity of ELISA tests for MAP exposure, ranging from 7% to 80% (McKenna et al., 2004; Sockett et al., 1992), depending on the stage of infection of the test population, numerous infected animals likely gave false negative test results. Specificity estimates of ELISAs for MAP exposure are also not ideal, ranging from 90-99% (Dargatz et al., 2001; McKenna et al., 2005), particularly when utilized on a large number of animals in a low prevalence population, leading to numerous false positive test results (low positive predictive values). The impact of these misclassifications, assuming they are not differential in nature (accuracy of ELISA is similar in high producing cows as compared to low producing cows), would likely bias these model estimates toward the null.

The effects of BLV, BVDV, and NC infection on production were also estimated. The results from multilevel linear regression analyses for BLV-, BVDV-, and NC-seropositivity (by lactation) on 305 d milk, 305 d protein, 305 d fat volume and ML_{10}SCC are shown in Tables 4.2-4.3.

When all lactations were pooled together, the cows in BVDV-seropositive herds had a lower 305 d milk volume of 368 kg/cow compared to cows in BVDV-seronegative

herds. NC-seropositivity in first lactation heifers was significantly associated with a lower 305 d milk volume of 158 kg. BLV-seropositive, 4+ lactation cows had a lower 305 d milk volume of 21 kg (data not shown), but this association was not statistically significant.

Cows in BVDV-seropositive herds had a marginally lower 305 d fat volume of 10.2 kg compared to cows in BVDV-seronegative herds. NC-seropositivity in first lactation heifers was significantly associated with a lower 305 d fat of 5.54 kg. However, after controlling for 305 d milk, BVDV- and NC-seropositivity were no longer associated with 305 d fat volume. There was no significant relationship between BLV-seropositivity and 305 d fat volume.

The cows in BVDV-seropositive herds also had a significantly lower 305 d protein of 9.35 kg compared to cows in BVDV-seronegative herds. NC-seropositivity in first lactation heifers was associated with a marginally lower 305 d protein of 3.30 kg. However, after controlling for 305 d milk, BVDV- and NC-seropositivity were no longer associated with 305 d protein volume. BLV-seropositivity was not significantly associated with 305 d protein volume.

The cows in BVDV-seropositive herds had a significantly higher $ML_{10}SCC$ of 0.096 compared to cows in BVDV-seronegative herds. NC-seropositivity in lactation 4+ cows was associated with a significantly lower $ML_{10}SCC$ of 0.094. BLV-seropositivity was not significantly associated with the $ML_{10}SCC$. A discussion of these co-infection results with respect to available scientific literature can be found in Chapter 4.

8.3 Culling impacts of MAP infection

The third objective of this thesis was to determine the culling impacts of MAP infection in Canadian dairy cattle and herds so that bovine practitioners and dairy farmers have a better understanding of the detrimental effects that MAP infection has on longevity in the Canadian dairy industry.

A subset of the nationally sampled herds for the prevalence study were utilized for this research, those herds that were on monthly milk testing and were tested early in the project, thereby allowing sufficient time to examine the effect of MAP-serpositivity on culling. The final dataset contained 134 herds with 3531 cows from NB, NS, PEI and SASK, all having serological results and production and culling data. For each tested animal, the culling and production data were gathered electronically from a central milk recording database for the follow-up period that extended from June, 1998 to Feb, 2002 for Atlantic herds, and from April, 2001 to Sept, 2002 for Saskatchewan herds because Atlantic and Saskatchewan herds were sampled in June-August, 1998 and April-May, 2001, respectively.

A total of 1981 (56.1%) cows were culled for all reasons out of 3531 tested cows. For all reasons of culling, MAP-seropositive cows had a 1.38 (1.05 – 1.81, 95% C.I.) times increased hazard of culling compared to MAP-seronegative cows. Among cows that were culled because of either decreased reproductive efficiency or decreased milk production or mastitis, MAP-seropositive cows had a 1.55 (1.12 – 2.15, 95% C.I.) times increased hazard compared to MAP-seronegative cows. These results support the results from chapter 4, where MAP-seropositivity was associated with a 0.098 increase in the lactational mean of $\log_{10}$ somatic cell count. MAP-seropositivity was also significantly

associated with 212 kg lower 305 d milk production in 4-plus lactation cows. A significant reduction in milk production was not detected in younger cows, although a trend toward lower milk production was seen.

The methods of this study, where there was an adjustment for herd clustering, controlling for confounding variables, and random sampling of herds, indicate that the impact of MAP-seropositivity on culling was likely a valid estimate for Canadian dairy herds. Wilson et al., (1993) and others (Goodle et al., 2000; Merkal et al., 1975; Wilson et al., 1993) found similar results utilizing either fecal culture or ELISA.

It is biologically plausible for MAP to lead to increased hazard of culling. MAP infection typically causes localized granulomatous lesions in lymph nodes and the lamina propria of the terminal ileum but granulomatous lesions can also be disseminated throughout the entire gastrointestinal (GI) tract (Chiodini et al., 1984). The gastroenteritis produces mucosal thickening that results in reduced absorption of nutrients from the intestine and alterations in cell-mediated immunity. Reduced GI absorption might cause or worsen a negative energy balance in early lactation, while alterations in cell-mediated immunity may decrease the resistance of cows towards other infectious diseases, possibly resulting in culling of MAP-seropositive cows due to decreased reproductive efficiency (Johnson et al., 2001) or decreased milk production (Goodell et al., 2000) or mastitis (McNab et al., 1991).

There was an additional interest in the effects of BLV, BVDV and NC infection on culling. BLV-, BVDV-, and NC-seropositivity were not associated with an increased risk of overall culling. However, among the cows that were culled because of reproductive inefficiency, NC-seropositive cows had a 1.43 (95% C.I., 1.15 – 1.79) times

greater hazard than NC-seronegative cows. Among cows that were culled because of decreased milk production, cows in BVDV-seropositive herds had a 1.86 (1.28 – 2.70 95% C.I.) times increased hazard compared to cows in BVDV-seronegative herds. BLV-seropositive cows did not have an increased risk of reason-specific culling as compared to BLV-seronegative cows. Again, a discussion of these co-infection results with respect to available scientific literature can be found in Chapter 5

8.4 Economic losses with MAP infection

Translating the production and culling impacts of MAP-infection in Canadian dairy cattle and herds into economic losses was the fourth objective of this thesis so that bovine practitioners and dairy farmers have a better understanding of how costly MAP infection is in the Canadian dairy industry.

The model used in this study was a stochastic, partial budget, simulation model, adapted from models published by Bennett et al. (1999) and Chi et al., (2002), and included the impacts of MAP-seropositivity on milk yield, mortality, culling and reproductive losses. Input values were obtained primarily from analyses of the national study of 373 Canadian dairy farms in 8 of 10 provinces presented in this thesis, with a few values coming from peer-reviewed literature. Considerations such as possible effects of MAP on human health, the ability of the farm to market livestock or other products, and other potential indirect costs were not included in the model.

For MAP-seropositive Canadian dairy herds, the mean loss per 61 cow herd, assuming a within-herd seroprevalence of 12%, was CD $2,992 (95% C.I., $143 – $9741) annually, or CD $49 per cow per year. Herd additional culling losses were responsible for

46% (CD $1374) of the total losses from MAP. Decreased milk production, mortality and reproductive losses accounted for 9% (CD $254), 16% (CD $488) and 29% (CD $875) of the losses, respectively, respectively.

Our results suggested a range (95% C.I.) of CD$ 18 to 123 lost per cow per year in MAP-infected herds. Ott et al., (1999) reported a range (90% C.I.) of US$ 2 to 120 per cow per year in MAP-infected herds (which had less than 10% of cull cows being culled due to clinical signs resembling Johne's disease). The American estimates (Ott et al., 1999) were considered similar to our results because more than 90% of positive herds in our study also had less than 10% of cows culled due to clinical signs resembling Johne's disease.

When considering only those cows that tested positive for MAP exposure, the cost estimates ranged (95% C.I.) from CD$ 197 to 613 per infected cow per year. These results were supported by reported cost estimates from previous studies. For example, the cost in subclinically infected cows ranged from US$ 123 to 696 per infected cow per year (Ott et al., 1999). Overlapping and similar ranges of cost estimates suggest very similar economics losses associated with MAP-infection in both the Canadian and American dairy industries.

Therefore, exposure to MAP bacteria is leading to substantial production and culling losses in infected Canadian dairy herds, even though the majority of infected herds detected in the study were not high prevalence herds. Losses would be expected to be much higher in high prevalence herds. Combined with the substantial prevalence of MAP infection in the Canadian dairy industry, these annual economic losses on infected

farms further demonstrate the strong need for a national Johne's disease control program in Canada to assist farmers to reduce MAP-prevalence.

8.5 Risk factors of MAP-infection

The final objective of this thesis was to quantify the important animal and herd level risk factors of MAP-seropositivity in the Canadian dairy industry so that bovine practitioners and dairy farmers have a better understanding of those factors that are most strongly associated with high MAP-seropositivity within Canadian dairy cattle management systems.

A subset of the nationally sampled herds were utilized for this research, those herds which: 1) were on monthly milk testing; 2) agreed to release production data; and 3) completed a questionnaire regarding management practices on the farm. Results from serum samples from approximately 30 randomly selected cows from 285 herds from 7 provinces (NB, NS, PEI, QUE, MAN, SASK and ALTA) were included in the study.

For each tested animal, demographic data, the actual and predicted (if more than 200 days in milk) 305-day milk, fat, and protein volumes, and somatic cell count (SCC) data (from the lactation in which the animals were tested for the 4 pathogens) were gathered electronically from a central milk-recording database.

The questionnaire was divided into following sections: identification and location; basic demographic information of the farm and farmer, herd population, housing, biosecurity (purchasing new animals; contact between domestic or wild animals and cattle or their water and food; contact with other people and their vehicles or equipment; transmission of disease through blood and prophylactic use of medications and vaccines),

calving and calf management, management of manure and possible concurrent diseases. The complete questionnaire description is in Chapter 7.

A zero-inflated negative binomial (ZINB) model was utilized to simultaneously determine the management factors associated with the number of MAP-seropositive cows in a herd, and the odds of herds having at least one MAP-seropositive cow as compared to having no MAP-seropositive cows in a herd. A logistic regression was also utilized to determine the risk factors associated with herds having at least two MAP-seropositive cows compared to one or no MAP-seropositive cow in a herd.

In the final ZINB model, significant risk factors included: "more than one cow in the maternity pen", "group housing in pre-weaned calves in winter", "purchasing open heifers" and "direct contact with beef cattle", associated with 1.69, 2.03, 2.34 and 1.27 times more MAP-seropositive cows, respectively.

There were two variables that were significant in the final logistic regression model but not in the final ZINB model (whole model shown in table 7.4). Herds having "total mixed ration (TMR) feeding" and "bedding that was not added frequently to calving areas (ie. for each calving)" were associated with 3.06 and 2.84 times greater odds of being MAP-seropositive herds, respectively.

Some of these risk factors have also been identified by other studies (Collins et al., 1994; Jakobsen et al., 2000; Obasanjo et al., 1997; Wells and Wagner, 2000) and are included as important elements of risk assessments for MAP transmission utilized in Johne's disease control programs. However, TMR feeding and contact with beef cattle have not previously been reported as risk factors in published literature.

Use of a total mixed ration (TMR) was associated with MAP-seropositivity in the logistic regression model. With herd size already forced in the model, the reason for TMR being in the model cannot be due to herd size. TMRs are generally not fed to calves and therefore are not likely to be a source of infection to calves. TMR may be a proxy for something else, such as a desire for labour-saving, which may also mean that farms with TMR use tractors or loaders, which may bring adult feces in proximity to calves. There is a need for future study on this possible association.

Contact with beef cattle was significantly associated with a higher number of MAP-seropositive cattle, suggesting that beef cattle could be a source of MAP to dairy cattle. MAP can also infect sheep, goats, farmed deer (Fawcett et al., 1995), alpacas (Cocito et al., 1994) and rabbits (Greig et al., 1997). The presence of farmed deer on dairy farms increased the risk of reporting paratuberculosis by 3772 dairy farmers in England and Wales (odds ratios ranged from 15.2 to 209) (Cetinkaya et al., 1997). Due to the low number of dairy farms in our study which had sheep, goats, farmed deer, alpacas or rabbits, our study was unable to confirm or reject the relationships of these risk factors with MAP-seroprevalence. However, there were 65 dairy farms that had contact with beef cattle, and these farms had higher numbers of MAP-seropositive cows.

The best management practices (BMPs) (based on the pathology and risk factor literature of Johne's disease) of the proposed national Johne's disease control program (see appendix - **A**) focus on modifying management factors related to calves and heifers for reducing the exposure of MAP, and our results support that this approach is warranted, given that the majority of the significant risk factors were related to calf and heifer management.

Herds that were "purchasing open heifers" were significantly associated with 4 times greater odds of being MAP-seropositive herds. This finding was expected because, like many infectious diseases, the MAP bacterium is likely to enter a herd by purchasing an infected animal. Purchasing other age groups of cattle, such as "purchasing bred heifers" or "purchasing milking cows", were highly correlated with "purchasing open heifers" in our dataset. In multiple variable regression analyses, often only one of a group of correlated variables will remain significant in the final model. However, the other correlated variables should be considered important as well, although perhaps of lesser consequence. This result confirms another of the BMPs associated with the proposed national Johne's disease control program that suggests that farmers either keep their herds closed or buy animals only from herds at an equal or better level of Johne's disease herd status.

8.6 Examination of co-infections

One of the major strengths of the studies in this thesis was that exposure to co-infections with other pathogens were examined simultaneously. Therefore, the impacts of MAP-seropositivity on production and culling could be controlled with respect to NC-, BLV- and BVDV-seropositivity. Also, interaction effects induced by co-infections with more than one of the pathogens could be examined.

As noted in section 8.2, while controlling for exposure to other pathogens, MAP-seropositivity was associated with reduced milk production in 4+ lactation cows, and increased somatic cell count in all lactations. NC-seropositivity was associated with reduced milk, fat and protein production in 1st lactation heifers, and lower somatic cell

counts in 4+ lactation cows, although this effect was diminished in younger cows. Cows in BVDV-seropositive herds had reduced milk, fat and protein production in all lactations, and higher somatic cell counts in all lactations. BLV-seropositive cows showed no significant effects on any of the milk production outcomes. When examining for interaction variables among the pathogens, there were no significant effect modifications among the four pathogens for any of the outcomes.

As noted in section 8.3, while controlling for exposure to other pathogens, MAP-seropositivity was associated with increased hazard of culling for all reasons. However, cows in BVDV-seropositive herds, and NC-seropositive cows were only associated with increased hazard of culling for low milk production and reproductive inefficiency, respectively. When examining for interaction variables among the pathogens, there again was no significant effect modification.

There are at least two reasons for this failure to detect interactions among the pathogens. 1) There was a limited number of animals that were seropositive for more than one pathogen. With the seroprevalence of MAP at only 3%, there were very few animals that were seropositive for MAP and another pathogen. 2) The significant effects of the 4 pathogens were only present in an age cohort of infected animals, leading to an even smaller population of animals that were seropositive for more than one pathogen and within those age cohorts. Therefore, there was a limited statistical power to test these hypotheses comprehensively.

The risk factors for MAP-seropositivity included not only management factors, but also exposure to the other three tested pathogens. "BVDV-seropositive herds" and "not having proper BVD vaccination in calves (i.e. animals receiving a killed BVDV

vaccine and not boostered 2-4 weeks after their first shot)" were associated with 1.41 and 1.77 times more MAP-seropositive cows, respectively, in the final ZINB model. The variable "herds having modified live BVDV vaccination in calves" was associated with 0.44 times fewer MAP-seropositive cows. In addition, the variable "NC-seropositivity" was marginally significantly associated ($P < 0.10$) with 1.36 times more MAP-seropositive cows in the ZINB model, and NC-seropositive herds had 2.9 times the odds of being MAP-seropositive ($P = 0.01$) in the final logistic model.

Interpreting these results, it would appear that infection with BVDV and NC appear to act as risk factors for MAP infection. The immunosuppressive effects of BVDV could act as a risk factor for establishment of infection or manifestation of paratuberculosis. The coexistence of NC with MAP infection could be the result of an unmeasured factor that could have introduced these two pathogens to a farm independent of each other (such as through the purchase of animals). Future research may prove or disprove these hypothesis.

8.7 Future research

This thesis, while answering some questions, leaves other questions unanswered. First, there may be value in additional MAP prevalence surveys in Canada in the future. While the accuracy of ELISAs for MAP exposure are poor, the recent evaluation of both ELISAs and fecal culture testing with respect to tissue culture positive cows (McKenna et al., 2005) provided a representative and accurate estimate of sensitivity for these tests. Therefore, the estimated true prevalence from our study is likely a valid estimate for the Canadian dairy industry, particularly considering the representativeness of the random

herd and cow selection processes. Therefore, a survey utilizing fecal culture is unlikely to produce a better estimate of true prevalence. However, in order to determine the progress towards controlling MAP infection on dairy farms in Canada, it would likely be useful to conduct another survey in 5-10 years.

Another reason for continuing to monitor the prevalence of MAP in cattle is to compare changes in MAP prevalence with changes in Crohn's disease incidence. Comparing these two could determine whether there are temporal and/or spatial similarities in their distributions, leading to additional evidence that the two diseases may both arise from exposure to MAP bacteria.

More accurate estimates of the impact of MAP-seropositivity on milk production parameters are still needed. Our estimates of this impact were from a random sample of Canadian dairy cattle and herds, but were likely very conservative in nature for a number of reasons, including: inaccuracy of the ELISA leading to substantial misclassification bias; and the small number of test positive cows, leading to low power to detect impacts within different lactations. A study utilizing fecal cultures would likely produce less misclassification bias and therefore more accurate estimates of impacts on milk production relevant to the Canadian dairy industry. However, a fecal culture study would still not determine if there are milk production effects in non-shedding infected cows. One way of identifying whether non-shedding cows have negative milk-production effects would be to conduct laparotomies and tissue cultures on springing heifers in a number of infected herds. One could then compare milk production between tissue culture positive and negative heifers for the rest of their lives, doing fecal cultures on them every 3 months to determine when they start shedding.

With regard to impacts on culling, one could argue that the culling study only utilized herds found in 4 out of 10 provinces, with those 4 provinces representing only a small portion of the dairy industry in Canada. Therefore, in a few years time, it would be desirable to analyse culling impacts of seropositivity for MAP, and the other pathogens, in all of the tested herds on monthly milk testing. This additional analysis with a larger number of cows could provide more accurate and representative estimates of the impact of MAP-seropositivity on culling, particularly for specific reasons of culling. The current study had limited power to detect an association between MAP-seropositivity and specific reasons for culling due to the limited number of culled MAP-seropositive cows within each of the categories for reasons of culling. Misclassification bias of the ELISA may have also lead to a biasing of the result toward the null, and therefore subsequent testing and assessments of culling impacts would likely be less conservative in nature if infected cows were identified using fecal culture.

With more accurate estimates of prevalence of infection, and more detailed estimates of impacts on productivity and culling, more precise estimates of direct economic losses should be estimated. Furthermore, monitoring farms enrolled in a national Johne's disease control program could provide additional data on which to monitor prevalence estimates across Canada and estimates of impacts on productivity and culling, and improve the precision of the estimates of economic losses associated with MAP infection.

The relative importance of controlling each risk factor in the prevention of MAP infections could be further elucidated. It is unclear why TMRs were associated with greater odds of MAP-seropositivity. Also, it is unclear whether certain environmental or

ecological characteristics may enhance or reduce the survivability and transmission of MAP among and between farms. For example, testing soil, water, and other environmental sampling from different areas of infected farms may assist in identifying specific risk factors of transmission. Monitoring the management practices of farms enrolled in the Canadian Johne's disease control program, both test-positive and test-negative, could provide additional data on which risk factors are more frequently associated with MAP infection. More studies should also be done to understand the relationship between age of exposure of the animal and the ability of MAP to infect animals and progress to sub-clinically infected cows (shedding MAP) and cows with clinical disease. Due to difficulties in quantifying this relationship using observational studies on commercial dairy farms, an experimental design with various infectious doses and ages of exposure would be preferred to answer this important question.

Regarding co-infections, we found that one risk factor for the count of MAP-seropositive cows was BVDV-seropositivity at the herd level, even after controlling for purchasing cattle. However, BVDV-seropositivity was not associated with MAP-seropositivity in the logistic regression analysis (for herds with ≥ 2 MAP-seropositive cows). Therefore, BVDV-seropositivity appears to be associated with an increased number of cattle with MAP infection on an infected farm, but not associated with the introduction of MAP into a herd. Further research would be useful to confirm this speculation, by identifying and monitoring herds with BVDV and MAP infection.

Impacts on productivity due to interactions between seropositivity for the different pathogens should also be examined further. While no interactions between pathogens were statistically significantly associated with impacts on milk production or

culling, both misclassification bias and limited sample size of MAP-seropositive cows likely limited the power to detect these interactions. Again, using the Canadian Johne's control program, identifying and monitoring herds with MAP infection (preferably using fecal culture testing) and other pathogens, particularly BVDV, would be useful in studying bovine infections further.

8.8 Reference list

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

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

Cocito, C., Gilot, P., Coene, M., de Kesel, M., Poupart, P., Vannuffel, P., 1994. Paratuberculosis. Clin. Microbiol. Rev. 7, 328-345.

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

Dargatz, D.A., Byrum, B.A., Barber, L.K., Sweeney, R.W., Whitlock, R.H., Shulaw, W.P., Jacobson, R.H., Stabel, J.R., 2001. Evaluation of a commercial ELISA for diagnosis of paratuberculosis in cattle. J Am. Vet Med. Assoc. 218, 1163-1166.

Fawcett, A.R., Goddard, P.J., McKelvey, W.A., Buxton, D., Reid, H.W., Greig, A., Macdonald, A.J., 1995. Johne's disease in a herd of farmed red deer. Vet Rec 136, 165-169.

Goodle, G.M., Hirst, H., Garry, F., Dinsmore, P., 2000. Comparison of cull rates and milk production of cilinially normal dairy cows grouped by ELISA *Mycobacterium avium parauberculosis* serum antibody results. Proceedings of The International Symposium of Vetrinary Epidemiology and Economics, Breckenridge, CO, USA.

Greig, A., Stevenson, K., Perez, V., Pirie, A.A., Grant, J.M., Sharp, J.M., 1997. Paratuberculosis in wild rabbits (Oryctolagus cuniculus). Vet Rec 140, 141-143.

Haddad, J.P., Dohoo, I.R., VanLeeuwen, J.A., Keefe, G.P., Tiwari, A., 2005. Seroprevalence and spatial distribution of Neospora caninum in Canadian dairy herds. Planned to Be Submiited to Can. Vet. Journal .

Jakobsen, M.B., Alban, L., Nielsen, S.S., 2000. A cross-sectional study of paratuberculosis in 1155 Danish dairy cows. Prev. Vet Med. 46, 15-27.

Johnson, Y.J., Kaneene, J.B., Gardiner, J.C., Lloyd, J.W., Sprecher, D.J., Coe, P.H., 2001. The effect of subclinical Mycobacterium paratuberculosis infection on milk production in Michigan dairy cows. J Dairy Sci 84, 2188-2194.

Kudahl, A., Nielsen, S.S., Sorensen, J.T., 2004. Relationship between antibodies against Mycobacterium avium subsp. paratuberculosis in milk and shape of lactation curves. Prev. Vet Med. 62, 119-134.

McKenna, S.L., Keefe, G.P., Barkema, H.W., McClure, J., Vanleeuwen, J.A., Hanna, P., Sockett, D.C., 2004. Cow-level prevalence of paratuberculosis in culled dairy cows in Atlantic Canada and Maine. J Dairy Sci 87, 3770-3777.

McKenna, S.L.B., Keefe, G.P., Barkema, H.W., Sockett, D.C., 2005. Evaluation of three ELISAs for *Mycobacterium avium* subsp. *paratuberculosis* using tissue and fecal culture as comparison standards. Accepted in Veterinary Microbiology .

McNab, W.B., Meek, A.H., Martin, S.W., Duncan, J.R., 1991. Associations between dairy production indices and lipoarabinomannan enzyme-immunoassay results for paratuberculosis. Can J Vet Res 55, 356-361.

Merkal, R.S., Larsen, A.B., Booth, G.D., 1975. Analysis of the effect of inapparent bovine paratuberculosis. Am. J Vet Res 36, 837-838.

Muskens, J., Barkema, H.W., Russchen, E., Van Maanen, K., Schukken, Y.H., Bakker, D., 2000. Prevalence and regional distribution of paratuberculosis in dairy herds in The Netherlands. Vet Microbiol. 77, 253-261.

NAHMS 1997. US Department of Agriculture, Animal and Plant Health Inspection Service, 1997. Johnes's Disease on US Dairy Operations, NAHMS Dairy '96, Center for Animal Health Monitoring, Report N245, 797.

Obasanjo, I.O., Grohn, Y.T., Mohammed, H.O., 1997. Farm factors associated with the presence of Mycobacterium paratuberculosis infection in dairy herds on the New York State Paratuberculosis Control Program. Prev. Vet Med. 32, 243-251.

Ott, S.L., Wells, S.J., Wagner, B.A., 1999. Herd-level economic losses associated with Johne's disease on US dairy operations. Prev. Vet Med. 40, 179-192.

Sockett, D.C., Conrad, T.A., Thomas, C.B., Collins, M.T., 1992. Evaluation of four serological tests for bovine paratuberculosis. J Clin. Microbiol. 30, 1134-1139.

Ward, M.P., Perez, A.M., 2004. Association between soil type and paratuberculosis in cattle herds. Am. J Vet Res 65, 10-14.

Wells, S.J., Wagner, B.A., 2000. Herd-level risk factors for infection with Mycobacterium paratuberculosis in US dairies and association between familiarity of the herd manager with the disease or prior diagnosis of the disease in that herd and use of preventive measures. J Am. Vet Med. Assoc. 216, 1450-1457.

Whitlock, R.H., Wells, S.J., Sweeney, R.W., Van Tiem, J., 2000. ELISA and fecal culture for paratuberculosis (Johne's disease): sensitivity and specificity of each method. Vet Microbiol. 77, 387-398.

Wilson, D.J., Rossiter, C., Han, H.R., Sears, P.M., 1993. Association of Mycobacterium paratuberculosis infection with reduced mastitis, but with decreased milk production and increased cull rate in clinically normal dairy cows. Am. J Vet Res 54, 1851-1857.

APPENDIX – A (Review Article)

Johne's Disease in Canada

Part II:

Disease Impacts, Risk Factors and Control Programs for Dairy Producers

Shawn L.B. McKenna, Greg P. Keefe, Ashwani Tiwari,

John VanLeeuwen, Herman W. Barkema

Department of Health Management

Atlantic Veterinary College, Prince Edward Island, 550 University Avenue,

Charlottetown, Prince Edward Island, C1A 4P3 Canada

Address correspondence to Shawn McKenna (slmckenna@upei.ca or address above)

Abstract

Part I of this two-part review examined the clinical stages, pathophysiology, diagnosis and epidemiology of Johne's Disease, providing information relevant to Canada, where available. In this second and final part of the series, a critical review of the economic impacts of the disease, risk factors, and important control measures are presented to enable Canadian bovine practitioners to successfully implement control strategies and participate in control programs. In ELISA-positive cattle, there is a 2.4 times increase in the risk of culling, and lactational 305d milk production is decreased by at least 370 kg. Reduced slaughter value and premature culling accounts for losses of CD$ 1330 per year per infected 50-cow herd. Research has failed to show a consistent association between *Mycobacterium avium* subsp. *paratuberculosis* test status and reduced fertility or risk of clinical/subclinical mastitis. Host level factors include age and level of exposure, along with source of exposure such as manure, colostrum or milk. Agent factors involve the dose of infectious agent and strains of bacteria. Environmental management factors influence the persistence of the bacteria and the level of contamination in the environment. Emphasizing a risk factor approach, various control strategies are reviewed, including a number of national control programs currently in place throughout the world, specifically Australia, The Netherlands and the United States. Information about the proposed Canadian Voluntary Johne's Control Program is also included. By reviewing the scientific literature about Johne's Disease, control of the disease could be pursued through informed implementation of rational biosecurity efforts and the strategic use of testing and culling.

Introduction

Johne's Disease (JD) is a chronic, infectious, enteritis of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). In the clinical stage of this disease, the infection causes severe diarrhea and wasting of the affected animal. The clinical aspects, pathophysiology, and the currently available diagnostic tests for JD were discussed in the initial paper of this two part series. In this second and final part of the series, the impact of the disease is discussed, including economic consequences on dairy production. Additionally, risk factors that are associated with the spread of the organism and the incidence of new infections on dairy farms are outlined. A review of control strategies that can be used to avoid new infections on a dairy farm is included. The proposed Canadian Voluntary Johne's Disease Control Program is presented here and is compared to current national control programs in other countries.

Canada has had success in controlling other mycobacterial diseases, specifically tuberculosis. Modern disease control programs for pathogens without clear zoonotic potential will have to be driven by economics of the disease at the individual and national herd level.

Methods

Due to substantial differences in management, production, and related control options between dairy and beef cattle, and the extensive literature on JD for both dairy and beef cattle, this paper focuses on dairy cattle. Also, because the intended audience for this paper is primarily Canadian veterinarians, we have emphasized Canadian references as much as possible.

Medline (accessed via PubMed from 1950 to present), The Commonwealth Animal Bureaux (CAB) (accessed via VetCD and ParasiteCD sets from 1973 to present) and Agricola, produced by the National Agricultural Library of the U.S. Department of Agriculture (from 1970 to present) were used to collect the references. The keywords used in the search of the databases were *Mycobacteria*, paratuberculosis, Johne's, Canada, Canadian, dairy and cattle. In addition, a small number of papers were identified from the reference lists of other papers, or personal knowledge of reports or conference proceedings.

All relevant material collected from the above process was included in the review, provided that it was pertinent to the methods of production within the Canadian dairy industry. Exclusion of material was only done if information was redundant or outdated and had been directly refuted. Otherwise, all available information was included.

Impacts on Productivity

Cattle that develop clinical JD have thickened intestinal mucosa, resulting in malabsorptive diarrhea and subsequent decreased intestinal absorption of nutrients. Reductions in milk production, infertility, premature culling, and lower slaughter value due to poor body condition are the common causes of economic loss associated with JD (1).

Losses associated with subclinical JD, defined as infected without overt clinical signs, have been more difficult to quantify because of the difficulty of accurately detecting subclinical paratuberculosis with the diagnostic tests currently available. Studies on subclinical impacts, as determined by identification of the organism through fecal culture, are less susceptible to misclassification bias than those using identification of an immune response to the organism (eg. enzyme linked immunosorbent assays - ELISAs), as explained below. However, due to the cost and long time lag between submission of samples and results for fecal culture, ELISAs are frequently used in the field to identify subclinically infected animals. Therefore, observational studies on impacts based on positive ELISA results have been conducted and are also critically evaluated in the following section. An estimate of subclinical economic losses is necessary for assessing the cost-effectiveness of control strategies at the farm, region, and national levels.

Reported economic losses attributable to subclinical JD include decreased milk production (1,2), decreased milk fat and protein yield (3,4), reduced slaughter weight at culling (5), and premature culling (6). Decreased fertility (6) and increased incidence of

clinical and/or subclinical mastitis (7) may also be associated with MAP infections. In the following sections, each of these areas of loss is critically evaluated and summarized in Table 1.

Milk production, fat and protein yield

In one of only two published Canadian studies looking at the impacts of subclinical infection in dairy cattle, records from 2395 randomly selected dairy cattle in 90 randomly selected herds were examined (8). Overall, 305-day milk production for ELISA-seropositive cows was lower than for seronegative cows. In their first and fifth lactation ELISA-seropositive animals produced 573 and 1273 kg less than seronegative cows, respectively. Similar milk production losses were found in ELISA-based studies in Wisconsin (7) and Colorado (9). The study from Wisconsin found positive cows had a 4% (376 kg) decrease in 305-day mature equivalent milk production (305ME) (7), and the study from Colorado found ELISA-positive cows had a 551 kg decrease in 305ME (9). Only two studies have reported an association between paratuberculosis and decreased milk fat and protein yield (3,4), costing approximately US$ 205 per cow per lactation (3). In a study based in Ontario (10), associations between results from an experimental ELISA and milk production were inconsistent, perhaps owing to the experimental nature of the test utilized.

When culled dairy cows were diagnosed positive by histopathology and culture of tissues, cows that showed no clinical signs but were positive had a 16% decrease in milk production in their last lactation compared to the lactation two years prior, and a 6% decrease in milk production compared with the lactation one year prior (1). However, there was no extensive look at production in uninfected cows for comparison. Assuming

a lactation which produces 8000 kg of milk, these estimates would equate to 1280 and 480 kg, respectively. Similar results of milk production losses associated with subclinical paratuberculosis were determined for cattle that were positive on fecal or tissue culture (1,3,6). In a dairy herd of 210 cows in the USA with a high prevalence of MAP infection, fecal culture-positive cows produced 590 kg and 1270 kg less milk in their third and fourth lactations, respectively, as compared to their fecal culture-negative herdmates (2).

There is potential for bias in each of these studies. In the cull cow study it is possible that cull cows would likely include older cows, which if infected, are close to entering the clinical stage of infection. Using cull cows instead of a random sample of cows in milking herds, potentially overestimates the overall impact on milk production among the general population. Similarly, cows from a herd with a high prevalence of infection (fecal culture study) would also more likely include cows entering the clinical stage of infection due to increased exposure to MAP as compared to the average farm.

However, there is a potential that this estimate of productivity loss may be an underestimation. The specificity of fecal culture is generally considered to be 100% and, therefore, all culture-positive cattle are assumed to be infected (no false-positives). However, because sensitivity is less than 100% (estimated at 50% (11)) there are false-negatives in the culture-negative group. It is likely that these false-negatives lower the average milk production of this group, bringing the mean productivity of the two groups closer, leading to an underestimation of the difference between test positive and test negative animals. Conversely, a herd with a high prevalence of infection may overestimate the milk production effects for the industry because heavy exposure to

calves in this herd could lead to earlier clinical signs than would be seen for the rest of the industry.

As shown by the above studies, the level of reduction in milk production in subclinically infected cows depends on a number of factors, including: stage of subclinical infection (1), parity, with infection in older cows having a larger negative impact (2), sensitivity and specificity of the test utilized for identifying infected cattle, which varies with the stage of infection (12), and farm management (e.g. cow comfort, concurrent infections) (8). Further studies are needed to identify the onset, progression and extent of milk production effects associated with JD in culture-positive animals, controlling for these and other confounding variables at the cow, herd and regional level. However, it is evident that current knowledge supports the fact that subclinical JD has a considerable negative effect on milk production and udder-health, the only difference being the magnitude of this effect.

Premature culling and reduced slaughter value

Premature culling associated with paratuberculosis is one of the major economic burdens of this disease (13). In the only Canadian study estimating culling risk based on serological testing, results from randomly sampled cattle among randomly selected Maritime Canadian dairy herds indicated that after controlling for parity, 305-day milk production and somatic cell count (SCC), the odds of being culled during the 3 years after testing was 2.3 times greater in ELISA-seropositive cows as compared to seronegative cows (14). While the owners in this study were informed of test results 1 year after sampling, the difference in odds of culling between seropositive and seronegative cattle

was not significantly different before and after the results were communicated, indicating that the difference was for biological reasons, not simply due to the test results. Similar results were found in a study comparing fecal culture-negative and culture-positive cows in one herd from New York, where the estimated loss due to premature culling for MAP for that herd was US$ 75 per cow per year (15). Age of culling in tissue culture-positive cows showing clinical signs, tissue culture-positive cows not showing clinical signs, and non-infected cows have been demonstrated to be 4.3, 4.9 and 7.7 years, respectively (16).

In addition to premature culling, slaughter value has also been shown to be affected by JD. Clinical JD has resulted in a reduced slaughter value of 20 to 30% in culled cattle (1). In addition, fecal culture-positive cattle without clinical signs have been shown to weigh approximately 59 kg less at slaughter, a loss of US$ 48 per head (5). Using economic modeling techniques, a loss of CD$ 1330 Canadian (CND) per 50 cow herd (assuming an average apparent prevalence of 7%) due to reduced cull value and premature culling associated with subclinical MAP seropositive cows was reported in Maritime Canadian dairy cattle (17). Further analysis is needed to verify that this estimate is appropriate for all of Canada.

Reduced fertility

Research has failed to show a consistent association between MAP test status and reduced fertility (18). In a study performed in a 900-cow Guernsey herd spanning a total of 10 years, data was collected on reasons for culling along with fecal, terminal ileum and associated lymph node culture (6). A greater percentage of infected cows (68.8% or 106/155) were culled for infertility than culture-negative cows (60.2% or 797/1324). The

reliability of producer-reported reasons for culling have been questioned due to the inability to identify more than one reason for culling in most dairy herd improvement (DHI) systems when multiple parameters often contribute to a culling decision (19). In the case of this 10-year study, however, they were not restricted to picking one reason for culling and multiple reasons were listed.

In 90 dairy herds from the Canadian Maritime provinces, seropositive first lactation heifers had an increase of 49 days open as compared to seronegative first lactation heifers, but no association was found in other parities (20). In a study done in Michigan, seropositive cows had a 28-day increase in days open; however, there was no significant association when infected cows were identified with fecal culture (18). Furthermore, studies done in six herds in New Zealand with fecal culture testing (21), and 304 herds from Ontario with ELISA testing (10) found no association between subclinical paratuberculosis and calving interval.

Overall, four studies (2 ELISA and 2 culture based) found JD had a negative effect on reproduction and three studies (1 ELISA and 2 culture based) found no negative effect on reproduction. As a result, it is difficult to draw strong conclusions regarding the impact of JD on fertility. From the available literature, seropositive cows appear to have some degree of diminished fertility; however this impairment is not evident when the classification of disease status is fecal culture. Therefore, the implications of reduced fertility due to JD may not have anything to do with whether the cow is actively shedding bacteria.

If there truly is an impact of subclinical paratuberculosis on fertility, it has been hypothesized that the mechanism may be related to the impaired immunological and

gastrointestinal absorptive capacity and accentuated negative energy balance sustained by infected cattle (18). Preliminary work has been performed looking at the effect of energy on metabolic and immune status of cows with JD around the time of parturition (22). In this study, fecal culture-positive cows were supplemented with a higher energy diet by means of "force feeding" through rumen cannulae. This study found that although supplemented cows had the same periparturient decline in neutrophil function, there was an increased *in vitro* immunoglobulin production and a less protracted return to normal lymphocyte proliferation activity. However, the major limitation of this study was that there were no uninfected cattle included for comparison.

Mastitis

There has also been an inability to show a consistent association between MAP test status and risk of clinical or subclinical mastitis. In 304 herds in Ontario, when an experimental ELISA was used to identify cattle with subclinical paratuberculosis (10), seropositivity was associated with higher SCC at the cow and herd level. Conversely, a study done in New Zealand (NZ) of six dairy herds found that subclinical, fecal culture-positive cows had significantly lower SCC compared to culture-negative cows (21). The differences in testing methodologies make it difficult to make direct comparisons between the two studies. However, the results from NZ may be specific to the six herds that were sampled, which are unlikely to be comparable to most dairy farms in Canada due to major differences in climate, management, and productivity between the two countries.

Results from a study in Maritime Canadian dairy cattle indicated that, after controlling for parity, 305-day milk production and linear score SCC, the odds of being culled because of either decreased milk production, mastitis or reproductive inefficiency was 2.9 times greater in MAP ELISA-positive cows as compared to ELISA-negative cows (14). These findings support previous reports where cull rates due to mastitis were higher for fecal culture-positive cows compared to negative cows (6). In the previously mentioned culling study using a Guernsey herd, the risk of culling due to mastitis in the culture-positive cows was 22.6% (45 of 199 cows) versus 3.6% (49 of 1361 cows) in the culture-negative cows (6). However, due to the potential unreliable nature of producer-reported reasons for culling in dairy herd improvement (DHI) data, mastitis-related culling evidence should be interpreted with caution. Nevertheless, in this case where there are such dramatic differences, there is likely a real association present.

Overall, there appears to be more evidence for than against the theory that JD negatively affects udder health (Table 1). However, further research is needed to clarify and quantify this impact. The pathophysiology of how subclinical paratuberculosis could affect mastitis remains unclear and also requires further research. Speculation includes negative energy balance and reduced cellular immunity, which have been shown to occur at least in periparturient cows with JD (22).

Total economic losses at the farm, regional and national level

Studies of annual economic losses associated with clinical and subclinical JD among dairy farms have indicated that there are substantial effects at the farm, regional, and national levels (15,17,21,23). As part of a survey by the USDA National Animal

Health Monitoring System (NAHMS) conducted in 1996, it was estimated that, averaged across all herds in the United States (US), JD cost the US dairy industry US$ 200-250 million annually (23). Subsequently, a study was conducted in Canadian Maritime region (17) which found that based on ELISA testing, the losses were CD$ 2472 per infected herd per year (average herd size of 50 cows with an average apparent prevalence of 7%) and CD$ 0.84 million per year for the Canadian Maritime provinces. Direct production losses included decreased milk production, increased culling risk, reduced cull value, mortality, treatment costs and reproductive loss. Assuming the prevalence of JD in the Maritimes were the same as the rest of Canada, the national cost of JD could be estimated at CD$ 15 million annually. However, these calculations most likely underestimated the actual losses associated with JD because of misclassification of infected cattle by the ELISA and the authors' decision to not recognize other potentially relevant economic effects, such as mastitis, decreased feed efficiency, and restrictions on market access. Further data and improved diagnostic methods are needed in order to accurately determine economic losses associated with JD for the Canadian dairy industry.

Risk Assessment

Various factors such as host susceptibility and environmental factors (mode of transmission) interact to determine the prevalence and severity of MAP infection (23).

Host factors

Level of exposure (dose of organisms) and age at the time of exposure are major factors in determining whether an animal eventually becomes infected with JD. Although there is a paucity of scientific evidence on these factors, there is consensus that younger animals require a lower infective dose than older animals (24-26), and it is quite unlikely for an adult animal to become infected, unless there is extreme environmental contamination (27). Poor nutrition, stress related to transport, lactation, parturition and immunosuppression by agents like bovine viral diarrhea virus have been proposed as biologically plausible factors accelerating or precipitating the onset of the clinical phase of infection (28).

Milk or colostrum may serve as the source of MAP organisms for neonates in two ways. Fecal contamination of these fluids may occur, allowing the milk or colostrum to act as the vehicle for infection. Additionally, MAP has been isolated from sterile collections of milk and colostrum from infected cows (29), indicating that there is potential for direct transmission through colostrum and milk from an infected dam (30). Infection can also occur directly across the placenta, as tissue-positive fetuses have been found in culled tissue-positive cows, although this occurrence is quite infrequent (31). This happens more often in cows displaying advanced clinical signs of JD, however it can occur in cows that are heavy fecal shedders, yet not displaying clinical signs of disease (32).

It is suspected that on rare occasions, certain animals that are exposed to MAP can generate a protective immune response resulting in full clearance of the MAP (27). It

is unclear whether this capacity, if it exists, is restricted to mature animals, or whether some young animals also have this capability.

Some studies have shown higher susceptibility of Jersey and Shorthorn cows for paratuberculosis (33,34). However, these observed differences were confounded by the fact that breed differences were actually linked to husbandry practices in specific regions (i.e. herds with these breeds in these particular studies, had worse hygiene). Due to these confounding circumstances, genetics and breed appear to be minor factors.

Agent factors

The specific number of organisms required to establish infection for specific age groups has not been determined. Infection can occur in calves with a dose of $1.6x10^7$ organisms, which would easily be surpassed in a 2-gram sample of heavily infected feces (35). However, this number is likely to increase with increasing age, as the resistance to infection of the animal increases (24).

Infection of animals may cause clinical disease, but this is not necessarily advantageous or essential to the organism. To survive, MAP only needs to colonize, replicate and be shed so that the rate of recruitment of new bacteria is equal to or greater than the loss of bacteria from the population. The presence of obvious clinical disease is not required for the spread of the organism in the animal population (30). It has been shown that, although the risk of individual cows being infected is higher on farms with clinical JD, there are still many herds that are infected, yet display no clinical signs of JD (36).

Different strains of MAP exist depending mainly on the species infected. Infections in cattle and sheep are considered to be caused by separate strains of MAP and are sometimes classified as type C and type S, respectively. There has been some evidence of cross-infection of animals between these species (37), but more research is needed to determine the degree to which these two MAP types can actually cross the species barrier. There is also evidence that wildlife species, particularly rabbits (38), may play a role in dispersion of MAP throughout the environment and cause contamination of feed for cattle (39).

Environmental Factors

The primary method of MAP transmission is believed to be a direct fecal-oral cycle. The process is quite similar to the transmission of other enteric infections whereby any exposure to manure from shedders can potentially lead to new infections. However, there is still a possibility of indirect transmission, such as through manure contamination of water bowls and machinery used for feed delivery. Therefore, any management activities that directly or indirectly lead to exposure of manure from shedding animals to susceptible animals could be considered risk factors of infection, and will be reviewed in detail in the section on disease control strategies. The efficiency of transmission by these pathways depends upon factors such as number of organisms shed in the feces and the organism's survival characteristics in the environment (40).

Factors that influence survival of the organism include substrate (feces, water, milk), temperature and pH. The MAP organism can persist in the environment for at least a year (40), but does not replicate in the environment. Although hardier than most

other pathogens, the bacterium is susceptible to long-term desiccation, repeated freeze-thaw cycles, exposure to sunlight, and soils with alkaline pH or low iron (41). MAP is more thermal resistant than other *Mycobacteria*, making pasteurization of milk and milk products somewhat problematic (42). Viable MAP was found in 2.1% of the pasteurized milk samples in Great Britain (43), but studies done in North America, including one using samples collected from retail stores and dairy plants in south-western Ontario, did not find viable MAP in pasteurized milk (44). This may be due to differences in pasteurization methods and temperature (45). However, recently, low numbers of viable organisms were found in 2.8% of 702 samples tested in commercially pasteurized milk purchased from stores in California, Minnesota and Wisconsin (46). While these data have not been published in peer-reviewed journals, these occurrences have called into question the validity of feeding pasteurized milk products to calves as a possible means of lowering the risk of MAP infection.

Although relationships are not well defined among the numerous combinations of host, agent, and environment factors found on different farm situations, known risk factors and routes of transmission must be recognized and addressed for implementation of a control program.

Herd Level Control Strategies

In general, every disease control program has three main objectives: decrease the number of new infections; decrease the number of clinically diseased or shedding animals, and decrease the duration of disease or its infective period. Understanding the

transmission and pathogenesis of the bacterium is crucial for achieving these objectives. For JD, the prolonged pre-clinical phase of the life-long infection and subsequent poor performance of diagnostic tests makes identification of subclinical shedders difficult. This in turn makes exposure of sub-clinical cows to the susceptible population the main risk factor for spreading infection (47). Furthermore, the ability of the bacterium to survive for more than a year in the environment makes it difficult to stop transmission within herds without stringent manure management control for young stock. Therefore, effective JD control programs involve two main objectives: to implement best management practices in order to decrease calf exposure to all manure (decrease incidence of new infections), and to reduce the number of infected animals that may shed bacteria in their manure (decrease prevalence of existing infections). We will review each in turn.

As with other herd health activities, a JD control program for a farm needs to be customized to the goals and resources of the farm. While all farms should have a plan for implementing best management practices for reducing fecal-oral transmission due to the challenges associated with the tests to identify infected animals, the intensity and focus of that plan will depend on the goals and resources of the farm. Table 2 summarizes recommended management practices for minimizing fecal-oral transmission of JD, and they are adapted from numerous sources (33, 48-52) including the USDA Johne's Disease website (www.aphis.usda.gov/vs/nahps/johnes).

Due to the many possible ways that calves can become exposed to MAP-infected manure, and the long interval between exposure and detectable disease, it is both very difficult and costly to conduct clinical trials to test and quantify the importance of

recommendations related to minimizing calf exposure to infected manure. However, it is universally accepted that poor manure management and hygiene around calves will lead to exposure and infection in herds with JD (51,52). Assessing and improving management practices that minimize calf exposure to infected manure will be cost-effective on most farms, not only for reducing the impacts of JD, but also for reducing the impacts of other fecal-orally transmitted diseases of cattle (e.g. *Escherichia coli, Campylobacter, Salmonella,* and *Cryptosporidium*).

The type of testing strategies utilized on a farm will depend not only on the farm goals and resources, but on which of three types a dairy farmer is categorized: 1) those that know they have a JD problem and want to decrease the prevalence; 2) those that have confirmed or suspected that they have JD but do not think it is present at a high prevalence; and 3) those that do not suspect they have JD and want it to remain that way.

Herds that have had at least one cow with clinical signs of JD and a positive fecal culture, likely have an infection prevalence of at least 15% (47). This assumption is based on the premise that the cow was not recently purchased (i.e. was born on the farm), and therefore at the time this cow became infected, there was a high likelihood that others were also infected. Additionally, the clinical cow has likely had heifer calves of her own that are still in the herd and could have infected those calves along with other in-contact heifer calves. This statement, though highly generalized and not accounting for herd size, compels one to consider the true implications of one clinically infected cow. For those farms with a moderate to high prevalence (>30%), regular testing of the herd is likely warranted to identify cows that are shedding and dramatically increasing the environmental load of MAP. On these farms, fecal culturing will identify a substantial

number of shedders. However, such farms should also consider using ELISA testing because the number of ELISA false-positives will not outweigh the number of true-positives, producing a good predictive value of a positive test.

The most appropriate testing strategy to use in high prevalence herds depends upon the goals of the producer and the time frame during which they want to accomplish those goals. If the goal is reduction of prevalence to close to zero within a few years, an aggressive approach of annual fecal culture testing of all cows 2 years and older may be warranted. However, if the herd size is large (>300 animals), more strategic measures may be necessary due to limitations of cost, time, and lab-space for fecal culture. For example, ELISA testing the whole herd and then fecal culture testing all ELISA-positives plus all cows with S/P ratios higher than one standard deviation below the cut-off value (cows that are more likely to be fecal culture-positive but ELISA-negative) may be an alternative approach (53). However this latter, less expensive approach will miss cows that are shedding MAP but have not mounted much of an immune response yet. With the improvements in fecal culture techniques during the last few decades, it is unlikely that fecal culture-negative cows are shedding significant numbers of bacteria in their feces. Simulation studies have reported that successful and simultaneous implementation of best management practices and strategic testing and culling leads to the largest and fastest reductions in infection levels (49).

In the past, the Dutch have attempted to decrease the number of clinically diseased animals on farms with high prevalence by use of a vaccination program using a killed vaccine on some farms so that they would not have to rely on imperfect tests to detect the subclinical animals (48). However, their observations with this program are

that producers often become less vigilant with other management-related control measures and too reliant on the vaccine. For this reason, if vaccination is considered, it should be restricted to high prevalence farms that have numerous clinical cases of JD.

If there has not been a confirmed clinical case of JD on a farm, and it is suspected, based on herd history, husbandry practises or testing, that the herd has a low to moderate prevalence (<30%), then individual fecal cultures will seem quite costly for the low number of shedding cows detected. Furthermore, using an ELISA with less than optimal specificity will result in interpretation difficulties of test positives. The likelihood of a positive test being a true positive is low in herds with low prevalence, and therefore a confirmatory test, such as a fecal culture, should always be performed. This approach can become quite costly in large herds and can be viewed as unrewarding, especially if the herd truly is negative and all confirmatory tests are negative. One viable strategy would be to perform pooled fecal cultures of the mature cows. The major benefit of pooling is the decreased testing cost, however diluting the sample with too many cows could lead to false-negatives. Additionally, there is need for subsequent confirmation testing of individual cows in positive pools, taking additional time before culling can be implemented. The use of broth media for cultures has reduced the time required for incubation from 16 to 6 weeks, which is one reason why this is becoming a viable option. The ideal number of cows per pool has not been well established, but available research would suggest pools of five would likely be adequate (54,55). This strategy has been shown to identify 87% of positive animals where individual culture found 96% of positive animals (54). However, cows with low level or intermittent shedding could be missed with pooled fecal cultures, and therefore management changes should be

implemented as well to minimize transmission between the missed cows and susceptible youngstock.

Another alternative for moderate to low prevalence herds would be to forego testing and focus on controlling the spread of the disease within the herd, focusing on the young animals. Implementing strict control measures along with a high turnover of cows should lead to a lower within herd prevalence after approximately five years. Considering the performance of the tests available, individual testing may be more difficult and costly than the benefits from such a program. Simply implementing control procedures will decrease the risk associated with a low number of cows spreading the disease within the herd, and eventually lead to a decreased prevalence.

For herds that have never identified a cow with clinical signs of JD on the farm, have had at least some of the herd tested for JD, and all tests produced negative results for JD, the assumption would be that the prevalence of infection on these farms is likely either zero or very low. However, with the poor sensitivity of current tests for identifying MAP-infected cattle, there is no method to definitively state that a farm is free of JD. With repeated negative tests over many years, producers and their health advisors may assume that they have disease-free status, leading to the temptation to relax within farm management practices and concentrate on keeping the disease out. However, because JD-free status cannot be guaranteed, continued vigilance is needed to minimize calf exposure to manure to avoid unknowingly spreading this insidious disease (52).

For those farms where the data suggest a high likelihood of being JD-free, along with on-farm biosecurity measures, it is likely more important to focus efforts onto keeping the disease out of the herd (56). This could be accomplished by implementing

all of the control points previously mentioned in the risk factor section (Table 2), and applying methods for keeping manure from cattle from other farms away from the herd, including: avoiding community or shared pasture; and restricting application of manure from other farms on the farm (49-52).

With the currently available tests for JD, the common movement of animals/equipment between herds, the difficulty of completely eliminating fecal-oral exposure of youngstock on a dairy farm, and the long incubation period, it is questionable whether JD can truly be eradicated from an infected farm.

National / Regional Control Programs

A number of countries have developed national, government-funded, animal health programs to provide logistical, administrative and funding support to control JD. National, government-funded, animal health programs are typically directed at exotic diseases and to the control of specific diseases of widely recognized economic or public health importance. Johne's Disease has emerged as a disease requiring a national control program due to mounting evidence and concern over production losses, and due to the possible restrictions to international movement of cattle as some countries require testing. In addition, it has been suggested that because DNA from MAP has been found in 69% of patients with Crohn's disease, that MAP may be a factor in the causation of the disease (57), although MAP may just be an opportunistic organism found in the intestines of Crohn's patients. If the relationship between JD and Crohn's Disease were scientifically confirmed, government funding of a control program would likely be forthcoming. A review into this potential relationship is beyond the scope of this paper. For further information, the reader is referred to the following website: http://www.crohns.org/.

Recently, in Canada a proposal for a nation-wide Voluntary Johne's Disease Control Program for dairy cattle has been developed by the authors of this review paper in response to a request by the Johne's Disease Consultation Group sub-committee of the Production Limiting Disease Committee. The proposal has received support in principle from the Canadian Animal Health Consultative Committee, Dairy Farmers of Canada and the Canadian Cattlemen's Association (58). The program builds on the strengths of national JD control programs developed in other countries. The current Alberta

voluntary JD control program is similar to the proposed national program and can be easily modified to reflect the proposed national program. The proposed program will be reviewed here, following brief reviews of the most recent editions of other established national control programs in Australia, The Netherlands, and the United States. The proposed Canadian program is described in some detail to demonstrate its strengths and weaknesses for Canadian veterinary practitioners and others.

Australian National Voluntary JD Control Program

Australia was among the first countries to implement a national JD control program, although many countries have a long history in JD education and extension. In 1996, the Australians launched the National Johne's Disease Market Assurance Program for Cattle (59,60). In this program, herds progress through levels of assurance on the basis of annual negative herd tests from Monitored Negative 1 (MN1) to Monitored Negative 3 (MN3), the highest level. The actual method of testing (ELISA, fecal culture, etc.) is not specified but is assessed for validity by the Chief Veterinary Officer (CVO). Annually, the supervising approved veterinarian also uses auditing procedures to monitor critical herd management aspects to control the spread of JD. Herds can opt to stay at a level by carrying out a maintenance test every two years where the entire herd, up to a maximum of 100 animals, is tested. Herds not participating in testing can be classified as a Non-Assessed Herd (NA), which is a herd with no history of JD or where any suspicion of infection has been resolved to the satisfaction of the CVO. A herd may be classified as a Suspect herd (SU) for numerous reasons including violations of the annual management

audit; however, no diagnostic confirmation of positive animals has occurred in these herds. Infected herds (IN) are herds with a confirmed infected animal. Restricted herds (RD) are herds that were previously IN herds, but are currently undertaking an approved test and control program under supervision of the CVO. In addition, RD herds have achieved one or more negative herd tests commencing at least 12 months after the last known infected animal was removed from the herd.

There has been a linear increase in herds participating in Australian JD control program from approximately 180 at the end of 1996, to around 1000 herds in 2000 (60), and by December 2003, 1623 herds were participating (61). There are some desirable components of this program which have been included in the proposed Canadian program, including maintenance testing every two years. However, the numerous different categories of herds were felt to be too confusing, and therefore, simplification of the categories was sought.

The Dutch National Voluntary JD Control Program

The original JD control program in The Netherlands began in 1991. It evolved into a pilot program in 1997 based on fecal culture of 125 herds that were tested every six months. After five rounds of testing (24 months), only 58 herds (46%) remained clear of infection (62). The lessons gained from this experiment were that although these herds had no clinical signs in the last 5 years, more than half were infected. Secondly, fecal culture, regarded as the "gold standard" was not sensitive enough to detect all infected animals. Finally, producers were disappointed to find out their herds were truly positive

and labelled as such. As a result, highly motivated producers felt they were penalized for participating.

The current Dutch JD control program, initiated in 1998, has an extensive program based on management assessment only and an intensive program based on pooled fecal cultures and management assessment. The extensive program was developed for the dairy industry, but has not yet been accepted by the industry as a whole (63). The rules of the management assessment are aimed at reducing the spread of infection to young calves. For example, pooled colostrum must not be used, milk replacer is required, and cows should calve separately in clean calving areas. There are also strict rules governing the purchase of animals and grazing practices, along with contact of animals of different species that may carry MAP.

In the intensive program, there are 10 levels of classification of herd status. The program is categorized to certify herds as free (level 10) or unsuspected (level 6-9) and provides a control program for infected herds. The program employs annual testing of adult cattle. Fecal samples are pooled in batches of five for status advancement and the ELISA is used at various levels individually for maintenance of levels. Positive ELISA results confirmed by positive fecal culture results lead to a decrease of a status level. There is a well-defined program to assist farms that have been identified as infected, which encourages farmers to participate. Additionally, there are funds provided to assist with the high cost of repetitive testing for farmers to re-enter the certification program (62).

From the original 350 infected herds, half are now unsuspected-free herds. There are now 1000 herds in the certification program for unsuspected-free herds and 250 are

classified as infected herds (63). Again, there are some specific desirable components of this program which have been included in the proposed Canadian program, including the pooled fecal culturing. However, due to limited funds currently available for the program in Canada, no financial assistance for testing costs will be provided.

United States National Voluntary JD Control Program

In order to address disparities between existing programs among states, and encourage non-participating states to participate, in April 2002, the USDA Veterinary Services section published the Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program (64). This program recommended an advisory committee in each State to assist the State veterinarian in establishing and operating a JD program. By the end of 2002, 40 states had established advisory committees for JD with federal representation on each committee (65).

The structure of the program has three parts. Part 1 is education of the producers using a means that is at the discretion of the state advisory committee. Part 2 is an assessment of on-farm risk and herd management plans. Part 3 of the program involves herd testing and classification into four levels. Under normal circumstances 10 months must pass before a herd can advance to the next level. If a herd does not test after 14 months, it reverts to a herd of unknown status or in some states a maximum risk herd. Testing in the initial stage is done on 30 randomly selected animals 36 months of age or older. The test used is specified as a screening test and is determined by the state administrator. At a recent US Animal Health Association-Johne's Committee meeting, a

resolution was passed to use environmental sampling as a potential screening test available to state administrators (66). The idea behind this is to decrease the cost of identifying positive herds without loss of herd sensitivity (67). However, if a herd is found positive, all animals must then be tested with an individual screening test. If an animal is found positive on the screening test, an appeal can be made to have that animal tested with an official Johne's test (either PCR or fecal culture, upon the discretion of the state administrator). If the official test is negative, the herd regains its test-negative status, but the animal that was retested must be submitted for testing at the next assessment if the animal still resides in the herd. If the appeal test is positive, the owner can request another appeal in which the animal must be either necropsied for further testing or undergo surgical biopsy of the ileum and lymph nodes. At the end of the testing and appeals process, if it is found that the animal is positive, the herd is assigned a positive status.

There is a Fast Track option in part 3 of the program that allows a herd to reach level 4 in two years with three tests, which was added at the insistence of the livestock industry. With this option, Level 1 is skipped with a signed declaration that no cows were seen or diagnosed with JD in the last five years (65).

At the end of 2002, approximately 2,675 herds were enrolled in JD control programs with herd management plans and/or risk assessments filed with State programs (65). As of the end of 2003, there were 4722 herds enrolled in JD control programs (68). Approximately 543 herds were enrolled in State-specific herd status programs in 2003, herds that test negative and are considered less likely to have JD than untested herds.

Again, there are some specific desirable components of this program that have been included in the proposed Canadian program, primarily the simple four-level system. However, the fast-track system was not included due to the experience of a Dutch study showing that more than half of herds that have never reported seeing clinical JD, in fact end up being infected. Additionally, due to the performance of ELISAs, particularly in low prevalence herds, the U.S. program's reliance on ELISA testing is less likely to be applicable to the majority of low prevalence herds in Canada (69) due to low herd level specificity. Herd-level specificity (HSp) is the probability that an uninfected herd yields a negative herd-test result, while herd-level sensitivity (HSe) is the probability that an infected herd yields a positive herd-test result. With 30 cows tested in a herd, HSe will be 66% and HSp will be 49% using the ELISA (assuming test Se and Sp of 45% and 98%, respectively). However, for fecal culture, the HSe will be 66% but the HSp will be 100% (assuming test Se and Sp of 45% and 100%, respectively). In 11 Dutch dairy herds, the reported HSe for fecal culture and pooled fecal-culture were 64 and 73% (54).

Alberta JD Control Program

In September 2001, Alberta Agriculture Food and Rural Development implemented a Voluntary Johne's Disease Herd Status Program. The testing protocol used in Alberta's program is similar to the American program, but is more specific about what test can be used at each level (70). There are also strong similarities between Alberta's program and the proposed Canadian program, and therefore Alberta's program is not described here. This congruence was done intentionally so that the Alberta program could seamlessly be modified to fit within the Canadian program. However, some

additional modifications have been made based on recent scientific information, such as, to not include ELISA testing for level advancement beyond level 1 due to the recent clearer understanding of the inaccuracies of the ELISA.

Proposed Canadian National Voluntary JD Control Program

The proposed Canadian National Voluntary Johne's Disease Control Program contains two major components (58). The first aspect of the program is a Best Management Practices (BMP) Assessment based upon a herd risk analysis of critical control points in the avoidance of transmission of MAP. The questions included in the assessment are a detailed evaluation of a herd's level of management with respect to the recommendations for decreasing the risk of new MAP infections. Table 2 summarizes BMPs for dairy herds, and recommendations and a BMP Assessment will be developed in the future that is specific to cow-calf operations. Herds may participate in this aspect of the program without taking part in the second component, the Voluntary Johne's Disease Herd Status Program.

The proposed Herd Status Program will have a total of five levels, zero through four (Figure 1). All herds entering the status program begin at level 0. In order to advance to level 1, a herd must have completed a recent BMP assessment and ELISA testing of 30 animals in their second or higher lactation. If all animals are ELISA-negative, the herd then advances to level 1. If some animals are ELISA-positive, the producer may elect to perform fecal cultures on those animals. If all fecal cultures are negative, the herd can advance to level 1. However, if any fecal cultures are positive or

the producer does not elect to do fecal culturing following positive ELISA results, the herd remains as Status Level 0.

There is a waiting period of a minimum 10 months before a producer can re-apply for Status Advancement. For a herd to advance from Level 1 to Level 2, a recent BMP assessment must be completed and pooled fecal cultures (currently pools of up to five animals will be accepted) are performed on all cows in their second or higher lactation. If all pools are negative, the herd advances to the next level. However, if at least one pool is positive, the producer can elect to: 1) do individual cow fecal cultures on animals contributing to the positive pool(s), and cull the infected cow(s) and remain at level one, or 2) allow the cow(s) in the positive pool(s) to remain in the herd and drop to level zero.

A producer that is at level one may opt to follow the Status Maintenance strategy rather than attempt to progress through the Status Advancement strategy. In order to maintain a herd status (i.e. not drop down to the next lower level), a producer must have a BMP assessment performed and have 30 cows in their second lactation or higher ELISA tested once during the two-year period subsequent to their previous test and BMP assessment. If all cows are negative, the herd maintains its status. If any cows are ELISA-positive, the producer can fecal culture those cows. If the fecal cultures are negative, the herd maintains its status. If a fecal culture is positive, the cow(s) must be culled in order to maintain status, otherwise the herd drops to level zero.

Advancement from Levels 2 to 3 and 3 to 4 follow the same protocol of a recent BMP assessment and pooled fecal culture. However, even if all positive cows are culled, the herd drops to Status Level 1. If the individual cows are not tested and positives culled, the herd drops to level zero.

For Status Maintenance at levels 2, 3 and 4, the same process applies, as described above for maintenance of level 1. The difference for maintaining these three levels versus level 1 is that if the culture is positive and the producer culls the infected cow(s), the herd still drops to level 1. If the producer does not do a fecal culture confirmation, or does not cull the test-positive cow, the herd is assumed to be infected and drops to level zero.

One strength of the proposed Canadian control program will be its ability to aid in the future research of JD. The program will provide data to monitor the proportion of enrolled herds with the disease, and the impacts of implementation of control measures in infected herds, enabling modifications as needed. It will also provide some indication on the prevalence of the disease in various regions of the country, which will be a vital aspect in understanding the epidemiology of the disease. The data will also allow on-going evaluation of the JD program and identification of strengths and weaknesses of the program and recommend modifications for maximum cost-benefit.

One weakness of the proposed program is that there are currently no funding mechanisms in place to subsidize testing and indemnification of fecal culture-positive cattle. Also, there is a need for market-based incentives to engage producers (i.e. higher prices for heifers from JD-negative certified herds) to participate in the program. Increased involvement of the producers and their governing organizations is going to be critical to any control program success.

Another possible incentive could be the incorporation within the program of specified best management practices for a wider range of fecal pathogens, to illustrate how a control program for JD will also have a positive impact on general calf health. These

incentives are being examined with respect to the proposed national JD control program for Canada. For a JD control program to be feasible and effective, it needs strong support from producers and governing agencies and the system must evolve with the needs of the program to continuously improve its effectiveness.

Conclusions

Johne's Disease continues to cause economic losses for dairy producers in Canada and worldwide, particularly with respect to lost milk production, premature culling, reduced slaughter value, and perhaps increased infertility and mastitis. The cost of JD to the Canadian dairy industry is estimated at CD$ 15 million annually. The control of JD nationally will be an immense task because of the insidious nature of the disease and the relatively poor performance of tests currently available. The industry must utilize knowledge of the biology of the disease and known risk factors in an attempt to control the spread of this disease through best management practices. Strategic testing can overcome the challenges with identifying infected cattle and herds. JD control programs initiated now will lead to lower control costs in the future, and will be seen as proactive steps for quality milk production if the link between Crohn's disease and JD is confirmed in the future.

References

1. Benedictus G, Dijkhuizen AA, Stelwagen J. Economic losses due to paratuberculosis in dairy cattle. Vet Rec 1987;121:142-146.
2. Wilson DJ, Rossiter C, Han HR, Sears PM. Association of *Mycobacterium paratuberculosis* infection with reduced mastitis, but with decreased milk production and increased cull rate in clinically normal dairy cows. Am J Vet Res 1993;54:1851-1857.
3. Collins MT, Nordlund K. Milk production levels in cows ELISA positive for serum antibodies to *M. paratuberculosis*. Proc. 3rd Int Colloq on Paratuberculosis, Orlando:1991;401-409.
4. Sweeny RW, Hutchinson LJ, Whitlock RH. Effects of *Mycobacterium paratuberculosis* infection on milk production in dairy cattle. Proc. 4th Int Colloq on Paratuberculosis 1994;133-139.
5. Whitlock RH, Hutchinson LT, Merkal RS, Glickman LT, Rossiter C, Harmon S et al. Prevalence and economic consideration of Johne's disease in the northeastern U.S. Proc of the US Anim Hlth Assoc 1985;89:484-490.
6. Merkal RS, Larsen AB, Booth GD. Analysis of the effect of inapparent bovine paratuberculosis. Am J Vet Res 1975;36:837-838.
7. Nordlund KV, Goodger WJ, Pelletier J, Collins MT. Associations between subclinical paratuberculosis and milk production, milk components, and somatic cell counts in dairy herds. J Am Vet Med Assoc 1996;208:1872-1876.

8. Vanleeuwen JA, Keefe GP, Tiwari A. Seroprevalence and Productivity effects of infection with Bovine Leukemia Virus, *Mycobacterium avium subspecies paratuberculosis*, and *Neospora caninum* in maritime Canadian dairy cattle. Bov Pract 2002;86-91.
9. Goodle GM, Hirst H, Garry F, Dinsmore P. Comparison of cull rates and milk production of clinically normal dairy cows grouped by ELISA *Mycobacterium avium paratuberculosis* serum antibody results. Proc. IX Int Symp of Vet Epidemiol and Econ, Breckenridge, 2000;897-899.
10. McNab WB, Meek AH, Martin SW, Duncan JR. Associations between dairy production indices and lipoarabinomannan enzyme-immunoassay results for paratuberculosis. Can J Vet Res 1991;55:356-361.
11. Sockett DC, Conrad TA, Thomas CB, and Collins MT. Evaluation of four serological tests for bovine paratuberculosis. J Clin Microbiol 1992;30:1134-1139.
12. Dargatz DA, Byrum BA, Barber LK, Sweeney RW, Whitlock RH, Shulaw WP, Jacobson RH, Stabel JR. Evaluation of a commercial ELISA for diagnosis of paratuberculosis in cattle. J Am Vet Med Assoc 2001;218:1163-1166.
13. Hutchinson LJ. Economic impact of paratuberculosis. Vet Clin North Am Food Anim Pract 1996;12:373-381.
14. Tiwari A, Vanleeuwen JA, Dohoo IR, Keefe GP. Effects of seropositivity for *Mycobacterium avium* subspecies *paratuberculosis* on risk of culling in Maritime Canadian dairy cattle. Proc 54th Ann Conv Can Vet Med Assoc, Halifax 2002:264.

15. Wilson DJ, Rossiter C, Han HR, Sears PM. Financial effects of *Mycobacterium paratuberculosis* on mastitis, milk production, and cull rate in clinically normal cows. Agri Pract 1995;16:12-18.

16. Buergelt CD, Duncan JR. Age and milk production data of cattle culled from a dairy herd with paratuberculosis. J Am Vet Med Assoc 1978;173:478-480.

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

18. Johnson-Ifearulundu YJ, Kaneene JB, Sprecher DJ, Gardiner JC, Lloyd JW. The effect of subclinical *Mycobacterium paratuberculosis* infection on days open in Michigan, USA, dairy cows. Prev Vet Med 2000;46:171-181.

19. Kelton, D and Lissemore K. Reasons that Dairy Cows Leave Home – Valid or not? Proc 30th Ann Conv Am Assoc of Bov Pract. Montreal,1997:177.

20. Tiwari A, Vanleeuwen JA, Dohoo IR, Stryhn H, Keefe GP. Effects of seropositivity for bovine Leukemia virus, *Mycobacterium avium* subspecies *paratuberculosis,* and *Neospora caninum* on calving to conception interval in maritime Canadian dairy cattle. Proc Soc Vet Epidemiol and Prev Vet Med, Warwick, 2003:243-252.

21. DeLisle GW, Milestone BA. The economic impact of Johne's disease in New Zealand. In: Milner A, Wood P, eds. Johne's disease. East Melbourne, CSIRO, 1989:41-45.

22. Stabel JR, Goff JP, Kimura K. Effects of supplemental energy on metabolic and immune measurements in periparturient dairy cows with Johne's disease. J Dairy Sci 2003;86:3527-3535.
23. Ott SL, Wells SJ, Wagner BA. Herd-level economic losses associated with Johne's disease on US dairy operations. Prev Vet Med 1999;40:179-192.
24. Chiodini RJ. Immunology: resistance to paratuberculosis. Vet Clin North Am Food Anim Pract 1996;12:313-343.
25. Chiodini RJ, Van Kruiningen HJ, Merkal RS. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. Cornell Vet 1984;74:218-262.
26. Hagan WA. Age as a factor in susceptibility to Johne's disease. Cornell Vet 1938;28:34.
27. Rankin JD. The experimental infection of cattle with *Mycobacterium johnei*. IV: adult cattle maintained in an infectious environment. J Comp Pathol 1962;72:133-117.
28. Kennedy DJ, Benedictus G. Control of *Mycobacterium avium* subsp. *paratuberculosis* infection in agricultural species. Rev Sci Tech 2001;20:151-179.
29. Streeter RN, Hoffsis GF, Bech-Nielsen S, Shulaw WP, Rings DM. Isolation of *Mycobacterium paratuberculosis* from colostrum and milk of subclinically infected cows. Am J Vet Res 1995;56:1322-1324.
30. Sweeney RW. Transmission of paratuberculosis. Vet Clin North Am Food Anim Pract 1996;12:305-312.

31. Seitz SE, Heider LE, Heuston WD, Bech-Nielsen S, Rings DM, Spangler L. Bovine fetal infection with *Mycobacterium paratuberculosis*. J Am Vet Med Assoc 1989;194:1423-1426.

32. Sweeney RW, Whitlock RH, Rosenberger AE. *Mycobacterium paratuberculosis* isolated from fetuses of infected cows not manifesting signs of the disease. Am J Vet Res 1992;53:477-480.

33. Cetinkaya B, Erdogan HM, Morgan KL. Relationships between the presence of Johne's disease and farm and management factors in dairy cattle in England. Prev Vet Med 1997;32:253-266.

34. Jakobsen MB, Alban L, Nielsen SS. A cross-sectional study of paratuberculosis in 1155 Danish dairy cows. Prev Vet Med 2000;46:15-27.

35. Waters WR, Miller JM, Palmer MV, Stabel JR, Jones DE, Koistinen KA, Steadham EM, Hamilton MJ, Davis WC, Bannantine JP. Early induction of humoral and cellular immune responses during experimental *Mycobacterium avium* subsp. *paratuberculosis* infection of calves. Infect Immun 2003;71:5130-5138.

36. Hirst HL, Garry FB, Morley PS, Salman MD, Dinsmore RP, Wagner BA, McSweeney KD, Goodell GM. Seroprevalence of *Mycobacterium avium* subsp *paratuberculosis* infection among dairy cows in Colorado and herd-level risk factors for seropositivity. J Am Vet Med Assoc 2004;225:97-101.

37. Muskens J, Bakker D, de Boer J, van Keulen L. Paratuberculosis in sheep: its possible role in the epidemiology of paratuberculosis in cattle. Vet Microbiol 2001;78:101-109.

38. Daniels MJ, Henderson D, Greig A, Stevenson K, Sharp JM, Hutchings MR. The potential role of wild rabbits *Oryctolagus cuniculus* in the epidemiology of paratuberculosis in domestic ruminants. Epidemiol Infect 2003;130:553-559.
39. Daniels MJ, Hutchings MR, Greig A. The risk of disease transmission to livestock posed by contamination of farm stored feed by wildlife excreta. Epidemiol Infect 2003;130:561-568.
40. Jorgensen JB. Survival of *Mycobacterium paratuberculosis* in slurry. Nord Vet Med 1977;29:267-270.
41. Lovell R, Levi M, Francis J. Studies on the survival of Johne's bacilli. J Comp Pathol 1944;54:120-129.
42. Lund BM, Donnelly CW, Rampling A. Heat resistance of *Mycobacterium paratuberculosis*. Lett Appl Microbiol 2000;31:184-185.
43. Grant IR, Ball HJ, Rowe MT. Incidence of *Mycobacterium paratuberculosis* in bulk raw and commercially pasteurized cows' milk from approved dairy processing establishments in the United Kingdom. Appl Environ Microbiol 2002;68:2428-2435.
44. Gao A, Mutharia L, Chen S, Rahn K, Odumeru J. Effect of pasteurization on survival of *Mycobacterium paratuberculosis* in milk. J Dairy Sci 2002;85:3198-3205.
45. Stabel JR. Johne's disease and milk: do consumers need to worry? J Dairy Sci 2000;83:1659-1663.
46. Ellingson J. *M. paratuberculosis* found in U.S. milk. 2004. Found at: http://www.johnes.org/ newsfiles/109216471862392.html

47. Whitlock RH. Preclinical and clinical manifestations of paratuberculosis (including pathology). Vet Clin North Am Food Anim Pract 1996:12:345-356.
48. Kalis CH, Hesselink JW, Barkema HW, Collins MT. Use of long-term vaccination with a killed vaccine to prevent fecal shedding of *Mycobacterium avium* subsp. *paratuberculosis* in dairy herds. Am J Vet Res 2001;62:270-274.
49. Wells SJ, Wagner BA. Herd-level risk factors for infection with *Mycobacterium paratuberculosis* in US dairies and association between familiarity of the herd manager with the disease or prior diagnosis of the disease in that herd and use of preventive measures. J Am Vet Med Assoc 2000;216:1450-1457.
50. Rossiter CA, Burhans WS. Farm-specific approach to paratuberculosis (Johne's disease) control. Vet Clin North Am Food Anim Pract 1996;12:383-415.
51. Johnson YJ, Kaneene JB. Management-related risk factors for *M. paratuberculosis* infection in Michigan, USA, dairy herds. Prev Vet Med 1998;37:41-54.
52. Goodger WJ, Collins MT, Nordlund KV, Eisele C, Pelletier J, Thomas CB et al. Epidemiologic study of on-farm management practices associated with prevalence of *Mycobacterium paratuberculosis* infections in dairy cattle. J Am Vet Med Assoc 1996;208:1877-1881.
53. Collins MT. Interpretation of a commercial bovine paratuberculosis enzyme-linked immunosorbent assay by using likelihood ratios. Clin Diagn Lab Immunol 2002;9:1367-1371.

54. Kalis CH, Hesselink JW, Barkema HW, Collins MT. Culture of strategically pooled bovine fecal samples as a method to screen herds for paratuberculosis. J Vet Diag Invest 2000;12:547-551.

55. Van Schaik G, CR Rossiter CR, Stehman SM, Shin SJ, Schukken YH. Longitudinal study to investigate variation in results of repeated ELISA and culture of fecal samples for *Mycobacterium avium* subsp. *paratuberculosis* in commercial dairy herds. Am J Vet Res 2003;64:479-484.

56. Benedictus G, Kalis CJ. Paratuberculosis: eradication, control and diagnostic methods. Acta Vet Scand 2003;44:231-241.

57. Sechi LA, Mura M, Tanda E, Lissia A, Fadda G, Zanetti S. *Mycobacterium avium* sub. *paratuberculosis* in tissue samples of Crohn's disease patients. New Microbiol 2004;27:75-7.

58. Canadian Animal Health Consultative Committee Annual Report for 2002. Found at: http://www.inspection.gc.ca/english/anima/heasan/cahcc/cahcc2002/adssc-rep-e.shtml

59. National Johne's Disease Program: Standard Definitions and Rules for Cattle. 2003. Found at: http://www.aahc.com.au/bjd/5th%20ed%20SDRS%20-%20August% 202003.pdf.

60. The New Market Assurance Programs for Johne's Disease. 2003. Australian Animal Health Association. Found at: http://www.aahc.com.au/jd/maps/.

61. Animal health Australia National Johnes disease control program, National coordinators quarterly report Oct-Dec 03 http://www.aahc.com.au/jd/njdcp_mar04.pdf.

62. Kalis CH, Hesselink JW, Russchen EW, Barkema HW, Collins MT, Visser IJ. Factors influencing the isolation of *Mycobacterium avium* subsp. *paratuberculosis* from bovine fecal samples. J Vet Diag Invest 1999;11:345-351.

63. Kalis CHJ. Gezondheidsdienst voor Dieren (Dutch Animal Health Service), personal communication, 2004.

64. Uniform Standards for the Voluntary U.S. Johne's Control Program. 2002. Found at: http://www.aphis.usda.gov/vs/nahps/johnes/.

65. Veterinary Services Annual Report: Johne's Program. Found at: http://www.aphis.usda. gov/vs/highlights/section3/section3-20.html

66. New National Johne's Disease Herd Prevalence Study. 2004. Found at: http://www.usaha.org/committees/resolutions/resolution01-2004.pdf

67. Raizman EA, Wells SJ, Godden SM, Bey RF, Oakes MJ, Bentley DC, Olsen KE. The distribution of *Mycobacterium avium* ssp. *paratuberculosis* in the environment surrounding Minnesota dairy farms. J Dairy Sci. 2004;87:2959-2966.

68. Carter MMA, National Johne's Disease Control Program Coordinator, Certification and Control Team, National Center for Animal Health Programs, USDA-APHIS-VS. 2004, personal communication.

69. VanLeeuwen JA, Keefe GP, Tremblay R, Power C, Wichtel JJ. Seroprevalence of infection with *Mycobacterium avium* subspecies *paratuberculosis*, bovine leukemia virus, and bovine viral diarrhea virus in maritime Canada dairy cattle. Can Vet J 2001;42:193-198.

70. An Integrated Johne's Disease Control Program for Cattle in Alberta.
http://www1.agric.gov.ab.ca/$department/newslett.nsf/all/ahf303?OpenDocument

3 **Table 1.** Affects of paratuberculosis on health, production, and culling.

Parameter	Diagnosis criteria	Affect of paratuberculosis	Reference
Milk Production	Tissue positive	16% decrease in last lactation compared to two years prior and 6% decrease compared to one year prior	1
	Fecal positive	590 kg and 1270 kg less milk in third and fourth lactations, respectively	2
	ELISA positive	4% (376 kg) decrease in 305ME production	7
		551 kg decrease in 305ME production	9
		573 and 1273 kg less milk in first and fifth lactations, respectively	8
Risk of Culling	Fecal positive	Greater culling rate cost infected herd US$ 75 per cow per year	15
	ELISA positive	Odds Ratio= 2.34 for risk of culling	14
Reduced Slaughter Value	Clinical JD	Reduced slaughter value of 20 to 30%	1
	Fecal positive	59 kg less weight at slaughter, for a loss of US$ 48 per head	5
	ELISA Positive	Estimated losses of CAN$ 1330 per infected 50 cow herd	17
Fertility	Tissue Positive	Higher fertility cull rate overall	6
	Fecal positive	No difference in fertility	21
	ELISA positive	49 day increase in days open in first lactation heifers	20
		No difference in fertility	21
Mastitis	Fecal positive	Lower Somatic Cell Count	21
		Cull rate for mastitis was 22.6% versus 3.6% in culture-negative cows	6

ELISA positive	Higher somatic cell count at the cow level and herd level	10
	Odds Ratio = 2.90 for risk of culling due to mastitis	14

Table 2. Recommendations for decreasing the risk of new infections of *Mycobacterium avium* subsp. *paratuberculosis* in a dairy operation.

Protect young stock from feces of mature cattle and feces-contaminated feed and water	Reduce the number of infected animals that may be shedding bacteria
a. Clean and disinfect maternity and calf pens after each use b. Calve cows in clean, dry, dedicated maternity pens c. Immediate removal of calves from maternity pen (while calf still wet) d. Collect colostrum from cleaned udders e. After colostrum feeding, use pasteurized milk or milk replacer f. Raise calves separate from the adult herd for first year of life g. Do not allow shared feed/water between adult cows and young stock h. Use separate equipment for handling feed and manure i. Feed-bunk and waterers should have no risk of fecal contamination j. Do not spread manure on grazing or hay land for young-stock	a. Immediate cull of animals with clinical signs of JD. b. Consider testing adult cows with ELISA or fecal culture; positive ELISA should be confirmed with fecal culture in clinically normal cows c. Cull fecal culture-positive cows; they are active shedders and are increasing the environmental challenge on the farm d. Maintain a closed herd or purchase animals only from source farms that have implemented similar or better control programs than purchasing farm (management practices and testing)

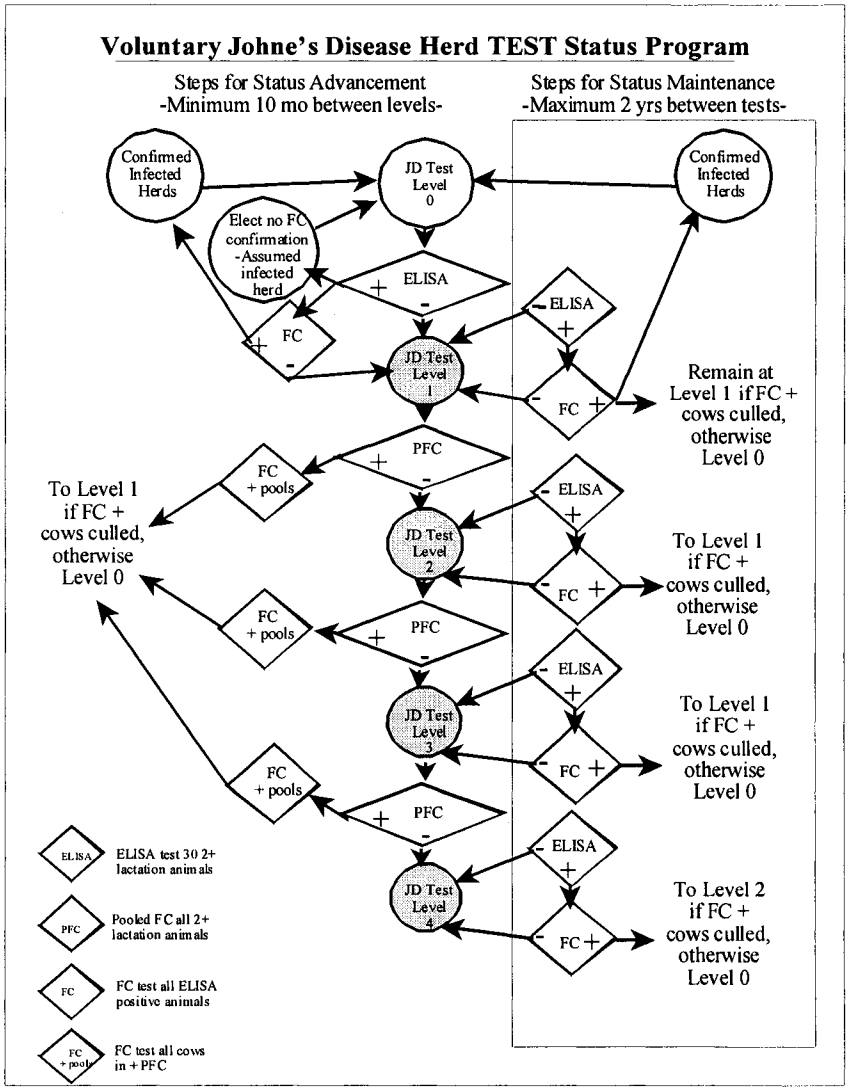


Figure 1. Schematic diagram of proposed Canadian Voluntary Johne's Disease Control Program for dairy cattle.

APPENDIX – B

Data Collection Farm - Risk factors for Neosporosis, Bovine viral diarrhea (BVD), Johne's Disease and Leukosis.

A. Location and identification

Name:	001	
DHI number:	002	
Quarter:	003	
Section:	004	
Township:	005	
CSD:	006	
County:	007	
Range:	008	
Meridian:	009	
Postal code:	010	

B. Farm and Farmer

Age (in years) of the primary person making day-to-day management decisions about the cows on the farm: ☐ 011 ☐

Province of the farm: 012 ☐

☐ [1]Alberta ☐ [4]New Brunswick ☐ [7]Ontario ☐ [10]Saskatchewan

☐ [2]British Columbia ☐ [5]Newfoundland ☐ [8]Prince Edward Island

☐ [3]Manitoba ☐ [6]Nova Scotia ☐ [9]Quebec

Area of the farm (in Acres), both owned and rented, in the last summer: 013 ☐ ☐

Area of pasture (grazing) (in Acres), both owned and rented, in the last summer: ☐ 014 ☐

Area of forage production (in Acres), both owned and rented, in the last summer: ☐ 015 ☐

Area of land used for non-forage agricultural(in Acres), both owned and rented, in the last summer: ☐ 016 ☐

Number of full-time employees, including family members, working directly in dairy production: ☐ 017 ☐

Number of part-time employees, including family members, working directly in dairy production: ☐ 018 ☐

Percentage of the total family income derived from dairy production: ☐ 019 ☐

Primary breed of your dairy cows (check one): 020 ☐

☐ [1]Holstein ☐ [2]Jersey ☐ [3]Ayrshire ☐ [4]Brown Swiss

☐ [5]Guernsey ☐ [6]Shorthorn ☐ [7]Other

C. Herd population

Please, fill in the table below (use an estimate, if exact numbers are unavailable) 021 to 050

	Pre-weaned calves	Open heifers	Bred heifers	Milk cows	Dry cows	Bulls
Number of animals on day of blood sampling	021	022	023	024	025	026
Number of animals sold for dairy purposes in the last 12 months	027	028	029	030	031	032
Number of animals culled in the last 12 months	033	034	035	036	037	038
Number of animals died in the last 12 months	039	040	041	042	043	044
Number of animals purchased in the last 12 months	045	046	047	048	049	050

How many of the cows (milking and dry) were raised on your farm: ☐ 051 ☐

How many of the cows (milking and dry) are registered: ☐ 052 ☐

D. Housing

Pre-weaned calves housing. Please, check all that apply for each season: 053 to 058

Barn type	Winter	Summer
Group pens	053	054
Individual pens	055	056
Hutches	057	058

Winter housing. Please, check all that apply for each group of animals on your farm: 059 to 073

Barn type	Open heifers	Bred heifers	Milk cows	Dry cows	Bulls
Tie-stall or Stanchion	059	060	061	062	063
Freestall	064	065	066	067	068
Loose housing	069	070	071	072	073

Summer housing. Please, check all that apply for each group of animals on your farm: 074 to 093

	Open heifers	Bred heifers	Milk cows	Dry cows	Bulls
Totally confined (in barn) 24 hrs/day	074	075	076	077	078
Spent some time grazing and met some of their nutritional requirement from pasture	089	090	091	092	093
Given access to a concrete or dirt (non-turf) surface exercise yard (outdoor) some time each day	079	080	081	082	083
Given access to a small field for the propose of exercise (not primarily for grazing)	084	085	086	087	088

If your heifers (open/bred) have access to pasture then answer the rest of the questions in this section

How did you manage the pastures that were used by **heifers** in the most recent grazing season: 094 ☐

☐ [1]continuous grazing (continuous access to the same pasture for the whole pasture season)

☐ [2]controlled access grazing (rotational or strip grazing)

Was any cattle manure mechanically spread on pastures that were used for grazing by **heifers**? ☐ [0]No ☐ [1]Yes 095 ☐

Were these pastures dragged or harrowed this year? ☐ [0]No ☐ [1]Yes 096 ☐

Were these pastures clipped at all this year? ☐ [0]No ☐ [1]Yes 097 ☐

Have you used lime on **heifer** pastures for reducing soil acidity during the last 5 years? ☐ [0]No ☐ [1]Yes 098 ☐

If YES, how often do the pasture fields receive lime? 099 ☐

☐ [1]every year

☐ [2]every 2 - 3 years

☐ [3]every 4 - 5 year

☐ [4]every 6 –10 years

☐ [5]never

E. Biosecurity – Purchase

Has the farm purchased any dairy animals in the last 5 years? ☐ [0]No ☐ [1]Yes 100 ☐

If Yes,

Percentage of purchased dairy animals directly from other producers: ☐ 101 ☐

Percentage of purchased dairy animals from private dealers: ☐ 102 ☐

Percentage of purchased dairy animals through an auction: ☐ 103 ☐

When animals are transported to your farm, do you only use your own trailer? 104 ☐

☐ 0No ☐ 1Yes

If YES, does other use your trailer to transport cows? ☐ 0No ☐ 1Yes 105 ☐

Before bringing cattle (either beef or dairy) on your farm, the farm normally requires:

- a negative test for BVDV from animal ☐ 0No ☐ 1Yes 106 ☐
- a negative test for Leucosis from animal ☐ 0No ☐ 1Yes 107 ☐
- a negative test for Neosporosis from the animal ☐ 0No ☐ 1Yes 108 ☐
- a negative test for Johne's disease from the animal ☐ 0No ☐ 1Yes 109 ☐
- a negative HERD test for Johne's disease ☐ 0No ☐ 1Yes 110 ☐
- a negative HERD HISTORY for Johne's disease ☐ 0No ☐ 1Yes 111 ☐
- a low somatic cell count from the cow ☐ 0No ☐ 1Yes 112 ☐
- a low bulk tank somatic cell count for the herd ☐ 0No ☐ 1Yes 113 ☐

F. Biosecurity – Contact

Please, fill in the table below to describe contact between your dairy animals and other animal species that are **on your farm**:

	Numbers on farm	Direct animal contact with dairy cattle ^{0}No /^{1}Yes	Contact with feed for dairy animals ^{0}No /^{1}Yes	Contact with water for dairy animals ^{0}No /^{1}Yes
Beef cattle	114	115	116	117
Sheep	118	119	120	121
Goats	122	123	124	125
Chicken or other poultry	126	127	128	129
Horses and other equines	130	131	132	133
Pigs	134	135	136	137
Deer or elk	138	139	140	141
Exotic ruminants (alpacas, llamas)	142	143	144	145
Domestic rabbits	146	147	148	149

114 to 149

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

- shared pasture (e.g.: community pasture) ☐ ^{0}No ☐ ^{1}Yes 168 ☐
- contract raising of young stock ☐ ^{0}No ☐ ^{1}Yes 169 ☐
- fence line contact while on pasture ☐ ^{0}No ☐ ^{1}Yes 170 ☐
- contact at fairs/exhibitions ☐ ^{0}No ☐ ^{1}Yes 171 ☐

- lending cows or bulls ☐ 0No ☐ 1Yes 172 ☐
- borrowing cows or bulls ☐ 0No ☐ 1Yes 173 ☐

Please, fill in the table below with estimates to describe the dogs and cats that live on your farm:

	Number of males	Number of spayed females	Number of not spayed females	Number of litters in the last 12 months	Usual Birthing locations
Dogs	151	152	150	153	154
Cats	156	157	155	158	159

(APPROXIMATION) 150 to 159

CODES FOR USUSAL BIRTHING LOCATION:

1-DAIRY BARN

2-FEEDSTORAGE AREAS

3- HOUSE

4- OTHER (PLEASE SPECIFY)

Compared with the previous years, has the number of litters of dogs in the last 12 months: 16 ☐ 0 ☐

☐ 1increased

☐ 2decreased

☐ 3continued to be the same

Compared with previous years, has the number of litters of cats in the last 12 months : 16 ☐ 1 ☐

☐ 1increased

☐ 2decreased

☐ 3continued to be the same

If there are NO dogs on the farm, how long ago (years) did one reside on the farm? ☐ 16 ☐ 2 ☐

In the last 12 months how often have the following animals been seen on the farm?

	Never	1 - 3 times/year	4 - 6 times/year	More than 6 times/year	
Coyotes/wolves					163 ☐
Foxes					164 ☐
Other dogs					165 ☐
Stray cats					166 ☐
Raccoons					167 ☐
Skunk					168 ☐

Does the farm use a footbath for disinfecting all visitor's boots before entering the cow and /or heifer barns ?

☐ 0No ☐ 1Yes 174 ☐

If YES, how many times is the disinfectant changed each month? ☐ 175 ☐

☐

During the past 12 months, give the number of times each category of people **actually entered your barn** and whether you felt their vehicles/equipment was properly cleaned.

	Number of times	Vehicles or equipment cleaned ^{0}No /^{1}Yes
other dairy farmers	176	177
other beef farmers	178	179
cattle dealers	180	181
AI technicians + sales reps	182	183
veterinarians	184	185
nutrition technicians/advisors + sales reps.	186	187
udder health advisers	188	189
hoof trimmers	190	191
dead stock collection	192	193
contract manure spreaders	194	195
DHI Technicians	194A	195A
Others (specify) e.g equipment sales rep	196	197

176 to 197

During the past 12 months, did you borrow equipment from other farmers that could have manure contact (e.g. foot trimming chute, manure spreader, tractor, cattle trailer) ? ☐ ^{0}No ☐ ^{1}Yes 198 ☐

If YES, did you always disinfect it before using it? ☐ ^{0}No ☐ ^{1}Yes 199 ☐

During the past year, did you lend equipment to other farmers that could have manure contact? ☐ 0No ☐ 1Yes 200 ☐

If YES, did you always disinfect it before using it again? ☐ 0No ☐ 1Yes 201 ☐

G. Biosecurity - Transmission of disease through biood.

Do you use a new needle for every injection? ☐ 0No ☐ 1Yes 202 ☐

If NOT, do you use a disinfected needle for every injection? ☐ 0No ☐ 1Yes 203 ☐

Do you use new syringe for every injection? ☐ 0No ☐ 1Yes 204 ☐

If NOT, do you use a disinfected syringe for every injection? ☐ 0No ☐ 1Yes 205 ☐

Usual method of dehorning: 206 ☐

☐ 1paste

☐ 2cutting (gougers, wire, etc)

☐ 3burning (electric, butane, etc)

If you use cutting equipment for dehorning, do you disinfect them between animals? ☐ 0No ☐ 1Yes 207 ☐

Are the instruments used for extra teat removal disinfected between animals? 208 ☐

☐ 0No ☐ 1Yes

Do people who artificially inseminate cows/heifers on your farm change rectal gloves between animals? ☐ 0No ☐ 1Yes 209 ☐

Do people who do other rectal exams (e.g. pregnancy check) change rectal gloves between animals? ☐ 0No ☐ 1Yes 210 ☐

Estimate the level of rodent infestation on your farm: 211 ☐

☐ 1low

☐ 2medium

☐ 3high

What is the primary method you use for insect control? 212 ☐

☐ 1spray

☐ 2bait

☐ 3adhesive tape

☐ 4other ☐ 5none

Is the equipment used for hoof trimming disinfected between animals? 213 ☐

☐ 0No ☐ 1Yes

H. Biosecurity - Vaccination and medication practices

Do you use coccidiostats/ionophores in calves/heifers/cows? ☐ 0No ☐ 1Yes 214 ☐

If YES, please fill in the table below(check that apply): 215 to 227

	Decoquinate in feed (Deccox)	Lasalocid in feed (Bovatec)	Monensin in feed (Rumensin) premix	Monensin in bolus (Rumensin CRC)
Pre-weaned calves	215	216	217	
heifers	218	219	220	221
dry cows		222	223	224
milk cows		225	226	227

Did you vaccinate any dairy animals on your farm for any disease in the last 12 months? ☐ 0No ☐ 1Yes 228 ☐

Did you vaccinate any dairy animals on your farm for BVD in the last 12 months? 229 ☐

☐ 0No ☐ 1Yes ☐ don't know

If yes, in their 1st year of vaccination, are animals boostered 2-4 weeks after their 1st shot? ☐ 0No ☐ 1Yes 230 ☐

If YES, are these 2 injections given after the animals are 6 months of age? ☐ 0No ☐ 1Yes 231

If you think you vaccinated your cows with BVD vaccine, indicate the vaccines you usually use in each group of animals in the table below (Check all that apply):

Vaccine Name	Cows	Heifers (+6 mo.)	Calves
Barvac 3, Barvac 3-BRSV, Barvac 3-Somnugen, Barvac 3-Somnugen-BRSV	232	233	234
Bovshield 3, Bovshield 4, Bovshield 4+L5	235	236	237
Breed back 9/Sumnagen	238	239	240
BRSV Vac 4, BRSV Vac 9	241	242	243
Cattlemaster BVD-K, Cattlemaster3, Cattlemaster 4, Cattlemaster 4+L5, Cattlemaster 4+VL5	244	245	246
Experess 5, Express 5 Somnugen, Express 10, Express 10 Somnugen	244A	245A	246A
Herd-vac 3	247	248	249
Horizon 1+vac3, Horizon 4, Horizon 9	250	251	252
IBR Plus 4	253	254	255
Journey 4	256	257	258
Preg-guard 9	259	260	261
Prism 4	259A	260A	261A
Pyramid MVL3, Pyramid MVL4, Pyramid 4+presponse, Pyramid 9	262	263	264
Reliant 3, Reliant 4, Reliant 8	265	266	267
Respishield 4, Respishield 4L5	268	269	270
Resvac 3/Somnuvac, Resvac 4/Somnuvac	271	272	273
Sentry 4, Sentry 4/Somnugen, Sentry 9, Sentry 9/Somnugen	274	275	276
Starvac 3 plus, Starvac 4 plus	274A	275A	276A
Triangle 1, Triangle 3, Triangle 4, Triangle 4+HS, Triangle 8, Triangle 9 (OR ANY OF THESE WITH TYPE II BVD)	277	278	279
Virabos 3, Virabos 4, Virabos 4+H. Somnus, Virabos 4 + VL5	280	281	282
OTHER	280A	281A	282A

Please provide the name of your vet if you do not know about the vaccination

.....

I. Calving and calf management

What is the usual amount of time after which your newborn heifer dairy calves are usually separated from their mothers (in hours)? ☐ 283 ☐

What percentage of heifer calves born on the farm remained with their dams for more than 24 hours? ☐ 298 ☐

What is the percentage of your newborn heifer dairy calves suckle their dam? ☐ 284 ☐

Are teats usually washed before the newborn heifer dairy calves nurse? 299a ☐

☐ [0]No ☐ [1]Yes

Are teats usually washed before colostrum is collected? ☐ [0]No ☐ [1]Yes 299b ☐

What percentage of your newborn heifer dairy calves receive colostrum : 285 ☐

[1]only from their mother ☐ %

[2]pooled from all cows ☐ % 286 ☐

[3]pooled from BLV negative cows ☐ % 287 ☐

[4]pooled from Johne's disease negative cows ☐ % 288 ☐

What percentage of your newborn heifer dairy claves receive: 289 ☐

[1]fresh colostrum ☐ %

[2]frozen colostrum ☐ % 290 ☐

[3]fermented colostrum ☐ % 291 ☐

[4]heat treated colostrum ☐ % 292 ☐

With regard to the primary source of milk given to calves, what percentage of milk fed to your heifer dairy calves is: 293 ☐

[1]milk replacer ☐ %

[2]pooled milk from all cows ☐ % 294 ☐

[3]pooled milk from negative for BLV cows ☐ % 295 ☐

[4]pooled from negative for Johne's disease cows ☐ % 296 ☐

[5]milk from mastitic (clinic or high SCC)cows or with antibiotic residue ☐ % 297 ☐

For calving indoor, if calving is always outdoors, skip to#308 and use code -999: 300 ☐

Was the calving area used as a hospital area for sick cows in the last 12 months?

☐ [0]No ☐ [1]Yes

Type of bedding used in calving areas. 301 ☐

☐ [1]straw

☐ [2]shavings/sawdust

☐ [3]other

☐ [4]none

Frequency of adding bedding to calving areas: 302 ☐

☐ [1]each calving

☐ [2]every 2-4 calvings

☐ [3]every 5 or more calvings

Frequency of removing surface manure from calving areas: 303 ☐

☐ [1]each calving

☐ [2]every 2-4 calvings

☐ [3]every 5 or more calvings

Frequency of removing ALL manure from calving areas: 304 ☐

☐ [1]each calving

☐ [2]every 2-4 calving

☐ [3]every 5 or more

After separation from the mother, but before weaning, do dairy heifer calves have physical contact (nose to nose) with other pre-weaned calves? 305 ☐

☐ [0]No

☐ [1]Yes

After separation from the mother, but before weaning, do dairy heifer calves 306 ☐

have physical contact (nose to nose) with heifers? ☐ [0]No ☐ [1]Yes

After separation from the mother, but before weaning, do dairy heifer calves have physical contact (nose to nose) with adult cows? ☐ [0]No ☐ [1]Yes 307 ☐

What percentages of pre-weaned dairy heifers calves are uniquely identified (e.g ear tags)? ☐ 308 ☐

Primary location of calving in the summer: 309 ☐

☐ [1]freestall

☐ [2]tie-stall/stanchion

☐ [3]loose housing

☐ [4]maternity pen

☐ [5]pasture

Primary Location of calving in the winter: 310 ☐

☐ [1]freestall

☐ [2]tie-stall/stanchion

☐ [3]loose housing

☐ [4]maternity pen

If maternity pens are used, what is the usual number of cows in the pens at one time. 311 ☐

☐ [1]always just a single cow in pen

☐ [2]sometimes multiple cows in the pen

If multiple cows are in the calving pen at a time, what is the percentage of calvings when multiple cows present: ☐ 312 ☐

Percentage of placentas partially or fully eaten by: 313 to 316

Dogs ☐ [1]Never ☐ [2]Sometimes ☐ [3]often 313

Cats ☐ [1]Never ☐ [2]Sometimes ☐ [3]often 314

Cows ☐ [1]Never ☐ [2]Sometimes ☐ [3]often

315

Wild animals ☐ [1]Never ☐ [2]Sometimes ☐ [3]often

316

Percentage of aborted fetuses partially or fully eaten by: 317

Dogs ☐ [1]Never ☐ [2]Sometimes ☐ [3]often to

317 319

Cats ☐ [1]Never ☐ [2]Sometimes ☐ [3]often

318

Wild animals ☐ [1]Never ☐ [2]Sometimes ☐ [3]often

319

Percentage of cows bred using artificial insemination: ☐ 320 ☐

Do you use embryo transfer on your farm? ☐ [0]No ☐ [1]Yes 321 ☐

If YES, number of embryos purchased outside the herd and implanted in the last 12 months: ☐ 322 ☐

If YES, number of embryos collected on farm and implanted in the last 12 months: ☐ 323 ☐

J. Feed, Water and Manure

Do you feed a TMR? ☐ [0]No ☐ [1]Yes 324 ☐

Do you feed greenchop? ☐ [0]No ☐ [1]Yes 325 ☐

How do you store your silage? 326 ☐

☐ [1]tower silo

☐ [2]bunker silo

☐ [3]plastic bags/wrap

☐ [4]none

Do dogs, cats or wildlife have access to stored grain? ☐ [0]No ☐ [1]Yes 327 ☐

Do you have an outdoor feed bunk or manger built for heifers? ☐ [0]No ☐ 328 ☐

[1]Yes

Do you have an outdoor feed bunk or manger built for milk cows? ☐ [0]No ☐ 329 ☐

[1]Yes

Do you have an outdoor feed bunk or manger built for dry cows? ☐ [0]No ☐ 330 ☐

[1]Yes

Method of manure removal from milk cow barn. 331 ☐

☐ [1]gutter cleaner

☐ [2]alley scraper (mechanical or tractor)

☐ [3]slatted floor

☐ [4]removed (with bucket, bulldozer, etc.) as bedded pack

☐ [5]alley flushed with water

☐ [6]other(specify).....

Method of storage of manure from milk cow barn: 332 ☐

☐ [1]pit (under barn)

☐ [2]open pile

☐ [3]earth lagoon

☐ [4]concrete lagoon

☐ [5]other (specify)

Distance (in feet) from milk cow manure storage area to nearest farm well? 333 ☐

☐

Distance (in feet) from milk cow manure storage area to stream, lake or pond? 334 ☐

☐

Do cows have access to a stream, lake or pond? ☐ [0]No ☐ [1]Yes 335 ☐

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

(check all that apply)

☐ [1]injection 336 ☐

☐ [2]spread with surface incorporation (e.g. plowing, disking) 337 ☐

☐ [3]spread without surface incorporation (e.g. plowing, disking) 338 ☐

How many days do you wait after applying manure to a field before heifers are allowed to graze the field or get fed green chop from the field? ☐ 339 ☐

In the last 12 months, what percentage of the grains you fed to heifers were homegrown? ☐ 340 ☐

In the last 12 months, what percentage of the roughages you fed to heifers were homegrown? ☐ 341 ☐

In the last 12 months, what percentage of the grains you fed to cows were homegrown? ☐ 342 ☐

In the last 12 months, what percentage of the roughages you fed to cows were homegrown? ☐ 343 ☐

Origin of drinking water by season; fill in for each group of animals on your farm: 344 to 351

1 - Surfaced water (stream, pond or lake)

2 - Well water

3 – Municipal water

Source of water in the...	Open heifers	Bred heifers	Dry cows	Milking cows
WINTER	344	345	346	347
SUMMER	348	349	350	351

How often is equipment that holds manure (e.g. bucket, spreader) also used to 352 ☐

handle feed fed to heifers?

☐ [1]regularly (at least weekly)

☐ [2]occasionally (less than once a week)

☐ [3]not a practice

How often is equipment that holds manure (e.g. bucket, spreader) also used to handle feed fed to cows? 353 ☐

☐ [1]regularly (at least weekly)

☐ [2]occasionally (less than once a week)

☐ [3]not a practice

Do heifers less than 12 months of age share a feed bunk with adult cattle? 354 ☐

☐ [0]No ☐ [1]Yes

Do heifers less than 12 months of age share a water trough with adult cattle? 355 ☐

☐ [0]No ☐ [1]Yes

K. Prevalence of disease

Please fill in the table based on the last 12 months, Give your best estimate: 3

(for disease monitoring, do not include animals tested as part of this research project) t

3

	Clinical Cases (sick animals)	Disease Monitoring * (sick and healthy animals)	
	Number of animals with the disease problem	Number of animals tested (blood, milk or fecal test)	Number of animals with positive tests results
BVD	356	357	358
Leukosis	359	360	361
Johne's Disease	362	363	365
Neosporosis	365	366	367
Retained afterbirth (> 24 hrs)	368		
Abortion less than 4 months	369		
Abortion 4 to 7 months	370		
Abortion greater than 7 months	371		

*** Do not include the tests and results from this research project.**

In the LAST 5 YEARS, how many cattle have been diagnosed with Johne's disease by: 3

	Number of animal tested	Number of positives
Fecal test	372	373
Blood test	374	375
Veterinary diagnosis		376

IN The LAST 12 MONTHS, how many of your CULLED COWS showed chronic diarrhea, normal appetite and weight loss that didn't respond to treatment? ☐ 377 ☐

What is done with apparently healthy cows that have a positive Johne's disease test? 378 ☐

☐ [1]immediately shipped

☐ [2]slaughtered at end of lactation

☐ [3]kept on farm but handled differently

☐ [4]nothing

Any other Johne's Disease's Control procedure? Describe. 379 ☐

Appendix C – Publication status of thesis chapters

Chapter 2. A. Tiwari, J. A. VanLeeuwen, S. L.B. McKenna, G. P. Keefe, H. W. Barkema. Johne's Disease in Canada. Part I: Clinical symptoms, Pathophysiology, Diagnosis and Prevalence in Dairy Herds. Accepted in Can. Vet. J.

Chapter 3. A. Tiwari, J. A. VanLeeuwen, I. R. Dohoo, G. P. Keefe, J. P. Haddad, R. Tremblay, H. M. Scott, T. Whiting, F. Markham. Seroprevalence and spatial distribution of bovine leukemia virus, bovine viral diarrhea virus and Mycobacterium avium subspecies paratuberculosis in Canadian dairy cattle. Planned Submitted to Vet Res.

Chapter 4. A. Tiwari, J. A. VanLeeuwen, I. R. Dohoo, G. P. Keefe, J. P. Haddad, R. Tremblay, H. M. Scott, T. Whiting. Effects of bovine leukemia virus, bovine viral diarrhea virus, *Mycobacterium avium* subspecies *paratuberculosis,* and *Neospora caninum* on milk, fat and protein production and somatic cell count. Submitted to J. Dairy. Sci.

Chapter 5. A. Tiwari, J. A. VanLeeuwen, I. R. Dohoo, H. Stryhn, G. P. Keefe, J. P. Haddad. Effects of seropositivity for bovine leukemia virus, bovine viral diarrhoea virus, Mycobacterium avium subspecies paratuberculosis, and Neospora caninum on culling in dairy cattle in four Canadian provinces. Vet. Microbiol. 109, 147-158.

Chapter 6. A. Tiwari, J. A. VanLeeuwen, I. R. Dohoo, J. P. Haddad, G.P. Keefe. Direct production losses from subclinical *Mycobacterium avium* subspecies *paratuberculosis* infection in Canadian dairy herds. Submitted to Vet. Microbilogy.

Chapter 7. A. Tiwari, J. A. VanLeeuwen, I. R. Dohoo, G. P. Keefe, J. P. Haddad, H. M. Scott, T. Whiting. Management risk factors associated with Mycobacterium avium subspecies paratuberculosis infection in Canadian dairy herds. Submitted to Prev. Vet. Med.

Appendix A. S. L.B. McKenna, G. P. Keefe, A. Tiwari, J. A. VanLeeuwen, H. W. Barkema. Johne's Disease in Canada - Part II: Disease Impacts, Risk Factors and Control Programs for Dairy Producers. Accepted in Can. Vet. J.