DEVELOPMENT AND IMPLEMENTATION OF A PATHOGEN SPECIFIC CLINICAL MASTITIS ECONOMIC DECISION MODEL

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DEVELOPMENT OF A PATHOGEN SPECIFIC CLINICAL MASTITIS ECONOMIC DECISION MODEL

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Clinical mastitis (CM) is a production limiting disease affecting dairy cattle worldwide. Cows suffering from CM may experience a loss in milk production, reduction in rate of conception an increased risk of mortality and culling. Direct costs to the dairy farmer include costs due to compromised milk quality, treatment and discarded milk costs if antibiotics are used. Given these effects, dairy farmers are often faced with the decision of what to do with their diseased cows; whether to keep or replace with a younger heifer. The economic model developed to assist dairy farmers with these decisions is based on dynamic programming. It was necessary to develop a pathogen specific model for several reasons: (1) The previous framework did not separate CM into the different pathogens that are causative, hence, in the past, the effects of pathogen specific CM were pooled while decisions may be pathogen specific and (2) Discarded milk due to treatment after adjusting for the loss of milk from disease was not accounted. Further, the previous framework did not have the flexibility to include additional pathogens easily. In developing this model, we estimated the effects of pathogen specific CM for inclusion in the model (i.e., risk of pathogen specific CM and risk of mortality (and culling)). Further, prior to developing a pathogen specific model, we expanded an existing generic CM model (i.e., where all CM pathogens were combined) to study the cost of 3 different types of CM i.e., gram-positive, gram-negative and other CM. Cows with more cases of CM in the previous lactation

were at greater risk of bacteria specific CM in the current lactation, e.g., among multiparous cows in wim ≥ 3, cows were 2.2 times more at risk of a first case of *E. coli* if they had 2 cases of CM (of any type) in the previous lactation compared with no cases in the previous lactation. Among multiparous cows, cows were at greater risk of a recurrent case within the first month after the previous case of CM, unlike primipara, where these cows were at greater risk of a recurrent case within 2 months of the previous case. Among first lactation cows, the presence of a first CM case generally exposed cows to a greater risk of mortality in the current month (compared with the absence of a first case). This was especially acute with a first case of *Klebsiella*, where cows were 4.57 (exp(1.52)= 4.57 [95% CI (2.75, 7.61)]) times more at risk of mortality, and with a first case of *E. coli* with cows 3.32 times more at risk (i.e., relative risk = exp(1.20)= 3.32, [95% CI (2.46, 4.48)]. In general the presence of a first or second case resulted in cows in parity ≥2 with a greater risk of mortality (compared with cows with no first or second case of bacteria specific CM in the current month in milk). In first parity cows, the risk of culling generally increased with the presence of a case of bacteria specific CM. Among cows of parity ≥2, when a cow contracted a case of *Streptococcus* spp., she was more likely to be culled one month after the case of CM (regardless of whether it was a first, second or third case of *Streptococcus* spp.). The average costs per case (USD) of gram-positive, gram-negative and other CM were 133.73, 211.03 and 95.31, respectively. This model provided a more informed decision making process in CM management for optimal economic profitability and determined that 93.1% of gram-positive CM cases, 93.1% of gram-negative CM cases and 94.6% of other CM cases should be treated. The main contributor to the total cost per case of gram-positive CM was treatment cost (51.5% of the total cost per case), milk loss for gram-negative CM (72.4%) and treatment cost for other CM (49.2%). From the pathogen specific economic model, we

determined the net returns per cow and year, with an incidence of CM (cases per 100-cow years) of 35.5 was 500 US$, of which 90.6% of cases were recommended to be treated under an optimal replacement policy. The cost per case of CM was 233.41. The average cost per case was greatest for *Klebsiella* (416), followed by other not treated cases (e.g., *Trueperella pyogenes*) (316), other treated cases (e.g. *pseudomonas*) (310), *Escherichia coli* (309), *Staphylococcus aureus* (298), *Staphylococcus* spp. (275), *Streptococcus* spp. (257) and Negative culture cases (151). Optimal recommended time for replacement was as great as 5 months earlier for cows with CM compared with cows without CM. This model provides economically optimal decisions depending on the individual characteristics of the cow and the specific pathogen causing CM. The parameter estimates may be altered so that the results are specific to a given farm

BIOGRAPHICAL SKETCH

Elva Cha was born in Sydney, Australia. She is the daughter of Nam Soo Cha and Seung Hee Cha and older sister of Della Cha. Her heritage is Korean, and she attended a Japanese Primary School, followed by high school at Monte Sant' Angelo Mercy College, then veterinary school at the University of Sydney.

Her passion has been veterinary medicine and the sciences which propelled her to pursue a career as a veterinarian. Following her fourth year of veterinary studies, she decided to take an honors year of research, studying biosecurity practices of exhibited pigs at agricultural shows. This experience clarified her interest in public health and epidemiology. Animal welfare has also always been a topic close to her heart, but she realized very early on that economics is often a conditional precedent to improving the lives of animals and people. This led to her sustained interest in studying economic modeling as the core component of her Ph.D.

Her interest in working in dynamic settings and science communication led her to be involved in extracurricular activities throughout veterinary school and also her Ph.D, including involvement in groups such as the Australian Veterinary Association, Australian Science Communicators and numerous student publications. Her passion rests in working on problems with high applicability and practical importance in multi-disciplinary settings.

내 사랑하는 가족

og til min bedste ven og mit livs kærlighed, Daniel

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The complexities of the dynamic program may have remained a black box to me had it not been for the incredibly helpful and insightful discussions and meetings I had with Dr Anders

Ringgaard Kristensen. Dr Kristensen not only helped me in the context of this Ph.D dissertation, but also introduced me to the world of programming and economic modeling. I have him to thank for my acquired skills in Java programming and just "thinking like a program" which was incredibly critical to the development of the economic model. He was always patient explaining new concepts to me, and I found myself looking forward to our meetings which were met with excitement and genuine curiosity as I would share the latest developments as well as trials and tribulations of the economic model.

I also thank Dr Doron Bar for sharing with me his development of the generic CM model and showing me the very basics of programming and economic modeling when I first arrived at Cornell. His answers to my emails in a relatively niche field was a great comfort to me as well as our conversations and his sense of humor.

My sincere thanks to Dr Ynte Schukken, for his insight into the statistical analyses I performed and his persistence in helping me with my analyses where I needed advice on iterative attempts at improving convergence of my models. His input and thought provoking questions relating to both statistical analysis and the economic model spurred me to be a better critical thinker and seek out alternative ways of modeling data to what I had planned to perform.

I would find myself looking forward to my meetings and discussions with Dr Loren Tauer because I knew I would leave learning something new about the results of my economic model and with ideas of how to change the parameters. His friendly demeanor always put me at ease and I felt free to discuss ideas, questions and uncertainties relating to economics, no matter how big or small the questions were.

I also thank Dr Frank Welcome for providing his knowledge in dairy medicine which was pertinent to the development of this economic model which would be representative of what is experienced in the real world. Despite his busy schedule, Dr Welcome always responded to me with the treatment protocols, drug indications and information relating to the study farms that were important to the parameterization of the economic model. Our discussions of what happens on farm and how dairy cows are managed was invaluable.

My thanks also to my field appointed member, Dr Martin Wiedmann, for helpful discussions relating to work opportunities in industry and his suggestions regarding the economic model and its applicability.

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CHAPTER 1

GENERAL INTRODUCTION

Clinical mastitis (CM) is a production limiting disease affecting dairy cattle worldwide. There are different pathogens that can cause CM (hereafter referred to as 'pathogen specific CM'). The risk of pathogen specific CM is dependent on factors such as the presence of disease (CM/other diseases), management and cow factors (Halasa et al., 2009; Petrovski et al., 2009; Sampimon et al., 2009). Cows suffering from CM may experience a loss in milk production (Gröhn et al., 2004; Bar et al., 2007; Schukken et al., 2009), reduction in rate of conception (Hertl et al., 2010), an increased risk of mortality and culling (Bar et al., 2008a; Hertl et al, 2011; Reksen et al., 2006;) and greater costs due to treatment (and discarded milk if antibiotics are used). By the process of milk culture, one can identify the pathogen involved. A rationale for identifying the exact pathogen causing CM is that treatment can then be tailored to the pathogen, which may result in less antibiotic use (as the antibiotic used will be tailored to the specific pathogen, increasing the probability of successful treatment), less discarded milk due to treatment (if the initial antibiotic treatment is not indicated for the pathogen involved, cows may need to be treated repeatedly resulting in more discarded milk) and a reduction in the overall cost per case of CM. In study by Ma et al., 2000, it was demonstrated that for high SCC milk, between 14 to 21 d post processing, sensory defects i.e., rancidity and bitterness were detected hence, adversely affecting the quality of pasteurized fluid milk. An elevated SCC also has implications on revenue; depending on the market, the acceptable range for the bulk tank SCC level varies. For example, while the regulatory limit in the USA is 750,000 cells/ml, the global

standard (also the limit in the European Union) is 400,000 cells/ml (Adkinson et al., 2001; Hoards Dairyman, 2011). As a result, these costs may affect a farm's net returns (Halasa et al., 2009).

Given the effects of CM on farm, dairy farmers are often faced with the decision of what to do with their diseased cows; whether to keep (and treat) or replace with a younger heifer. These decisions are dependent on a number of factors, namely the effects of CM on the cow e.g., if a cow is kept and is suffering from CM and the reduction in milk yield which would occur. With CM occurring in a cow the farmer is faced with a number of questions. Is the detrimental impacts of CM in a cow offset by the overall high milk producing capacity of the dairy cow, in which case it is better not to replace the cow immediately? Would it be better to keep the cow in the herd for an additional month before replacement? Essentially these questions can be summarized into the question of at what time is the value of replacing the cow greater than keeping the cow?

Previous studies have demonstrated that production limiting effects of CM (which contribute to decision making) are dependent on the pathogen involved. For example, the milk loss for CM attributed to *Escherichia coli* is not the same as that for *Staphylococcus spp* (Gröhn et al., 2004). A focus of this thesis is to identify whether the risk of these pathogens causing repeated CM cases, the risk of mortality and culling, reduction in conception and treatment cost are also dependent on the pathogen involved.

The economic model we developed to assist dairy farmers with these decisions is based on dynamic programming. One of the basic elements of dynamic programming is the sequential approach to decision making, which aligns well with sequential decisions in animal production,

including replacement of animals where at regular time intervals management decisions are made i.e., whether the animal should be replaced, inseminated or kept for another time period (Kristensen et al., 2010). The development of dynamic programming dates to the 1950s. The application of this technique to animal replacement models was illustrated by Jenkins and Halter (1963); where the trait of lactation number (12 levels) was all that was included, but served the purpose of showing dynamic programming could indeed be applied to such a problem. In 1966, Giaever illustrated with various solution techniques (value iteration, policy iteration and linear programming) the feed intake and production of a dairy cow, then Smith (1971) was able to achieve a state space of 15 000, as opposed to the 106 states which was the upper limit in the Giaever study. Kristensen and Østergaard (1982) and van Arendonk (1985, 1986) and van Arendonk and Dijkhuizen (1985) studied the effect of prices and conditions on the optimal replacement policy (Kristensen, 2010).

A problem with working with such large models is that as the state-space expands, it may become prohibitive to solve large problems, frequently termed the "curse of dimensionality". A contribution to the solution of dimensionality was made by Kristensen (1988; 1991), where the computational advantages of the value iteration method and the exactness and efficiency of the policy iteration method were combined. This makes it possible to give exact solutions to models with even extremely large state spaces (Kristensen, 2010). It is this solver and Multi level Hierarchic Markov Process (MLHMP) software that is used as the application program interface to develop the pathogen specific model in this study (Kristensen, 2003).

Optimal replacement models can be used to provide dairy farmers with guidance on what action to take with their animals. The advantage of these models is that they can assimilate large

amounts of information on health status, age of the cow, milk production etc., and provide the optimal action to be taken.

Dairy cattle replacement models have been developed (De Vries et al. 2006; Nielsen et al., 2010; Demeter et al., 2011), with inclusion of information relating to disease (Houben et al., 1994; Bar et al., 2008a). The model developed by Bar et al. (2008a) incorporated information on generic CM (i.e., CM that is not differentiated by causative agent), extending and building upon the assumptions of the optimal replacement model developed by Houben et al. (1994) and earlier asset replacement principles (Perrin, 1972). Because the focus of this dissertation is CM, subclinical mastitis (SC) was not included. SC has been modeled before; in a study by Yalcin and Stott (2001), 11 somatic cell count states were incorporated into a model with a time horizon of 20 years, 12 lactations and 15 milk yield states. In this study, the expected net present value was £285.50 and the percentage of cows with a SCC ≤400, 000 cells/ml was 76.90.

To the authors' knowledge, there are very few studies that examine the cost of pathogen-specific CM (Østergaard et al., 2005; Sørensen et al., 2010). In the study by Sørensen et al. (2010), economic values for pathogen-specific CM were estimated using a stochastic simulation model (SimHerd IV). The simulations were conducted over time with weekly time increments, where other diseases were included as well as severity of CM.

The basis of this Ph.D dissertation was the development of a larger model which encompasses pathogen specific CM. It was necessary to develop this model for several reasons:

1. The previous framework did not separate CM into the different pathogens that are causative or differentiate between different cases of CM, the associated properties of each pathogen i.e., risk of CM, milk loss (Hertl et al., *unpublished*), conception rate, mortality

risk and treatment cost were not accounted for individually. Rather, in the past, these estimates were pooled either at the generic CM level, or gram-specific level (i.e., gram positive, gram negative or other CM). The importance of pathogen information arises in more specific situations, e.g., when the profile of a cow results in a borderline decision (Østerås et al., 1999). That is, additional knowledge of the exact pathogen involved may assist in decision making, especially In those cases where one only knows the cow is suffering from CM (but not the pathogen involved) and it is uncertain which decision is optimal.

2. We would like to assess whether the risk of CM differs depending on whether the cow has had a case of CM in the preceding lactation i.e., carry over effect, and if that is the case, to include this information in the economic model.
3. Discarded milk due to treatment after adjusting for the loss to milk due to disease was not previously accounted. This would have an impact on the estimated cost per case of CM.
4. We have additional information relating to the risk of CM by case, carryover and pathogen, milk loss by case and pathogen, and conception rate, mortality risk and treatment cost by pathogen and at the cow level which were not available before.
5. The previous framework did not have the flexibility to include additional pathogens or additional diseases easily; the new framework affords the versatility to expand the model for further research purposes.
6. We also allow more than one event to occur as a cow transitions from one month to another; e.g., a transition in CM status, pregnancy and milk yield are now all concurrently possible, thus, providing a more realistic representation of what happens in the real life of dairy cows.

In order to develop a pathogen specific CM economic model, the first step was to decide on the classification of the pathogen specific CM for inclusion. A total of eight culture result classes were identified for the purpose of this thesis: (1) *Staphylococcus* spp., (2) *Staphylococcus aureus*, (3) *Streptococcus* spp., (4) *Escherichia coli*, (5) *Klebsiella*, (6) Other treated (these included *Enterobacter*, *Enterococcus*, *Citrobacter*, *Serratia*, *Pasteurella*, *Corynebacterium* species, *Pseudomonas*, *Proteus*, *Corynebacterium bovis*, *Gram+ bacillus*, *Gram- bacillus*, fungus, *Strep.* group 'C', mold and *Nocardia*), (7) Other not treated (these included *Trueperella pyogenes*, *Mycoplasma*, *Prototheca* and yeast), and (8) Negative culture, contamination (more than two bacterial species on the culture plate) and no significant organisms. The latter, no significant organisms, was defined as no bacterial growth of either *Staphylococcus aureus* or *Streptococcus agalactiae* while the culture plate contained more than two different species. These cases exhibited clinical signs of mastitis.

Clinical mastitis pathogens can also be classified based on gram-staining i.e., gram-positive, gram-negative and other CM. This classification is not as specific or as comprehensive as above, however, is more valuable than not knowing anything about the CM causing pathogen. Gram-staining classification can also form a component of on-farm treatment protocols.

The overall objectives of this Ph.D research were:

(1) to estimate the risk of pathogen specific CM

(2) to estimate the effect of pathogen specific CM on mortality and culling

(3) to expand an existing generic CM (i.e., where all CM pathogens are combined) economic model to estimate the cost of three different types of CM i.e., gram-positive, gram-negative and other CM,

(4) to develop an economic optimization model which would incorporate the previously defined classes of pathogens that cause CM, as well as account for whether the CM was a first, second or third case in the current lactation and whether the cow had a previous case of CM in the preceding lactation and

(5) to develop a model that would be versatile enough to add more pathogens, diseases or other factors in the future without significant alterations to the basic structure of the model.

By developing a pathogen specific CM model, the model would provide economically optimal decisions depending on the individual characteristics of the cow and the specific pathogen or group of pathogens causing CM. We also elucidate the cost of each pathogen causing CM, and undertake sensitivity analyses of how these costs are affected by changes in milk price, pregnancy rate and replacement cost.

CHAPTER 2

RISK OF A FIRST CASE AND RECURRENT CASES OF BACTERIA SPECIFIC CLINICAL MASTITIS IN DAIRY COWS

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ABSTRACT

The objective of this study was to estimate the risk of a first case and recurrent cases of bacteria specific clinical mastitis (CM) in Holstein dairy cows. The pathogens studied were *Streptococcus* spp., *Staphylococcus aureus*, *Staphylococcus* spp., *Escherichia coli*, *Klebsiella* spp., and *Trueperella pyogenes*. A total of 40,864 lactations (9,873 cows) were analyzed of which 17,265 were primiparous and 23,599 were multiparous lactations, in 5 large, high milk producing dairy herds in New York State. There were 12,725 first cases, 4,535 second cases and 1,798 third cases of CM. Generalized linear mixed models with a Poisson error distribution were used to study the effects of parity, calving diseases, milk yield from 2 wks before the current week in milk (previous milk yield), current season, number of cases of CM in the previous lactation and previous cases of bacteria specific CM within the lactation on the risk of a first case and the conditional risks for second and third cases of bacteria specific CM. The first 2 weeks in milk (wim) and wim ≥ 3 were analyzed separately as the former analysis focused on calving diseases as risk factors and previous milk yield was not included. Cows with more cases of CM in the previous lactation were at greater risk of bacteria specific CM in the current lactation, e.g., among multiparous cows in wim ≥ 3, cows were 2.2 times more at risk of a first case of *E. coli* if they had 2 cases of CM (of any type) in the previous lactation compared with no cases in the previous lactation. In this same group of cows, those with a first case of mastitis caused by *Staphylococcus* spp. were 3.8 times more likely to have a second case of mastitis caused by *Staphylococcus* spp. than if the first case was not caused by *Staphylococcus* spp.; in general, multiparous cows were at greater risk of a second case of CM if they had suffered from a first

case of CM that was caused by the same pathogen as the second case. For third cases, however, a second case of CM caused by both the same and a different pathogen as the third case generally put cows at greater risk of a third case, compared with if they had either the same or a different pathogen as the second case. Among multiparous cows, cows were at greater risk of a recurrent case within the first month after the previous case of CM, unlike primipara, where those cows were at greater risk of a recurrent case within 2 months of the previous case. With reference to recurrent CM cases, there was no evidence for protective immunological memory due to a previous exposure to the same pathogen; cows that had suffered from the same pathogen previously were found to be at greater risk of a recurrent case of CM from the same pathogen. The risks from this study will be used to parameterize an economic model which will provide dairy farmers with economically optimal decisions for their diseased cows.

Key words: bacteria specific, risk, recurrent, repeated, mastitis

INTRODUCTION

Mastitis is a disease of importance in dairy cows due to its biological and economic effects. Cows with mastitis experience a drop in milk yield (Gröhn et al., 2004; Bar et al., 2007; Schukken et al., 2009), an increased risk of culling (Reksen et al., 2006; Bar et al., 2008a; Hertl et al, 2011), and reduced fertility (Hertl et al., 2010). It is also an expensive disease, as these biological effects, coupled with the fact that mastitic cows may be treated with antibiotics, incur

not only a treatment cost, but also an economic loss due to milk that is rendered unsalable. Further, cows with subclinical or clinical mastitis with their accompanying increased somatic cell count (SCC) will be contributing to the bulk tank. As a result, these costs will affect a farm's net returns (Halasa et al., 2009), profitability and management as well as optimal treatment, culling and replacement policies (Bar et al., 2008b; Bar et al., 2009c; Cha et al., 2011).

The risk of mastitis has been studied as a function of other factors such as disease, management and cow factors by different classifications: (1) generic (Rajala, P.J. and Gröhn, Y.T., 1998; Steeneveld et al., 2008), (2) gram-positive, gram-negative or other (Hertl et al., *unpublished*) and (3) bacteria specific mastitis (Barkema et al., 1998; Sargeant et al., 1998; Riekerink et al., 2008). To analyze the effect of mastitis on herd profitability, it is necessary to distinguish the different pathogens causing mastitis. This is essential as the losses to mastitis (milk yield, decreased conception risks), prognosis, cost of culture and cost of treatment depend on the specific agent causing clinical mastitis (CM). Inclusion of information relating to previous case(s) may also be useful; this could help explain whether a previous infection from one pathogen provides protection from a subsequent case of CM of the same pathogen, or if cows with a subsequent CM infection are actually more likely to suffer from the same bacteria as experienced in the previous case, suggesting persistence of infection. The persistence of infection can be examined within the same lactation and across lactations. A study by Green et al. (2002) found that the probability of a quarter succumbing to CM in the next lactation increased when *Streptococcus dysgalactiae, Streptococcus faecalis*, *Escherichia coli* or *Enterobacter* spp. were cultured at drying off and the risk of mastitis for specific pathogens increased if the same species of bacteria that had caused mastitis was isolated at least twice in the late dry and post-calving samples. Within lactation, Döpfer et al. (1999) demonstrated that the occurrence of recurrent

episodes of CM caused by *E. coli* in any quarter of a cow is high (13.04% of all episodes of mastitis caused by *E. coli* in the study). Intracellular survival and replication is an important attribute for the maintenance of intracellular infections (Dogan et al., 2006). Dogan et al. (2006) explored the possibility that persistent strains are better able to invade, survive and replicate within cultured mammary epithelial strains than transient strains. The authors discovered that all persistent *E. coli* strains but only one transient strain, were able to survive and replicate intracellularly in MAC-T cells over 48 h. *Staphylococcus aureus* is reported to be a mastitis pathogen often involved in chronic cases and persistence in udder tissues with an intermittent shedding pattern (low sensitivity to diagnostic tests) (Vaarst and Enevoldsen, 1997). Atalla et al. (2008), reported for the first time the isolation of *S. aureus* small colony variants (SCV) from the milk of cows with persistent bovine mastitis, suggesting that SCV strains may be important contributors to persistent bovine intramammary infections.

To the authors' knowledge, there are no studies which have estimated the risk of bacteria specific mastitis as a function of cow factors, with inclusion of information on whether a previous case of CM was the same, or different to the current case, the time delay between cases and the number of cases of CM in the previous lactation. While the effects of cow characteristics on the risk of CM have been studied extensively, the strength of this study is the new information that contributes to our knowledge of bacterial interactions and persistence.

The objective of this study was to estimate the effects of parity, milk yield from 2 wk before the current week in milk ("previous milk yield"), calving diseases, current season, previous bacteria specific CM in the current lactation, time since previous CM case and number

of cases of CM from the previous lactation ("carryover") on the risk of a first and recurrent cases of bacteria specific CM.

MATERIALS AND METHODS

Herd Descriptions

We collected and analyzed data from 40,864 lactations (17,265 of parity 1 and 23,599 of parity 2 and greater in 9,873 cows). The data in this study were collected from 2003/2004 until 2011 (7-8 years) from 5 large dairy herds in New York State. The 305-d rolling herd average milk production ranged from 11,260 to 13,123 kg/cow per year, and the monthly mean SCC ranged from 137,000 to 262,000cells/ml. The monthly mean SCC is calculated summing all the SCC contributed to the bulk tank by each cow (which is calculated by (milk produced by the cow) x (SCC that particular cow contributes/ml)) on the monthly test day.

All herds performed a full milking routine which included fore stripping, pre dipping, wiping teats clean and dry with single use towels and dipping all teats at the end of milking. The order of fore stripping and pre dip applications varied on some farms.

Cows were stratified by lactation, production, and reproductive status into feeding groups which were fed a Total Mixed Ration. Cows were milked 3 times a day and the milking units automatically recorded milk production. Lactation, reproductive and medical information were entered into DairyComp305 herd management software (Valley Agricultural Software, Tulare, CA) by herd personnel. Information on parity, diseases, drying off, calving and exit from all

herds were available as this information was used by herd personnel for management of the dairy (Bar et al., 2008; Hertl et al., 2011).

The variables relating to milk yield, mastitis culture results, diseases and reproduction that were necessary to conduct this study were outputed to ASCII files from DairyComp305 and imported into SAS v. 9.2 (2008). The quality of the data was assessed through preliminary descriptive analyses of the variables of interest.

Eligibility criteria

All lactating cows in the 5 herds were eligible for inclusion in the study.

Case definition and unit of observation

Cows were identified as having CM based on (1) milkers observing clinical signs of CM, i.e. a warm, swollen udder or changes in milk consistency; otherwise, the remaining cases which were missed by milkers were identified by (2) herdspersons who examined cows due to elevated milk electrical conductivity in addition to a sudden concurrent milk loss as indicated by the farm computer system.

The unit of observation was week (for first case analysis) or month (for recurrent case analysis) within the lactation of a cow. Some cows had two types of bacteria specific CM (at the quarter level) that were isolated within the same lactation within a few days of each other. If the second pathogen was isolated in the same quarter 5 or fewer days after the first pathogen (regardless of the pathogen isolated) or occurred within 14 d with the same pathogens isolated, it

was considered to be the same case of mastitis. Any mastitis case after 14 d since the previous mastitis case was considered a new case (Barkema et al., 1998).

If a cow had multiple quarter infections at the same time (e.g., *E. coli* in the left rear quarter and *Staphylococcus* spp. in the right front quarter, or both pathogens in the same quarter), she was considered to have both *E. coli* and *Staphylococcus* spp. isolated within the one case (Hertl et al., 2011). These 'mixed' cases were excluded in our statistical analyses as this would render the outcome variable to be unclear i.e., it is unclear which pathogen came first, hence, which pathogen is the risk factor for the other pathogen. In Figure 2.1, however, these mixed cases are included.

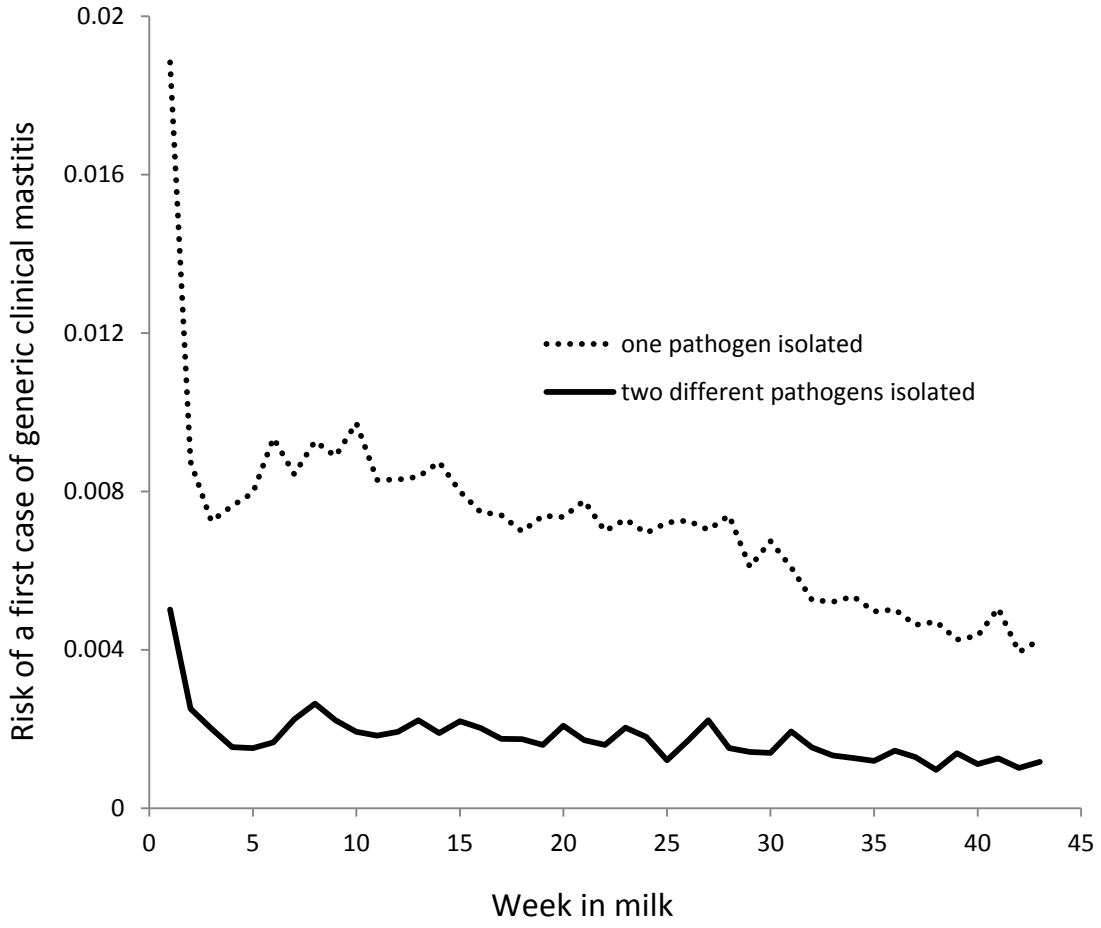

Figure 2.1. Weekly risk of a first case of generic clinical mastitis by only one pathogen isolated or 2 different pathogens isolated in the same week, in the first 43 weeks of lactation (all lactations included), in 5 New York State Holstein herds

Sampling

Cows with CM were segregated from pen mates immediately after milking for sampling and further evaluation. Milk samples from clinical quarters were collected aseptically. Teats were cleaned, dried then sanitized with and alcohol swab. The first several squirts of milk were discarded prior to collection of the diagnostic sample. Samples were labeled and refrigerated

immediately after collection. Samples were collected daily during the week (weekend samples were collected the following Monday) by the Quality Milk Production Services at Cornell University and transported to the laboratory under refrigeration. The culture procedures are described in Gröhn et al. (2004). Briefly, milk samples were plated by streaking 0.01mL on trypticase soy agar II with 5% sheep blood and 0.1% esculin (BBL; Becton Dickinson Microbiology Systems, Cockeyville, MD). Plates were incubated at 37°C for 48 h. Following observation of colony morphology and hemolytic patterns on blood agar, isolates were examined by means of 3% KOH, gram-staining organisms, catalase and oxidase testing, and additional biochemical and metabolic evaluations as required. Colony morphology on Mac Conkey agar and the BBL Crystal ID System (Becton Dickinson) identified gram-negative organisms. Streptococci that had a negative CAMP reaction were classified as *Streptococcus* spp. Staphylococci with β or αβ hemolytic patterns that had a positive tube test for free coagulase were classified as *Staph. aureus.* Nonhemolytic staphylococci with a positive tube coagulase test were further identified with the API Staph System (bio-Merieux Vitek, Hazelwood, MO). Coagulase-negative staphylococci were classified as *Staphylococcus* spp. Single quarter samples were used. Contamination was defined as more than two organisms on a plate.

Treatment protocol

The treatment protocol for diseased cows was similar across the 5 dairy herds and throughout the study. Treatment protocols were determined by the herd veterinarians and applied to clinical cows based on the identified pathogen and/or severity of signs displayed by the affected cow. All intramammary treatments involved the use of FDA approved commercially

available medications. Animals displaying systemic signs of mastitis also received supportive treatment which included systemic antibiotics, IV and oral fluids and anti-inflammatory drugs (flunixin meglumine) according to the farm protocols. Duration of treatment varied with the drug selection and signs exhibited by the cow.

Contagious mastitis pathogens are well controlled in all study herds. The vast majority of clinical cases was environmental in origin and was considered to be mild to moderate in severity. Mild clinical cases exhibited only signs of abnormal milk. Cows with moderate CM displayed abnormal milk and the affected quarter showed signs of inflammation (pain and/or swelling) but no systemic signs of illness. Individuals displaying fever (>103.5^{o}F), dehydration, decreased rumen motility (<1 rumen contraction per minute) or loss of appetite and/or depression were considered to have severe acute CM and received additional supportive therapy.

Bacteria specific CM

The bacteria causing CM studied were *Streptococcus* spp., *Staphylococcus aureus*, *Staphylococcus* spp., *Escherichia coli, Klebsiella* and *Trueperella pyogenes*.

Other Diseases

Five other diseases (milk fever, retained placenta, metritis, ketosis and displaced abomasum (DA)) were included as potential risk factors. They were defined as follows: 1) milk fever occurred if a cow was unable to rise or had cool extremities and sluggish rumen motility near the time of calving, but was treated successfully with calcium, 2) retained placenta was

retention of fetal membranes for at least 24 h post calving, 3) metritis involved a febrile state accompanying a purulent or fetid vaginal discharge or diagnosis of an enlarged uterus by veterinary palpation, 4) ketosis was diagnosed by a drop in feed intake and milk production with detection of ketones in milk, urine or breath and no other concomitant diseases and with response to treatment, and 5) DA occurred when the abomasum was enlarged with fluid, gas or both and was mechanically trapped in either the left or right side of the abdominal cavity; nearly every DA case was confirmed by surgery, but cows removed from the herd without treatment were also recorded. Written disease definitions were disseminated to participating dairy producers and veterinarians to ensure that disease definition and diagnostic criteria were consistent across study farms (Hertl et al., 2011).

Carryover

The carryover variable represented the number of cases of CM a parity ≥2 cow had in her previous lactation (ranging from 0 to ≥3).

Previous milk yield

Primipara and multipara were analyzed separately and the previous milk yield (milk yield from 2 wk before the current week in milk) was stratified into five quintiles. The first 2 wim of lactation that were recorded for the cow were also analyzed separately, as this analysis focused on calving diseases as a risk factor; previous milk yield was not included.

Cases of bacteria specific CM

The outcome variable was bacteria specific CM of case 1, case 2 or case 3. The risk for cases 2 and 3 were conditional on the previous case of CM. Due to a very small number of second and third cases in the wim=1 and wim=2 analysis, only the risk of a first case of bacteria specific CM was estimated in the models for CM in the first two weeks in milk. Every effort was made to study the effects of interest for each bacteria specific CM and case; however, if solution convergence could not be attained, generic CM was the outcome variable (i.e., for primiparous cows of wim ≥3). All the models that reached convergence are included in our results.

Previous CM exposure and months since the previous case

For cases 2 and 3, the previous case (pathogen) was included in the model. In addition to the bacteria specific CM, other CM (i.e. pathogens other than *Streptococcus* spp., *Staphylococcus aureus*, *Staphylococcus* spp., *Escherichia coli*, *Klebsiella* and *Trueperella pyogenes)*, and no important growth (no bacterial growth above the level which could be detected from our microbiological procedures observed in the culture sample) were included as a previous case (pathogen). This variable had 3 levels: (1) the previous case (pathogen) could be the same as the outcome of interest, (2) the previous case (pathogen) could be different to the outcome of interest or (3) the cow may have had both (1) and (2).

Statistical Methods

The GLIMMIX procedure of SAS (SAS Institute, 2008) was used to study the risk of the bacteria specific (or generic) CM of interest occurring due to various factors. Variables for

inclusion were selected based on univariate analysis where a P-value below ≤0.20 was considered significant; variables of biological importance were also kept. These variables were then all included in the model and stepwise backward elimination was performed until all the variables included were significant at a P-value ≤0.05 or were considered biologically significant (i.e., calving diseases for first 2 weeks in milk analyses, previous CM history for recurrent CM analyses). The form of the generalized linear mixed model used was

$$\text{Ln}\,(\mathbf{Y}) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \ldots + \beta_k x_k + \mathbf{Z}\gamma + \varepsilon, \qquad [1]$$

where Ln is a link function, here the natural log of the probability of a cow contracting the bacteria specific CM in a week for first case analyses and in a month for second and third case analyses; **Y** is the vector of observations (either presence or absence of bacteria specific CM in a given week or month); β_0 is the regression parameter for the intercept, and $\beta_1, \beta_2, \beta_3 \ldots, \beta_k$ are the regression coefficients for the fixed effects $x_1, x_2, x_3 \ldots, x_k$ (described below); γ is an unknown vector of random-effect parameters with known design matrix **Z**, and ε, a presumed independent random Poisson distributed residual term. Fixed effects in our models were parity with 3 levels (i.e., second, third and fourth and greater in lactation), stage of lactation (for first case analysis, ranging from wim 1 to wim 43, and for second and third case analyses, months 1, 2 and 3 and greater); current season with four levels (summer i.e., June->Aug, fall i.e., Sep->Nov, winter i.e., Dec->Feb and spring i.e., Mar->May), carryover effect (CM cases in the previous lactation) with four levels (for multiparous cows only, i.e., 0, 1, 2, 3 and greater), other diseases with 2 levels (only for first case analysis; if she contracted the disease in the first two weeks –

presence/absence), milk weight with five levels (only for wim≥3), and her previous CM exposure (in the current lactation) with 3 levels (only for second and third case analyses, i.e., she had the same bacteria, a different bacteria, or both in her most recent previous case of CM). Herd was a random effect unless otherwise specified (if model fit improved with herd as fixed effect this was the model used, which was noted). We assumed that all cases of CM occurred at the end of the risk period; therefore an equal weight of 1 was assigned to every observation.

The unit of analysis was relatively short; therefore the distinction between risk and rate diminishes. Hence, from here on the risk per cow-week (first case analysis) or cow-month (second and third case analyses) was used as a measure of CM occurrence. The relationship between risk (cumulative incidence) and rate is given by:

$CI = 1 - \exp(-I * \Delta t)$, where CI is cumulative incidence and I is incidence rate. Δt would be the time measured in cow-months. For small CI, a good approximation is $I*\Delta t$, and as Δt in our models equals 1, essentially CI and I can be used interchangeably (Rothman, 1986).

Because we were not interested in specific herds, but rather herds in general with the common characteristics of being large, high milk producing dairy herds with a low incidence of contagious mastitis, we always attempted to include herd as a random (intercept) effect. Model fit was evaluated by comparing the Pearson chi-square (where herd was a fixed effect) or Generalized chi-square (where herd was a random effect) with the remaining degrees of freedom as well as assessing the -2 log pseudo-likelihood value to determine residual variability in the marginal distribution of the data (SAS Institute, 2006). A ratio of 1 (Pearson or Generalized chi-square/df) indicates good model fit. There was one model where the negative binomial

distribution provided a better fit, in which case the deviance statistic was provided in addition to the Pearson-chi square/df to assess model fit. Cows were censored at 44 wim (to approximate a 305 day lactation), or when they contracted a fourth case of CM, or the cow died or was culled (whichever one came first). A sample coding scheme for four cows with a second case of CM is illustrated in Table 2.2.1.

This particular example is of a dataset used to study a second case of CM which happens to be *E. coli*. Therefore, cows in this dataset are either free of CM, or have had a first case of CM (of any type) and are censored from the wim they have a second CM case which is identified as *E. coli* only (no concurrent infections with another pathogen were included). Hence, each bacteria by case (1, 2 or 3) had its own separate dataset for analysis.

For the dataset with only wim 1 and 2, 2 models were fitted: (1ai) effects of risk factors (other diseases, season) on the risk of bacteria specific CM in primipara; (1bi) effects of risk factors (parity, carryover, other diseases, season) on risk of bacteria specific CM in multipara. Week of lactation was not included as wim 1 and 2 were combined into 1 time step.

Table 2.2.1. Covariate coding scheme used in this study for 4 example cows with a second case of CM. This particular example is of a dataset used to study the risk of a second case of *E. coli* in dairy cows. [1]

Cow ID	Week in milk	CM1[2]: *Staph* spp.	CM1: *Staph aureus*	CM1: *Strep* spp.	CM1: *E. coli*	CM1: *Klebsiella*	CM1: *T. pyogenes*	CM1: Other	CM1: No imp. growth	Previous CM exposure[3]	Months since CM1[4]	CM2: *Staph* spp.	CM2: *Staph aureus*	CM2: *Strep* spp.	CM2: *E. coli*	CM2: *Klebsiella*	CM2: *T. pyogenes*
1	4	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
1	8	0	0	0	1	0	0	0	0	1	2	0	0	0	0	0	0
1	12	0	0	0	1	0	0	0	0	1	3	0	0	0	1	0	0
2	5	0	0	1	0	1	0	0	0	2	1	0	0	0	0	0	0
2	9	0	0	1	0	1	0	0	0	2	2	0	0	0	0	0	0
2	13	0	0	1	0	1	0	0	0	2	3	0	0	0	0	0	0
2	17	0	0	1	0	1	0	0	0	2	3	0	0	0	0	0	0
2	21	0	0	1	0	1	0	0	0	2	3	0	0	0	0	0	0
2	25	0	0	1	0	1	0	0	0	2	3	0	0	0	0	0	0
2	29	0	0	1	0	1	0	0	0	2	3	0	0	0	0	0	0
2	33	0	0	1	0	1	0	0	0	2	3	0	0	0	0	0	0
2	37	0	0	1	0	1	0	0	0	2	3	0	0	0	0	0	0
3	13	0	1	0	1	0	0	0	0	3	1	0	0	0	0	0	0
3	17	0	1	0	1	0	0	0	0	3	2	0	0	0	0	0	0
3	21	0	1	0	1	0	0	0	0	3	3	0	0	0	0	0	0
3	25	0	1	0	1	0	0	0	0	3	3	0	0	0	0	0	0
3	29	0	1	0	1	0	0	0	0	3	3	0	0	0	0	0	0
3	33	0	1	0	1	0	0	0	0	3	3	0	0	0	0	0	0
3	37	0	1	0	1	0	0	0	0	3	3	0	0	0	1	0	0
4[5]	10	0	0	0	0	0	1	1	0	2	1	0	0	0	0	0	0
4	14	0	0	0	0	0	1	1	0	2	2	0	0	0	0	0	0
4	18	0	0	0	0	0	1	1	0	2	3	0	0	0	0	0	0
4	22	0	0	0	0	0	1	1	0	2	3	0	0	0	0	0	0
4	26	0	0	0	0	0	1	1	0	2	3	0	0	0	0	0	0
4	30	0	0	0	0	0	1	1	0	2	3	0	0	0	0	0	0
4	34	0	0	0	0	0	1	1	0	2	3	0	0	0	0	0	0

[1] The dataset contained only cows (all of which experienced a 1st case of CM of any type) that had *E. coli* as their second case of CM and cows that did not have a second case of CM (of any type)

[2]CM1 = is the first case of CM. Because this is an example dataset for a second case of CM, the first record kept for this analysis would be the week the cow had her first case of CM. Note that the time step is months since the week the cow had her first CM.

[3]Previous CM exposure had 3 levels: 1 = CM1 is due to the same pathogen as CM2 (second case of CM); 2 = CM1 is due to a different pathogen than CM2; 3 =

CM1 was due to both the same and different pathogens as CM2. Cow 3 is an example of level 3 i.e., her second case of CM is due to *E. coli*, and her first case of CM were both *Staph aureus* and *E. coli*. Hence, her previous risk is coded as '3'.

[4] Months since CM1 had 3 levels: 1 = 1 month since her first case of CM, 2 = 2 months since her first case of CM, 3 = 3 and or greater than 3 months since her first case of CM.

[5]While we did not model 'other' and 'no important growth' as outcome variables, these were included as risk factors. Cow 4 demonstrates how these cases were treated.

For the dataset with wim≥3, for primipara, generic CM models were fitted: (1aii) effects of risk factors (week of lactation, other diseases, season, previous milk yield) on the risk of a first case of generic CM in primipara; (2a) effects of risk factors (current season, months since first CM) on risk of a second case of generic CM in primipara and (3a) effects of risk factors (current season, months since second CM) on risk of a third case of generic CM in primipara. For multipara: (1bii) effects of risk factors (lactation, week of lactation, carryover, other diseases, season, previous milk yield) on the risk of a first case of bacteria specific CM in multipara, (2b) effects of risk factors (lactation, carryover, previous CM exposure, months since first CM, current season) on risk of a second case of bacteria specific CM in multipara and (3b) effects of risk factors (lactation, carryover, previous CM exposure, months since second CM, current season) on risk of a third case of bacteria specific CM in multipara. Not all models retained the variables listed in brackets; a P-value below 0.05 was considered significant.

RESULTS

Descriptive findings

A total of 40,864 lactations (9,873 cows) were analyzed of which 17,265 were primiparous and 23,599 were multiparous lactations, in 5 large, high milk producing dairy herds in New York State. There were 12,725 first cases, 4,535 second cases and 1,798 third cases of CM. The lactational incidence risk and median week in milk (wim) was 45.1% (19) for generic CM, 3.0% (20) for *Staphylococcus* spp., 2.7% (23) for *Staph. aureus*, 10.5% (20) for *Strep.* spp., 10.2% (17) for *E. coli*, 3.9% (19) for *Klebsiella*, 0.9% (11) for *T. pyogenes*, 7.1% (19) for other and 11.3% (19) for no important growth. The summation of the pathogen specific lactational incidence risks (49.6%) are slightly greater than the generic lactational incidence risk (45.1%), as the cases with multiple pathogens are included as one in the generic lactational incidence risk, but individually in the pathogen specific lactational incidence risks. The most common pathogens found across lactation and cases were *Strep.* spp., *E. coli* and no important growth (Table 2.2).

Table 2.2. Distribution of pathogens causing clinical mastitis (CM) in 5 New York State dairy herds, in first and second and higher lactations[1]

Pathogen	First lactation (17,265 lactations)			Second and higher lactation (23,599 lactations)		
	1st CM case (3576) (%)[6]	2nd CM case (941) (%)	3rdCM case (299) (%)	1st CM case (9149) (%)	2nd CM case (3594) (%)	3rd CM case (1499) (%)
Staph. spp.	281 (7.9)	73 (7.8)	25 (8.4)	602 (6.6)	256 (7.1)	108 (7.2)
Strep. spp.	906 (25.3)	199 (21.1)	48 (16.1)	2374 (25.9)	916 (25.5)	383 (25.6)
Staph. aureus	272 (7.6)	74 (7.9)	32 (10.7)	379 (4.1)	233 (6.5)	96 (6.4)
Klebsiella	159 (4.4)	66 (7.0)	14 (4.7)	765 (8.4)	336 (9.3)	147 (9.8)
E. coli	807 (22.6)	153 (16.3)	42 (14.0)	2320 (25.4)	634 (17.6)	219 (14.6)
T. pyogenes	92 (2.6)	31 (3.3)	7 (2.3)	191 (2.1)	51 (1.4)	17 (1.1)
Other[2]						
Enterobacter	3 (0.08)	3 (0.3)	1 (0.3)	15 (0.2)	3 (0.08)	2 (0.1)
Citrobacter	8 (0.2)	2 (0.2)	1 (0.3)	11 (0.1)	7 (1.9)	1 (0.07)
C. bovis	18 (0.5)	9 (1.0)	5 (1.7)	13 (0.1)	7 (1.9)	3 (0.2)
Prototheca	0 (0)	0 (0)	0 (0)	3 (0.03)	0 (0)	0 (0)
Mycoplasma	60 (1.7)	9 (1.0)	2 (0.7)	12 (0.1)	4 (0.1)	2 (0.1)
Pseudomonas	9 (0.3)	2 (0.2)	0 (0)	8 (0.09)	5 (0.1)	4 (0.3)
Pasteurella	63 (1.7)	16 (1.7)	8 (2.7)	34 (0.4)	12 (0.3)	8 (0.5)
Yeast	57 (1.6)	14 (1.5)	4 (1.3)	95 (1.0)	34 (0.9)	15 (1.0)
G+ bacillus	8 (0.2)	5 (0.5)	1 (0.3)	34 (0.4)	13 (0.4)	8 (0.5)
Contamination	34 (1.0)	22 (2.3)	7 (2.3)	87 (1.0)	48 (1.3)	30 (2.0)
Other[3]	403 (11.3)	105 (11.1)	33 (11.0)	1041 (11.4)	422 (11.7)	174 (11.6)
No imp. growth[4]	772 (21.6)	248 (26.4)	90 (30.1)	2210 (24.2)	1017 (28.3)	457 (30.5)
Unknown[5]	253 (7.1)	99 (10.5)	32 (10.7)	544 (5.9)	273 (7.6)	115 (7.7)

[1] Total number of CM cases by case number in which the pathogen was identified. Each cow may have more than one lactation and in each case there may be more than one organism involved

[2] This classification used in our analyses comprised all the organisms other than the main 6 that were modeled as outcome variables (*Staph* spp., *Strep* spp., *Staph. aureus*, *Klebsiella*, *E. coli* and *T. pyogenes*)

[3] This classification could be one of several pathogens, hence it was grouped into 'other'

[4] No bacterial growth (above the level which can be detected from our microbiological procedures) observed in the culture sample

[5] The etiologic agent was not identified in the cultured sample

[6] Percentage of cases

All models described below had a Generalized Chi-square or Pearson Chi-square/df fit ranging from 0.84 to 1.20 unless otherwise specified in the tables of results.

Risk of generic CM (one pathogen isolated or two different pathogens isolated for first case) by week in milk (Figure 2.1)

Because we did not model cases of CM where a cow could have two pathogens isolated as an outcome, we illustrated this as a first case of generic CM in Figure 2.1. This figure demonstrates the risk of a first case of CM by the presence of only one pathogen or the presence of two pathogens. In calculating the risk of only one pathogen identified (two pathogens identified), cows were censored when they reached wim 44, died or contracted two pathogens (only one pathogen identified); whichever one came first. The risk of a first case of generic CM was greater where one pathogen was isolated, compared with two different pathogens throughout lactation, but also particularly at the beginning of lactation (i.e., wim ≤ 3).

Risk of a first case of bacteria specific CM by week in milk (Figures 2.2.1 and 2.2.2)

The risk of a first case of bacteria specific CM was generally greater at the beginning of lactation (*Strep.* spp., *Staph. aureus*, *Staph.* spp., *Klebsiella*, *T. pyogenes*) or peak lactation (*E. coli*), then decreased and leveled out as wim increased (Figures 2.2.1 and 2.2.2). The same trend was seen for generic CM where only one bacteria was isolated in case 1 and where two different bacteria were isolated in case 1.

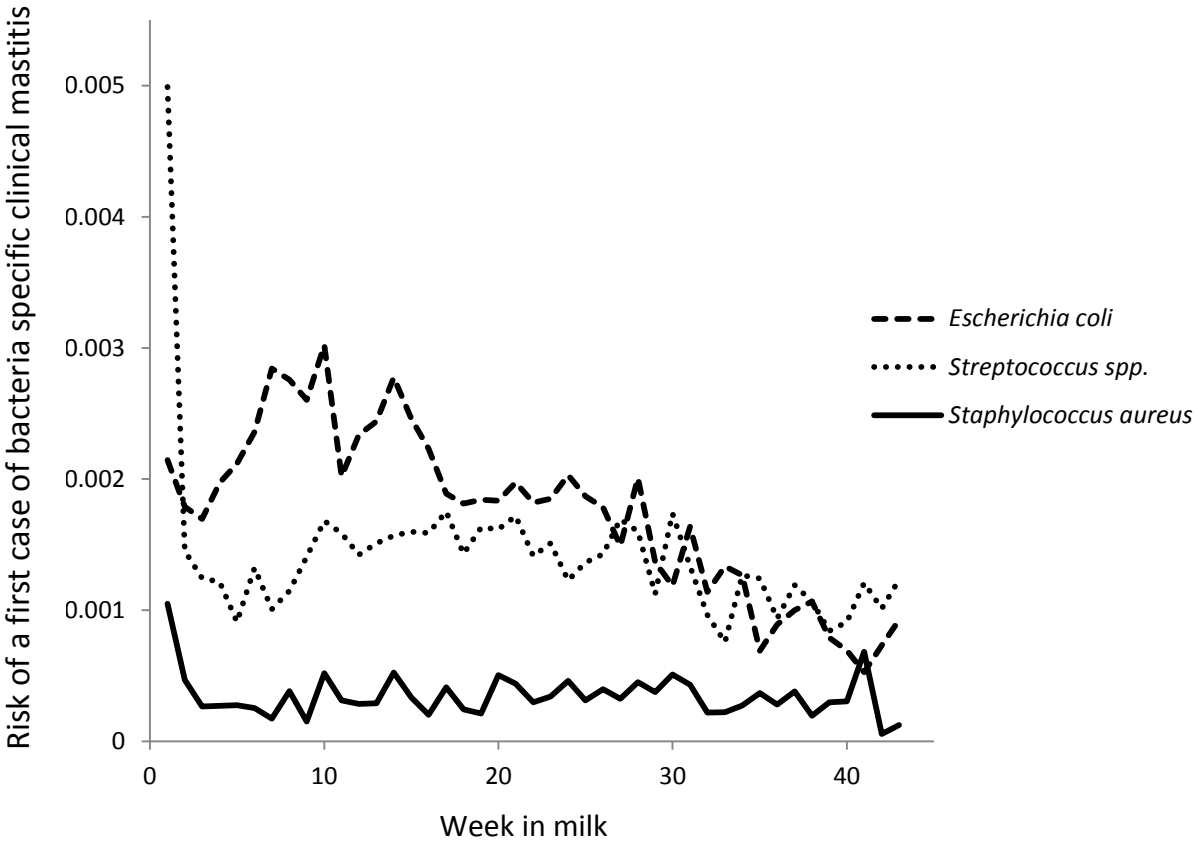

Figure 2.2.1. Weekly risk of a first case of bacteria specific clinical mastitis (*E. coli*, *Strep.* spp. and *Staph. aureus*) by week in milk, in the first 43 weeks of lactation, in 5 New York State Holstein herds.

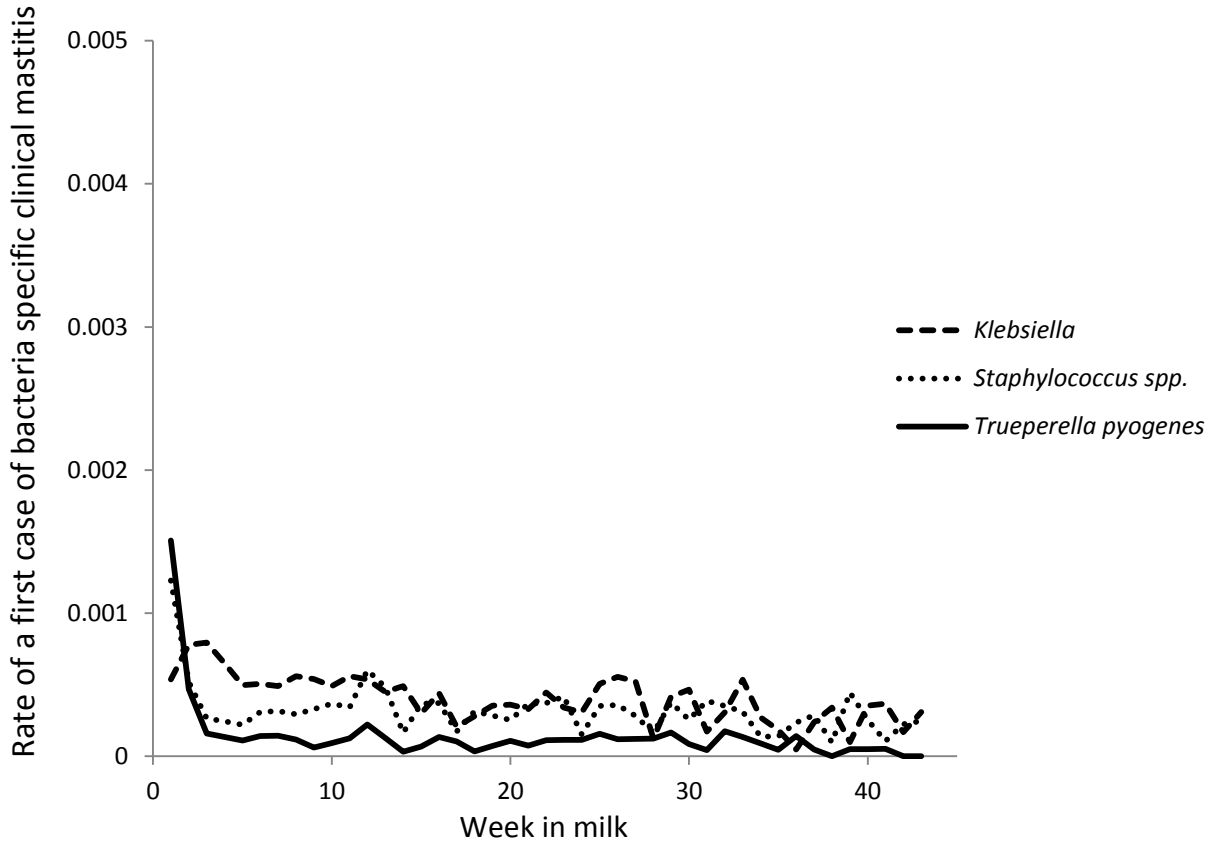

Figure 2.2.2. Weekly risk of a first case of bacteria specific clinical mastitis (*Klebsiella*, *Staph. spp.* and *T. pyogenes*) by week in milk, in the first 43 weeks of lactation, in 5 New York State Holstein herds.

Risk of a first case of bacteria specific CM in the first two weeks of lactation

First lactation cows with retained placenta within the first two weeks were found to be exp(1.11)=3.03 [95% CI (2.05, 4.48)] times more at risk of a first case of *E. coli* than cows without retained placenta. In multipara, cows in a higher lactation were found to be more at risk of bacteria specific CM than cows in a lower lactation. Cows with more cases of CM in the previous lactation (carryover) were at greater risk of a first case of bacteria specific CM (Table 2.3).

Table 2.3. Parameter estimates and standard errors for the generalized linear mixed models used to estimate the effects of parity, retained placenta and carryover on the risk of first occurrence of bacterial specific clinical mastitis (CM) in the first two weeks of lactation in multiparous cows (23,563 lactations) in 5 New York State dairy herds[1]

Parameter	Estimate (SE) for risk of bacteria specific CM				
	Streptococcus spp.	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella* spp.	*Trueperella pyogenes*
Intercept	-5.02 (0.36)***	-7.03 (0.43)***	-5.43 (0.26)***	-6.32 (0.47)***	-6.20 (0.43)***
Week in milk in the model (estimates not shown)					
Parity					
2 (baseline)	-0.79[2] (0.22)***	-0.46 (0.51)	-0.50 (0.24)*	-0.56 (0.36)	-1.16 (0.44)***
3	-0.27 (0.21)	0.25 (0.47)	-0.18 (0.24)	-0.28 (0.36)	-0.09 (0.34)
≥4	0	0	0	0	0
Retained placenta	--	--		--	--
No (baseline)			0		
Yes			0.72 (0.23)***		
Carryover from previous lactation[3]					
0 (baseline)	0	0	0	0	0
1	0.52 (0.21)***	1.13 (0.41)***	0.29 (0.25)	0.96 (0.34)***	0.53 (0.39)
2	0.46 (0.33)	-0.19 (1.04)	0.69 (0.33)**	0.47 (0.61)	0.74 (0.53)
3	0.93 (0.33)***	0.94 (0.77)	0.71 (0.39)**	1.78 (0.45)***	1.51 (0.47)***

[1]Herd was a random effect
[2]The risk ratio is calculated as exp(-0.79) = 0.45
[3]The carryover is the number of cases of CM from the previous lactation: 0= none, 1= 1 case of CM, 2= 2 cases of CM and 3= ≥3 cases of CM.
*** p≤0.05
** p≤0.10
* p≤0.15

Risk of a first case of bacteria specific CM in wim ≥3 of lactation

A generic CM model for a first case in primipara (Table 2.4) and a bacteria specific CM model for a first case in multipara were estimated (Table 2.5). In primipara, cows were at a greater risk of contracting a first case of CM in summer [exp(0.14)=1.15; 95% CI (1.08, 1.22)] compared with winter (baseline). The same seasonal effect was observed among multipara for a first case of *Staph. aureus* [exp(0.34)=1.40; 95% CI (1.16, 1.70)], *Staph.* spp. [exp(0.40)=1.49; 95% CI (1.26, 1.77)], *E. coli* [exp(0.20)=1.22; 95% CI (1.14, 1.31)] and *Klebsiella* [exp(0.64)=1.90; 95% CI (1.63, 2.20)]. The trends in parity and carryover among multipara were the same in wim ≥3 as for wim 1 and 2 described above. A significant effect of previous milk yield on a first case of *E. coli* demonstrated that cows with a larger previous milk yield were at greater risk of a first case of *E. coli*.

Risk of a second case of bacteria specific CM in wim ≥3 of lactation

The risk of a second case of bacteria specific CM was higher within 1 month after the first case, then decreased as more months passed (Figures 2.3.1 and 2.3.2). A generic CM model for a second case in primipara (Table 2.4) and a bacteria specific CM model for a second case in multipara were estimated (Table 2.6).

Table 2.4. Parameter estimates and standard errors for the generalized linear mixed models used to estimate the effects of current season and previous milk yield on the risk of first occurrence of clinical mastitis (CM) in primiparous cows (16,554 lactations) in 5 New York State dairy herds[1] and effects of current season and months since previous CM, on the risk of a second occurrence of CM in primiparous cows (3,197 lactations) and third occurrence of CM in primiparous cows (754 lactations) in wim ≥ 3 in 5 New York State dairy herds[1]

Parameter	Estimate (SE) for risk of CM		
	CM1	CM2	CM3
Intercept	-5.39 (0.23)***	-3.12 (0.18)***	-2.41 (0.17)***
	Week in milk In the model (estimates not shown)		
Current season			--
Fall	-0.11 (0.06)*	-0.09 (0.13)	
Spring	-0.005 (0.06)	0.35 (0.12)***	
Summer	0.14 (0.06)***	0.22 (0.12)**	
Winter (baseline)	0	0	
Previous milk yield[2]		NA	NA
Level 1	0		
Level 2	-0.17 (0.07)***		
Level 3	-0.18 (0.07)***		
Level 4	-0.20 (0.07)***		
Level 5	-0.11[3] (0.07)*		
Months since previous CM case	NA		
1 (baseline)		0	0
2		0.19 (0.12)*	0.07 (0.20)
3		-0.10 (0.13)	-0.60 (0.28)**
4+		-0.59 (0.11)***	-0.95 (0.23)***

[1]Herd was a random effect

[2]Milk yield from 2 weeks before current wim; levels are quintiles of milk yield (1=lowest, 5=highest).

[3]The risk ratio is calculated as exp (-0.11) = 0.90

*** p<0.05

** p<0.10

* p<0.15

Table 2.5. Parameter estimates and standard errors for the generalized linear mixed models used to estimate the effects of parity, carryover, current season and previous milk yield on the risk of first occurrence of bacterial specific clinical mastitis (CM) in multiparous cows (23,017 lactations) in wim ≥ 3 in 5 New York State dairy herds[1]

Parameter	Estimate (SE) for risk of bacteria specific CM				
	Streptococcus spp.	*Staphylococcus aureus*	*Staphylococcus* spp.	*Escherichia coli*	*Klebsiella* spp. [2]
Intercept	-6.27 (0.26)***	-8.68 (0.60)***	-8.16 (0.55)***	-6.37 (0.24)***	-7.82 (0.33)***
Week in milk in the model (estimates not shown)					
Parity					
2	-0.23 (0.07)***	0.05 (0.17)	-0.56 (0.15)***	-0.41 (0.07)***	-0.31 (0.15)***
3	-0.19 (0.08)***	-0.05 (0.19)	-0.49 (0.16)***	-0.10 (0.07)	0.10 (0.15)
≥4 (baseline)	0	0	0	0	0
Carryover[3]					
0 (baseline)	0	0	0	0	0
1	0.51 (0.07)***	0.70 (0.16)***	0.29 (0.16)**	0.34 (0.07)***	0.48 (0.14)***
2	0.77 (0.10)***	0.42 (0.30)	0.76 (0.23)***	0.81 (0.10)***	0.20 (0.27)
3	0.89 (0.14)***	1.25 (0.29)***	1.19 (0.25)***	0.79 (0.13)***	1.23 (0.23)***
Current season					
Fall	-0.23 (0.08)***	0.06 (0.20)	0.04 (0.19)	0.03 (0.08)	0.03 (0.17)
Spring	0.04 (0.07)	0.06 (0.19)	0.08 (0.18)	-0.05 (0.08)	-0.29 (0.18)*

Summer	-0.13[4] (0.08)*	0.34 (0.19)**	0.40 (0.17)***	0.20 (0.07)***	0.64 (0.15)***
Winter (baseline)	0	0	0	0	0
Previous milk yield[5]	--	--	--		--
Level 1 (baseline)				0	
Level 2				0.30 (0.13)***	
Level 3				0.53 (0.12)***	
Level 4				0.52 (0.12)***	
Level 5				0.70 (0.12)***	

[1] Herd was a random effect unless otherwise specified.
[2] Herd was a fixed effect.
[3] The carryover is the number of cases of CM from the previous lactation: 0= none, 1= 1 case of CM, 2= 2 cases of CM and 3= ≥3 cases of CM.
[4] The risk ratio is calculated as exp (-0.13)=0.88
[5] Milk yield from 2 weeks before current wim; levels are quintiles of milk yield (1=lowest, 5=highest).
*** p<0.05
** p<0.10
* p<0.15

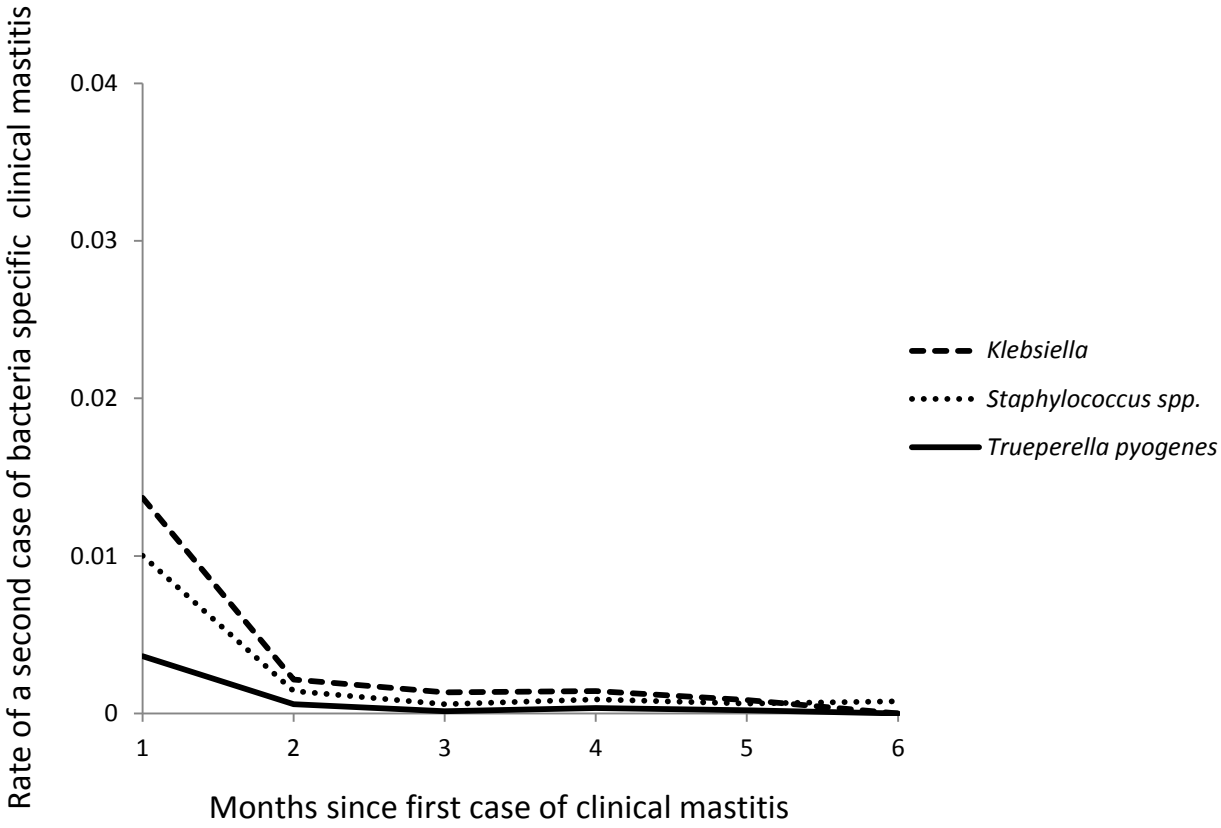

Figure 2.3.1. Monthly risk of a second case of bacteria specific clinical mastitis (*Klebsiella*, *Staph*. spp. and *T. pyogenes*) by months since the first case of clinical mastitis, in 5 New York State Holstein herds.

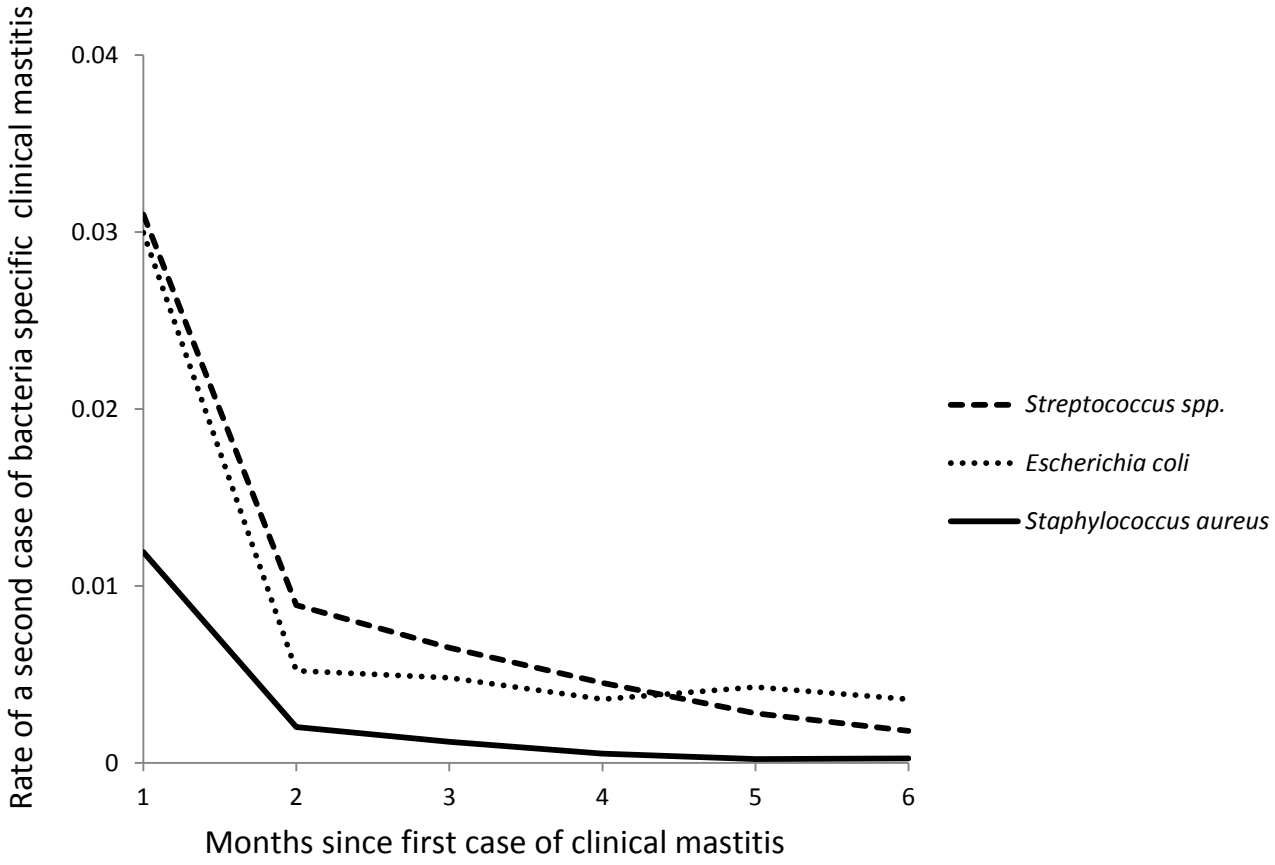

Figure 2.3.2. Monthly risk of a second case of bacteria specific clinical mastitis (*Strep.* spp., *E. coli* and *Staph. aureus*) by months since the first case of clinical mastitis, in 5 New York State Holstein herds.

Table 2.6. Parameter estimates and standard errors for the generalized linear mixed models used to estimate the effects of parity, carryover, previous clinical mastitis (CM) exposure, months since first CM and current season, on the risk of a second occurrence of bacterial specific clinical mastitis (CM) in multiparous cows (8,417 lactations) in wim ≥ 3 in 5 New York State dairy herds[1]

Parameter	Estimate (SE) for risk of 2nd case of bacteria specific CM					
	Streptococcus spp.[2]	*Staphylococcus aureus*[3]	*Staphylococcus* spp.[4]	*Escherichia coli*	*Klebsiella* spp.[5]	*Trueperella pyogenes*[6]
Intercept	-3.71 (0.19)***	-4.89 (0.39)***	-4.79 (0.44)***	-3.89 (0.18)***	-4.85 (0.32)***	-5.62 (0.40)***
Parity						
2	-0.09 (0.12)	0.31 (0.25)	-0.04 (0.25)	0.06 (0.14)	0.23 (0.20)	-0.09 (0.42)
3	-0.11 (0.13)	0.08 (0.27)	-0.18 (0.27)	0.22 (0.14)*	0.004 (0.21)	-1.03 (0.59)**
≥4 (baseline)	0	0	0	0	0	0
Carryover[7]						--
0 (baseline)	0	0	0	0	0	
1	0.36[8] (0.12)***	-0.02 (0.24)	0.17 (0.25)	0.41 (0.13)***	-0.02 (0.21)	
2	0.40 (0.17)***	0.20 (0.34)	0.26 (0.35)	0.28 (0.19)	0.60 (0.25)***	
3	0.84 (0.17)***	0.41 (0.37)	0.68 (0.35)**	0.80 (0.19)***	0.76 (0.27)***	
Previous CM exposure (associated with 1st CM case)						
Different pathogen	0	0	0	0	0	0
Same pathogen	0.80 (0.11)***	1.78 (0.27)***	1.33 (0.32)***	0.52 (0.12)***	1.48 (0.21)***	2.73 (0.50)***
Both	0.22 (0.18)	0.67 (0.53)	0.38 (0.49)	0.09 (0.22)	0.56 (0.37)	1.91 (1.00)**
Months since first case of CM						
1 (baseline)	0	0	0	0	0	0
2	-1.18 (0.14)***	-1.71 (0.32)***	-2.51	-1.66 (0.19)***	-1.80 (0.28)***	-1.64 (0.61)***

			(0.49)***			
3	-1.45 (0.18)***	-2.09 (0.44)***	-2.73	-1.64 (0.21)***	-2.13 (0.38)***	-4.90 (3.30)*
			(0.62)***			
4+	-2.21 (0.16)***	-3.76 (0.58)***	-2.43	-2.06 (0.16)***	-2.94 (0.34)***	-3.72 (1.06)***
			(0.33)***			
Current season	--	--	--	--		--
Fall					0.43 (0.25)*	
Spring					0.12 (0.26)	
Summer					0.53 (0.23)***	
Winter (baseline)					0	

[1] Herd was a random effect

[2] Fit statistics: -2 log pseudo-likelihood= 218408.5; Gener. Chi-Square/df= 0.74

[3] Fit statistics: -2 log pseudo-likelihood= 278804.3; Gener. Chi-Square/df= 0.54

[4] Fit statistics: -2 log pseudo-likelihood= 272734.1; Gener. Chi-Square/df= 0.61

[5] Fit statistics: -2 log pseudo-likelihood= 257412.6; Gener. Chi-Square/df= 0.51

[6] Fit statistics: -2 log pseudo-likelihood= 359.25; Pearson Chi-Square/df= 0.33. Herd was fixed effect.

[7] The carryover is the number of cases of CM in the previous lactation: 0= none, 1= 1 case of CM, 2= 2 cases of CM and 3= ≥3 cases of CM.

[8]The risk ratio is calculated as exp (0.36)=1.43

*** $p<0.05$

** $p<0.10$

* $p<0.15$

In the statistical models, the analyses are stratified by lactation (i.e., primipara and multipara). In Figures 2.3.1 and 2.3.2, the lactations are combined. For generic CM in primipara, cows were found to be at a greater risk of a second case of CM between 1-2 months after a first case of CM [exp(0.19)=1.21; 95% CI (1.07, 1.36); Table 2.4]. In multipara, however, we discovered cows were at greater risk within 1 month since the first case of CM. The effect of carryover was the same as for first case of bacteria specific CM where applicable. Cows were at greater risk of a second case of pathogen specific CM if they had the same pathogen in their first case as in the second case, compared with a different pathogen in their first case. There was a greater risk of cows contracting a second case of *Klebsiella* in the summer compared with other seasons.

Risk of a third case of bacteria specific CM in wim ≥3 of lactation

As above, the risk of a third case of bacteria specific CM was greater within 1 month after the second case, then decreased as more months passed (Figures 2.4.1 and 2.4.2). A generic CM model for a third case in primipara (Table 2.4) and a bacteria specific CM model for a third case in multipara were estimated (Table 2.7).

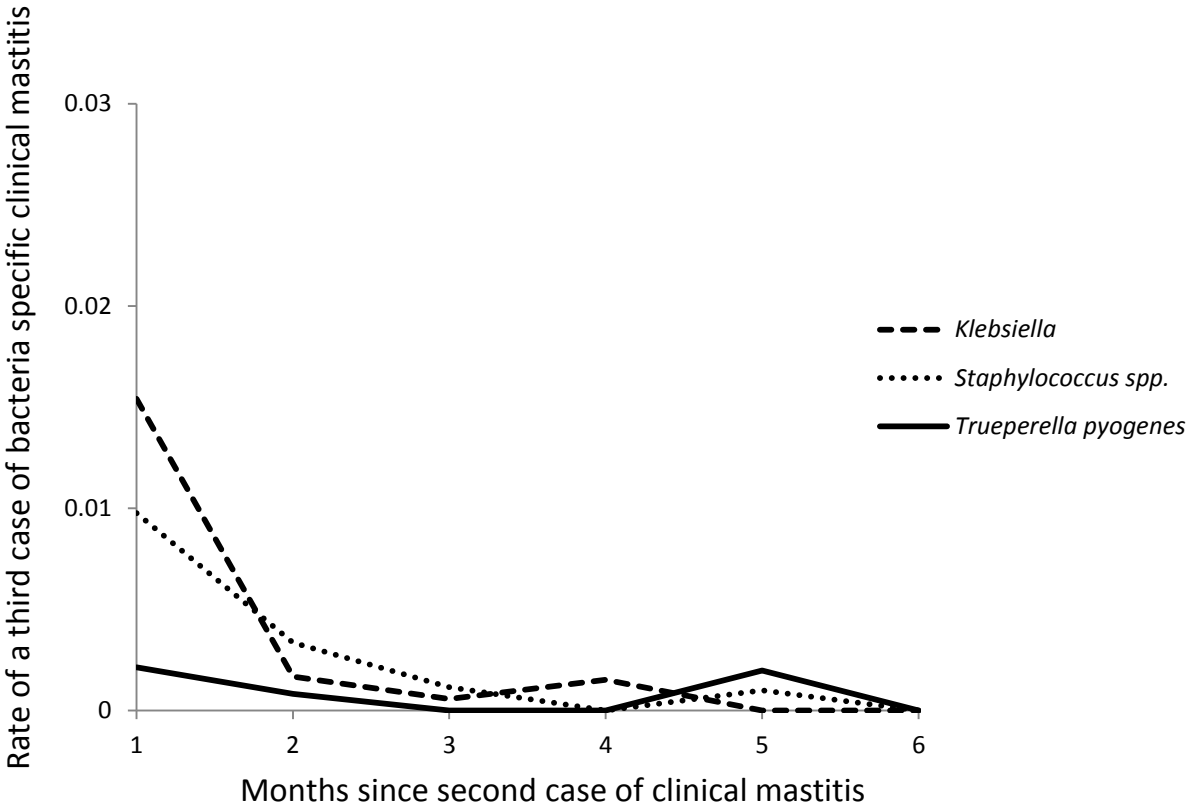

Figure 2.4.1. Monthly risk of a third case of bacteria specific clinical mastitis (*Klebsiella*, *Staph*. spp. and *T. pyogenes*) by months since the second case of clinical mastitis, in 5 New York State Holstein herds.

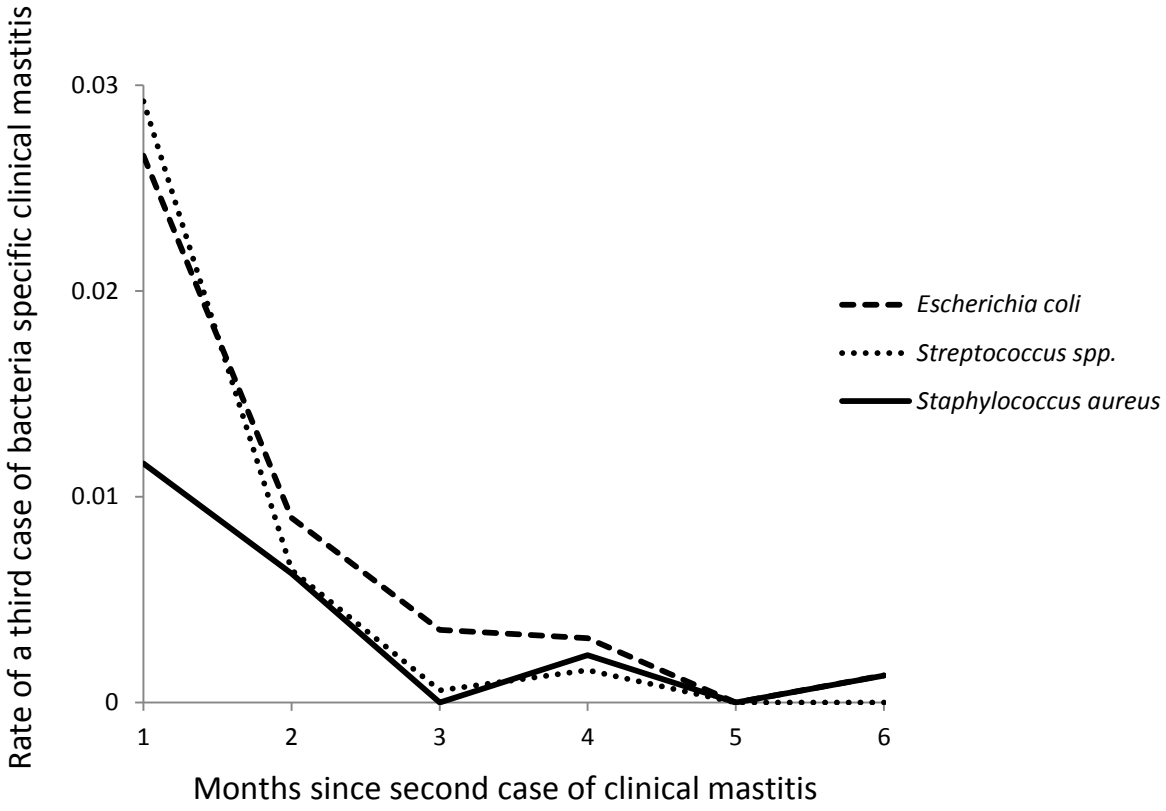

Figure 2.4.2. Monthly risk of a third case of bacteria specific clinical mastitis (*E. coli*, *Strep.* spp. and *Staph. aureus*) by months since the second case of clinical mastitis, in 5 New York State Holstein herds.

Table 2.7. Parameter estimates and standard errors for the generalized linear mixed models used to estimate the effects of parity, carryover, previous clinical mastitis (CM) exposure, months since second CM and current season, on the risk of a third occurrence of bacterial specific CM in multiparous cows (3,031 lactations) in wim ≥ 3 in 5 New York State dairy herds[1]

Parameter	Estimate (SE) for risk of 3rd case of bacteria specific CM					
	Streptococcus spp.[2]	*Staphylococcus aureus*[3]	*Staphylococcus* spp.[4]	*Escherichia coli*	*Klebsiella* spp.	*Trueperella pyogenes*[5]
Intercept	-3.59 (0.25)***	-5.92 (0.51)***	-6.08 (0.65)***	-3.69 (0.26)***	-4.60 (0.44)***	-5.85 (0.73)***
Parity						
2	0.24 (0.26)	0.64 (0.41)*	-0.21 (0.40)	-0.36 (0.26)	-0.13 (0.33)	-0.45 (0.95)
3	0.03 (0.28)	0.06 (0.48)	0.01 (0.40)	-0.07 (0.25)	-0.15 (0.34)	0.21 (0.85)
≥4 (baseline)	0	0	0	0	0	0
Carryover[6]		--				--
0 (baseline)	0		0	0	0	
1	0.24[7] (0.24)		0.21 (0.38)	0.05 (0.25)	0.70 (0.33)**	
2	-0.73 (0.47)*		-0.36 (0.56)	-0.09 (0.36)	0.76 (0.41)**	
3	0.20 (0.34)		0.31 (0.46)	0.32 (0.32)	0.78 (0.43)**	
Previous CM exposure (associated with 2nd CM case)					--	
Different pathogen	0	0	0	0		0
Same pathogen	-0.13 (0.28)	1.97 (0.34)***	0.27 (0.54)	0.39 (0.27)		3.66 (0.86)***
Both	-1.50 (0.72)**	1.15 (0.54)***	0.47 (0.53)	0.66 (0.34)**		3.87 (1.14)***
Months since second case of CM						
1 (baseline)	0	0	0	0	0	0
2	-1.36 (0.31)***	-0.38 (0.35)	-1.39 (0.48)***	-0.96 (0.28)***	-2.27 (0.59)***	-0.43 (0.84)

3	-3.45 (1.0)***	-5.63 (5.09)	-1.96 (0.73)***	-1.74 (0.47)***	-2.99 (1.00)***	-6.01 (14.22)
4+	-3.10 (0.59)***	-1.96 (0.61)***	-3.59 (1.01)***	-2.99 (0.59)***	-3.0 (0.72)***	-1.61 (1.10)*
Current season	--	--	--	--		--
Fall					0.46 (0.41)	
Spring					-0.64 (0.51)	
Summer					0.71 (0.38)**	
Winter (baseline)					0	

[1] Herd was a random effect

[2] Fit statistics: -2 log pseudo-likelihood= 64833.70; Gener. Chi-Square/df= 1.26

[3] Fit statistics: -2 log pseudo-likelihood= 416.56; Pearson Chi-Square/df= 0.58 and herd as fixed effect

[4] Fit statistics: Deviance=0.0363; Pearson Chi-Square/df= 1.5 and herd as fixed effect. Negative binomial distribution employed to attain reasonable fit.

[5] Fit statistics: -2 log pseudo-likelihood= 95.34; Pearson Chi-Square/df= 0.36 and herd as fixed effect

[6] The carryover is the number of cases of CM in the previous lactation: 0= none, 1= 1 case of CM, 2= 2 cases of CM and 3= ≥3 cases of CM.

[7]The risk ratio is calculated as exp (0.24)= 1.27

*** $p<0.05$

** $p<0.10$

* $p<0.15$

As for second case, the Figures 2.4.1 and 2.4.2 combined lactations, unlike the statistical models whereby we separated by primipara and multipara. In primipara, we found a cow's risk of a third case was greater 1-2 months since her second case. In multipara, however, the risk was greater within the first month since her second case. The effect of carryover was the same as for first case of bacteria specific CM where applicable. The bacteria specific CM of her second case was again found to be significant; however, this time, cows were at greater risk if they had both (the same pathogen and a different pathogen) as the second case, followed by the same pathogen in their third case as second case, followed by a different pathogen. As for second case, there was a greater risk of cows contracting a third case which happened to be *Klebsiella* in the summer compared with other seasons.

DISCUSSION

The relationship between cow characteristics i.e., parity, carryover, previous milk yield, previous CM exposure, history of diseases near calving and season on the risk of a first, second and third case of bacteria specific CM were estimated. The effects of cow characteristics on the risk of CM have been previously extensively studied; the value of this study is the new information that contributes to our knowledge of bacterial interactions and persistence. The reason for including diseases (other than CM) was because they are among the most common clinical conditions that are universally a problem in dairy cows (Gröhn et al., 2004). Clinical mastitis infections may be described as 'recurrent' and this can be explained by two different processes (1) the cases are really recurrent cases otherwise (2) these cases are chronic cases that are persistent and appear to be recurrent cases. Throughout this paper we distinguish recurrent

cases from infection persistence where recurrent cases are multiple CM cases occurring in the same animal in the same lactation whereas infection persistence refers to the infection process that may be the underlying cause for recurrent clinical cases.

Compared with our recent previous publications on gram positive, gram negative and other CM (Schukken et al., 2009; Hertl et al., 2010; Hertl et al., 2011), we had approximately 30% more data which allowed us to examine relationships at the bacteria specific level. A key contribution of this study is findings which elucidate the effects of carryover cases of CM from the previous lactation, and previous cases of CM within the lactation on the risk of recurrent cases of bacteria specific CM.

Studies have shown that higher milk producing cows are at a greater risk of CM (Gröhn et al., 1990; Houben et al., 1993). We also know that cows with CM will begin losing milk weeks before CM is identified, and how far back the loss begins is dependent on the pathogen involved (Gröhn et al., 2004). Therefore, we analyzed primipara and multipara separately and previous milk yield was defined as milk yield from 2 wk before the current week in milk.

While the statistical models were developed by parity, the figures in this paper are based on pooled observations (not predictive values from the statistical models). This was done to observe general trends without stratification of the data and to also compare the results from the statistical models with the raw data.

We found the most common pathogens isolated were *Strep.* spp., *E. coli* and no important growth. This mirrors the ranking of the most common pathogens identified from our previous studies (Schukken et al., 2009; Hertl et al., 2010; Hertl et al., 2011). And as shown in other studies, we found cows were at greater risk of CM in general as they grew older (Sargeant et al., 1998; Steeneveld, 2008; Petrovski et al., 2009).

Our results indicate that cows with more cases of CM in the previous lactation were at greater risk of bacteria specific CM in the current lactation. Similarly, Houben et al. (1993) reported findings of an increased risk of mastitis infections due to infections in the previous lactation ranging from a factor of 2.0 (one mastitis case) to 2.9 (3 or more cases). In that study however, unlike our study, CM cases were defined differently and at the quarter level. The effect of carryover we see in our study may be due to infections from the dry period that are having an effect in the subsequent lactation (Green et al., 2002); however, because we did not stratify carryover cases by when they occurred in lactation, it is unclear if this is the sole reason for our findings.

The pathogens involved in the persistence of CM infections and the effect these have on subsequent infections have been studied extensively (Vaarst and Enevoldsen, 1997; Döpfer et al., 1999; Green et al., 2002). In our study, we examine repeat cases of CM based on the bacteria causing the previous case and the time delay in between. We found multiparous cows were at greater risk of a second case of CM if they had suffered from a first case of CM that was caused by the same organism as the second case. For third cases, however, a second case of CM caused by both the same and a different pathogen as the third case generally put cows at greater risk of a third case, compared with if they had either the same or a different pathogen as the third case. For multiparous cows, the general trend we found was that cows were at greater risk of a recurrent case within the first month after the previous case of CM, unlike primipara, which were at greater risk of a recurrent case within 2 months of the previous case. Our results clearly suggest that a previous case of CM does not protect against a subsequent case. This is supported by the findings of Schukken et al. (2009); a previous case of gram-negative or gram-positive mastitis did not protect against a subsequent case of CM with the same Gram-stain classification.

For both types of CM, the incidence approximately doubled when a previous case was experienced, indicating there is no evidence for a protective immunological memory due to a previous exposure to an organism in the same generic class. In the study by Steeneveld et al., (2008), the incidence risk of CM for a multiparous cow in month ≥2 increased with increases in the accumulated number of CM cases in (1) the previous month in lactation [0 vs ≥2 cases; OR=3.12; 95% CI (1.35-8.81)] and also (2) the month in lactation before the previous month in lactation [0 vs ≥2 cases; OR=1.82; 95% CI (1.63-5.07)]. The specific pathogen involved in the previous case was not delineated; however, the results show that previous cases within the same lactation were positive risk factors for the incidence of CM.

Similar conclusions have also been drawn from infections involving *S. uberis* and *S. aureus* mastitis (Zadoks et al., 2001a; Zadoks et al., 2001b). Our results indicate that at the cow level, those cows with a previous case of the same pathogen or previous case with both the same and different pathogens put those cows at greater risk of a subsequent case. Zadoks et al. (2001a) found that quarters that had recovered from *S. uberis* (Zadoks et al., 2001b) or *S. aureus* mastitis had a higher rate of infection compared with quarters that had not experienced infection before, indicating that recovery from infection does not confer immunity to reinfection with the same pathogen, and in fact, was a risk factor for reinfection with the same and also different bacterial species. Although persistence of single cases of CM were not addressed directly in this study, it may be that the clinical signs observed were a part of an underlying subclinical process that was chronic in nature, which could be applicable to some of the *Strep.* spp. cases in our study. In a study by Zadoks et al., (2003), it was shown that infections starting as clinical cases are significantly shorter (median of 13 d) compared with those infections that start subclinically (median of 45 d), and infections with a subclinical onset and clinical flare-ups are even longer

(median=90 d). Under our case definition of CM, it is therefore possible that the separate cases we observed are indications of a disease process that is chronic in nature. This may also lead to a longer window of opportunity for spread of infection from cow-to-cow (Zadoks et al., 2003), and in part explain the large numbers of *Strep.* spp. cases seen in this study. Further, Milne et al., (2005) showed that 96% (24 out of 25) of the cases of *S. uberis* that failed to respond to conventional treatment were persistent infections with one strain rather than reinfections with different strains. The persistent infections of *Strep.* spp. observed in our study could be attributable to strains that are different to recurrent infections; however, because we did not examine the *Streptococcus* isolates at the species or strain level this is not conclusive.

Much attention has been given to the reason for persistent infections, specifically the biological mechanisms that facilitate an infection from being persistent compared with being transient. Dogan et al. (2006) found that the number of all persistent *E. coli* strains and one transient strain almost doubled during 48 h whereas transient *E. coli* strains and the non-pathogenic *E. coli* strain DH5α decreased over 48 h within cultured mammary epithelial cells. The results of this study support an intracellular niche in persistent *E. coli* mastitis; invasion of cultured mammary epithelial cells involved host cell cytoskeletal rearrangement and intracellular signaling cascades. White et al. (2009) modeled the dynamics of intramammary *E.coli* infections in dairy cows with the aim of understanding the mechanisms that distinguish transient from persistent infections, where the risk of movement of *E. coli* to and from milk to intra-cellular reservoirs were used to parameterize each type of infection. The authors found the predicted minimum level of bacteria present was sensitive to changes in the duration of survival in the intracellular reservoir; long durations of survival corresponded with higher bacterial counts following the acute phase, indicating a failure to spontaneously clear the infection which then led

to a persistent infection. The authors proposed that bacterial strains that result in persistent infections are more effective at surviving in an intra-cellular reservoir in mammary epithelial cells. A study by Vaarst and Enevoldsen (1997) found that *Streptococcus dysgalactiae* causing mastitis (comprising 9% of cases) was typically persistent, virulent and manifested in periods of lower cow resistance; quarter abnormalities before the incident were positively related to mastitis cases involving this organism, suggesting that it caused severe damage to the udder tissue.

Atalla et al. (2008) argue that the difficulty in eliminating *Staphylococcus aureus* mastitis may be due partly to the ability of this organism to exist as small colony variants (SCVs). The persistence of the intramammary infection may be related to the ability of SCVs to survive within the host cells causing minimal perturbation in cell physiology and minimal stimulation of the host defense system. It is protected against the host defense system and antimicrobial treatment by internalization of SCV within host cells. Although the slow-growing bacteria may release small amounts of antigens, it is not enough to induce an appropriate immune response, aiding the SCV to establish persistent infection within its host. These underlying biological mechanisms may explain the results we see in recurrent cases of *S. aureus* CM which can be translated to persistent infections across time.

The findings in our study relating to the absence of immunological memory across cases of pathogen specific CM is not directly comparable with the premise behind vaccine development. The purpose of vaccines is to elicit an immune response which confers the cow to being protected against the pathogen of interest. The same losses to production and damage to the mammary gland are not elicited by a vaccination, the same way that it is in a real life infection. Due to the detrimental effects of *Staphylococcus aureus* mastitis, many attempts have

been made in developing a vaccine (Calzolari et al., 1997; Leitner et al., 2003; Wallemacq et al., 2012). Cows suffering from *S. aureus* mastitis experience damage to the mammary gland and a reduction in the quantity and quality of milk. Intracellular invasion protects the pathogen from the humoral immune response. Vaccination strategies to increase herd resistance to *S. aureus* exist, however, for these vaccines to be of real use, they need to work at increasing immunity without generating the same production limiting effects of the pathogen (Leitner et al., 2003; Wallemacq et al., 2012). The *Escherichia coli* J5 vaccine which has received much attention has been known to reduce the incidence of intramammary infections in addition to the associated clinical signs in first lactation heifers (Hogan et al., 1999) and multiparous cows (González et al., 1989; Hogan et al., 1992). In the study by Hogan et al., (1999), the severity and duration of clinical signs following intramammary challenge with a heterologous strain of *E. coli* were reduced in vaccinated heifers compared with placebo-injected heifers; this is just one example of the value of vaccines in generating an appropriate immune response, which cannot be directly compared with the lack of immunological immunity following a real infection. The effect of combinations of pathogens in a CM case, and their added risk to subsequent CM cases, as demonstrated in our study, suggests that the effects of pathogens are interactive and we may gain greater understanding of their effects by considering their synergy instead of viewing them as independent organisms in assessing their contribution to future CM cases.

A greater risk of recurrent cases of CM, may therefore be due to an immune-compromised state that cows may experience post-real life CM infection, but not post-vaccination. Depending on the pathogen involved, the effect on the cow will vary. Therefore, it makes sense that this risk was greater when cows had experienced either the same pathogen before, or the same pathogen in combination with a different pathogen; the previous exposure to

the same pathogen has damaged the udder in such a way that infection with that pathogen again is less likely to be overcome. Further, we did not examine individual strains; it is possible that at the strain level, the same pattern of absence of immunological memory does not hold.

The advantage of developing generalized linear mixed models with an assumed Poisson error distribution, as in this study, is that the risk of CM can be easily calculated from the parameter estimates in the tables by taking the exponential of the summation of the intercept + parameter estimates of interest. As an example, referring to Table 2.3, the risk of a first case of *Strep* spp. within the first 2 weeks of lactation 3 with 2 cases of CM in her previous lactation (carryover=2) is calculated by exp(-5.02 -0.27 + 0.46)= 0.008 or 0.8%.

A practical application of this study is that the risk of bacteria specific CM by case can be calculated depending on cow characteristics, while accounting for variability about the estimates. This can guide farmers in identifying which pathogens are more pervasive and require particular attention. To quantify the economic consequences of these bacteria causing CM, we are currently developing an economic model. The risk of bacteria specific CM from this study will be used to parameterize this economic model which will provide dairy farmers with economically optimal decisions for their diseased cows.

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CHAPTER 3

THE EFFECT OF REPEATED EPISODES OF BACTERIA SPECIFIC CLINICAL MASTITIS ON MORTALITY AND CULLING IN HOLSTEIN DAIRY COWS

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ABSTRACT

The objective of this study was to estimate the effect of a first and repeated cases of bacteria specific clinical mastitis (CM) on the risk of mortality and culling in Holstein dairy cows. The pathogens studied were *Streptococcus* spp., *Staphylococcus aureus*, *Staphylococcus* spp., *Escherichia coli*, *Klebsiella* spp., *Arcanobacterium pyogenes*, others and no growth. A Total of 50,166 lactations were analyzed from 5 large, high milk producing dairy herds in New York State from 2003/2004 until 2011. Generalized linear mixed models with a Poisson error distribution were used to study the effects of parity, month of lactation, CM, calving diseases, pregnancy status, current season and economic values on the risk of mortality and culling. Among first lactation cows, the presence of a first CM case generally exposed cows to a greater risk of mortality in the current month (compared with the absence of a first case). This was especially acute with a first case of *Klebsiella*, where cows were 4.57 (exp(1.52)= 4.57 [95% CI (2.75, 7.61)]) times more at risk of mortality, and with a first case of *E. coli* with cows 3.32 times more at risk (i.e., relative risk = exp(1.20)= 3.32, [95% CI (2.46, 4.48)]. In general the presence of a first or second case resulted in cows in parity ≥2 with a greater risk of mortality (compared with cows with no first or second case of bacteria specific CM in the current month in milk). This was greatest for cows with a first case of *Klebsiella*,; (exp (1.39)= 4.01 [95% CI (3.53, 4.57)]), followed by a second case of *Klebsiella*, (exp(1.14)=3.13 [95% CI (2.39, 4.10)]), then a first case of *E. coli* (exp(1.11) = 3.03 [95% CI (2.75, 3.35)]). In first parity cows, the risk of culling generally increased with the presence of a case of bacteria specific CM. This was observed among cows with a first case of *T. pyogenes*, (exp (2.46)= 11.70 [95% CI (9.03, 15.18)]), a first case of *Klebsiella*, (exp(2.30)= 9.97 [95% CI (7.92, 12.55)]) and a first case of

Staphylococcus aureus, (exp(1.74)= 5.70 [95% CI (4.57, 7.10)]). Among cows of parity ≥2, when a cow contracted a case of *Streptococcus* spp., she was more likely to be culled one month after the case of CM (regardless of whether it was a first, second or third case of *Streptococcus* spp.). The risk of culling depending on the cow's characteristics can be easily calculated from the parameter estimates in provided tables.

Key words: mortality, culling, bacteria specific, mastitis

INTRODUCTION

Mastitis is one of the top three reasons for culling dairy cows, alongside lameness and failure to conceive (Esslemont and Kossaibati, 1997). The effects of mastitis include reduced conception (Hertl et al., 2010), losses to milk yield (Schukken et al., 2009), a higher risk of mortality (Bar et al., 2008a; Hertl et al., 2011) and, depending on the pathogen involved, significant treatment costs. In addition to the presence of disease, when making a decision, the dairy farmer considers milk yield, conception status, stage of lactation and parity (Gröhn et al., 1998; Rajala-Schultz and Gröhn, 1999). From this perspective, studies have been performed to identify the cost of mastitis and optimal economical management decisions (Halasa et al., 2009; Sørensen et al., 2010). Farmers may justify that it is economically optimal to replace their diseased cows with heifers, in anticipation of accrued losses to production over their projected lifespan (Houben et al., 1994; Bar et al., 2008b; Cha et al., 2011) and management of milk quality.

In order to estimate the cost of mastitis, it becomes necessary to separate those events that are beyond the control of the dairy farmer from those events that are within the dairy farmer's control. Mortality, i.e., death, is a forced event that occurs with a given probability, whereas culling, i.e., selling a cow, is a decision that may or may not be economically optimal for a dairy farmer to make depending on the specific cow involved (Houben et al., 1994; Bar et al., 2008a; Bar et al., 2008b).

The risk of mortality and culling is dependent on the pathogen that is causing the clinical mastitis (CM), the repeatability of cases, seasonality and cow characteristics. For example, a study of mortality among Danish dairy cattle showed that the most frequent specific diagnosis among udder/teat disorders resulting in death was septic mastitis caused by *Escherichia coli* (Thomsen et al., 2004). Hazlett et al. (1984) similarly found that *E. coli* followed by *Klebsiella* were the most common causes of fatal mastitis. The severity of mastitis has been shown to be time dependent; Bradley and Green (2001) demonstrated that *E. coli* mastitis had a tendency of being more severe between October and March than between April and September and a trend towards more severe mastitis occurring in the first 90 days of lactation than in the latter periods of lactation. The authors reason that this may be due to alterations in pathogenicity of the organisms present among environmental pathogens at various times of the year. This parallels the findings of Lehtolainen et al. (2003) where cows' responses, defined by local and systemic signs to intramammary infused *E. coli* endotoxin were significantly more severe in early lactation than late lactation. We have studied the risk of repeated cases of bacteria specific CM and have found that a previous case with the same pathogen will put cows at greater risk of a subsequent case (E. Cha, unpublished data). Earlier, Bradley and Green (2001) reported similar results i.e., repeat cases of CM occurred in 46 (16.4%) of affected quarters, and in 24 (8.6%) the

repeat cases were due to the same species of pathogens as the initial case. Although previous studies have examined the effects of repeated episodes of non-bacteria specific CM (Bar et al., 2008a), repeated episodes of gram-positive, gram-negative and other CM (Hertl et al., 2011) and a first case of pathogen specific CM on mortality and culling in dairy cows (Hazlett et al., 1984; Gröhn et al., 2005), no study has investigated the role of repeated cases of pathogen specific CM. Specifically, it is unclear if repeated cases of the same pathogen and if a shorter time interval between repeated cases put cows at a greater risk of being culled.

The objective of this study was to estimate the effect of a first and repeated cases of bacteria specific CM on the risk of mortality and culling in Holstein dairy cows.

MATERIALS AND METHODS

Herd Descriptions

Data from 50,166 lactations were assembled (18,420 of parity 1 and 31,746 of parity 2 and greater) from 23,409 cows. The data in this study were collected from 2003/2004 until 2011 (7-8 years) from 5 large dairy herds in New York State. The 305-d rolling herd average milk production ranged from 11,260 to 13,123 kg/cow per year, and the monthly mean SCC ranged from 137,000 to 262,000 cells/ml.

Cows were housed in freestalls. Feed was provided in the feed alleys with headlocks which facilitated the treatment and examination of cows. Cows were stratified by lactation, production, and reproductive status into feeding groups which were fed a total mixed ration. Cows were milked 3 times a day and the milking units automatically recorded milk production. Lactation, reproductive and medical information were entered into DairyComp305 herd management software (Valley Agricultural Software, Tulare, CA) by herd personnel. Information on parity, diseases, drying off, calving and exit from all herds was available as it was used by herd personnel for management of the dairy (Bar et al., 2008a; Hertl et al., 2011).

The variables relating to milk yield, mastitis culture results, diseases and reproduction necessary to conduct this study were exported to ASCII files from DairyComp305 and imported into SAS v. 9.2 (2008). The quality of the data was assessed through preliminary descriptive analyses of the variables of interest.

Case definition

All lactating cows in the 5 herds were eligible for inclusion in the study. Cows were identified as having CM based on (1) milkers observing clinical signs of CM, i.e., a warm, swollen udder or changes in milk color or consistency; otherwise, the remaining cases which were missed by milkers were identified by (2) herdspersons who examined cows due to elevated milk electrical conductivity in addition to a sudden concurrent milk loss as alerted by the farm computer system. The treatment protocol for diseased cows was similar across the 5 dairy herds and throughout the study. Herd personnel collected milk samples from quarters with signs of CM for microbiological culturing at the Quality Milk Production Services laboratories located in NY

state. The culture procedures are described in more detail Gröhn et al. (2004). Briefly, milk samples were plated by streaking 0.01mL on trypticase soy agar II with 5% sheep blood and 0.1% esculin (BBL; Becton Dickinson Microbiology Systems, Cockeyville, MD). Plates were incubated at 37°C for 48 h. Following observation of colony morphology and hemolytic patterns on blood agar, isolates were examined by means of 3% KOH, gram-staining organisms, catalase and oxidase testing, and additional biochemical and metabolic evaluations as required. Colony morphology on Mac Conkey agar and the BBL Crystal ID System (Becton Dickinson) identified gram-negative organisms. Streptococci that had a negative CAMP reaction were classified as *Streptococcus* spp. Staphylococci with β or αβ hemolytic patterns that had a positive tube test for free coagulase were classified as *Staph. aureus.* Nonhemolytic staphylococci with a positive tube coagulase test were further identified with the API Staph System (bio-Merieux Vitek, Hazelwood, MO). Coagulase-negative staphylococci were classified as *Staphylococcus* spp.

The unit of observation was month within the lactation of a cow. Some cows experienced two types of bacteria specific CM that were isolated within the same lactation within a few days of each other. If the second pathogen was isolated in the same quarter 5 or fewer days after the first pathogen (regardless of the pathogen isolated) or occurred within 14 d with the same pathogens isolated, it was considered to be the same case of mastitis. Any mastitis case after 14 d since the previous mastitis case was considered a new case (Barkema et al., 1998).

If a cow had multiple quarter infections at the same time (e.g., *E. coli* in the left rear quarter and *Staphylococcus* spp. in the right front quarter, or both pathogens in the same quarter), she was considered to have both *E. coli* and *Staphylococcus* spp. isolated within the one case (Hertl et al., 2011).

Bacteria specific CM

The bacteria causing CM studied were *Streptococcus* spp., *Staphylococcus aureus*, *Staphylococcus* spp., *Escherichia coli, Klebsiella, Trueperella pyogenes,* other CM (i.e., pathogens other than those listed above*),* and no growth. No growth was defined as either no growth on the culture plate, contaminated sample (more than two bacterial organisms on same plate) or no significant organisms. The latter, no significant organisms, were defined as no bacterial growth of either *Staphylococcus aureus* or *Streptococcus agalactiae* while the culture plate contained more than two different species.

Other Diseases

Five other diseases were included as potential confounders. These diseases are among the most common clinical conditions universally problematic in dairy cows (Gröhn et al., 2004). The 5 diseases included were milk fever, retained placenta, metritis, ketosis and displaced abomasum (DA). They were defined as follows: 1) milk fever occurred if a cow was unable to rise or had cool extremities and sluggish rumen motility near the time of calving, but was treated successfully with calcium, 2) retained placenta was retention of fetal membranes for at least 24 h post calving, 3) metritis involved a febrile state accompanying a purulent or fetid vaginal discharge or diagnosis of an enlarged uterus by veterinary palpation, 4) ketosis was diagnosed by a drop in feed intake and milk production with detection of ketones in milk, urine or breath and no other concomitant diseases and with response to treatment, and 5) DA occurred when the abomasum was enlarged with fluid, gas or both and was mechanically trapped in either the left or

right side of the abdominal cavity; nearly every DA case was confirmed by surgery, but cows removed from the herd without treatment were also recorded. Every effort was made to ensure that disease definition and diagnostic criteria were consistent across herds and written disease definitions were provided to dairy producers and veterinarians involved to assist with diagnosis.

Statistical Methods

The GLIMMIX procedure of SAS v. 9.2 (SAS Institute, 2008) was used to study the effect of bacteria specific CM on the risk of mortality and culling. Variables for inclusion were selected based on univariate analysis where a P-value ≤ 0.20 was considered significant using stepwise barkward elimination; variables of biological importance were also kept. The form of the generalized linear mixed model used was

$$\text{Ln}(\mathbf{Y}) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \ldots + \beta_k x_k + \mathbf{Z}\gamma + \varepsilon, \qquad [1]$$

where Ln is a link function, here the natural log of the probability of a cow dying or being culled in a month; **Y** is the vector of observations (either dying or being culled in a given month); β_0 is the regression parameter for the intercept, and $\beta_1, \beta_2, \beta_3 \ldots, \beta_k$ are the regression coefficients for the fixed effects $x_1, x_2, x_3 \ldots, x_k$ (described below); γ is an unknown vector of random-effect parameters with known design matrix **Z**, and ε, a presumed independent random Poisson distributed residual term. Fixed effects in our models were parity with 3 levels (for multiparous cows, i.e., second, third and fourth and greater in lactation), stage of lactation (months 1 to 10);

current season with four levels (Summer (Jun to Aug), Fall (Sep to Nov), Winter (Dec to Feb) and Spring (Mar to May)), pregnancy status (yes, no), other diseases with 2 levels (presence or absence), and non-bacteria specific CM indices. The selection of these periods are informative but also based upon needs of a later economic model to be constructed using the estimates. For the culling analysis, fixed effects included monthly profitability and monthly net replacement cost. Monthly profitability was defined to be monthly milk price – monthly operating cost. Milk prices were obtained from the USDA (USDA-National Agricultural Statistics Service, 2012) for each month of observation in the dataset, and are wholesale prices ($/cwt.) received by New York farmers. Operating costs were obtained from the USDA (USDA-Economic Research Service, 2010; USDA-Economic Research Service, 2012) for each month of observation and are the monthly dairy costs of production per hundredweight of milk sold for New York farmers. Monthly net replacement cost was defined as monthly purchase price of replacement – monthly cull price of cow. Purchase prices of replacements were obtained from the USDA (USDA-National Agricultural Statistics Service, 2012) for each month of observation. More details can be found in Hertl et al. (2011).

For the mortality analyses, we were particularly interested in estimating the risk of mortality based on the presence or absence of pathogen specific CM by case (1, 2 and 3) in the current month in milk, whereas in the culling analysis, we were also interested in observing whether the risk of culling was not only greatest in month of bacteria specific CM occurrence by case, but also how the risk varied as months passed since the case of bacteria specific CM. Models with a binomial, poisson and negative binomial distribution, as well as covariate coding schemes in accordance with the above were developed. In instances where such a detailed covariate coding scheme did not allow model convergence in any of these 3 different

distributions, alternative covariate coding schemes were explored. The best models were selected, based on whether convergence could be attained and model fit (-2 Log likelihood and Pearson or Generalized Chi Square/df). The models with the best fit are presented in this paper (with fit statistics reported in the results section).

For the mortality analysis of primiparous cows, each bacteria specific CM had two separate binary variables (a) 1 = presence of a 1st case of bacteria specific CM in the current month of lactation and 0 = absence of that bacteria specific CM in the current month of lactation and (b) 1 = presence of a second or third case of bacteria specific CM in the current month of lactation and 0 = absence of a second or third case of bacteria specific CM in the current month of lactation. For the mortality analysis of multiparous cows, each bacteria specific CM by case had a binary variable i.e. 1 = presence of a 1st case of bacteria specific CM in the current month of lactation and 0 = absence of that bacteria specific CM in the current month of lactation (Table 3.3.1).

For the culling analysis of primiparous cows, each case of bacteria specific CM had 2 binary variables i.e., (a) 1 = presence of a 1st case of bacteria specific CM in the current month of lactation and 0 = absence of that bacteria specific CM in the current month of lactation and (b) 1 = one or more months have passed since the 1st case of bacteria specific CM and 0 = the cow had a 1st CM case of that bacteria specific CM (or if it is before that CM) or the cow did not have that CM.

Lastly, the bacteria specific CM indices for the culling analysis of multiparous cows had, for each CM index variable and each case (e.g., *Streptococcus* spp. case 1, *Streptococcus* spp. case 2, *Streptococcus* spp. case 3, *Staphylococcus aureus* case 1, *Staphylococcus aureus* case 2, *Staphylococcus aureus* case 3, *Staphylococcus* spp. case 1, *Staphylococcus* spp. case 2,

Staphylococcus spp. case 3 etc), 3 levels as for the non-bacteria specific CM levels for the mortality analysis described above (Table 3.3.2).

Table 3.3.1. Covariate coding scheme used in the (multiparous cows) mortality analysis: data for 3 example cows with bacteria specific clinical mastitis. (Only *Escherichia coli* and *Staphyloccoccus* spp. index variables are shown due to space restrictions)

Cow ID[1]	Month in milk	Month of first CM case	1st case pathogen[1]	Month of second CM case	2nd case pathogen[1]	Month of third CM case	3rd case pathogen[1]	Escherichia coli case 1[2]	Escherichia coli case 2[2]	Escherichia coli case 3[2]	Staph spp. case 1[2]	Staph spp. case 2[2]	Staph spp. case 3[2]	Month died	Died
1	1	2	EC	2	SP	-	NA	0	0	0	0	0	0	-	0
1	2	2	EC	2	SP	-	NA	1	0	0	0	1	0	-	0
1	2	2	EC	2	SP	-	NA	0	0	0	0	0	0	-	0
1	3	2	EC	2	SP	-	NA	0	0	0	0	0	0	-	0
1	4	2	EC	2	SP	-	NA	0	0	0	0	0	0	-	0
2	1	1	SP	-	NA	-	NA	0	0	0	1	0	0	4	0
2	2	1	SP	-	NA	-	NA	0	0	0	0	0	0	4	0
2	3	1	SP	-	NA	-	NA	0	0	0	0	0	0	4	0
2	4	1	SP	-	NA	-	NA	0	0	0	0	0	0	4	1
3	1	-	NA	-	NA	-	NA	0	0	0	0	0	0	-	0
3	2	-	NA	-	NA	-	NA	0	0	0	0	0	0	-	0
3	3	-	NA	-	NA	-	NA	0	0	0	0	0	0	-	0
3	4	-	NA	-	NA	-	NA	0	0	0	0	0	0	-	0
3	5	-	NA	-	NA	-	NA	0	0	0	0	0	0	-	0
3	6	-	NA	-	NA	-	NA	0	0	0	0	0	0	-	0
3	7	-	NA	-	NA	-	NA	0	0	0	0	0	0	-	0
3	8	-	NA	-	NA	-	NA	0	0	0	0	0	0	-	0
3	9	-	NA	-	NA	-	NA	0	0	0	0	0	0	-	0
3	10	-	NA	-	NA	-	NA	0	0	0	0	0	0	-	0

[1]Note that a max of 2 pathogens is listed because of space limitations. EC= *Escherichia coli*; SP= *Staphylococcus* spp. and NA= none

[2]The index variable for cases of CM had 2 levels: 0 = absence of that pathogen and case in the current month of lactation; 1 = presence of that pathogen and case in the current month of lactation

Table 3.3.2. Covariate coding scheme used in the (multiparous cows) culling analysis: data for 4 example cows with bacteria specific clinical mastitis (CM indices; only *Streptococcus* spp. case 2, *Streptococcus* spp. case 3, *Staphylococcus* spp. case 1, *Escherichia coli* case 2, *Klebsiella* case 1, no growth case 1, no growth case 2 and other case 1 are shown due to space restrictions)

Cow ID	Month in milk	Month of first CM case	Month of second CM case	Month of third CM case	Month sold	Index variable for *Streptococcus* spp. CM case 2[1]	Index variable for *Streptococcus* spp. CM case 3	Index variable for *Staphylococcus* spp. CM case 1	Index variable for *Escherichia coli* CM case 2	Index variable for *Klebsiella* CM case 1	Index variable for no growth CM case 1	Index variable for no growth CM case 2	Index variable for other CM case 1	Sold
1	1	1	2	-	-	10	10	10	10	10	1	10	10	0
1	2	1	2	-	-	10	10	10	1	10	2	10	10	0
1	3	1	2	-	-	10	10	10	2	10	3	10	10	0
1	4	1	2	-	-	10	10	10	3	10	3	10	10	0
1	5	1	2	-	-	10	10	10	3	10	3	10	10	0
1	6	1	2	-	-	10	10	10	3	10	3	10	10	0
1	7	1	2	-	-	10	10	10	3	10	3	10	10	0
1	8	1	2	-	-	10	10	10	3	10	3	10	10	0
1	9	1	2	-	-	10	10	10	3	10	3	10	10	0
1	10	1	2	-	-	10	10	10	3	10	3	10	10	0
2	1	2	2	3	4	10	10	10	10	10	10	10	10	0
2	2	2	2	3	4	10	10	10	10	10	1	1	10	0
2	3	2	2	3	4	10	1	10	10	10	2	2	10	0
2	4	2	2	3	4	10	2	10	10	10	3	3	10	1
3	1	-	-	-	3	10	10	10	10	10	10	10	10	0
3	2	-	-	-	3	10	10	10	10	10	10	10	10	0
3	3	-	-	-	3	10	10	10	10	10	10	10	10	1
4	1	3	3	8	-	10	10	10	10	10	10	10	10	0
4	2	3	3	8	-	10	10	10	10	10	10	10	10	0
4	3	3	3	8	-	1	10	10	10	10	10	10	1	0
4	4	3	3	8	-	2	10	10	10	10	10	10	2	0
4	5	3	3	8	-	3	10	10	10	10	10	10	3	0
4	6	3	3	8	-	3	10	10	10	10	10	10	3	0
4	7	3	3	8	-	3	10	10	10	10	10	10	3	0
4	8	3	3	8	-	3	1	10	10	10	10	10	3	0
4	9	3	3	8	-	3	2	10	10	10	10	10	3	0
4	10	3	3	8	-	3	3	10	10	10	10	10	3	0

[1] The index variable for cases of bacteria specific CM (*Streptococcus* spp., *Staphylococcus aureus*, *Staphylococcus* spp., *Escherichia coli*, *Klebsiella*, *Trueperella pyogenes*, no growth, other by case, although not all are shown here) had 3 levels: 1 = case of CM occurring in the current month of lactation; 2 = case of CM occurring in the previous month (in the same lactation); 3 = case of CM occurring ≥ 2 months ago (in the same lactation)

We analyzed the occurrence of CM per cow-month at risk. Because we used such a short time-period as our unit of analysis, the distinction between risk and rate diminishes. The relationship between risk (cumulative incidence) and rate is given by:

Cumulative Incidence = 1 – exp(-Incidence * delta t), where delta t would be the time unit of cow-months. For a small cumulative incidence, a good approximation is Incidence*delta t, and where delta t in our models equals 1, and where essentially the cumulative incidence and incidence can then be used interchangeably. For that reason we will use risk as a measure of disease occurrence throughout this paper (Rothman, 1986).

Because we were not interested in specific herds, but rather herds in general with the common characteristics of being large, high-milk producing dairy herds with a low incidence of contagious mastitis, we always attempted to include herd as a random effect. Observation periods were censored at a typical lactation of 10 months, or when cows died or were culled. A sample coding scheme for the mortality (culling) analysis is illustrated in Table 3.3.1 (Table 3.3.2).

For the mortality dataset, 2 models were fitted: (1a) effects of risk factors (current season, month of lactation, bacteria specific CM cases, pregnancy status, other diseases) on the risk of mortality in primipara; (1b) effects of risk factors (parity, current season, month of lactation, bacteria specific CM cases, pregnancy status, other diseases) on risk of mortality in multipara. For the culling dataset, 2 models were fitted: (2a) effects of risk factors (month of lactation, bacteria specific CM cases including time since previous case, pregnancy status, other diseases, economic values) on the risk of culling in primipara; (2b) effects of risk factors (parity, season,

month of lactation, bacteria specific CM cases, pregnancy status, other diseases, economic values) on the risk of culling in multipara. Not all models retained the variables listed in brackets; a P-value below 0.05 was considered significant.

RESULTS

Descriptive findings

The number of observations were 416,016 months, from, 50,166 lactations (18,420 of parity 1 and 31,746 of parity 2 and greater in 23,409 cows) from 5 large dairy herds in New York State. In primipara (multipara) there were 3,712 (11,388) first cases, 959 (4,352) second cases and 300 (1,816) third cases of CM. The median day in milk for mastitis occurrence for each case was greater for primiparous cows compared with multiparous cows. This was also the trend for the median day in milk of mortality (culling); in primipara, 100 (269) compared with 74 (207) in multipara (Table 3.3). The most common pathogens in both groups were *Streptococcus* spp., *E. coli* and no growth (Table 3.4).

Table 3.3. Number of lactations, and median day in milk of occurrence of a first, second, or third case of clinical mastitis (CM), mortality (dying) or being culled (sold) in the first 10 months of lactation, in 5 New York State Holstein herds

	First lactations (n=18,420)		Second and greater lactations (n=31,746)	
	Number	Median day in milk	Number	Median day in milk
First case of CM	3,712	128	11,388	103
Second case of CM	959	200	4,352	159
Third case of CM	300	226	1,816	190
Mortality	727	100	2,855	74
Culling	2,523	269	9,476	207

Table 3.4. Distribution of pathogens causing clinical mastitis (CM) in 5 New York State dairy herds by lactation (1st lactation and ≥2 lactations) [1]

Lactation and pathogen	Number (Lactation incidence risk %)
First lactation (n= 18,420) [2]	
Streptococcus spp.	1,185 (6.4)
Staphylococcus aureus	393 (2.1)
Staphylococcus spp.	392 (2.1)
Escherichia coli	1,046 (5.7)
Klebsiella	278 (1.5)
Trueperella pyogenes	139 (0.8)
Citrobacter	0 (0)
Enterobacter	1 (0.005)
Pseudomonas	10 (0.05)
Mycoplasma	73 (0.4)
Corynebacterium bovis	30 (0.2)
Yeast	87 (0.5)
Miscellaneous	677 (3.7)
Contamination	61 (0.3)
Unknown[3]	378 (2.1)
No growth[4]	1,141 (6.2)
Second and greater lactations (n= 31,746) [5]	
Streptococcus spp.	4,275 (13.5)
Staphylococcus aureus	974 (3.1)
Staphylococcus spp.	1,119 (3.5)
Escherichia coli	4,040 (12.7)
Klebsiella	1,844 (5.8)
Trueperella pyogenes	332 (1.0)
Citrobacter	35 (0.1)
Enterobacter	50 (0.2)
Pseudomonas	22 (0.07)
Mycoplasma	76 (0.2)
Corynebacterium bovis	36 (0.1)
Yeast	216 (0.7)
Miscellaneous	2,133 (6.7)
Contamination	218 (0.7)

Unknown[2]	1,073 (3.4)
No growth[3]	4,363 (13.7)

[1]Total number of CM cases (first, second and third) in which the pathogen was identified. A cow may have more than one pathogen in one case. In the table
above, both are accounted for.
[2]The number of cows that started their first lactation
[3]The etiologic agent was not identified in the cultured sample
[4]No bacterial growth above the level of detection of our microbiological procedures was observed in the cultured sample; however, these cases exhibited clinical signs of mastitis
[5]The number of cows that started their second or greater lactation

Effect of bacteria specific CM on mortality in primiparous cows

The fit statistics for the model estimated were as follows: -2 Log likelihood of 6744.03 and Pearson chi-square/df of 0.95. The presence of a first case in the current month in milk (compared with the absence of a first case) generally put cows at greater risk of mortality in that month; this was seen particularly with a first case of *Klebsiella*, where cows were exp(1.52)= 4.57 [95% CI (2.75, 7.61)] times more at risk of mortality than cows without a first case of *Klebsiella* (Table 3.5). This was followed by a first case of *E. coli* (relative risk = exp(1.20)= 3.32 [95% CI (2.46, 4.48)] compared with the baseline of the absence of a first case of *E. coli* in the current month in milk). Cows with a second or third case of *E. coli* were exp (1.1)= 3.00 [95% CI (1.42, 6.36)] times more at risk of mortality compared with cows without a second or third case of *E. coli* in the current month in milk. Cows with retained placenta or displaced abomasum were also at greater risk of mortality i.e., exp(1)= 2.72 [95% CI (2.32, 3.19)] and exp(1.10)= 3.00 [95% CI (2.59, 3.49)], respectively. Pregnancy had a protective effect i.e., exp(-1.34)= 0.26 [95% CI (0.23, 0.30)].

Table 3.5. Effects of the first 3 pathogen specific clinical mastitis (CM) cases and other factors on mortality in primiparous cows (18,420 lactations) in 5 New York State dairy farms over the first 10 months of lactation[1]

Parameter	Estimate (SE)
Intercept	-4.61 (0.13)***
Fall[2]	-0.08 (0.12)
Spring	-0.43 (0.13)***
Summer	-0.05 (0.11)
Winter (baseline)	0
Month 1 of lactation (baseline)	0
Month 2 of lactation	-1.16 (0.14)***
Month 3 of lactation	-1.57 (0.20) ***
Month 4 of lactation	-1.09 (0.18) ***
Month 5 of lactation	-1.69 (0.26) ***
Month 6 of lactation	-0.83 (0.20) ***
Month 7 of lactation	-1.04 (0.22) ***
Month 8 of lactation	-0.78 (0.21) ***
Month 9 of lactation	-0.77 (0.22) ***
Month 10 of lactation	-0.60 (0.22) ***
First CM case (*Streptococcus.* spp.) occurring in the current month of lactation	0.22 (0.42)
No first CM case (*Streptococcus.* spp.) in the current month (baseline)	0
Second or third CM case (*Streptococcus.* spp.) occurring in the current month of lactation	-3.17 (6.10)
No second or third CM case (*Streptococcus.* spp.) in the current month (baseline)	0
First CM case (*Escherichia coli*) occurring in the current month of lactation	1.20 (0.3)***
No first CM case (*Escherichia coli*) in the current month (baseline)	0

Second or third CM case (*Escherichia coli*) occurring in the current month of lactation	1.10 (0.75)*
No second or third CM case (*Escherichia coli*) in the current month (baseline)	0
First CM case (*Klebsiella*) occurring in the current month of lactation	1.52 (0.51)***
No first CM case (*Klebsiella*) in the current month (baseline)	0
Second or third CM case (*Klebsiella*) occurring in the current month of lactation	1.35 (1.04)
No second or third CM case (*Klebsiella*) in the current month (baseline)	0
First CM case (no growth[3]) occurring in the current month of lactation	-0.49 (0.71)
No first CM case (no growth) in the current month (baseline)	0
Second or third CM case (no growth) occurring in the current month of lactation	0.84 (0.72)
No second or third CM case (no growth) in the current month (baseline)	0
First CM case (other[4]) occurring in the current month of lactation	0.65 (0.35)**
No first CM case (other) in the current month (baseline)	0
Second or third CM case (other) occurring in the current month of lactation	1.06 (0.64)**
No second or third CM case (other) in the current month (baseline)	0
Pregnant (yes vs. no)	-1.34 (0.14) ***
Retained placenta (yes vs. no)	1.0 (0.16) ***
Displaced abomasum (yes vs. no)	1.10 (0.15) ***
Farm 1	-0.10 (0.18)

Farm 2	0.46 (0.12) ***
Farm 3	0.41 (0.13) ***
Farm 4	0.18 (0.15)
Farm 5	0

[1]Estimates were obtained from a generalized mixed model with a Poisson distribution
[2]The rate ratio is calculated as exp(-0.08)=0.92
[3]No bacteriological growth (i.e., no bacterial growth above the level of detection of our microbiological procedures) was observed in the cultured sample; however, these cases exhibited clinical signs of mastitis
[4]These included *Mycoplasma*, *Corynebacterium bovis*, yeast, miscellaneous, contamination and unknown CM
*** p<0.05 ** p<0.10 * p<0.15

Effect of bacteria specific CM on mortality in multiparous cows

The fit statistics for the model estimated were as follows: -2 Log likelihood of 24572.08 and Pearson chi-square/df of 0.98. A first or second case of *Streptococcus spp.* had a protective effective on the risk of mortality (evidenced by negative parameter estimates) (Table 3.6). In general, however, the presence of a first or second case put cows at greater risk of mortality (compared with the absence of a first or second case). This was greatest for cows with a first case of *Klebsiella* in the current month in milk (exp (1.39)= 4.01 [95% CI (3.53, 4.57)]), followed by a second case of *Klebsiella*, (exp(1.14)=3.13 [95% CI (2.39, 4.10)]), a first case of *E. coli* (exp(1.11)= 3.03 [95% CI (2.75, 3.35)]), a first case of *T. pyogenes* (exp(1.08)= 2.94 [95% CI (2.27, 3.82)]) and a third case of other CM (exp(1.03)= 2.80 [95% CI (1.92, 4.10)]). As for the primiparous cow analysis, pregnancy had a protective effect and other diseases (retained placenta, displaced abomasum and milk fever) increased the risk of mortality. The risk was greatest in the summer (compared with winter); exp(0.16) = 1.17, [95% CI (1.12, 1.23)].

Table 3.6. Effects of the first 3 pathogen specific clinical mastitis (CM) cases and other factors on mortality in multiparous cows (31,746 lactations) in 5 New York State dairy farms over the first 10 months of lactation[1]

Parameter	Estimate (SE)
Intercept	-4.51 (0.07)***
Parity 2 (baseline)	0
Parity 3	0.42 (0.05)***
Parity 4	0.72 (0.05)***
Fall[2]	-0.15 (0.06)***
Spring	-0.10 (0.06)*
Summer	0.16 (0.05)***
Winter (baseline)	0
Month 1 of lactation (baseline)	0
Month 2 of lactation	-0.86 (0.07)***
Month 3 of lactation	-0.86 (0.08) ***
Month 4 of lactation	-0.89 (0.09) ***
Month 5 of lactation	-0.86 (0.09) ***
Month 6 of lactation	-0.92 (0.10) ***
Month 7 of lactation	-0.72 (0.10) ***
Month 8 of lactation	-0.68 (0.11) ***
Month 9 of lactation	-0.74 (0.12) ***
Month 10 of lactation	-0.57 (0.11) ***
First CM case (*Streptococcus.* spp.) occurring in the current month of lactation[2]	-0.64 (0.22) ***
No first CM case (*Streptococcus.* spp.) in the current month (baseline)	0
Second CM case (*Streptococcus.* spp.) occurring in the current month of lactation	-0.74 (0.51)*
No second CM case (*Streptococcus.* spp.) in the current month (baseline)	0

Third CM case (*Streptococcus.* spp.) occurring in the current month of lactation	-2.37 (1.94)
No third CM case (*Streptococcus.* spp.) in the current month (baseline)	0
First CM case (*Staphylococcus aureus*) occurring in the current month of lactation	0.64 (0.28) ***
No first CM case (*Staphylococcus aureus*) in the current month (baseline)	0
Second CM case (*Staphylococcus aureus*) occurring in the current month of lactation	0.29 (0.59)
No second CM case (*Staphylococcus aureus*) in the current month (baseline)	0
Third CM case (*Staphylococcus aureus*) occurring in the current month of lactation	-2.33 (3.59)
No third CM case (*Staphylococcus aureus*) in the current month (baseline)	
First CM case (*Staphylococcus* spp.) occurring in the current month of lactation	0.29 (0.27)
No first CM case (*Staphylococcus* spp.) in the current month (baseline)	0
Second CM case (*Staphylococcus* spp.) occurring in the current month of lactation	1.02 (0.42)***
No second CM case (*Staphylococcus* spp.) in the current month (baseline)	0
Third CM case (*Staphylococcus* spp.) occurring in the current month of lactation	1.00 (0.74)
No third CM case (*Staphylococcus* spp.) in the current month (baseline)	0
First CM case (*Escherichia coli*) occurring in the current month of	1.11 (0.10) ***

lactation	
No first CM case (*Escherichia coli*) in the current month (baseline)	0
Second CM case (*Escherichia coli*) occurring in the current month of lactation	0.70 (0.24) ***
No second CM case (*Escherichia coli*) in the current month (baseline)	0
Third CM case (*Escherichia coli*) occurring in the current month of lactation	-0.21 (0.73)
No third CM case (*Escherichia coli*) in the current month (baseline)	0
First CM case (*Klebsiella*) occurring in the current month of lactation[2]	1.39 (0.13) ***
No first CM case (*Klebsiella*) in the current month (baseline)	0
Second CM case (*Klebsiella*) occurring in the current month of lactation	1.14 (0.27) ***
No second CM case (*Klebsiella*) in the current month (baseline)	0
Third CM case (*Klebsiella*) occurring in the current month of lactation	0.77 (0.48)*
No third CM case (*Klebsiella*) in the current month (baseline)	
First CM case (*Trueperella pyogenes*) occurring in the current month of lactation	1.08 (0.26) ***
No first CM case (*Trueperella pyogenes*) in the current month (baseline)	0
Second CM case (*Trueperella pyogenes*) occurring in the current month of lactation	1.17 (0.72)*
No second CM case (*Trueperella pyogenes*) in the current month (baseline)	0
Third CM case (*Trueperella pyogenes*) occurring in the current month of lactation	-2.44 (8.51)

No third CM case (*Trueperella pyogenes*) in the current month (baseline)	0
First CM case (no growth[3]) occurring in the current month of lactation	-0.14 (0.21)
No first CM case (no growth) in the current month (baseline)	0
Second CM case (no growth) occurring in the current month of lactation	-0.18 (0.36)
No second CM case (no growth) in the current month (baseline)	0
Third CM case (no growth) occurring in the current month of lactation	0.72 (0.41)**
No third CM case (no growth) in the current month (baseline)	0
First CM case (other[4]) occurring in the current month of lactation	0.54 (0.13) ***
No first CM case (other) in the current month (baseline)	0
Second CM case (other) occurring in the current month of lactation	0.09 (0.29)
No second CM case (other) in the current month (baseline)	0
Third CM case (other) occurring in the current month of lactation	1.03 (0.38)***
No third CM case (other) in the current month (baseline)	0
Pregnant (yes vs. no)	-1.17 (0.07) ***
Retained placenta (yes vs. no)	0.68 (0.07) ***
Displaced abomasum (yes vs. no)	0.48 (0.08) ***
Milk fever (yes vs. no)	1.69 (0.10) ***
Farm 1	-0.06 (0.09)
Farm 2	0.65 (0.06)***
Farm 3	0.67 (0.06)***
Farm 4	0.28 (0.07)***
Farm 5	0

[1]Estimates were obtained from a generalized mixed model with a Poisson distribution
[2]The rate ratio is calculated as exp(-0.15)=0.86
[3]No bacteriological growth (i.e., no bacterial growth above the level of detection of our microbiological procedures) was observed in the cultured sample; however, these cases exhibited clinical signs of mastitis
[4]These included *Mycoplasma*, *Corynebacterium bovis*, yeast, miscellaneous, contamination and unknown CM
*** p<0.05 ** p<0.10 * p<0.15

Effect of bacteria specific CM on culling in primiparous cows

The fit statistics for the model developed were as follows: -2 Log likelihood of 14790.97 and Pearson chi-square/df of 0.89. The risk of culling generally increased with the presence of a case of bacteria specific CM (compared with an absence of bacteria specific CM) (Table 3.7). This was observed among cows with a first case of *T. pyogenes* (exp (2.46)= 11.70 [95% CI (9.03, 15.18)]), a first case of *Klebsiella* (exp(2.30)= 9.97 [95% CI (7.92, 12.55)]), a first case of *Staphylococcus aureus* (exp(1.74)= 5.70 [95% CI (4.57, 7.10)]), a first case of *E. coli* (exp(1.21) =3.35 [95% CI (2.80, 4.01)]) and a third case of *Klebsiella* (exp (1.48)= 4.39 [95% CI (2.72, 7.10)]). We also observed a greater risk of culling 1 month after a case of CM (compared with (a) the cow not having experienced that CM, or (b) the cow was in the time period at or before that CM). For example, a month after a first case of *T. pyogenes*, the risk of culling was (exp(1.44)= 4.22 [95% CI (3.29, 5.42)]) times greater than the comparison group as described above. Pregnancy had a protective effect (exp(-1.87)= 0.15 [95% CI (0.14, 0.17)]) and cows with displaced abomasum were (exp(0.49)= 1.63 [95% CI (1.45, 1.84)]) times more at risk of being culled compared with cows without displaced abomasum. Culling risk was greater as cows were further in their lactation.

Table 3.7. Effects of the first 3 pathogen specific clinical mastitis (CM) cases and other factors on culling in primiparous cows (18,420 lactations) in 5 New York State dairy farms over the first 10 months of lactation[1]

Parameter	Estimate (SE)
Intercept	-4.42 (0.13)***
Fall[2]	0.0015 (0.08)
Spring	-0.11 (0.08)
Summer	0.07 (0.07)
Winter (baseline)	0
Month 1 of lactation (baseline)	0
Month 2 of lactation	0.07 (0.10)
Month 3 of lactation	0.10 (0.11)
Month 4 of lactation	-0.04 (0.12)
Month 5 of lactation	0.39 (0.12)***
Month 6 of lactation	0.30 (0.13)***
Month 7 of lactation	0.71 (0.12)***
Month 8 of lactation	0.74 (0.12) ***
Month 9 of lactation	0.87 (0.12) ***
Month 10 of lactation	1.06 (0.12) ***
First CM case (*Streptococcus* spp.) occurring in the current month of lactation	1.14 (0.18)***
No first CM case (*Streptococcus* spp.) in the current month (baseline)	0
1 or more months since first CM case (*Streptococcus* spp.)	0.57 (0.12) ***
It's not 1 or more months since first CM case (*Streptococcus* spp.) (baseline)	0
Second CM case (*Streptococcus* spp.) occurring in the current month of lactation	0.006 (0.60)
No second CM case (*Streptococcus* spp.) in the current month (baseline)	0

1 or more months since second CM case (*Streptococcus* spp.)	0.23 (0.23)
It's not 1 or more months since second CM case (*Streptococcus* spp.) (baseline)	0
Third CM case (*Streptococcus* spp.) occurring in the current month of lactation	0.72 (0.76)
No third CM case (*Streptococcus* spp.) in the current month (baseline)	0
1 or more months since third CM case (*Streptococcus* spp.)	-1.17 (0.73)*
It's not 1 or more months since third CM case (*Streptococcus* spp.) (baseline)	0
First CM case (*Staphylococcus aureus*) occurring in the current month of lactation	1.74 (0.22) ***
No first CM case (*Staphylococcus aureus*) in the current month (baseline)	0
1 or more months since first CM case (*Staphylococcus aureus*)	0.84 (0.18) ***
It's not 1 or more months since first CM case (*Staphylococcus aureus*) (baseline)	0
Second CM case (*Staphylococcus aureus*) occurring in the current month of lactation	-0.55 (1.03)
No second CM case (*Staphylococcus aureus*) in the current month (baseline)	0
1 or more months since second CM case (*Staphylococcus aureus*)	0.29 (0.33)
It's not 1 or more months since second CM case (*Staphylococcus aureus*) (baseline)	0
Third CM case (*Staphylococcus aureus*) occurring in the current month of lactation	0.90 (0.81)
No third CM case (*Staphylococcus aureus*) in the current month (baseline)	0

1 or more months since third CM case (*Staphylococcus aureus*)	0.55 (0.52)
It's not 1 or more months since third CM case (*Staphylococcus aureus*) (baseline)	0
First CM case (*Staphylococcus* spp.) occurring in the current month of lactation	0.28 (0.36)
No first CM case (*Staphylococcus* spp.) in the current month (baseline)	0
1 or more months since first CM case (*Staphylococcus* spp.)	0.35 (0.20)**
It's not 1 or more months since first CM case (*Staphylococcus* spp.) (baseline)	0
Second CM case (*Staphylococcus* spp.) occurring in the current month of lactation	-0.49 (1.02)
No second CM case (*Staphylococcus* spp.) in the current month (baseline)	0
1 or more months since second CM case (*Staphylococcus* spp.)	0.63 (0.31)***
It's not 1 or more months since second CM case (*Staphylococcus* spp.) (baseline)	0
Third CM case (*Staphylococcus* spp.) occurring in the current month of lactation	-4.52 (12.5)
No third CM case (*Staphylococcus* spp.) in the current month (baseline)	0
1 or more months since third CM case (*Staphylococcus* spp.)	-1.25 (1.03)
It's not 1 or more months since third CM case (*Staphylococcus* spp.) (baseline)	0
First CM case (*Escherichia coli*) occurring in the current month of lactation	1.21 (0.18) ***
No first CM case (*Escherichia coli*) in the current month (baseline)	0
1 or more months since first CM case (*Escherichia coli*)	0.45 (0.13) ***

It's not 1 or more months since first CM case (*Escherichia coli*) (baseline)	0
Second CM case (*Escherichia coli*) occurring in the current month of lactation	1.02 (0.37) ***
No second CM case (*Escherichia coli*) in the current month (baseline)	0
1 or more months since second CM case (*Escherichia coli*)	0.61 (0.23) ***
It's not 1 or more months since second CM case (*Escherichia coli*) (baseline)	0
Third CM case (*Escherichia coli*) occurring in the current month of lactation	0.96 (0.55)**
No third CM case (*Escherichia coli*) in the current month (baseline)	0
1 or more months since third CM case (*Escherichia coli*)	-0.66 (0.48)
It's not 1 or more months since third CM case (*Escherichia coli*) (baseline)	0
First CM case (*Klebsiella*) occurring in the current month of lactation[2]	2.30 (0.23) ***
No first CM case (*Klebsiella*) in the current month (baseline)	0
1 or more months since first CM case (*Klebsiella*)	0.30 (0.25)
It's not 1 or more months since first CM case (*Klebsiella*) (baseline)	0
Second CM case (*Klebsiella*) occurring in the current month of lactation	0.27 (0.73)
No second CM case (*Klebsiella*) in the current month (baseline)	0
1 or more months since second CM case (*Klebsiella*)	0.38 (0.32)
It's not 1 or more months since second CM case (*Klebsiella*) (baseline)	0
Third CM case (*Klebsiella*) occurring in the current month of	1.09 (0.77)

lactation	
No third CM case (*Klebsiella*) in the current month (baseline)	0
1 or more months since third CM case (*Klebsiella*)	1.48 (0.48) ***
It's not 1 or more months since third CM case (*Klebsiella*) (baseline)	0
First CM case (*Trueperella pyogenes*) occurring in the current month of lactation	2.46 (0.26) ***
No first CM case (*Trueperella pyogenes*) in the current month (baseline)	0
1 or more months since first CM case (*Trueperella pyogenes*)	1.44 (0.25) ***
It's not 1 or more months since first CM case (*Trueperella pyogenes*) (baseline)	0
Second CM case (*Trueperella pyogenes*) occurring in the current month of lactation	1.39 (0.72)**
No second CM case (*Trueperella pyogenes*) in the current month (baseline)	0
1 or more months since second CM case (*Trueperella pyogenes*)	0.60 (0.43)
It's not 1 or more months since second CM case (*Trueperella pyogenes*) (baseline)	0
Third CM case (*Trueperella pyogenes*) occurring in the current month of lactation	0.83 (1.12)
No third CM case (*Trueperella pyogenes*) in the current month (baseline)	0
1 or more months since third CM case (*Trueperella pyogenes*)	0.16 (1.01)
It's not 1 or more months since third CM case (*Trueperella pyogenes*) (baseline)	0
First CM case (no growth[3]) occurring in the current month of lactation	1.07 (0.24) ***
No first CM case (no growth) in the current month (baseline)	0

1 or more months since first CM case (no growth)	0.80 (0.13) ***
It's not 1 or more months since first CM case (no growth) (baseline)	0
Second CM case (no growth) occurring in the current month of lactation	0.92 (0.31) ***
No second CM case (no growth) in the current month (baseline)	0
1 or more months since second CM case (no growth)	0.44 (0.20) ***
It's not 1 or more months since second CM case (no growth) (baseline)	0
Third CM case (no growth) occurring in the current month of lactation	1.11 (0.47) ***
No third CM case (no growth) in the current month (baseline)	0
1 or more months since third CM case (no growth)	0.06 (0.35)
It's not 1 or more months since third CM case (no growth) (baseline)	0
First CM case (other[4]) occurring in the current month of lactation	0.71 (0.20) ***
No first CM case (other) in the current month (baseline)	0
1 or more months since first CM case (other)	0.52 (0.12) ***
It's not 1 or more months since first CM case (other) (baseline)	0
Second CM case (other) occurring in the current month of lactation	0.41 (0.36)
No second CM case (other) in the current month (baseline)	0
1 or more months since second CM case (other)	0.04 (0.20)
It's not 1 or more months since second CM case (other) (baseline)	0
Third CM case (other) occurring in the current month of lactation	1.13 (0.41) ***
No third CM case (other) in the current month (baseline)	0
1 or more months since third CM case (other)	0.13 (0.34)
It's not 1 or more months since third CM case (other) (baseline)	0

Pregnant (yes vs. no)	-1.87 (0.07)***
Displaced abomasum (yes vs. no)	0.49 (0.12)***
Farm 1	-0.11 (0.10)
Farm 2	-0.11 (0.08)
Farm 3	0.42 (0.07)***
Farm 4	-0.002 (0.09)
Farm 5	0

[1]Estimates were obtained from a generalized mixed model with a Poisson distribution; monthly net replacement cost was also included but estimates are not shown

[2]The rate ratio is calculated as exp(0.0015)=1.00

[3]No bacteriological growth (i.e., no bacterial growth above the level of detection of our microbiological procedures) was observed in the cultured sample; however, these cases exhibited clinical signs of mastitis

[4]These included *Mycoplasma*, *Corynebacterium bovis*, yeast, miscellaneous, contamination and unknown CM

*** p<0.05 ** p<0.10 * p<0.15

Effect of bacteria specific CM on culling in multiparous cows

The fit statistics for the model developed were as follows: -2 Log likelihood of 1652172 and Generalized chi-square/df of 0.79. The risk of culling was greater for cows as they progressed through lactations, i.e., cows in parity 4+ were exp (0.71) = 2.03 [95% CI (1.9,2.2)] times more likely to be culled compared with cows in lactation 2 (the baseline) (Table 3.8). As for primiparous cows, the culling risk was greater as cows were further in their lactation. When a cow contracted a case of *Streptococcus* spp., she was more likely to be culled one month after the case of CM (regardless of whether it was a first, second or third case of *Streptococcus* spp.). Similar trends were seen for *Staphylococcus aureus* and no growth, whereas a first or second case of *E. coli* or *Klebsiella* put cows at risk of being culled in the same month of CM occurrence. Pregnant cows were less likely to be culled compared with non-pregnant cows and ketosis and displaced abomasum were risk factors for culling. As monthly profitability increased, the likelihood of a cow being culled increased slightly. A $1 increase resulted in a rise in risk of culling from exp(-3.48)= 0.031 to exp (-3.48 + 0.008 * 1) = 0.0311. As net replacement cost increased, the probability of culling decreased slightly. A $100 increase in net replacement cost demonstrated a reduction in risk of culling from exp(-3.48)= 0.031 to exp (-3.48 -0.0005 * 100) = 0.029.

Table 3.8. Effects of the first 3 pathogen specific clinical mastitis (CM) cases and other factors on culling in multiparous cows (31,746 lactations) in 5 New York State dairy farms over the first 10 months of lactation[1]

Parameter	Estimate (SE)
Intercept	-3.48 (0.14)***
Parity 2 (baseline)	0
Parity 3	0.38 (0.03)***
Parity 4+	0.71 (0.03)***
Fall	-0.06 (0.03)
Spring	-0.12 (0.03)**
Summer	-0.09 (0.03)*
Winter (baseline)	0
Month 1 of lactation (baseline)	0
Month 2 of lactation	-0.25 (0.05) ***
Month 3 of lactation	-0.06 (0.05)
Month 4 of lactation	0.18 (0.05) **
Month 5 of lactation	0.53 (0.05) ***
Month 6 of lactation	0.66 (0.05) ***
Month 7 of lactation	0.88 (0.05)***
Month 8 of lactation	1.14 (0.05)***
Month 9 of lactation	1.24 (0.05)***
Month 10 of lactation	1.24 (0.06)***
First CM case (*Streptococcus.* spp.) occurring in the current month of lactation[2]	0.33 (0.09)**
First CM case (*Streptococcus.* spp.) occurring in previous month	0.62 (0.08)***
First CM case (*Streptococcus.* spp.) occurring ≥2 months ago	0.31 (0.05)***
No first CM case (*Streptococcus.* spp.) in the current month (baseline)	0
Second CM case (*Streptococcus.* spp.) occurring in the current month of lactation	-0.14 (0.14)
Second CM case (*Streptococcus.* spp.) occurring in previous month	0.30 (0.13)*
Second CM case (*Streptococcus.* spp.) occurring ≥2 months ago	0.18 (0. 09)
No second CM case (*Streptococcus.* spp.) in the current month (baseline)	0

Third CM case (*Streptococcus.* spp.) occurring in the current month of lactation	-0.02 (0.20)
Third CM case (*Streptococcus.* spp.) occurring in previous month	0. 72 (0.17)**
Third CM case (*Streptococcus.* spp.) occurring ≥2 months ago	-0.38 (0.16)*
No third CM case (*Streptococcus.* spp.) in the current month (baseline)	0
First CM case (*Staphylococcus aureus*) occurring in the current month of lactation	0.81 (0.16)***
First CM case (*Staphylococcus aureus*) occurring in previous month	1.12 (0.14)***
First CM case (*Staphylococcus aureus*) occurring ≥2 months ago	0.11 (0.11)
No first CM case (*Staphylococcus aureus*) in the current month (baseline)	0
Second CM case (*Staphylococcus aureus*) occurring in the current month of lactation	0.62 (0.20)**
Second CM case (*Staphylococcus aureus*) occurring in previous month	0.60 (0.22)*
Second CM case (*Staphylococcus aureus*) occurring ≥2 months ago	0.22 (0.17)
No second CM case (*Staphylococcus aureus*) in the current month (baseline)	0
Third CM case (*Staphylococcus aureus*) occurring in the current month of lactation	0.14 (0.35)
Third CM case (*Staphylococcus aureus*) occurring in previous month	0.37 (0.34)
Third CM case (*Staphylococcus aureus*) occurring ≥2 months ago	0.08 (0.25)
No third CM case (*Staphylococcus aureus*) in the current month (baseline)	0
First CM case (*Staphylococcus* spp.) occurring in the current month of lactation	0.64 (0.14)***
First CM case (*Staphylococcus* spp.) occurring in previous month	0.33 (0.17)
First CM case (*Staphylococcus* spp.) occurring ≥2 months ago	0.13 (0.10)
No first CM case (*Staphylococcus* spp.) in the current month (baseline)	0
Second CM case (*Staphylococcus* spp.) occurring in the current month of lactation	-0.31 (0.29)
Second CM case (*Staphylococcus* spp.) occurring in previous month	0.23 (0.24)
Second CM case (*Staphylococcus* spp.) occurring ≥2 months ago	-0.15 (0.17)
No second CM case (*Staphylococcus* spp.) in the current month (baseline)	0
Third CM case (*Staphylococcus* spp.) occurring in the current month of lactation	0.44 (0.30)
Third CM case (*Staphylococcus* spp.) occurring in previous month	0.05 (0.39)
Third CM case (*Staphylococcus* spp.) occurring ≥2 months ago	-0.71 (0.41)
No third CM case (*Staphylococcus* spp.) in the current month (baseline)	0
First CM case (*Escherichia coli*) occurring in the current month of lactation	1.02 (0.07)***

First CM case (*Escherichia coli*) occurring in previous month	0.97 (0.07)***
First CM case (*Escherichia coli*) occurring ≥2 months ago	0.38 (0.05)***
No first CM case (*Escherichia coli*) in the current month (baseline)	
Second CM case (*Escherichia coli*) occurring in the current month of lactation	0.68 (0.11)***
Second CM case (*Escherichia coli*) occurring in previous month	0.61 (0.13)***
Second CM case (*Escherichia coli*) occurring ≥2 months ago	-0.14 (0.11)
No second CM case (*Escherichia coli*) in the current month (baseline)	0
Third CM case (*Escherichia coli*) occurring in the current month of lactation	0.51 (0.18)*
Third CM case (*Escherichia coli*) occurring in previous month	0.74 (0.20)**
Third CM case (*Escherichia coli*) occurring ≥2 months ago	0.004 (0.15)
No third CM case (*Escherichia coli*) in the current month (baseline)	0
First CM case (*Klebsiella*) occurring in the current month of lactation[2]	1.75 (0.07)***
First CM case (*Klebsiella*) occurring in previous month	1.30 (0.10)***
First CM case (*Klebsiella*) occurring ≥2 months ago	0.44 (0.08)***
No first CM case (*Klebsiella*) in the current month (baseline)	0
Second CM case (*Klebsiella*) occurring in the current month of lactation	0.95 (0.12)***
Second CM case (*Klebsiella*) occurring in previous month	0.56 (0.17)**
Second CM case (*Klebsiella*) occurring ≥2 months ago	0.06 (0.12)
No second CM case (*Klebsiella*) in the current month (baseline)	0
Third CM case (*Klebsiella*) occurring in the current month of lactation	0.61 (0.18)**
Third CM case (*Klebsiella*) occurring in previous month	0.67 (0.23)*
Third CM case (*Klebsiella*) occurring ≥2 months ago	0.05 (0.19)
No third CM case (*Klebsiella*) in the current month (baseline)	0
First CM case (*Trueperella pyogenes*) occurring in the current month of lactation	1.80 (0.14)***
First CM case (*Trueperella pyogenes*) occurring in previous month	1.81 (0.16)***
First CM case (*Trueperella pyogenes*) occurring ≥2 months ago	0.97 (0.13)***
No first CM case (*Trueperella pyogenes*) in the current month (baseline)	0
Second CM case (*Trueperella pyogenes*) occurring in the current month of lactation	0.88 (0.28)**
Second CM case (*Trueperella pyogenes*) occurring in previous month	1.58 (0.26)***

Second CM case (*Trueperella pyogenes*) occurring ≥2 months ago	-0.04 (0.31)
No second CM case (*Trueperella pyogenes*) in the current month (baseline)	0
Third CM case (*Trueperella pyogenes*) occurring in the current month of lactation	1.08 (0.37)*
Third CM case (*Trueperella pyogenes*) occurring in previous month	0.41 (0.90)
Third CM case (*Trueperella pyogenes*) occurring ≥2 months ago	-0.36 (0.54)
No third CM case (*Trueperella pyogenes*) in the current month (baseline)	0
First CM case (no growth[3]) occurring in the current month of lactation	0.65 (0.09)***
First CM case (no growth) occurring in previous month	0.72 (0.09)***
First CM case (no growth) occurring ≥2 months ago	0.24 (0.05)***
No first CM case (no growth) in the current month (baseline)	0
Second CM case (no growth) occurring in the current month of lactation	0.15 (0.13)
Second CM case (no growth) occurring in previous month	0.58 (0.12)***
Second CM case (no growth) occurring ≥2 months ago	0.07 (0.08)
No second CM case (no growth) in the current month (baseline)	
Third CM case (no growth) occurring in the current month of lactation	-0.12 (0.20)
Third CM case (no growth) occurring in previous month	1.02 (0.14)***
Third CM case (no growth) occurring ≥2 months ago	-0.12 (0.12)
No third CM case (no growth) in the current month (baseline)	0
First CM case (other[4]) occurring in the current month of lactation	0.59 (0.08)***
First CM case (other) occurring in previous month	0. 51 (0.09)***
First CM case (other) occurring ≥2 months ago	0.17 (0.06)**
No first CM case (other) in the current month (baseline)	0
Second CM case (other) occurring in the current month of lactation	0.38 (0.11)*
Second CM case (other) occurring in previous month	0.66 (0.11)***
Second CM case (other) occurring ≥2 months ago	0.08 (0.09)
No second CM case (other) in the current month (baseline)	
Third CM case (other) occurring in the current month of lactation	0.48 (0.15)**
Third CM case (other) occurring in previous month	0.46 (0.19)*
Third CM case (other) occurring ≥2 months ago	-0.21 (0.15)

No third CM case (other) in the current month (baseline)	0
Pregnant (yes vs. no)	-1.66 (0.03)***
Retained placenta (yes vs. no)	-0.37 (0.11)*
Ketosis (yes vs. no)	0.14 (0.04)*
Displaced abomasum (yes vs. no)	0.26 (0.05)**

[1]Estimates were obtained from a generalized mixed model with a Poisson distribution and random herd effect; monthly profitability, and monthly net replacement cost were also included but estimates are not shown
[2]The rate ratio is calculated as exp(0.33)=1.39
[3]No bacteriological growth (i.e., no bacterial growth above the level of detection of our microbiological procedures) was observed in the cultured sample; however, these cases exhibited clinical signs of mastitis
[4]These included *Mycoplasma*, *Corynebacterium bovis*, yeast, miscellaneous, contamination and unknown CM
*P <0.05 ; **P<0.01; *** P<0.001

The risk of culling depending on the cow's characteristics can be easily calculated from the parameter estimates in our tables by taking the exponential of the summation of the intercept + parameter estimates of interest. As an example, referring to Table 3.8, the risk of culling a cow that is in parity 4, month in milk 5, in summer with a first case of *E. coli* which occurred 1 month ago and a second case of *E. coli* in the current month in milk that is pregnant and without any other disease is calculated by exp(-3.48+0.71+0.53-0.09+0.97+0.68-1.66)= 0.096 or 9.6%.

DISCUSSION

The effect of CM on mortality and culling has been studied previously (Neerhof et al. 2000; Thomsen et al. 2004; Hertl et al., 2011); however, very few studies have examined the effect of bacteria specific CM on mortality and culling, and in particular, the effect of repeated cases of bacteria specific CM. While addressing these questions, we also took this one step further by demonstrating how culling risks are affected based on historical knowledge of the exact pathogens involved and cases a cow has experienced, e.g., the risk of culling for a cow that had a first case of *E. coli* that occurred 3 months ago and a second case of *E. coli* in the current month of lactation. This knowledge can help farmers identify which pathogens need particular attention and are responsible for the greatest culling. We have been able to quantify for the first time the combined effect of different cases of pathogens on the risk of culling, while also accounting for time since case of bacteria specific CM.

Based on the milk loss estimates for each pathogen by case which we have previously observed (J. Hertl, unpublished data), among multiparous cows, as a general rule, milk losses were greatest in the same week, or soon after the occurrence of any bacteria specific CM. The culling estimates of a cow that has a first or second case of *E. coli* or *Klebsiella* indicate greater risks of culling in the month in milk these occur, i.e., a farmer is less likely to wait to cull these cows, which reflect an action taken due to the immediate milk losses experienced or the anticipated losses. It is difficult to compare directly, as the milk loss models had a weekly time step, unlike the culling models which have a monthly time step. Hence it is possible that a case of CM could have occurred at the beginning or end of the month and the milk loss associated

with the CM case could be seen either within the same month, or soon after in the following month depending on when the CM occurred.

In primipara and multipara, displaced abomasum was a risk factor for mortality, as well as milk fever (among multipara). Among all cows, displaced abomasum was a risk factor for culling. We also found that the risks of culling among multipara were greater for the coliforms compared with *Staphylococcus* and *Streptococcus* spp. These findings are similar to those of Thomsen et al. (2004); based on data from the Danish Cattle Database and a questionnaire study, the authors identified the most frequent diagnosis among udder/teat disorders resulting in unassisted death or euthanasia to be septicemic mastitis caused by *E. coli.* Milk fever constituted the majority of diagnoses among metabolic disorders and left and right displaced abomasum constituted the majority of diagnoses among digestive disorders.

The increase in the risk of culling we observe from approximately month 6 (Tables 3.7 and 3.8) could be explained by pregnancy status having an effect on culling; however, in our current study, we were unable to estimate an interaction between month in milk and pregnancy status due to lack of convergence of our statistical models. It is, therefore, unclear if the increase in risk of culling is definitively due to the additional effect of conception status. In a study by Rajala-Schultz and Gröhn (1999), the authors identified that the later a farmer knew a cow was pregnant, the higher her risk of being culled. Dechow and Goodling (2008) showed that as lactation progressed, reproduction became a more common reason for disposal (and the proportion of cows that died or were injured declined). In a study by Esslemont and Kossaibati (1997), poor fertility was the single most important reason for involuntary culling regardless of the cow's age, with 36.5% of disposals due to poor fertility.

The culling risks that are seen at the beginning of lactation are likely to be due to the incidence of disease at the beginning of lactation. This is in agreement with the increase in risk of CM often seen at the beginning of lactation, soon after the dry period (Green et al., 2002). Dechow and Goodling (2008) found most cows culled by 60 DIM had compromised health or were injured. The increase in mortality also seen at the beginning of lactation may also be explained by the composition of cases in that period of time. For example, Bradley and Green (2001) reported a trend towards more severe mastitis occurring in the first 90 days of lactation (14 of 167 cases) than in the rest of lactation (4 of 140) (P=0.09).

We observed that the cows with CM in parity 1 had a drop in risk of culling between months 3 to 6. Similarly, Neerhof et al. (2000) report that the risk of culling due to mastitis increased with an increase in the stage of lactation, with the exception of diseased cows in parity 1 which had the lowest risk of culling in the second stage (60 – 180d) of lactation.

We did not include milk yield as it is an intervening variable between mastitis and culling, and in early lactation, cows have higher milk production so culling is less likely compared with late lactation when milk production tapers off (Gröhn et al., 2005). Although milk yield was not included in our models, by including the stage of lactation i.e., month in milk, this variable incorporated many factors including milk yield (Gröhn et al., 2005). From our parameter estimates, we observe that as month in milk increases, so does risk of culling.

The reason for creating binary variables for the mortality analysis (both primipara and multipara) and culling analysis (primipara) was because when we created a bacteria specific CM variable encompassing all cases, we discovered that we were not able to attain model convergence. The advantage of the latter coding scheme, however, is that the baseline remains

the same within a bacteria specific CM. For this reason, throughout this paper we have specified what baseline the relative risks are in comparison to, because depending on the pathogen and case, the baseline is not always the same.

CONCLUSIONS

This study quantifies the effect of bacteria specific CM, by case, on mortality and culling among primiparous and multiparous cows. This provides farmers with information relating to which specific pathogens contribute to mortality and culling and where focus needs to be placed for improvements in management practices. Increases in the risk of mortality and culling were observed across all pathogens, but particularly among cases of *Klebsiella*, *E. coli* and *T. pyogenes*, buttressing our understanding of the severity of these pathogens and their effects on the bovine udder. The mortality risks of these models will be incorporated into a bacteria specific economic optimization model, aimed at calculating the cost of bacteria specific CM, and providing economically optimal decisions for dairy farmers with diseased cows.

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CHAPTER 4

THE COST OF THREE DIFFERENT TYPES OF CLINICAL MASTITIS IN DAIRY COWS ESTIMATED BY DYNAMIC PROGRAMMING

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ABSTRACT

The objective of this study was to estimate the cost of 3 different types of clinical mastitis (CM) (due to gram-positive bacteria, gram-negative bacteria and other organisms) at the individual cow level and thereby identify the economically optimal management decision for each type of mastitis. We made modifications to an existing dynamic optimization and simulation model, studying the effects of various factors (incidence of CM, milk loss, pregnancy rate and treatment cost) on the cost of different types of CM. The average costs per case (USD) of gram-positive, gram-negative and other CM were 133.73, 211.03 and 95.31, respectively. This model provided a more informed decision making process in CM management for optimal economic profitability and determined that 93.1% of gram-positive CM cases, 93.1% of gram-negative CM cases and 94.6% of other CM cases should be treated. The main contributor to the total cost per case of gram-positive CM was treatment cost (51.5% of the total cost per case), milk loss for gram-negative CM (72.4%) and treatment cost for other CM (49.2%). The model affords versatility as it allows for parameters such as production costs, economic values and disease frequencies to be altered. Therefore, cost estimates are the direct outcome of the farm specific parameters entered into the model. Thus, this model can provide farmers economically optimal guidelines specific to their individual cows suffering from different types of CM.

Key Words. mastitis, gram-positive, gram-negative, dynamic programming

INTRODUCTION

Mastitis reduces dairy farm profitability with losses stemming from both milk production decreases and discarded milk, and treatment and culling costs (Gröhn et al., 2005). The specific inflammatory response from a mastitis incident is dependent on the bacterial species involved (Bannerman, 2008). Depending on the pathogen involved, the impact may vary, so studies determining which pathogens have the greatest impact on cow health, production and profitability are valuable (Gröhn et al., 2004).

Treatment for mastitis is the most common cause of antibacterial use on dairy farms. There are public concerns, however, of the possible health hazards posed by the presence of antibiotic residues and other drugs in milk (Erksine et al., 2003). This is despite all bulk tanks being tested for antibiotics. Antibiotic use also raises questions of reduced animal welfare and biosecurity (Sørensen et al., 2010).

A fundamental component of mastitis control programs is the identification of pathogens in mastitis samples. For example, the ability to determine whether a cow is suffering from gram-positive or gram-negative CM would help determine the choice of antimicrobial therapy (Waage et al., 1994) and potentially reduce unnecessary use of antibiotics.

Most pathogens which cause mastitis can be classified as gram-positive or gram-negative bacteria and determined by on-farm culturing, which is generally faster and more convenient than sending the milk sample to a commercial laboratory (Hertl et al., 2010). The on-farm culture

has an approximate 24 h turn-around time. The development of cow-side tests identifying whether a case of mastitis is gram-positive or gram-negative is ongoing (Waage et al., 1994; Yazdankhah, 2001). The objective of this study was to estimate the cost of different types of clinical mastitis (**CM**) (due to gram-positive bacteria, gram-negative bacteria and other organisms) and to determine the optimal management decision of whether it may or may not be economically optimal for a cow to be (1) replaced with a heifer, (2) kept in the herd (and treated if she has a CM case), but not inseminated or (3) kept (and treated if she has a CM case) and inseminated, for each type of CM. We did this by modifying an existing dynamic programming model previously used to study CM and other diseases in dairy cows (Bar et al., 2008a; Cha et al., 2010).

MATERIALS AND METHODS

Clinical mastitis categorization

We classified CM into 3 categories: (1) CM due to gram-positive bacteria, (2) CM due to gram-negative bacteria and (3) CM due to other organisms (hereafter, referred to as gram-positive CM, gram-negative CM and other CM).

Gram-positive CM included *Streptococcus* spp., *Staphylococcus aureus* and *Staphylococcus* spp. Gram-negative CM included *Escherichia coli*, *Klebsiella* spp., *Citrobacter* spp. and *Enterobacter* spp. Other CM included *Trueperella pyogenes*, *Mycoplasma* spp., *Corynebacterium bovis, Pseudomonas* spp. and yeast.

Replacements and inseminations optimization and simulation model

Software. The model was built using the Multi Level Hierarchic Markov Process (MLHMP) software as the application program interface (Kristensen, 2003). We modified an existing optimization and simulation model which was first developed to study the cost of generic CM in dairy cows, then 3 different types of lameness in dairy cows (Bar et al., 2008a; Cha et al., 2010).

The model. The model was constructed as a 3-level hierarchic Markov process comprised of: the founder (parent) level containing state variables of permanent traits throughout the cow's life span, the child level divided into stages representing one whole lactation, and the grandchild level divided into stages of one month during lactation. The possible actions in a given month of lactation that could occur at the final level are: (1) replace the cow with a calving heifer, (2) keep the cow for another month without insemination and treat her if she has CM or (3) keep the cow for another month and inseminate her and treat her if she has CM (Bar et al., 2008a). Figure 4.1 is a schematic representation of the model used in the current study on CM. At the founder level, 5 milk yield categories (kg) were modeled as: -5, -2.5, 0, +2.5, and +5 from the mean level of milk production per day; these represented the cow's genetic potential. At the child level, 8 possible whole lactation stages were modeled. At the grandchild level, 20 lactation stages (mo) were modeled. In each stage the cow was described by one level within each of the following states: 5 temporary (i.e., daily) milk yield levels, 9 pregnancy states (0 = open, 1-7 = 1-7 mo pregnant and milking and 8 = last 2 mo of pregnancy and dry (not milking)), 1 involuntarily culled state and 13 CM states. The CM states were defined as: 0 = no CM, 1 = first occurrence of gram-positive CM (observed at the end of the stage enabling immediate culling with no loss to treatment or production), 2, 3 and 4 corresponding to 1, 2, 3 and more mo after the first case of gram-positive CM (this does not mean reoccurrence, but rather time horizon since the first case

of gram-positive CM), respectively, 5 = first occurrence of gram-negative CM and 9 = other CM (with numbers from 6-8 and 10-12 corresponding to 1, 2, 3 and more mo after the first case of the CM type, respectively, and again, this does not mean reoccurrence, but rather time horizon since the first case of gram-negative or other CM, respectively).

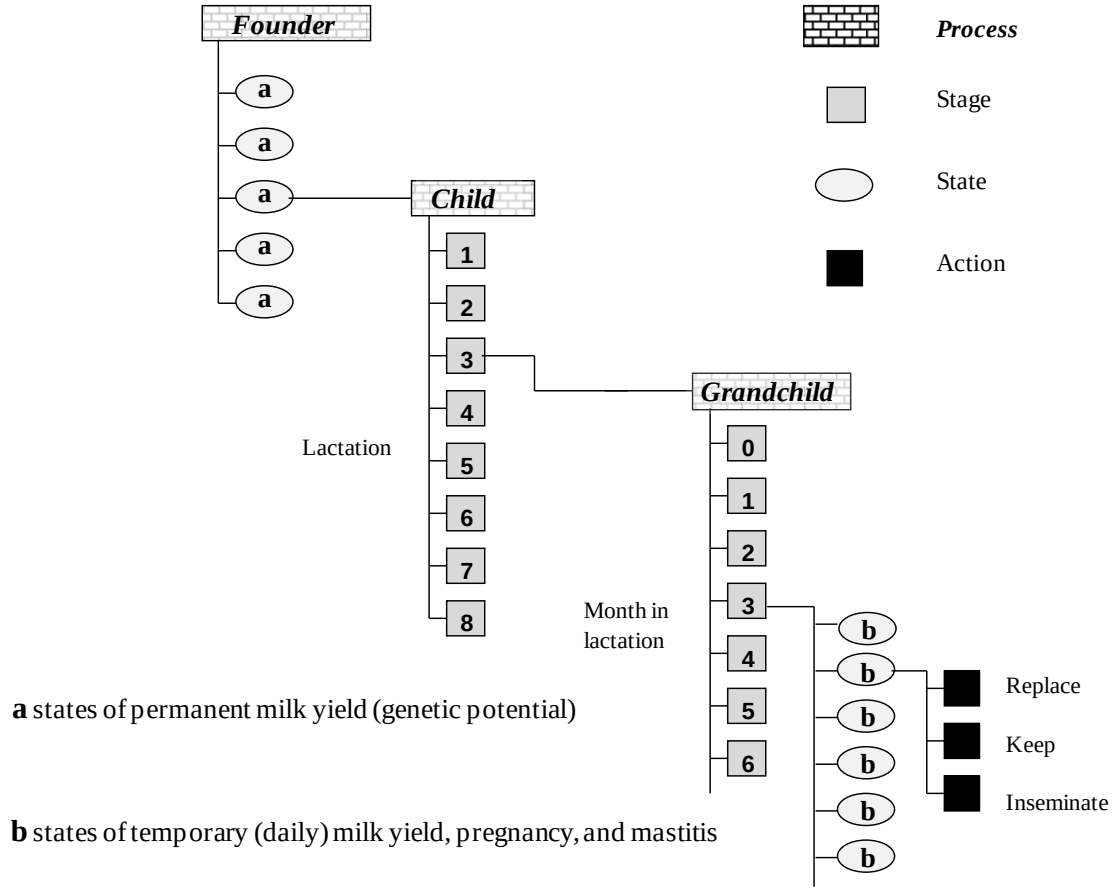

Figure 4.1. Schematic representation of the structure of the multi-level hierarchic Markov process optimization and simulation model, to determine the average cost of clinical mastitis in dairy cows

In the case of a reoccurrence, if a cow has reoccurrence of e.g. gram-positive CM, she will return to state 1, when she has a reoccurrence of gram-negative CM, she will return to state 5, and in the case of other CM, she will return to state 9.

The objective function maximized by the model was the discounting criterion (Kristensen, 2003), which maximizes the net present value of the cow using a yearly interest rate of 8% (De Vries, 2006; Bar et al., 2008a; Federal Reserve Bank of Kansas City, 2011).

Optimization technique. By combining the advantages of the two types of iteration methods used to solve the Markov Process (namely value iteration and policy iteration), a new notion of a hierarchic Markov process was developed by Kristensen (1988; 1991), which forms the basis of our dynamic program. This solution approach allows us to obtain solutions to large state space problems as described below (Kristensen, 1996).

Value iteration is performed to identify the decision that maximizes the total expected discounted rewards when the process starts from state *i* and continues for *n* stages before ending. Policy iteration involves choosing an arbitrary set of decision rules for each state at each stage and solving a set of simultaneous linear equations describing the expected future rewards of a process starting from state *i* and running over an infinite number of stages until the same optimal decision is reached (Kristensen, 1996; Cha et al., 2010). Our model is structured in such a way that a cow can be replaced until time infinity, hence at the founder (parent) level, we have an

infinite time horizon. At the subprocess (child and grandchild) levels, however, we have a finite time horizon (i.e., the lifespan of a specific cow).

Kristensen (1988; 1991) combined the benefits of both policy and value iteration, by applying value iteration to the subprocesses and using these results in the final step of the policy iteration method of the main process. Hence, in our model, at the founder level, we used policy iteration, and at the child and grandchild levels, value iteration (Figure 4.1). More details of the mathematics pertaining to this technique can be found in Cha et al., 2010.

Model parameters

Description of data. Model parameters were obtained from analyses of data from 7 dairy herds in New York State. These 7 herds were followed for approximately 4 years, and contained a total of 23,902 lactations in 14,208 cows.

Parameters. Model parameters specific to the 3 different types of CM are given in Table 4.4.1.

Table 4.4.1. Model parameters specific to 3 different types of clinical mastitis.

Parameter		Time period	Gram-positive	Gram-negative	Other	Reference
Milk loss (kg/d)		1st lactation, 1st month after CM	3.1	5.4	3.38	Schukken et al., 2009
		1st lactation, 2nd month after CM	2.25	3.35	2.03	
		1st lactation, following months	1.0	2.6	1.7	
		2nd &3rd lactation, 1st month after CM	3.43	7.58	2.88	
		2nd &3rd lactation, 2nd month after CM	1.48	3.65	1.23	
		2nd &3rd lactation, following months	0.9	2.4	0.6	
Pregnancy rate adjusted by odds ratios:			0.76	0.65	0.84	Hertl et al., 2010
Treatment cost ($)			73.50	35.50	49.50	See materials and methods
Risk (%)	1st case	1st lactation	0.019,0.005,0.006, 0.006[1]	0.008, 0.006, 0.005, 0.005[1]	0.015, 0.008, 0.007, 0.006[1]	See materials and methods
		2nd lactation	0.017, 0.01, 0.013, 0.01	0.018, 0.02, 0.02, 0.013	0.022, 0.015, 0.016, 0.013	
		3rd lactation	0.02, 0.012, 0.016, 0.013	0.024, 0.026, 0.027, 0.017	0.027, 0.019, 0.02, 0.016	
		4+ lactation	0.026, 0.013, 0.02, 0.016	0.027, 0.03, 0.03, 0.02	0.032, 0.023, 0.023, 0.02	
	2nd case	1st lactation	0.035, 0.015, 0.013, 0.01	0.03, 0.02, 0.015, 0.01	0.04, 0.02, 0.01, 0.01	
		2nd lactation	0.042, 0.03, 0.024, 0.02	0.046, 0.044, 0.026, 0.017	0.065, 0.048, 0.035, 0.031	
		3rd lactation	0.043, 0.03, 0.025,	0.054, 0.052, 0.044,	0.077, 0.074, 0.042,	

	4+ lactation	0.02 0.039, 0.03, 0.023, 0.02	0.021 0.051, 0.049, 0.029, 0.019	0.037 0.075, 0.056, 0.041, 0.037	
Involuntary culling risk[2]		0.006, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07	0.024, 0.4, 0.08, 0.12, 0.15, 0.15, 0.15, 0.15	0.006, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07	Bar et al., 2008a and unpublished data
Mortality risk			0.02[3] 0.04[4]		Gröhn et al., 2005

[1]According to month 1, 2, 3 and 4+
[2] Monthly involuntary culling risks for lactations 1 through 8
[3] primipara
[4] multipara

A decision to treat a cow with gram-positive CM was associated with a cost (USD) of 73.50. This cost was an estimated average from antibiotics (8), the decreased value of 5d worth of discarded milk from an average production cow (20), 50% of cows receiving anti-inflammatory drugs and fluid IV or per os (15.50), labor (20) and culturing (10). A decision to treat a cow with gram-negative CM was associated with a cost of 35.50. This cost was an estimated average from 50% of cows receiving anti-inflammatory drugs and fluid IV or per os (15.50), labor (10) and culturing (10). The decision to treat a cow with other CM was associated with a cost of 49.50. This was an estimated average from antibiotics (4), 50% of cows receiving anti-inflammatory medication and fluid IV or per os (15.50), labor (20) and culturing (10); we assumed the discarded milk could be used in place of milk replacer for calves. Recognizing that the cost of treatment varies by farm (depending on drug administration, days of discarded milk due to drug use, etc.), a sensitivity analysis (described later below) of the cost of treatment was also performed.

Pregnancy risk was set to 0.21 per month. Odds ratios which would reduce the rate of conception for each type of CM were applicable only for the first month after the cow got CM (i.e. CM states 1, 5 and 9) and also if she got another case of the same type of CM (where she would return to state 1, 5 or 9 for a recurrent case of gram-positive, gram-negative or other CM, respectively). If a cow contracted CM, her probability of going into the pregnancy state the following month was multiplied by this formula: (pregnancy rate*conception odds ratio for type of CM/(1-pregnancy rate+pregnancy rate*conception odds ratio for type of CM)). The voluntary waiting period was 60 d. The maximum calving interval was 20 mo and the involuntary culling risk at calving was 2%.

The monthly risk estimates (first case and recurrent cases), by lactation and CM type, were obtained from generalized linear mixed models with a random herd effect. The monthly risks for repeated cases were an average of the monthly risks for the second and third CM occurrence. The monthly risk estimates for the second CM occurrence in multiparous cows meant the cow could have had any type of CM within the lactation (and no CM in the previous lactation). The monthly risk estimates for the third CM occurrence in multiparous cows referred to cows that had already experienced 2 cases of CM (of any type) within the lactation and without CM in the previous lactation.

The cost of a calving first lactation animal (all costs in USD) was 1,600, average monthly cow maintenance cost was 150 and insemination cost/month of insemination was 20. The average price for a calf born was 200. The milk price was $0.31/kg and the feed cost/kg of dry matter was $0.20. The cull price for voluntarily culled cows was $0.74/kg of body weight.

Other parameters and prices and costs were taken from Bar, (2007), De Vries (2006) and Bar et al. (2008a). The milk yields, transition probabilities (the probabilities describing the different states a cow can transition to from one month to another), exit from the herd and effects of CM are described in Bar et al. (2008a).

Estimating CM cost

The average net returns per cow per year for a herd without CM were compared with the average net returns per cow per year for a herd with CM (by type), while keeping other parameters constant. The profit or loss was divided by the CM incidence to generate the herd average cost per case of CM. As the cost of CM was minimized under optimal treatment decisions, it is possible that these values differ from actual farm figures.

The effects of milk loss, decreased fertility and treatment cost on the average cost of a CM case were also determined by obtaining the net present values of the model with and without the CM type and effect in question, then dividing by the incidence of CM.

The net present value (NPV) is the current value of actions where the benefits and costs of the actions are calculated until the end of the time horizon. This is achieved by discounting the various benefits and costs by an annual interest rate over that time period. An interest rate of 8% was used (De Vries, 2006; Bar et al., 2008a; Federal Reserve Bank of Kansas City, 2011). The discounting factor (β) is equal to exp(-r) where r = 0.08, i.e. β = 0.92. The retention payoff (RPO) value is the NPV of retaining a cow compared with the NPV of her replacement (Bar et al., 2008b), i.e. $NPV_{retaining} - NPV_{replacing}$.

Exit from the herd

Exit from the herd can be due to two reasons: (1) voluntary culling based on what the model recommends or (2) due to what is commonly referred to as involuntary culling. Involuntary culling can be due to euthanasia or cows sold for slaughter because of reasons other

than milk yield, pregnancy or CM (i.e. reasons not determined directly from the model). The values used for the probability of involuntary culling are discussed in Bar et al. (2008a). As the probability of involuntary culling of gram-negative mastitic cows was approximately 4 times that of healthy cows, this was reflected in the monthly involuntary culling values used in our model for gram-negative CM (unpublished data). The mortality of gram-negative CM was simplified to be 2% and 4% for primipara and multipara, respectively (Gröhn et al., 2005).

Sensitivity analyses

Given that economic values such as milk price, replacement cost and treatment cost can vary from time to time and farm to farm, a sensitivity analysis was performed to evaluate how an increase and decrease of 20% in each of these values individually affected the percentage of CM cases in the herd and the average cost per case. Further, we also measured the effect of halving the incidence of all 3 different types of CM, and also the effect of increasing the pregnancy rate by 20% (from 0.21 to 0.25) to determine which of these two management measures have the most beneficial effect on the average cost/case of CM.

RESULTS

The cost of different types of CM

The effects of each different type of CM on net return, incidence of CM, percent of CM cases treated, average cost of CM and average cost per case, are shown in Table 4.4.2.

Table 4.4.2. The effects of different types of clinical mastitis (CM) (gram-positive, gram-negative, other) on net return, CM cases, % of CM cases treated, average cost of CM and average cost per case, following an optimal replacement policy (all costs in USD)

	Net return[1]	CM cases[2]	% of CM cases treated[3]	Average cost of CM	Average cost per case[4]
No CM [5]	426.05				
All [6]	357.35	44.3	93.6	68.70	155.08
Gram-negative and other [7]	374.20				
Only gram-positive [8]		12.6	93.1	16.85	133.73
Gram-positive and other	390.06				
Only gram-negative		15.5	93.1	32.71	211.03
Gram-positive and gram-negative	372.79				
Only other		16.2	94.6	15.44	95.31

[1] net returns in USD per cow and year
[2] incidence of CM (cases per 100 cow years)
[3] percent of treated CM cows per all CM cows
[4] average cost per CM case
[5] CM incidences set to 0
[6] all 3 different types of CM
[7] incidences of gram-negative and other CM included only
[8] the added effects of gram-positive CM only

The monetary values correspond to averaging over cow characteristics (parity, month of lactation, etc.). The average cost per case (USD) was greatest for gram-negative CM at 211.03 (32.71/0.155) (where 32.71 is the average cost (=390.06-357.35) and 0.155 is the incidence of gram-negative CM), followed by gram-positive CM at 133.73 (16.85/0.126), and other CM at 95.31 (15.44/0.162). The percentage of mastitic cows recommended to be treated, following an optimal replacement policy, was 93.1, 93.1 and 94.6 for gram-positive, gram-negative and other CM, respectively. For the remainder of cows, the recommended policy was to cull immediately.

The effects of exogenous factors on the cost of different types of CM

We quantified how penalties associated with each type of CM, i.e., the milk loss, decreased fertility and treatment cost, contribute to the average cost per case of each type of CM. For gram-positive CM, the total cost (133.73) was comprised mostly of the treatment cost (68.89; 51.5% of the total cost), followed by milk loss (49.64; 37.1%) and decreased fertility (15.20; 11.4%). For gram-negative CM, the total cost (211.04) was primarily from the milk loss (152.76; 72.4%), followed by treatment cost (32.74; 15.5%) and decreased fertility (25.54; 12.1%). For other CM, the same trend was seen as for gram-positive CM, i.e. the treatment cost (46.86; 49.2%) contributed most to the total cost (95.30), followed by milk loss (38.64; 40.5%) and decreased fertility (9.80; 10.3%).

We increased and decreased the milk price by 20%, to observe how sensitive the average cost/case was to milk prices for each type of CM (Table 4.4.3).

Table 4.4.3. Effects of increasing and decreasing milk price replacement cost and treatment cost by 20%, halving the incidence of all 3 different types of clinical mastitis (CM) and increasing pregnancy rate by 20% on CM cases and the average cost/case for all CM, and each different type of CM.

Scenario	All[1]		Gram-positive[2]		Gram-negative		Other	
	CM cases[3]	Average cost/case[4]	CM cases[3]	Average cost/case[4]	CM cases[3]	Average cost/case[4]	CM cases[3]	Average cost/case[4]
Milk price +20%	43.5	173.23	12.4	145.36	15.1	240.63	15.9	105.08
Milk price -20%	45.3	137.91	12.8	123.49	15.9	183.37	15.9	90.10
Replacement cost +20%	45.1	163.23	12.8	138.70	15.8	225.15	16.5	99.05
Replacement cost -20%	43	148.67	12.3	130.58	15	200.06	15.8	93.13
Treatment cost +20%	44.2	164.97	12.6	147.60	15.5	218.57	16.2	104.10
Treatment cost -20%	44.3	145.59	12.6	120.13	15.5	203.96	16.2	86.84
Halving incidence of all 3 different types of CM	23	158.17	6.5	141.42	8	218.93	8.5	100.41
Increasing pregnancy rate by 20%	45.7	150.35	12.9	131.55	16.1	205.90	16.7	92.70

[1] all 3 different types of CM
[2] gram-positive CM only
[3] incidence of CM (cases per 100 cow years)
[4] average cost per CM case

When we increased the milk price by 20%, the average cost/case of all CM increased by 11.7% (from 155.08 to 173.23), and decreased by 11.1% (from 155.08 to 137.91) when we decreased milk price by 20%. Gram-negative CM was most sensitive to these changes; the average cost per case increased by 14% (from 211.03 to 240.63) and decreased 13.1% (from 211.03 to 183.37) when milk price was increased and decreased by 20%, respectively.

When we increased and decreased the replacement cost by 20%, the average cost/case of CM increased by 5.3% (from 155.08 to 163.23) and decreased by 4.1% (from 155.08 to 148.67), respectively (Table 4.4.3). Gram-negative CM was most sensitive to these changes; the average cost/case increased by 6.7% (from 211.03 to 225.15) and decreased by 5.2% (from 211.03 to 200.06) when replacement cost was increased and decreased by 20%, respectively.

When we increased and decreased the treatment cost by 20%, the greatest change in cost/case was seen for gram-positive CM (increase of 10.4%, from 133.73 to 147.60, and decrease of 10.2%, from 133.73 to 120.13, respectively), followed by other CM (increase of 9.2%, from 95.31 to 104.10, and decrease of 8.9% from 95.31 to 86.84), and gram-negative CM (increase of 3.6%, from 211.03 to 218.57, and decrease of 3.4% from 211.03 to 203.96) (Table 4.4.3).

The average cost per case increased when the incidence of all different types of CM was halved. The greatest increase was in the other CM category (from 95.31 to 98.47, a 3.3% increase) (Table 4.4.3).

When pregnancy rate was increased by 20%, the average cost per case decreased. Of the 3 categories of CM, the largest decrease was seen in the other category (from 95.31 to 92.70, a 2.7% decrease) (Table 4.4.3).

Retention payoff of open healthy and mastitic cows

Our economic model calculates the retention payoff for cows, dependent on their individual characteristics. Figures 4.2 and 4.3 are hypothetical examples of retention payoffs under an optimal policy for cows free of CM and with different types of CM, specific to an open (non-pregnant), second lactation cow with average milk yield per 305 day lactation, and with permanent milk yield of 1,500 kg per 305 day lactation less than the average in the herd, respectively. The optimal policy recommended by the model (keep but not inseminate, keep and inseminate or replace) is also illustrated by the symbols on the graph.

In Figure 4.2, the RPO (USD) of cows at calving was 1,227, 1,091, 1,053 and 933 for no CM, other CM, gram-positive CM and gram-negative CM, respectively. The average cost at calving was calculated by subtracting the RPO for the different types of CM from the RPO for no CM. The average cost at calving was 136 (1,227-1,091), 174 (1,227-1,053) and 294 (1,227-933) for other CM, gram-positive CM and gram-negative CM, respectively. When the RPO is negative, it is more profitable to cull the cow than keep her. This was observed at month 12 for no CM, month 11 for other CM, and month 10 for gram-positive and gram-negative CM. Our figure illustrates the recommended policy until month 14; cows in month 14 and onwards were all recommended to be replaced.

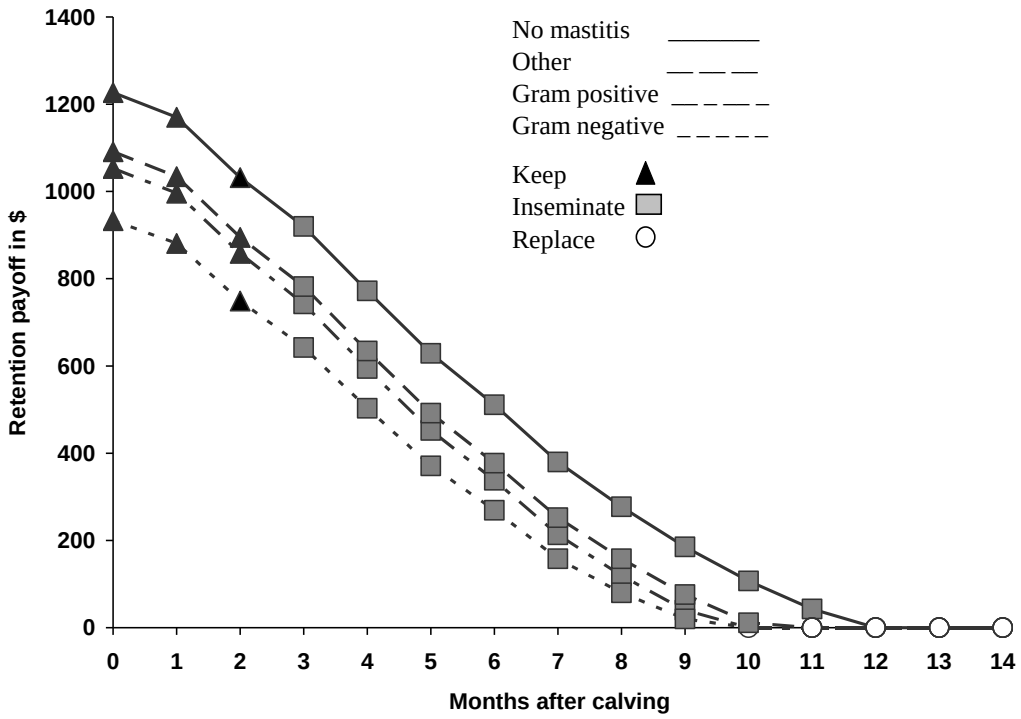

Figure 4.2. Retention payoffs under an optimal policy for hypothetically open (non pregnant) cows free of clinical mastitis (CM) and with different types of CM, specific to a second lactation cow with average milk yield per 305 day lactation

In Figure 4.3, it can be seen that the culling recommendation has shifted forward, i.e., culling was recommended at month 9 for a cow without CM, and at month 7 for cows with gram-positive, gram-negative and other CM. The RPO of these cows at calving was 626, 518, 481 and 422, for no CM, other CM, gram-positive and gram-negative CM, respectively. Therefore, the average cost at calving was 108 (626-518), 145 (626-481) and 204 (626-422) for other CM, gram-positive and gram-negative CM, respectively. Our figure illustrates the recommended policy until month 12; cows in month 12 and onwards were all recommended to be replaced.

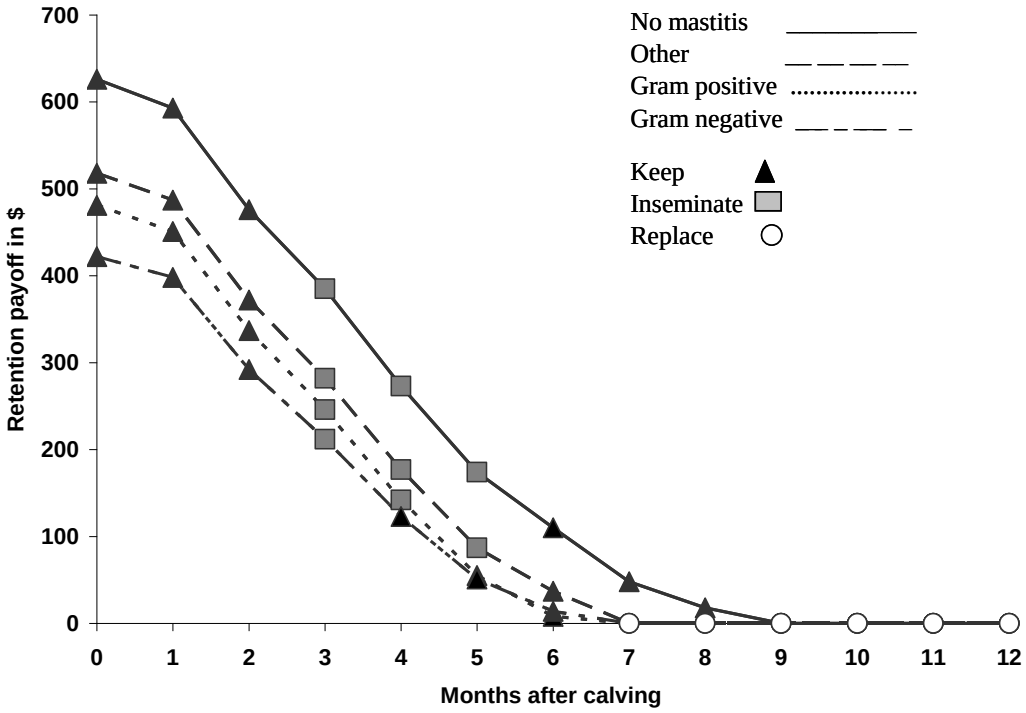

Figure 4.3. Retention payoffs under an optimal policy for hypothetically open (non pregnant), second lactation cows, with permanent milk yield of 1,500 kg per 305 day lactation less than the average in the herd, free of clinical mastitis (CM) and with different types of CM(note: gram-positive and gram-negative CM graphs overlap from month 5)

Endogenous factors affecting the cost of CM

Tables 4.4 and 4.5 are a cross-sectional view of Figures 4.2 and 4.3 at 4 and 8 months after calving, respectively (but with more information than the figures, i.e., Tables 4.4 and 4.5 also include cows of high permanent milk yield potential and pregnant cows).

Table 4.4. Average costs (in USD) of 3 types of clinical mastitis (CM) in cows with different levels (low, average, high) of permanent (genetically determined) milk yield potential 4 mo after calving, obtained by the insemination and replacement optimization model

	Permanent milk yield potential																	
	Low						Average						High					
	Open			Pregnant			Open			Pregnant			Open			Pregnant		
Lactation	GP[1]	GN[2]	O[3]	GP[1]	GN[2]	O[3]	GP[1]	GN[2]	O[3]	GP[1]	GN[2]	O[3]	GP[1]	GN[2]	O[3]	GP[1]	GN[2]	O[3]
1	136	150	126	125	140	115	167	216	154	125	154	115	152	245	160	125	171	115
2	131	150	96	123	147	90	178	269	139	124	165	91	206	352	164	124	189	91
3	116	136	86	121	151	92	156	240	127	122	180	93	176	323	148	124	219	95
6	105	117	76	102	112	74	114	149	84	115	159	86	137	229	109	116	196	85

[1] Gram-positive CM
[2] Gram-negative CM
[3] Other CM

Table 4.5. Average costs (in USD) of 3 types of clinical mastitis (CM) in cows with different levels (low, average, high) of permanent (genetically determined) milk yield potential 8 mo after calving, obtained by the insemination and replacement optimization model

	Permanent milk yield potential																	
	Low						Average						High					
	Open			Pregnant			Open			Pregnant			Open			Pregnant		
Lactation	GP[1]	GN[2]	O[3]	GP[1]	GN[2]	O[3]	GP[1]	GN[2]	O[3]	GP[1]	GN[2]	O[3]	GP[1]	GN[2]	O[3]	GP[1]	GN[2]	O[3]
1	15	15	15	125	137	115	151	193	142	125	150	115	170	248	164	125	167	115
2	18	18	18	69	69	69	158	197	120	123	153	91	210	308	165	124	177	91
3	21	21	21	30	30	30	133	153	103	122	157	93	177	264	146	123	196	94
6	0	0	0	0	0	0	46	46	46	0	0	0	125	153	95	115	161	87

[1] Gram-positive CM
[2] Gram-negative CM
[3] Other CM

The cost of CM is dependent on endogenous factors, i.e., permanent (genetic) milk yield potential, pregnancy status and lactation (Tables 4.4 and 4.5). The general trends are discussed below.

For a cow 4 months after calving (Table 4.4), we found that the average cost of CM was greater in open cows compared with pregnant cows. Also, the average cost of CM was greater in younger cows compared with older cows.

The average cost was greatest for gram-negative CM, followed by gram-positive CM, and other CM, for each permanent milk yield potential and pregnancy status combination. Also, the cost was greatest for cows that were high milk producing, followed by average and low producing.

At 8 months after calving (Table 4.5), the average cost was generally greater for cows suffering from gram-negative CM, and this was followed by gram-positive CM and other CM. Also, in the low permanent milk yield potential category, pregnant cows had a higher average cost of CM compared with open cows, but this was reversed in the average and high permanent milk yield potential categories. Similar to the trend at 4 months after calving, younger cows had a higher average cost of CM than older cows and the higher the permanent milk yield potential of the cow, the greater the average cost of CM (Table 4.5).

Exit from the herd (voluntary culling and involuntary culling)

When all the different types of CM were included in the model, the percentage exit from the herd was 35.5 (comprised of 17% from voluntary culling and 18.5% from involuntary culling). This increased to 38.7 (20.8, 17.9) when milk price was increased by 20%, and decreased to 33.1 (13.8, 19.3) when milk price was reduced by 20%. When replacement cost was increased by 20%, herd exit decreased to 33.6 (14.4, 19.2) and increased to 39.3 (17.5, 21.8)

when replacement cost was reduced by 20%. When the incidences of CM were halved, herd exit decreased to 34.4 (15.7, 18.7), and when pregnancy rate was increased by 20%, it decreased to 33.4 (13.8, 19.6).

DISCUSSION

When a cow contracts mastitis, the dairy farmer needs to decide whether treatment is warranted, and if so, what treatment is most appropriate. These decisions are ideally made based on the organism causing mastitis. In determining how to treat a cow, one common way of grouping these organisms is to separate them into gram-positive and gram-negative mastitis. These two groups of organisms cause mastitis of different symptoms and severity. This classification can form the basis of on-farm treatment protocols (Hertl et al., 2010).

The importance and reliance on classifications of mastitis has become prevalent in the literature. For example, a study conducted by Neeser et al. (2006) found that there were significant reductions in the amount of antimicrobial use when on-farm culture systems were employed. Most producers treated gram-positive mastitis with antibiotics, whereas gram-negative mastitis treatment varied. They concluded that the reduction in antimicrobial use could lead to several advantages, such as decreases in discarded milk and antimicrobial residues in milk, and improved treatment outcome due to targeted treatment. From our study, we found the average cost per case (USD) of gram-negative CM (211.03) was due mostly to milk loss, which is logical given that the milk loss was greatest for gram-negative CM out of the 3 types of CM (see also Schukken et al. (2009)). For gram-positive CM, this cost was primarily due to the treatment cost, which is also intuitive, given that the treatment cost was greatest for gram-

positive CM, of all 3 types of CM. Similarly, treatment cost contributed most to the average cost per case of other CM.

The average cost per case with all 3 different types of CM in the model was 155.08, which is lower than that found in the study by Bar et al. (2008a) for generic CM, where the average cost per case was 179. This difference is due to a number of reasons: our model was more detailed in that generic mastitis was differentiated into types and data in our study were updated from Bar et al. (2008a), In Bar et al. (2008a) the parameter values used in the model (risk, treatment cost, involuntary culling, etc.) were for generic CM and not groups of CM, and we did not include a carryover state from the previous lactation. Unlike the generic CM case, if we were to include a carryover state, we would need to model all the different combinations of carryover effects possible (e.g. gram-positive CM in previous lactation, gram-negative CM in current lactation, or gram-positive CM in previous lactation, gram-positive CM in current lactation, etc.). This would cause the state-space of the model to grow considerably, increasing the time and computer capacity necessary to calculate an optimal solution. The inclusion of carryover effect is an area of future research.

Although a few studies have examined the cost of CM in dairy cows, none have quantified this cost at the individual cow level for 3 different types of CM. The study that comes closest to examining such costs was conducted by Sørensen et al. (2010). In that study, the authors estimated the costs related to 5 different pathogen-specific mastitis traits and unspecific mastitis using a stochastic simulation model (SimHerd IV). Costs ranged from 189.42USD to 724.64USD per case (converted on 20Aug2010 from €149 and €570, respectively), and were greater for contagious pathogens, compared with environmental pathogens.

In our study, not only did we estimate the cost of different types of CM, but also the sensitivity of these costs from parameter changes. When we increased the milk price, the average cost per case of CM increased, as the milk losses associated with each type of CM were higher valued. The reverse was seen when milk price was reduced. Again, as expected, the average cost of gram-negative CM was most sensitive to this change. The same pattern was observed when replacement cost was increased.

We increased and decreased treatment cost by 20% to account for differences across farms in e.g. the use of antibiotics and associated discarded milk. What we found was, despite these changes, the order of the cost of CM from most costly to least costly did not change (i.e. gram negative was always most expensive, followed by gram positive then other CM).

Between the two scenarios of increasing pregnancy rate or halving the incidence of CM, it was apparent that the former case led to a reduction in the average cost/case of CM, indicating the benefits to farmers of focusing on improving their breeding programs. In interpreting the results of the model, we emphasize that the cost of CM calculated by the model should be interpreted as the lowest cost possible following the optimal insemination and culling policy, under stable prices, and in a steady state. The best insemination and culling policy is assuming a constant number of cows on the farm and immediate availability of replacement heifers. As a result, if the incidence of CM is halved, fewer cows are culled because of CM. As the number of cows is constant, this means that when fewer cows are culled because of CM (hence, less need for young replacement heifers), the cows in the herd will be getting older on average, and these older cows will be more prone to other diseases. Decreased culling rate means there is also less intensive genetic improvement (as we assume each generation to be more genetically advanced

than the current genetic average). Therefore, our results show a slight decrease in the marginal net return per reduction of CM incidence. In reality, the decreased model related culling would be translated to inner expansion or better selection of replacement heifers. As such, the value calculated from our model slightly underestimates the cost of CM.

Both Figures 4.2 and 4.3 illustrate that cows with CM should be replaced earlier than cows without CM, and that cows with lower milk yield should be replaced earlier than cows with higher milk yield. From Figure 4.3, it can be seen that cows with gram-negative CM are recommended to be inseminated only once compared with cows having gram-positive and other CM; this can be attributed to the greater milk loss from gram-negative CM (Schukken et al., 2009), making it less economically optimal to spend the money on inseminating them from that one point onwards. The cross sectional views of the figures (Tables 4.4 and 4.5) quantified what one would expect in the average cost/case of CM, as permanent milk yield, age, type of CM and pregnancy status vary. For example, as permanent milk yield potential increased from low to high, the average cost/case of CM increased. As expected, the older the cow is, the lower the average cost/case of CM, as an older cow has less lifespan remaining, than a younger cow, for the cow to succumb to the detrimental effects of disease (and for these to be translated into monetary losses). Gram-negative CM generated the highest cost, as has been the case so far. Generally, CM cases were more costly in open cows, as they have the added effect of reduced fertility (unlike pregnant cows, as they are already with calf). Among cows 8 mo after calving (Table 4.5), in the low permanent milk yield potential category, however, pregnant cows had a higher average cost of CM compared with open cows, which is due to these cows being further into pregnancy, and a greater probability of going to term (unlike cows at 4 mo, where the opposite trend was seen in average cost/case of CM).

As anticipated, when milk price increased, culling (voluntary) increased as well, due to the increased cost from milk loss and the greater expected profit of a replacement heifer. When the incidence of CM was halved, and pregnancy rate increased, culling (voluntary) percentages decreased. When replacement cost increased, culling (voluntary) was reduced, as it was more expensive to replace than to keep a cow.

In our model, we use a monthly time step, where we assume that e.g. all CM cases occurring 152 – 183 days after calving occur at day 183, enabling the decision to cull (and not treat) before incurring the costs of disease. The only exception to this is the first stage after calving which has a length of only 3 days, i.e. we assume that all cows that have mastitis shortly after calving have it by the third day after calving (Bar et al., 2008a). This is also because we estimate a greater risk for CM in these days.

Our study focuses on decisions for individual animals, and as such is an individual based model. All modeling techniques have their advantages which need to be weighed with their disadvantages in selecting the technique most appropriate for the study. The limitations of our individual cow model are that we cannot include herd dynamics, e.g. infectivity of CM, and see the effects of this at the individual cow level. If the latter were the focus of our study, then another modeling technique would be appropriate.

Our research was specific to cow characteristics which allow us to undertake a comprehensive analysis of the costs of CM by type. Further, the cost of disease depends on the fate of the cow. If the cow is to be culled, milk loss effects and fertility effects are not applicable. If the cow is pregnant, disease effects on fertility are not applicable. Pregnant cows were almost always recommended to be kept in the herd until the next lactation. Because the CM losses in

these cows are only the treatment cost and milk loss and these were assumed to be the same for both high yielding cows and low yielding cows, the cost of CM is the same for all these pregnant cows. Intuitively, one would assume that a high producing cow loses more milk to CM (compared with an average or low producer); however, we have assumed this to be the same. While we know that high milk production is a risk factor for mastitis (Gröhn et al., 1990; Gröhn et al., 1995), we have not investigated whether these losses are different for low or high milk producing cows, though this would not be unexpected. Because we do not included this risk factor in our model, and assume that milk loss is consistent across all milk production levels, it is possible that there may be more variability in the results than currently shown in our model.

Further, we did not model seasonality and milk component variations, or the exact shape of the lactation curves beyond 10 mo, as these issues were beyond the scope of our study objectives. A further limitation includes the assumption that the farmer has complete knowledge of cow traits, and that a replacement heifer immediately enters the milking herd following a cow replacement, which is not always the case (Bar et al., 2008a).

The 'treat' decision which our economic model can recommend does not take into account how effective the treatment may be. And given that in our current model, CM is divided into 3 categories of gram-positive, gram-negative and other CM, the treatment policies for each type of bacteria in each category are assumed to be the same. Admittedly, the success and type of treatment for bacteria within each group, or even the same bacterial species between different strains, can differ; however, the focus of our economic model was not to assess the success of different types of treatment options.

This model, therefore, serves as a decision tool to aid farmers when deciding what to do with their diseased cows. The economic values, production costs and disease frequencies can be altered; hence, the results can be made applicable to individual farms, although our used values are representative.

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CHAPTER 5

OPTIMAL DECISIONS TO MINIMIZE THE COST OF PATHOGEN SPECIFIC CLINICAL MASTITIS IN DAIRY COWS

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ABSTRACT

Intensive dairy farming is characterized by maximizing revenue through the sale of consumable milk, milk products and beef. In this industry, mastitis is a serious production limiting disease, having effects on milk yield, milk quality, conception rate and an increase in the risk of mortality and culling. Our objective was twofold: (1) to develop an economic optimization model which would incorporate all the different types of pathogens that cause CM categorized into 8 classes of culture results, as well as account for whether the CM was a first, second or third case in the current lactation and whether the cow had a previous case of CM in the preceding lactation and (2) to develop a model which would be versatile to add more pathogens, diseases or other factors in the future as more information becomes available without significant alterations to the basic structure of the model. The model provides economically optimal decisions depending on the individual characteristics of the cow and the specific pathogen causing CM. The net returns in US$ per cow and year for the basic scenario (with all CM included) was 500, where the incidence of CM (cases per 100-cow years) was 35.5, of which 90.6% of cases were recommended to be treated under an optimal replacement policy. The cost per case of CM was 233.41. The CM cases were comprised of (all in %, totaling 35.5%) *Staphylococcus* spp. (1.6), *Staphylococcus aureus* (1.8), *Streptococcus* spp. (6.9), *Escherichia coli* (8.1), *Klebsiella* (2.2), Other treated cases (e.g., *pseudomonas*) (1.1), Other not treated cases (e.g., *Trueperella pyogenes*) (1.2) and Negative culture cases (12.7). The average cost per case even under optimal decisions was greatest for *Klebsiella* (416), followed by other not treated cases (bacteria like *T. pyogenes*) (316) which was similar to the other treated cases (bacteria like *pseudomonas*) (310)

and *Escherichia coli* (309). This was followed by the gram-positive pathogens, with the greatest cost per case being due to *Staphylococcus aureus* (298), then *Staphylococcus* spp. (275) and *Streptococcus* spp. (257). Negative culture cases had the lowest cost (151). Most CM cases (by pathogen), were recommended to be treated (i.e. all over 85%), and this was greatest for other treated cases (94.4%) and lowest for *Staphylococcus aureus* cases (86.4%). In general, the optimal recommended time for replacement was as great as 5 months earlier for cows with CM compared with cows without CM. Further, while the parameter estimates implemented in this model are specific to the dairy farms in this study, they may be altered so that the results are specific to any other farm.

Keywords: mastitis, pathogen, cost, dynamic programming

INTRODUCTION

Intensive dairy farming is characterized by maximizing revenue through the sale of consumable milk, milk products and beef. In this industry, mastitis is a serious production limiting disease, having effects on milk yield (Gröhn et al., 2004; Bar et al., 2007; Schukken et al., 2009), milk quality, conception rate (Hertl et al., 2010) and an increase in the risk of mortality and culling (Bar et al., 2008a; Hertl et al, 2011; Cha et al., 2012b). Further, treatment of cows may be necessary depending on the pathogen causing mastitis which creates a loss of saleable milk during and following treatment due to the required withholding period.

The economic model we developed is based on dynamic programming. One of the basic elements of dynamic programming is the sequential approach to decision making, which aligns well with sequential decisions in animal production, including replacement of animals where at regular time intervals management decisions are made such as whether the animal should be replaced, inseminated or kept for another period. Optimal replacement models can be used to provide dairy farmers with guidance on what action to take with their animals. The advantage of these models is that they can assimilate large amounts of information on health status, age of the cow, milk production etc., and provide the optimal action to be taken.

An animal replacement model of value would need to include information that is considered to be necessary in identifying the optimal decision. Given that the goal of intensive dairy farming is typically to maximize revenue, one optimality criterion would logically be the maximization of net returns. Dairy cattle replacement models have been developed (De Vries et al. 2006; Nielsen et al., 2010; Demeter et al., 2011), with inclusion of information relating to disease (Houben et al., 1994; Bar et al., 2008a; Cha et al., 2011).

Bar et al. (2008a) developed an animal replacement model, incorporating information on generic clinical mastitis (**CM**), extending and building upon the assumptions of the optimal replacement model developed by Houben et al. (1994) and earlier asset replacement principles (Perrin, 1972). Cha et al. (2011) modified the original model by Bar et al. (2008a) to study 3 groupings of CM (gram positive, gram negative and other).

In the current study, we were motivated to develop a larger model for several reasons:

1. The previous framework did not separate CM into the different pathogens that are causative or, in the case of Cha et al. (2011), differentiate between different cases of CM.

Specifically, CM is caused by a variety of different pathogens, and depending on the pathogen involved as well as whether it is the first, second or third case in the lactation, the risk of CM (Cha et al., 2012a), milk loss (Hertl et al., *unpublished*), conception rate, mortality risk (Cha et al, 2012b) and treatment cost will differ. The importance of pathogen information arises in more specific situations, e.g., when the profile of a cow results in a borderline decision (Østerås et al., 1999).

2. We have additional information relating to the risk of CM by case, carryover and pathogen (Cha et al., 2012a), milk loss by case and pathogen (Hertl et al., *unpublished*), and conception rate, mortality risk (Cha et al., 2012b) and treatment cost by pathogen.
3. The previous framework did not have the flexibility to include additional pathogens or additional diseases easily; the new framework affords the versatility to expand the model for further research purposes.
4. By allowing more than one event to occur as a cow transitions from one month to another, a transition in CM status, pregnancy and milk yield will be concurrently possible.

Our objective was twofold: (1) to develop an economic optimization model which would incorporate the different types of pathogens that cause CM categorized into 8 culture classes, as well as account for whether the CM was a first, second or third case in the current lactation and whether the cow had a previous case of CM in the preceding lactation and (2) to develop a model which would be versatile to add more pathogens, diseases or other factors without significant alterations to the basic structure of the model. Further, the model will need to provide economically optimal decisions depending on the individual characteristics of the cow and the specific pathogen causing CM. We will also elucidate the cost of each pathogen causing CM

under optimal decisions, and undertake sensitivity analyses of how these costs are affected by changes in milk price, pregnancy rate and replacement cost.

MATERIALS AND METHODS

Clinical mastitis categorization

We classified CM into 8 categories: (1) *Staphylococcus* spp., (2) *Staphylococcus aureus*, (3) *Streptococcus* spp., (4) *Escherichia coli*, (5) *Klebsiella*, (6) Other treated (these included *Enterobacter*, *Enterococcus*, *Citrobacter*, *Serratia*, *Pasteurella*, *Corynebacterium* species, *Pseudomonas*, *Proteus*, *Corynebacterium bovis*, *Gram+ bacillus*, *Gram- bacillus*, fungus, *Strep.* group 'C', mold and *Nocardia*), (7) Other not treated (these included *Trueperella pyogenes*, *Mycoplasma*, *Prototheca* and yeast), and (8) Negative culture, contamination (more than two bacterial species on the culture plate) and no significant organisms. The latter, no significant organisms, was defined as no bacterial growth of either *Staphylococcus aureus* or *Streptococcus agalactiae* while the culture plate contained more than two different species. These cases exhibited clinical signs of mastitis.

Replacements and inseminations optimization and simulation model

Software. The model was built using the Multi Level Hierarchic Markov Process (MLHMP) software as the application program (Kristensen, 2003). We made significant additions and changes to the structure of an existing optimization and simulation model which was first developed by Bar et al. (2008a) to study the cost of generic CM in dairy cows, then

modified to study 3 different types of lameness in dairy cows (Cha et al., 2010). In the current model, we included a pathogen class, where parameters specific to each pathogen can be changed in the user graphical interface, allowing for the study of the effect of different parameters and prices on the cost of CM and optimal replacement decisions.

The model. The model was constructed as a 3-level hierarchic Markov process comprised of: the founder (parent) level containing state variables of permanent traits throughout the cow's life span, the child level divided into stages representing one whole lactation, and the grandchild level divided into stages of one month during lactation. The lactation number and stage of lactation are known properties from the hierarchical model structure, therefore, these are not included directly as state variables. The possible actions in a given month of lactation that could occur at the final level are: (1) replace the cow with a calving heifer, (2) keep the cow for another month without insemination and treat her if she has CM or (3) keep the cow for another month and inseminate her and treat her if she has CM (Bar et al., 2008a). Further, an alternative possible decision for CM cows is to keep without treatment; however, because we did not have access to detailed information on such cases, we did not have the parameter values necessary to model this. Figure 5.1 is a schematic representation of the model used in the current study on CM. At the founder level, 5 permanent milk yield categories were modeled as: -5, -2.5, 0, +2.5, and +5 (kg) from the mean level of milk production per day; these represented the cow's genetic potential. At the child level, 5 possible whole lactation stages were modeled. We also included a carryover state from lactation 2 onwards i.e., whether the cow had a case of CM in the preceding lactation (Y/N). At the grandchild level, 20 lactation stages (months) were modeled. In each stage the cow was described by one level within each of the following states: 5 temporary (i.e., daily) milk yield levels, 9 pregnancy states (0 = open, 1-7 = 1-7 mo pregnant and milking and 8

= last 2 mo of pregnancy and dry (not milking)), 1 involuntarily culled state and CM states. The CM states were defined as: 0 = no CM, 1 = *Staphylococcus* spp., 2 = *Staphylococcus aureus*, 3 = *Streptococcus* spp., 4 = *Escherichia coli*, 5 = *Klebsiella*, 6 = Other treated, 7 = Other not treated and 8 = Negative culture. We also introduced a history variable 'H' which ranged from 0 to 3, where H indicates the case number if the cow has CM. H=3 means a case that is ≥3. For example, CM=2 and H=1 means the cow has a first case of *Staphylococcus aureus*. CM=0, H=0 indicates that the cow has never had CM since calving. We also introduced a variable to indicate the number of months that have passed since the previous case of CM (if the cow had a previous CM case), termed 'PM'. This variable ranged from 0 to 3, where 3 indicates months ≥3. The resulting total state space is 2,095,425 combinations. The graphical user interface of the dynamic program is shown by Figure 5.2.

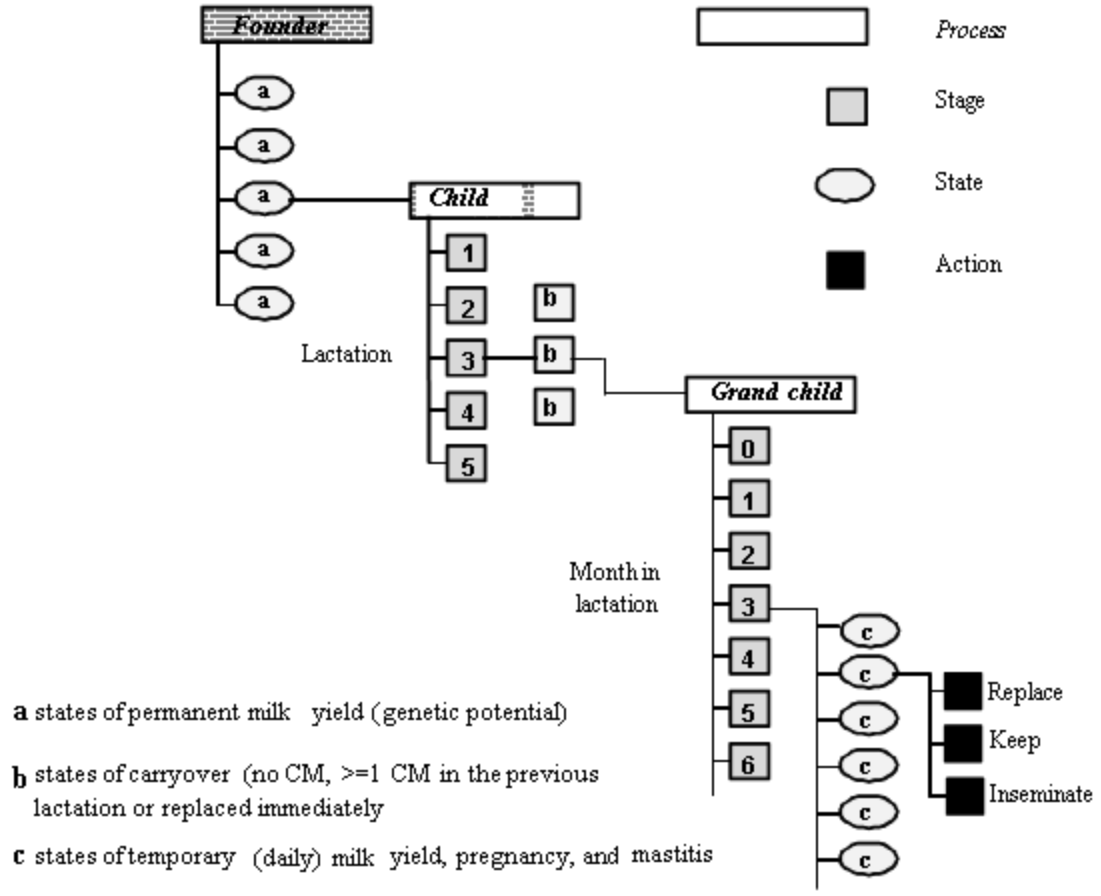
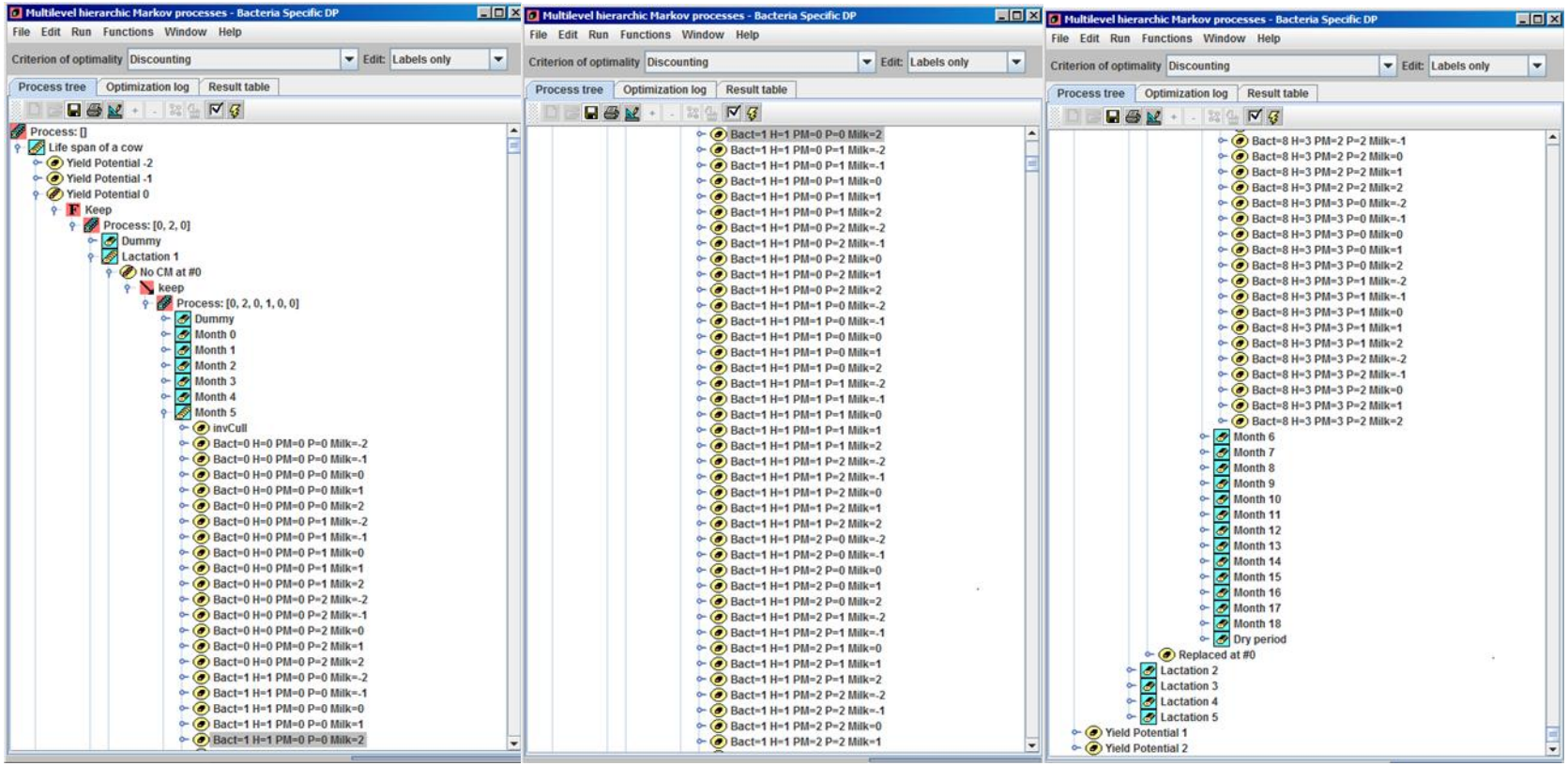


Figure 5.1. Schematic diagram of the DP structure. CM = clinical mastitis.



A) **B)** **C)**

Figure 5.2. Graphical user interface of the dynamic program, with A) showing permanent milk yield 0, lactation 1, month 5 has been selected and the first several clinical mastitis states are shown, with additional selected states shown by B) and C)

This model assumed that there were replacement heifers readily available, as well as a beef calf market, thus maintaining a constant herd size. We also assumed the decision was made at the end of the stage and the effects of the disease were accounted for in the following stage, depending on the decision taken. Also, when a cow made a transition from one state to another, we allowed for more than one change in her state at any one point in time.

The objective function maximized by the model was the discounting criterion (Kristensen, 2003), which maximizes the net present value of the cow using a yearly interest rate of 8% (De Vries, 2006; Bar et al., 2008a).

Optimization technique. By combining the advantages of the two types of iteration methods used to solve the Markov Process (namely value iteration and policy iteration), a new notion of a hierarchic Markov process was developed by Kristensen (1988; 1991) and later generalized by Kristensen and Jørgensen (2000). The notion forms the basis of solving this dynamic program. This solution approach allows us to obtain solutions to large state space problems as described below.

Value iteration is performed to identify the decision that maximizes the total expected discounted rewards when the process starts from state *i* and continues for *n* stages before ending. Policy iteration involves choosing an arbitrary set of decision rules for each state at each stage and solving a set of simultaneous linear equations describing the expected future rewards of a process starting from state *i* and running over an infinite number of stages until the same optimal decision is reached (Kristensen et al., 2010). Our model is structured in such a way that a cow can be replaced until time infinity, hence at the founder (parent) level, we have an infinite time horizon. At the subprocess (child and grandchild) levels, however, we have a finite time horizon

(i.e., the lifespan of a specific cow).

Kristensen (1988; 1991) combined the benefits of both policy and value iteration, by applying value iteration to the subprocesses and using these results in the final step of the policy iteration method of the main process. Hence, in our model, at the founder level, we used policy iteration, and at the child and grandchild levels, value iteration (Figure 5.1).

Model parameters

Description of data. Model parameters were obtained from analyses of data from 5 dairy herds in New York State. These 5 herds were followed for approximately 7-8 years (2003/2004-2011), and contained a total of 50,166 lactations in 23,409 cows.

Parameters. Model parameters specific to the 8 different pathogens causing CM are given in Tables 5.1 to 5.3.

Table 5.1. Monthly probability of pathogen specific clinical mastitis (CM) by case number, carryover[1] (if applicable) and parity (Cha et al., 2012a). For cases 2 and 3, months are months since previous case of CM

CM case	Carry-over	Parity	Month	*Staphylococcus* spp.	*Staphylococcus aureus*	*Streptococcus* spp.	*Escherichia coli*	*Klebsiella*	Other treated	Other not treated	Negative culture
1st case		1	0	0.0007	0.0006	0.0020	0.0009	0.00017	0.0004	0.0009	0.0012
			1	0.0019	0.0019	0.0050	0.0030	0.0005	0.0062	0.0020	0.0060
			2	0.0004	0.0006	0.0017	0.0047	0.0003	0.0016	0.0005	0.0066
			3+	0.0007	0.0010	0.0028	0.0034	0.0005	0.0005	0.0006	0.0041
	0	2+	0	0.0003	0.0003	0.0016	0.0005	0.0002	0.0001	0.0005	0.0015
			1	0.0009	0.0008	0.0054	0.0073	0.0032	0.0008	0.0020	0.0093
			2	0.0014	0.0011	0.0049	0.0112	0.0027	0.0006	0.0010	0.0133
			3+	0.0011	0.0012	0.0058	0.0077	0.0017	0.0008	0.0008	0.0093
	>0	2+	0	0.0007	0.0006	0.0033	0.0016	0.0006	0.0003	0.0015	0.0054
			1	0.0030	0.0031	0.0127	0.0134	0.0064	0.0015	0.0032	0.0228
			2	0.0021	0.0021	0.0094	0.0207	0.0047	0.0028	0.0015	0.0271
			3+	0.0030	0.0030	0.0124	0.0128	0.0035	0.0016	0.0012	0.0197
2nd case*	n/a	1	1	0.0050	0.0084	0.0208	0.0203	0.0072	0.0003	0.0028	0.0216
			2	0.0020	0.0016	0.0044	0.0028	0.0000	0.0019	0.0031	0.0205
			3+	0.0003	0.0004	0.0013	0.0016	0.0004	0.0007	0.0013	0.0093
		2+	1	0.0118	0.0136	0.0344	0.0343	0.0166	0.0003	0.0020	0.0314
			2	0.0012	0.0023	0.0104	0.0063	0.0027	0.0010	0.0029	0.0394
			3+	0.0008	0.0005	0.0042	0.0042	0.0008	0.0005	0.0011	0.0202
3rd case*	n/a	1	1	0.0041	0.0136	0.0245	0.0232	0.0054	0.0040	0.0027	0.0402
			2	0.0058	0.0039	0.0080	0.0060	0.0000	0.0000	0.0000	0.0421
			3+	0.0006	0.0000	0.0006	0.0019	0.0006	0.0006	0.0013	0.0131
		2+	1	0.0110	0.0117	0.0416	0.0277	0.0219	0.0000	0.0018	0.0404
			2	0.0028	0.0072	0.0182	0.0096	0.0033	0.0016	0.0022	0.0563
			3+	0.0006	0.0009	0.0049	0.0022	0.0013	0.0002	0.0021	0.0299

[1]The number of cases of CM the cow had in the immediate preceding lactation

*The monthly risk estimates for the second (and third) CM occurrence applied to cows that had already experienced 1 (2) case(s) of CM (of any type) within the lactation.

Table 5.2. Average daily (kg) milk loss since onset of pathogen specific clinical mastitis, by case, parity and months passed (positive values are gains and losses are for the month(s) since CM as specified) (Hertl et al., 2012).

CM case	Parity	Month	*Staphylococcus* spp.	*Staphylococcus aureus*	*Streptococcus* spp.	*Escherichia coli*	*Klebsiella*	Other treated	Other not treated	Negative culture
1st case	1	1	0.07	-7.19	-6.35	-8.36	-5.87	-1.93	-12.35	-2.22
		2	0.41	-2.81	-1.07	-0.90	-1.14	-0.79	-2.81	-0.98
		3+	0.38	-1.84	-0.73	-0.27	-0.65	-0.52	-1.72	-0.80
	2	1	-0.34	-9.72	-5.84	-11.27	-10.11	-1.49	-12.16	-2.62
		2	-0.35	-2.90	-0.75	-0.99	-3.14	-0.33	-1.81	0.29
		3+	-0.16	-2.30	-0.39	-0.19	-1.97	-0.39	-0.73	0.66
	3+	1	0.95	-3.67	-6.30	-12.57	-12.22	-0.99	-13.60	-5.16
		2	-0.37	-1.39	-0.49	-0.80	-2.93	-0.38	-3.48	-0.50
		3+	-0.11	-0.78	0.01	0.48	-1.56	-0.97	-1.89	0.13
2nd case	1	1	0.73	0.26	-6.13	-11.21	-3.51	-1.03	-10.96	-4.87
		2	0.06	-1.60	-1.67	-3.12	-1.61	0.85	-3.82	-1.38
		3+	-1.45	-1.02	-1.05	-1.87	-2.0	1.24	-2.73	-1.29
	2	1	-0.79	-6.44	-4.31	-12.40	-11.94	-2.11	-10.02	-5.54
		2	-2.27	-4.60	0.06	-0.26	-1.22	-0.98	-3.76	-0.60
		3+	-1.92	-4.52	-0.08	0.53	-0.53	-1.06	-2.43	0.02
	3+	1	-3.36	-4.64	-5.55	-10.11	-12.88	-1.72	-13.32	-6.71
		2	0.07	-1.99	-0.33	0.24	-1.47	-1.27	-3.60	-1.16
		3+	1.65	-1.65	0.64	0.61	0.06	-1.27	-2.47	-0.54
3rd case	1	1	1.79	-2.65	-6.22	-9.64	-2.45	-5.44	-13.68	-4.63
		2	-0.90	-1.38	-1. 60	-1.76	3.86	-0.48	-5.27	-0.27
		3+	-0.34	-2.32	0.15	-1.14	7.50	-0.16	-2.87	0.69
	2	1	-3.33	-3.68	-4.91	-9.01	-11.11	-0.38	-2.96	-7.47
		2	-1.16	-1.69	1.52	-1.73	-1. 30	-1.32	0.40	-1.11
		3+	-1.79	-1.27	1.84	-0.94	-0.41	-0.88	1.77	-0.03
	3+	1	-2. 50	-2.22	-5.18	-12.15	-6.96	-1.90	-6.06	-7.04
		2	-0.03	-1.13	-1.86	-2.02	-1.38	-0.59	-2.43	-0.90
		3+	-0.82	-1.0	-0.51	0.72	-1.90	0.52	-2.59	-0.26

Table 5.3. Treatment costs (USD), discarded milk days, odds ratios (for pregnancy rate adjustments) and probability of involuntary culling by pathogens causing clinical mastitis

Item	*Staphylococcus* spp.	*Staphylococcus aureus*	*Streptococcus* spp.	*Escherichia coli*	*Klebsiella*	Other treated	Other not treated	Negative culture	References
Treatment cost	65.50	70.5	70.5	35.5	35.5	51.5	35.5	35.5	See footnote 3 below.
Discarded milk days	10	8.5	8.5	0	0	3.5	0	0	As above
Odds ratio[1] (for pregnancy rate adjustment)	0.76	0.76	0.76	0.65	0.65	0.84	0.84	0.84	Hertl et al., 2010
Risk of involuntary culling[2]	0.006, 0.01, 0.02, 0.03, 0.04	0.004, 0.01, 0.008, 0.009, 0.01	0.004, 0.00096, 0.00645, 0.011, 0.00313	0.02, 0.03, 0.04, 0.04, 0.03	0.04, 0.05, 0.10, 0.10, 0.11	0.005, 0.02, 0.02, 0.13, 0.13	0.002, 0.02, 0.05, 0.03, 0.04	0.003, 0.007, 0.01, 0.01, 0.003	Cha et al., 2012b

[1] In calculating the pregnancy rate for CM cows by pathogen, this is the odds ratio by which pregnancy rate (for a cow without CM) was adjusted

[2] Monthly involuntary culling risks for lactations 1 through 5

[3] By determining the cost of drugs for a specific protocol times the number of recommended treatments (on manufacturers label) or the protocol defined by the farm plus the value of the discarded milk at the current milk price. The cost of drugs was identified by researching three on-line drug sales companies (where prices would be similar for most products). Websites included Animart <http://www.animart.com/store/mastitis-tubes-lactatingtreatments/> Animal Livestock Supply Inc. <http://www.americanlivestock.com/cattle.html> and Dairy Health USA <http://www.pbsanimalhealth.com/category/Dairy/Mastitis-Treatments/D80200.html#cat_top>

The monthly involuntary culling risks for lactations 1 through 5 for healthy cows were 0.003, 0.005, 0.008, 0.01 and 0.01 (pathogen specific involuntary culling risks are in Table 5.3). The treatment costs are displayed in Table 5.3. These treatment costs included costs that are for palliative care i.e., not necessarily only antibiotic treatments, but also the cost of sending samples for culturing. The cost of *Streptococcus* spp. and *Staphylococcus aureus* treatment (all in USD) was comprised of antibiotics (25), 50% of cows receiving anti-inflammatory drugs and fluids intravenously per os (15.50), labor (20) and cost of culturing (10) for a total of 70.50.

The cost of *Staphylococcus* spp. treatment was comprised of antibiotics (20), 50% of cows receiving anti-inflammatory drugs and fluids intravenously per os (15.50), labor (20) and cost of culturing (10), for a total of 65.50. The treatment cost of *Escherichia coli, Klebsiella,* negative culture and other not treated was comprised of 50% of cows receiving anti-inflammatory drugs and fluids intravenously per os (15.50), labor (10) and culturing (10), for a total of 35.50. Lastly, the treatment cost of the other treated category was comprised of antibiotics (6), 50% of cows receiving anti-inflammatory drugs and fluid intravenously per os (15.50), labor (20) and culturing (10), for a total of 51.50.

The probability of pregnancy was set to 0.21 if the decision was to inseminate (Bar et al., 2008a). Odds ratios which would reduce the rate of conception for each type of CM were applicable only for the first month after the cow got CM. If a cow contracted CM, her probability of transitioning into the pregnancy state the following month was multiplied by this formula: (pregnancy rate*conception odds ratio for type of CM/(1-pregnancy rate+pregnancy rate*conception odds ratio for type of CM)). The voluntary waiting period was 60 d. The maximum calving interval was 20 months and the involuntary culling risk at calving was 2%.

The cost of a calving first lactation animal (all costs in USD) was 1,600, average monthly cow maintenance cost was 150 and insemination cost/month of insemination was 20. The average price for a calf born was 200. The milk price was $0.31/kg and the feed cost/kg of dry matter was $0.20. The cull price for voluntarily culled cows was $0.74/kg of body weight.

The milk yield estimation procedure used here is described in Bar et al. (2008a). Other parameters and prices and costs were taken from Bar (2007), De Vries (2006) and Bar et al. (2008a).

Estimating CM cost

The average net returns under optimization per cow per year for a herd without CM were compared with the average net returns per cow per year for a herd with CM (by pathogen), while keeping other parameters constant. The profit or loss was divided by the CM incidence to generate the herd average cost per case of CM. As the cost of CM was reduced under optimal treatment decisions, it is possible that these values differ from actual farm figures.

The net present value (NPV) is the current value of actions where the benefits and costs of the actions are calculated until the end of the time horizon. This is achieved by discounting the various benefits and costs by an annual interest rate over that time period. The discounting factor (β) is equal to exp(-r) where r = 0.08 (interest rate), i.e. $\beta = 0.92$. The retention payoff (RPO) value is the NPV of retaining a cow compared with the NPV of her replacement (Bar et al., 2008b), i.e. $NPV_{retaining} - NPV_{replacing}$.

Estimating components of CM cost

The components contributing to the average cost per case i.e., reduced milk production due to CM, reduced conception, treatment cost, milk discarded due to antibiotic treatment and risk of mortality are estimated by sequentially adding the effects of these components and comparing the difference in the average cost/case.

Exit from the herd

Exit from the herd can be due to two reasons: (1) voluntary culling based on what the model recommends or (2) due to involuntary culling. Involuntary culling can be due to euthanasia or cows sold for slaughter because of reasons other than milk yield, pregnancy or CM (i.e. reasons not determined directly from the model).

Sensitivity analyses

Given that economic values such as milk price, pregnancy rate and replacement cost can vary from time to time and farm to farm, a sensitivity analysis was performed to evaluate how an increase and decrease of 20% in each of these values individually affected the percentage of CM cases in the herd and the average cost per case.

RESULTS

The Cost of Pathogen Specific CM

The net returns in US$ per cow and year for the basic scenario (with all CM included) was 500, where the incidence of CM (cases per 100-cow years) was 35.5, of which 90.6% of cases were recommended to be treated under an optimal replacement policy (Table 5.4).

Table 5.4. The effects of different pathogens causing clinical mastitis (CM) on CM cases, % of CM cases treated, average cost of CM and average cost per case, following an optimal replacement policy (all costs in US$)

Item	CM cases[1]	Percent of CM cases treated[2]	Average costs of CM per cow in the herd	Average cost per case[3]
Basic scenario (incl. all CM)	35.5	90.6	83	233
Staphylococcus spp.	1.6	91.4	4	275
Staphylococcus aureus	1.8	86.4	5	298
Streptococcus spp.	6.9	87.3	18	257
Escherichia coli	8.1	90.7	25	309
Klebsiella	2.2	90.2	9	416
Other treated cases	1.1	94.4	3	310
Other not treated cases	1.2	89.5	4	316
Negative culture cases	12.7	92.6	19	151

[1]Incidence of CM (cases per 100-cow years).
[2]Percentage of treated CM cows per all CM cows
[3]Average cost per CM case

The cost per case of CM was 233.41 (82.86/0.355). The CM cases (total of 35.5 per 100 cows) were comprised of *Staphylococcus* spp. (1.6), *Staphylococcus aureus* (1.8), *Streptococcus* spp. (6.9), *Escherichia coli* (8.1), *Klebsiella* (2.2), Other treated cases (1.1), Other not treated cases (1.2) and Negative culture cases (12.7). The average cost per case was greatest for *Klebsiella* (416), followed by other not treated cases (316) which was similar to the other treated cases (310) and *Escherichia coli* (309). This was followed by the gram-positive pathogens, with the greatest cost per case being due to *Staphylococcus aureus* (298), then *Staphylococcus* spp. (275) and *Streptococcus* spp. (257). Negative culture cases had the lowest cost (151).

Most CM cases (by pathogen), were recommended to be treated (i.e. all over 85%), and this was greatest for other treated cases (94.4%) and lowest for *Staphylococcus aureus* cases (86.4%).

The components contributing to the average cost per case i.e., reduced milk production due to CM, reduced conception, treatment cost, milk discarded due to antibiotic treatment and risk of mortality are presented in Table 5.5.

Table 5.5. Breakdown of components (milk loss, reduction in conception, treatment cost, milk discarded due to antibiotic treatment and risk of mortality) contributing to the average cost per case by pathogen specific clinical mastitis (CM)

	Components contributing to average cost per case of CM in USD (% of average cost/case)					
Pathogen	Milk loss	Reduced conception	Treatment cost	Discarded milk	Risk of mortality	Average cost/case (USD)
Staphylococcus spp.	2 (1)	35 (13)	110 (40)	64 (23)	64 (23)	275
Staphylococcus aureus	62 (21)	33 (11)	113 (38)	55 (18)	35 (12)	298
Streptococcus spp.	26 (10)	43 (17)	113 (44)	55 (21)	20 (8)	257
Escherichia coli	58 (19)	61 (20)	57 (19)	0	132 (43)	309
Klebsiella	25 (6)	25 (6)	56 (14)	0	257 (62)	416
Other treated	22 (7)	80 (26)	85 (27)	22 (7)	101 (33)	310
Other not treated	107 (34)	56 (18)	57 (18)	0	97 (31)	316
Negative culture	25 (17)	35 (23)	59 (39)	0	31 (21)	151

The Effects of Changes in Exogenous Factors on the Cost of Pathogen Specific CM

In general, a 20% increase in milk price resulted in an increase in the average cost/case of CM. This same trend was observed as replacement cost and pregnancy rate increased by 20% (Table 5.6). The incidence of CM increased overall when replacement cost and pregnancy rate increased by 20%.

Table 5.6. Effects of increasing and decreasing milk price, replacement cost and pregnancy rate by 20% on clinical mastitis (CM) cases and the average cost/case for all CM and for each pathogen causing CM

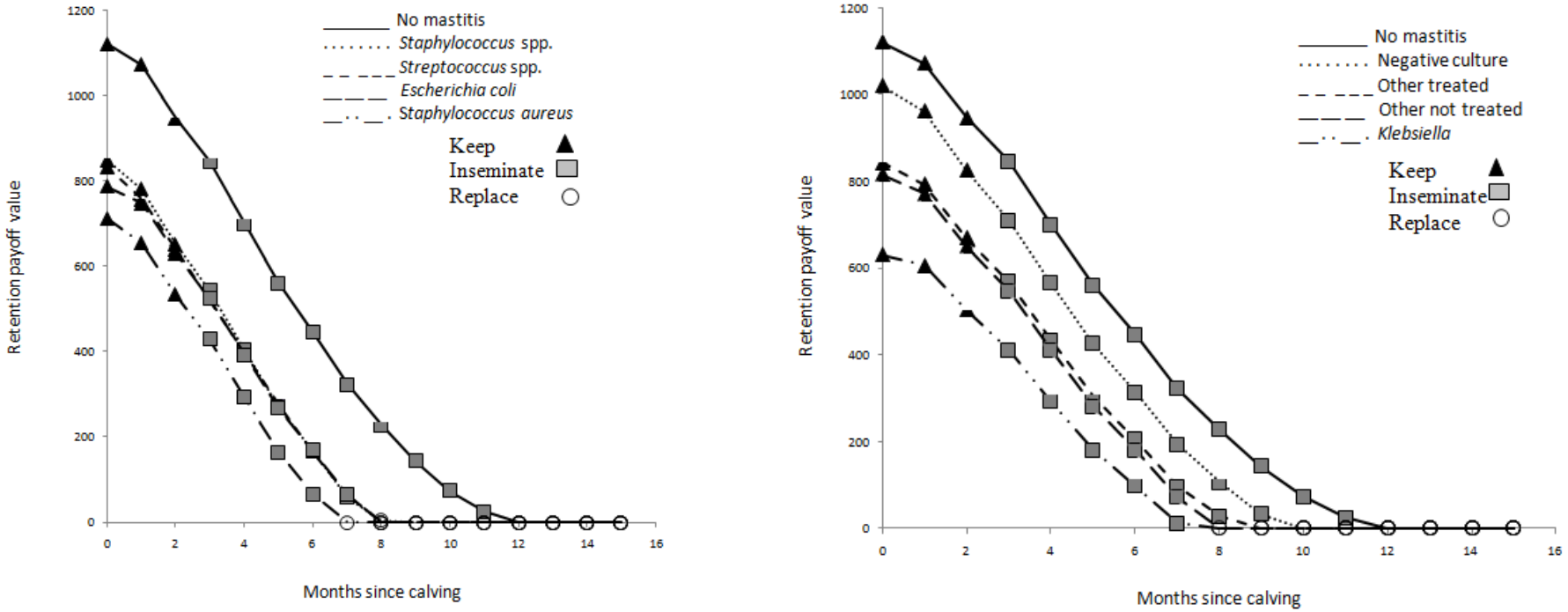
Item	*Staphylococcus* spp.		*Staphylococcus aureus*		*Streptococcus* spp.		*Escherichia coli*		*Klebsiella*		Other treated		Other not treated		Negative culture	
	CM cases[1]	cost/ case[2]	CM cases[1]	cost/ case[2]	CM cases[1]	cost/ case[2]	CM cases[1]	cost/ case[2]	CM cases[1]	cost/ case[2]	CM cases[1]	cost/ case[2]	CM cases[1]	cost/ case[2]	CM cases[1]	cost/ case[2]
Milk price +20%	1.6	303	1.8	333	6.8	291	8.0	344	2.2	456	1.1	345	1.2	360	12.5	170
Milk price -20%	1.6	247	1.8	259	7.0	222	8.2	270	2.3	361	1.1	277	1.2	272	12.9	131
Replacement cost +20%	1.6	288	1.8	307	7.0	258	8.1	332	2.2	465	1.1	331	1.2	337	12.8	154
Replacement cost -20%	1.6	260	1.8	286	6.8	252	7.9	288	2.2	367	1.1	292	1.2	296	12.4	148
Pregnancy rate +20%	1.6	284	1.8	301	7.0	260	8.2	310	2.3	413	1.1	320	1.2	326	12.9	153
Pregnancy rate -20%	1.6	266	1.8	297	6.7	256	7.8	308	2.1	415	1.1	306	1.2	310	12.3	149

[1]Incidence of CM (cases per 100 cow-years)

[2]Average cost/case (USD)

Retention Pay Off Value of Open Healthy and Mastitic Cows

Our economic model calculates the RPO for cows, dependent on the cow's individual characteristics. Figures 5.3.1 and 5.3.2 are an example of a hypothetical cow's RPO under an optimal policy for cows free of CM and with CM by pathogen, specific to an open (non-pregnant), second-lactation cow with >= 1 case of CM in the previous lactation and average permanent and temporary milk yields per 305-d lactation. The optimal policy recommended by the model [(1) keep (and treat if CM) and not inseminate, (2) keep (and treat if CM) and inseminate, or (3)



A) **B)**

Figure 5.3.1 and 5.3.2. Retention payoff values for a hypothetical cow in lactation 2 with >= 1 case of clinical mastitis in the previous lactation, with average permanent and temporary milk yields, by no mastitis or pathogen (first case only. 2A: *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli* and *Staphylococcus aureus*; 2B: Negative culture, Other treated, Other not treated and *Klebsiella*).

replace] is also illustrated by the symbols in the figures.

In Figures 5.3.1 and 5.3.2, the RPO (US$) of cows at calving was $1,121, $849, $833, $787, $713, $1,020, $843, $815 and $631 for no CM, *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Staphylococcus aureus*, Negative culture, Other treated, Other not treated and *Klebsiella*, respectively. The average cost at calving was calculated by subtracting the RPO for the pathogen specific CM from the RPO for no CM. The average cost at calving was $272 ($1,121 – $849), $288 ($1,121 – $833), $334 ($1,121 – $787), $408 ($1,121 – $713), $101 ($1,121 – $1,020), $278 ($1,121 – $843), $306 ($1,121 – $815) and $490 ($1,121 – $631) for *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Staphylococcus aureus*, Negative culture, Other treated, Other not treated and *Klebsiella*, respectively. When the RPO is negative, it is profitable to cull the cow than to keep her. This was observed in mo 12 for no CM, mo 10 for Negative culture, mo 9 for *Staphylococcus* spp. and Other treated, mo 8 for *Streptococcus* spp., *Escherichia coli*, *Klebsiella* and Other not treated and mo 7 for *Staphylococcus aureus.* Figures 5.3.1 and 5.3.2 illustrate the recommended policy until mo 15; the model recommended that cows in mo 15 and onward be replaced.

Endogenous Factors Affecting the Cost of CM

The cost of CM is dependent on endogenous factors, such as the permanent (genetic) milk yield potential of the cow, pregnancy status and lactation as shown in Table 5.7.

Table 5.7. Average cost (in US$) of a first case of pathogen specific clinical mastitis (CM) in cows with no CM in the previous lactation and different levels (low, average, high) of permanent (genetically determined) milk yield potential 4 mo after calving and open (i.e., not pregnant) or pregnant, obtained by the insemination and replacement optimization model[1]

Permanent milk yield potential

Low

	Open								Pregnant							
Lactation	Sta	S.aur	Str	E.coli	Kleb	Otht	Othn	Negc	Sta	S.aur	Str	E.coli	Kleb	Otht	Othn	Negc
1	123	123	123	123	123	123	123	123	257	260	260	235	241	235	260	233
2	208	249	249	174	201	166	186	95	295	382	337	260	309	246	267	163
4	184	229	220	157	195	163	198	145	265	317	291	231	281	250	276	209

Average

	Open								Pregnant							
Lactation	Sta	S.aur	Str	E.coli	Kleb	Otht	Othn	Negc	Sta	S.aur	Str	E.coli	Kleb	Otht	Othn	Negc
1	328	450	394	365	457	299	388	287	349	434	398	361	440	308	379	293
2	358	469	363	372	478	330	351	193	371	458	384	383	483	345	363	221
4	327	361	311	280	397	391	327	215	350	370	328	308	452	470	341	236

High

	Open								Pregnant							
Lactation	Sta	S.aur	Str	E.coli	Kleb	Otht	Othn	Negc	Sta	S.aur	Str	E.coli	Kleb	Otht	Othn	Negc
1	378	488	431	505	714	336	403	307	394	466	430	471	647	341	392	311
2	416	530	371	519	709	431	450	225	424	509	397	505	675	432	446	252
4	416	393	346	383	629	667	415	238	420	397	359	387	625	684	401	256

[1]Sta = Staphylococcus spp.; S. aur = Staphylococcus aureus; Str = Streptococcus spp.; E. coli = Escherichia coli; Kleb= Klebsiella; Otht = Other treated; Othn = Other not treated; Negc = Negative culture

These average costs are for a cow 4 months after calving with no CM in the previous lactation. The average cost was greater in pregnant cows compared with open cows. Younger cows also had a greater average cost compared with older cows. The average cost

increased as permanent milk yield potential increased. While the pathogen with the greatest average cost varied by pregnancy status and genetic milk yield potential, *Klebsiella* appeared to have the largest average cost overall. This reflects the average cost/case trend seen in Table 5.5.

Exit from the Herd (Voluntary and Involuntary Culling)

The percentage of involuntary cullings over a year was 10.4%, and the percentage of voluntary cullings, 20.2%, for a total exit of 30.6% when all CM was included in the model. The total exit increased to 33.0% (10.3% involuntary and 22.7% voluntary cullings) when milk price increased by 20%, and reduced to 28.6% (10.5% and 18.1%) when milk price was decreased by 20%. The total exit reduced to 29.1% (10.5% and 18.6%) when replacement cost was increased by 20%, and increased to 33.3% (10.2% and 23.1%) when replacement cost was decreased by 20%. When pregnancy rate was increased by 20%, the total exit reduced to 28.8% (10.7% and 18.1%) and increased to 33.4% (9.9% and 23.5%) when pregnancy rate was reduced by 20%.

DISCUSSION

The objective of this study was to develop and solve an economic optimization model which provides dairy farmers optimal decisions for management of their cows suffering from CM. The economic value of one decision compared with another is quantified by the calculation of retention pay off values. We analyzed the effect of different scenarios by making changes to input parameter values and comparing the net returns. A limitation of including other diseases aside from CM is that the economic model becomes prohibitively large as the state space expands, and also, not all the parameters required to run such a model are available. Because it

may be possible to include other diseases in the near future as data are available, we developed this model from the beginning to have a relatively versatile structure, allowing for relatively easy expansion.

By stratifying by pathogen, we were able to identify that mortality and treatment cost contribute largely to the cost of pathogen specific CM, and the effect of reduced milk production due to CM was actually smaller than what we observed when CM was combined into one group (Bar et al., 2008), or stratified by gram-groups (Cha et al., 2011). This is largely attributed to the data being different across the time periods (we have collected more data with each progressive study). Also, in this study, we identified that other CM (i.e. those that are not gram-positive or gram-negative CM) actually have a high cost/case comparable with gram-positive CM cases. The total percent of cows recommended to be culled in our study was lower than that in Cha et al. (2011) i.e., 30.6%, mainly due to a lower involuntary culling percentage. The percentage of cows recommended to be culled by the economic model i.e., voluntary cullings, however, was similar.

As expected, the average cost of CM generally increased as cows were in greater milk yield potentials and decreased as they progressed across lactations. The latter is explained by cows getting older, hence, having less time remaining for losses to production from CM to take effect. While we observed similar trends in average costs across different permanent milk yield levels and across lactations as reported by Cha et al. (2011), we found the average costs were lower for open cows compared with pregnant cows. This is because in the previous study, the effects of reduced conception were allowed to span for 3 months since the case of CM; however, in this study, we have accounted for reduced conception for 1 month after a case of CM, which

we have found is when the reduced conception effects following CM occurrence have the most effect (Hertl et al., *unpublished*).

It is difficult to validate animal replacement economic models as other existing models often differ in their structure and parameter values. Interestingly, the net return/year from our study (500 USD) is comparable with the expected net present value of £285.50 (approx. 457 USD) from a study by Yalcin and Stott (2001); however, the latter model had a time horizon of 20 years, 12 lactations, 15 milk yield states and 11 somatic cell count states. The net return in our current study is greater than that from the generic CM study by Bar et al., (2008) i.e., 355 USD and the gram-positive, gram-negative and other CM study by Cha et al., (2011) i.e., 357.35. Differences in these values are attributed to the varying structure and parameter estimates adopted. In the model by Bar et al. (2008), CM was included as one group, not separated by culture classes. This meant that the effects of CM i.e., repeated risks, milk loss, mortality risks and reduced conception were all for generic CM and not culture classes as in the case of our model. Also, the withholding period due to antibiotic treatment was not incorporated as an adjustment to the already lower milk production among CM cows.

To the authors' knowledge, there are very few studies that examine the cost of pathogen-specific CM (Østergaard et al., 2005; Sørensen et al., 2010). In the study by Sørensen et al. (2010), economic values for pathogen-specific CM were estimated using a stochastic simulation model (SimHerd IV). The simulations were conducted over time with weekly time increments, other diseases were included as well as severity of CM, and an annual interest rate of 4% was used (unlike 8% in our study). While we found the average cost/case was in general greater for *Escherichia coli* and *Klebsiella,* the study by Sørensen et al. (2010) found the opposite trend, where the cost per case in €(USD) was greater i.e., 570 (725) for *Staphylococcus aureus* and 380

(483) for CNS, and less i.e., 206 (262) for *Escherichia coli* and 149 (189) for *Streptococcus dysgalactiae* and *Streptococcus uberis*. The model by Sørensen et al. (2010) did not model contagious spread between herd mates, hence, this assumption is the same as our dynamic program. The difference in the costs/case may be due to the mortality risks we adopted (Cha et al., 2012b), and based on this, it did not come as a surprise that risk of mortality contributed largely to the cost per case in our study, especially for *Escherichia coli* and *Klebsiella*.

The cost of CM (Table 5.4) calculated by the economic model following optimization and simulation reflect what has been observed on average across our study farms (Cha et al., 2012a). The average cost per case for each pathogen is an economic consequence of the assimilation of the pathogen specific CM effects (i.e., milk loss, reduced conception, treatment cost, discarded milk days and mortality risk) with which we parameterized our model. By including discarded milk due to treatment after adjusting for the loss to milk due to CM, our results were more accurate than if the discarded milk was accounted for without considering the initial drop in milk due to CM. Through scenarios analyses, we were able to break down the contribution of each effect to the average cost per case by pathogen (Table 5.5). It may seem counterintuitive that some pathogens may be treated with antibiotics given that the milk loss due to days of discarded milk post antibiotic therapy is greater than the effects of reduced conception and reduced milk production combined. For example, for CM attributed to *Staphylococcus* spp., there is less cost to a reduction in milk production due to CM (0.8% of the total average cost/case) and reduced conception (12.8%) combined, compared with the cost of discarded milk (23.1%). A reason for treating these cows suffering from CM attributed to *Staphylococcus* spp. Regardless of our empirical results, may be because the overall quality of the milk is compromised, resulting in milk price penalties. However, we did not model poor milk quality

directly. In study by Ma et al., 2000, it was demonstrated that for high SCC milk, between 14 to 21 d post processing, sensory defects i.e., rancidity and bitterness were detected which resulted in lower quality ratings. These defects were found to be consistent with higher levels of lipolysis and proteolysis, adversely affecting the quality of pasteurized fluid milk. A study by Wilson et al. (1997) showed that cows suffering from CM due to *Staphylococcus* spp. infection had a linear score of SCC of 3.7, corresponding to 162,000 cells/ml (Western Canadian Dairy Herd Improvement Services, accessed Nov. 9, 2012). While the regulatory limit in the USA is 750,000 cells/ml, the global standard (and also the European Union limit) is 400,000 cells/ml, highlighting the economic ramifications of CM and why treatment is often warranted (Adkinson et al., 2001; Hoards Dairyman, 2011). Milk loss for *Escherichia coli* cases in this study is representative of farms that use a J5 vaccine which has been demonstrated to reduce milk loss due to *Escherichia coli* (Wilson et al., 2007).

The sensitivity analyses of milk price, replacement cost and pregnancy rate were performed to identify the effect of these factors on the direction and magnitude of the average cost/case and incidence of CM. When milk price was increased by 20%, the average cost per case increased because the production limiting effects of CM have a greater impact at higher milk prices. The incidence of CM was reduced when replacement cost was decreased by 20%, as an increase in replacement would lead to an overall younger herd of animals with an overall lower incidence of CM compared with an older herd of animals. The increased level of CM when pregnancy rate was increased by 20% is explained by there being more cows being kept in the herd, as pregnancy adds value to the cow, thereby lending to more older cows in the herd, and a greater incidence of CM compared with a younger group of animals. These trends are in agreement with those from our previous studies (Bar et al., 2008a; Cha et al., 2011).

From month 3, the first month cows can be inseminated, we observed that until the optimal policy is replacement (the latest being at month 12), the cows are recommended to be inseminated regardless of CM status (Figures 5.3.1 and 5.3.2). Because the length of each lactation was a maximum of 20 months, and any cows that became pregnant from month 13 would be automatically replaced at the end of the lactation, all cows from month 12 onwards were recommended to be replaced. The point at which cows were recommended to be replaced could be different if the maximum number of months were changed to another value; however, it was necessary to have a maximum stopping point for the lactation. We also used a constant pregnancy rate of 21% throughout lactation for primiparous and multiparous cows; if we had modeled pregnancy rate to decrease throughout lactation, it is possible that the optimal decision would not have been to inseminate each month until cows are replaced. This would depend on the value of the decision to keep the cow compared with the decision to inseminate her, which is a function of the probability of pregnancy, the expected gain from pregnancy and the cost of insemination.

Given that the quantity of milk produced is by far one of the key determinants of net returns in a dairy operation, it would be ideal for an economic optimization model to contain very detailed information on the lactation curve of the dairy cows, while accounting for seasonality. While the replacement heifers in this study do follow a skewed distribution toward genetic improvement which is a reflection of what is taking place on farms, we do not account for each successive replacement to be an improvement since the previous replacement. These considerations were beyond the scope of this study, and we are working toward expanding this model further, and to include more diseases of importance as well as other significant production effects.

Key assumptions of the model include (1) that a constant herd size is maintained, (2) if an animal is replaced a replacement heifer is readily available and (3) there is a market for beef calves. In reality, these assumptions may not always be true. These assumptions are the same as those applicable to the generic CM model (Bar et al., 2008a). Unlike the generic CM model, we included 5 lactations (as opposed to 8 lactations), as 5 lactations includes over 98% of cows in the herd (Cha et al., 2012b). Another motivation was that by reducing the number of lactations, we could also reduce the prohibitive effects of a large state space, otherwise known as the curse of dimensionality (Kristensen et al., 2010). Even though inclusion of a carryover state significantly increased the state space of the model, it became necessary to include this information as we have identified that the risk of CM differs depending on whether a cow suffered from cases of CM in the previous lactation (Cha et al., 2012a). This prohibitive effect would also be in effect if more stratifications of CM or additional diseases (if such information were available) were included.

In our study, the stage length adopted was 1 month, hence, milk production parameters were specific across 1 month, although we did have access to daily milk yields. A daily time step would have expanded the state space enormously and it is not clear whether the benefits would have been sufficient given the benefits of modeling more detail elsewhere as we did. Nielsen et al. (2010) developed a model with a daily time step with Bayesian updating to predict the performance of cows in the herd; however, their focus was milk yield and not dairy cattle diseases.

In addition to calculating the cost of disease and optimal replacement decisions, this model can be used to investigate the value of different control strategies as has been explored by

the generic CM model (Bar et al., 2008b). A next step would be to assess different control strategies specific to each pathogen specific CM using the current model.

CONCLUSION

The dynamic programming model developed and solved generates optimal economic decisions to make with the diseased cows experiencing CM. The recommendations are specific to an individual cow, and hence, are dependent on the permanent milk yield potential, lactation, month of lactation, pathogen causing CM, temporary milk yield and pregnancy status. In general, the average cost per case was greatest for *Escherichia coli* and *Klebsiella* CM. Optimal recommended time for replacement was as great as 5 months earlier for cows with CM compared with cows without CM. Further, while the parameter estimates implemented in this model are specific to the dairy farms in this study, results should prove useful to farmers with cows of the modeled traits. In addition, the cows characteristics and model parameters may be altered so that the results are specific to any farm. Inclusion of additional information pertaining to other diseases of importance in dairy cattle, detail to the milk yield distribution and genetic improvements would also add value to the existing model and forms the basis for future research efforts. Further, we are currently studying the value of information i.e., monetary losses or gains to having information on CM at the pathogen-specific or gram-specific level, compared with knowing the cow is suffering from non pathogen-specific CM. This information would provide the value of pathogen specific tests.

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CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

The advantage of animal replacement models is that they can include a vast amount of information that is available to dairy farmers that one would otherwise not be able to completely assimilate when making informed decisions. Often times, dairy farmers employ rules of thumb when deciding what to do with their diseased cows. The economic model developed in this study is not designed to override the decisions dairy farmers make, but rather, provide an adjunct to this decision process. Our model provides economically optimal decisions based on the probability of events that are projected into the future and expected future losses due to CM. The recommended decision is specific to a cow dependent on the cow's characteristics and stage of lactation while maximizing net returns in general.

In developing this model, we needed to parameterize the model with the risks of bacteria specific CM and their production limiting effects. For the risk and mortality and culling analyses, generalized linear mixed models (GLMM) with an assumed random Poisson distributed error were developed. We decided to adopt this method of analysis because a key reason for estimating the risk and mortality estimates was for the purpose of parameterizing the economic model. For the economic model, probabilities were needed, and from the parameter estimates of these GLMM, the risks can be easily calculated. This made the GLMM approach favorable to other methods of analyzing the data in this study e.g., survival analysis or logistic regression.

Interestingly, we found that cows with a second or third case of CM that had been exposed to the same pathogen previously were at greater risk of that same pathogen (compared with cows that had been exposed to a previous case of CM that was different to the second or third case in question). The findings in our study relating to the absence of immunological memory across cases of pathogen specific CM is not directly comparable with the premise behind vaccine development. The purpose of vaccines is to elicit an immune response which confers the cow to being protected against the pathogen of interest. The same losses to production and damage to the mammary gland are not elicited by a vaccination, the same way that it is in a real life infection. In the study by Hogan et al., (1999), the severity and duration of clinical signs following intramammary challenge with a heterologous strain of *E. coli* were reduced in vaccinated heifers compared with placebo-injected heifers; this is just one example of the value of vaccines in generating an appropriate immune response, which cannot be directly compared with the lack of immunological immunity following a real infection. A greater risk of recurrent cases of CM, may therefore be due to an immune-compromised state that cows may experience post-real life CM infection, but not post-vaccination.

In the mortality and culling analyses, the time step was 1 month, meaning it is assumed that the cow survives, dies or is culled at the end of each month. One could argue that this is a large time step and that weekly time steps would be more accurate, as there is a chance that we are underestimating the risk of mortality and culling. A reason for using a 1 month time step, however, was because the economic model had this time step to control dimensionality to produce a solvable model and the parameter estimates were required to be consistent to the economic model as detailed above.

In the mortality analysis, the CM indices were specific to the month in lactation as we were interested in immediate death due to CM. This is unlike the culling analysis, where it is possible that dairy farmers will wait 1 or even 2 months after the CM episode before deciding to replace a cow. The CM indices were, therefore, reflected to be able to ascertain whether the risk of culling was greatest in the month of CM occurrence or since 'x' number of months after, and while this differed by pathogen, it was not uncommon for farmers to wait 1 month since the occurrence of CM before culling the cow. This may be due to the observed effects of CM not being seen immediately, but accumulated over a time delay of 1 month.

Our results apply to large, well managed herds in New York State. The size of these herds allowed us access to a large number of observations; however, the external validity extends only to herds of these types. This is true of the risk and mortality and culling analyses, but with reference to the economic model, if the parameter estimates were altered to be specific to herds of another type, then the results would be reflective of that herd. In this way, the economic model can be applicable to a wider range of situations.

Within the datasets, it is possible that there were discrepancies in the way that the information was collected. While every efforts was made to make consistent the definition of CM cases as recorded in Dairy Comp 305 ® herd management software (Valley Agricultural Software, Tulare, CA), it is possible that inconsistencies were introduced across the farms and herdspersons involved.

Dairy cattle replacement models have been previously developed (De Vries et al. 2006; Nielsen et al., 2010; Demeter et al., 2011), with inclusion of information relating to disease (Houben et al., 1994; Bar et al., 2008a; Cha et al., 2011). The model by Bar et al. (2008a)

incorporated information on generic CM, extending and building upon the assumptions of the optimal replacement model developed by Houben et al. (1994) and earlier asset replacement principles (Perrin, 1972). Cha et al. (2011) modified the original model by Bar et al. (2008a) to study 3 groupings of CM (gram positive, gram negative and other).

Our motivation to develop this economic model was primarily due to the fact that the previous framework did not separate CM into the different pathogens that are causative or, in the case of Cha et al. (2011), differentiate between different cases of CM. This meant that the effects of CM were pooled. The influence of this is seen in more specific situations, e.g., when the profile of a cow results in a borderline decision (Østerås et al., 1999). Since the publication of the generic CM study, we had access to 30% more data, enabling us to estimate the effects of CM at the pathogen specific CM level. Discarded milk due to treatment after adjusting for the loss to milk due to disease was not previously accounted for, but now, we were able to quantify as a percentage the effect of discarded milk on the average cost per case. Also, by allowing more than one event to occur as a cow transitions from one month to another, our model better represented what happens in the real world.

As with any model, however, this economic model is a simplification of reality. A study by Ben-Ari et al. (1983) formulated the limitations of animal production modeling in this framework which apply to our economic model, namely (1) uniformity – the traits of the animal are difficult to define and measure and the random variation in traits is large, (2) the production of an animal is cyclic – one needs to decide in which cycle as well as when within a cycle to replace the animal and (3) there is only a limited supply of replacements available (Kristensen, 2010). Evidently, in reality, these conditions will not always be met, nor are always true.

Further, we did not model seasonality or an improvement in the genetic potential of replacement heifers over time. While the replacement heifers in our study were slightly better than average, the fact that replacement heifers generally improve with successive generations was not modeled.

The focus here has been clinical mastitis. Subclinical mastitis (mastitis which does not exhibit clinical signs) is also a big contributor to the overall cost of mastitis. If subclinical mastitis were to be included in the economic model, this could be achieved by introducing additional states. The complexity of this is two-fold; firstly, we would need parameter estimates for these subclinical CM levels i.e., losses to milk yield, reduction in conception etc., but also, as we include more states in the economic model, the model becomes prohibitive, at least, to run on the computers that we have available now. A way around this would be running this model on a supercomputer, but if we want the finished product to be directly accessible to dairy farmers in the future, we need to keep in mind the technology that is realistically available to them.

The problem described above relating to the expansion of the state-space which may be prohibitive is termed the "curse of dimensionality". The solver in the Multilevel Hierarchic Markov Process (MLHMP) software which is used to develop the pathogen specific CM model makes it possible to give exact solutions to models with extremely large state spaces (Kristensen, 2010).

To show more clearly how this came to be, it is necessary to provide some history to the development of dynamic programming, which dates all the way back to the 1950s. In 1957, Bellman published a book which described the theory of a numerical method for solving sequential decision problems, known as the *Bellman Principle of Optimality*. This was followed

by Howard in 1960, who combined dynamic programming with the theory of the Markov chain, resulting in the *Markov Decision Process*. Howard also introduced *policy iteration*, a solution to infinite time horizon problems, as an alternative to the backward contraction method of *value iteration*.

The application of this technique to animal replacement models was illustrated by Jenkins and Halter (1963); in this study, the trait of lactation number (12 levels) was all that was included, but served the purpose of showing that this technique could indeed be applied to such a problem. It was the work of Giaever (1966) that was groundbreaking, as he illustrated with various optimization techniques (value iteration, policy iteration and linear programming) the feed intake and production of a dairy cow. Studies since Giaever (1966) have contributed in ways other than methodology; for example, Smith (1971) was able to achieve a state space of 15 000, as opposed to the 106 states which was the upper limit in the Giaever study. Kristensen and Østergaard (1982) and van Arendonk (1985, 1986) and van Arendonk and Dijkhuizen (1985) studied the effect of prices and conditions on the optimal replacement policy (Kristensen, 2010).

Returning to the idea of the "curse of dimensionality", in developing this economic model, it was always a balance of including detailed information being weighed against the increased risk of the model becoming solution prohibitive. This meant we needed to choose very carefully the stages and states to include. One of the criteria was ensuring these pieces of information were influential in deciding the optimal management decision for cows affected with CM. This became especially important in the founder state where policy iteration, which is computationally demanding, is performed.

At the founder level, we included the permanent milk yield potential of the cow which we have termed a genetic property. This feature was preserved from generic CM model developed by Bar et al. (2008a). In our model, this permanent milk yield potential was account for as deviations from an average milk curve. The meaning ascribed to permanent milk yield potential, however, could be very different. For example, rather than these deviations, this state could have been described by an index encompassing an array of features which determine the cow's genetic potential. This could have been an udder index or an estimated breeding value (EBV) for instance.

This economic model has a time step of 1 month, which meant that the effects of diseases and decisions all applied to 1 month. A smaller time step would have led to more precise decision making but would result in an even larger state-space, and it is unclear whether the benefits of the detail would be sufficient given the benefits of modeling more detail elsewhere as we did.

The value of treatment decisions is overestimated, as we have not accounted for the time delay in obtaining culture results or the efficacy of treatment. Further, within the current framework, it is not possible to account for infectivity between cows with contagious CM, however, the risk estimates for these pathogens reflect the incidence of CM which indirectly includes the fact that these pathogens have had a greater likelihood of having spread within the herd. Further, the economic values e.g., net return, average costs etc are point estimates. Standard errors around these point estimates could be generated with additional work at the model level, however, this was beyond the scope of this study.

When parameterizing this economic model with treatment costs for each pathogen, we accounted for palliative treatment for *Escherichia coli* and *Klebsiella* (gram-negative CM) cases. Not treating with antibiotics for these cases is not uncommon, however, recent research has demonstrated the benefits of treating non severe clinical gram-negative mastitis with ceftiofur hydrochloride (Schukken et al., 2011). In this study, it was demonstrated that treated animals clinically improved significantly more than control cows, and while there was no significant difference in milk production between the two groups, treated animals left the study at a lower rate than control animals. Inclusion of this information, in addition to a withholding period for treatment with anti-inflammatories (as part of palliative care) will be a focus of future work. By comparing two protocols i.e., antibiotic treatment of gram-negative CM with less mortality and no antibiotic treatment, we will be able to determine the economic value of antibiotic treatment of gram-negative CM.

An application of this model is for dairy farmers to include parameter estimates specific to their farm and generate optimal management decisions tailored to their cows. Following optimization, the value of cows can be determined, and based on this, cows may be ranked according to their value, assisting decisions concerning how to down-size, or which cows to replace as necessary (Kristensen, 1989). It would probably be most effective for these results to be communicated to dairy farmers by personnel who have been trained to understand the results of the model and provide consulting services.

Identifying whether dairy farmers need information relating to CM at the generic, gram-positive, gram-negative or other or pathogen specific level will form the basis of future research. It would be ideal to integrate the time delay in asking for more information e.g., gram-positive,

gram-negative and other CM identification can be done on-farm immediately, compared with pathogen specific identification which can take days.

An advantage of this economic model is the versatile structure, which allows for relative easy inclusion of additional diseases or replacement of current CM indexes with other diseases, for instance, calving diseases or other production limiting diseases such as lameness. The structure also allows for the relatively easy inclusion of new disease effects.

CONCLUSIONS

The model developed provides dairy farmers with optimal economic decisions to make for diseased cows experiencing clinical mastitis depending on the stage of lactation the cow is in and her individual characteristics. By altering the parameter estimates of the model, the results can be specific to any farm, providing estimates of the cost of CM, optimal replacement decisions and cow rankings. This model affords flexibility so that inclusion of additional information pertaining to other diseases of importance in dairy cattle, details of the milk yield distribution and genetic improvements can be introduced relatively easily. Changes can also be made at the founder level to permanent genetic milk yield potential and at the level of the stages e.g., inclusion of a subclinical CM index. Future work would include improvements in the aforementioned areas, as well as studying the cost and benefits of different control strategies targeted for the pathogen specific CM and evaluating the value of information i.e., monetary losses or gains to having information on CM at the pathogen-specific or gram-specific level, compared with merely knowing the cow is suffering from non pathogen-specific CM.

BIBLIOGRAPHY

Adkinson, R.W., Gough, R.H., Graham, R. and Yilmaz, A. 2001. Implications of Proposed Changes in Bulk Tank Somatic Cell Count Regulations. J. Dairy Sci. 84: 370-374

Animart 2013. http://www.animart.com/store/mastitis-tubes-lactating-treatments/ (accessed 6 Jan 2012)

Animal Livestock Supply Inc. http://www.americanlivestock.com/cattle.html (accessed 6 Jan 2012)

Atalla, H., Gyles, C., Jacob, C.L., Moisan, H., Malouin, F. and Mallard, B. 2008. Characterization of a *Staphylococcus aureus* small colony variant (SCV) associated with persistent bovine mastitis. Foodborne Pathogens and Disease. 5:785-799.

Bannerman, D. D. 2008. Pathogen-dependent induction of cytokines and other soluble inflammatory mediators during intramammary infection of dairy cows. J. Anim. Sci. 87(Suppl. 1):10-25.

Bar, D. 2007. Cost of generic clinical mastitis in dairy cows. PhD Thesis. Cornell University, Ithaca, NY.

Bar, D., Y. T. Gröhn, G. Bennett, R. N. González, J. A. Hertl, H. F. Schulte, L. W. Tauer, F. L. Welcome, Y. H. Schukken. 2007. Effect of repeated episodes of generic clinical mastitis on milk yield in dairy cows. J. Dairy Sci. 90: 4643-4653.

Bar D, Gröhn YT, González RN, Hertl JA, Schulte HF, Bennett GJ, Tauer L, Schukken YH. 2008a. Effect of repeated episodes of generic clinical mastitis on mortality and culling in dairy cows. *J Dairy Sci* 91: 2196-2204.

Bar, D. L. W. Tauer, G. Bennett, R. N. González, J. A. Hertl, Y. H. Schukken, H. F.Schulte, F. L. Welcome, Y. T. Gröhn. 2008b. The cost of generic clinical mastitis in dairy cows as estimated by using dynamic programming. J. Dairy Sci. 91: 2205-2214.

Bar, D. L. W. Tauer, G. Bennett, R. N. González, J. A. Hertl, H. F. Schulte, Y. H. Schukken, F. L. Welcome, Y. T. Gröhn. 2008c. Use of a dynamic programming model to estimate the value of clinical mastitis treatment and prevention options utilized by dairy producers. Agricultural Systems 99: 6-12.

Barkema, H. W., Y. H. Schukken, T.J. Lam, M. L. Beiboer, H. Wilmink, G. Benedictus, and A. Brand. 1998. Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts. J. Dairy Sci. 81: 411-419.

Bellman, R. 1957. Dynamic programming. Princeton Univ. Press, Princeton. NJ.

Ben-Ari, Y., I. Amir, and S. Sharar. 1983. Operational replacement decision model for dairy herds. J. Dairy Sci. 66:1747–1759.

Bradley, A. J., and Green, M. J. 2001. Aetiology of clinical mastitis in six Somerset dairy herds. The Veterinary Record. 148: 683-686

Calzolari, A., J. A. Giraudo, H. Rampone, L. Odierno, A. T. Giraudo, C. Frigerio, S. Bettera, C. Raspanti, J. Hernández, M. Wehbe, M. Mattea, M. Ferrari, A. Larriestra, and R. Nagel. 1997. Field trials of a vaccine against bovine mastitis. 2. Evaluation in two commercial dairy herds. J. Dairy Sci. 80: 854-858.

Cha, E, J. A. Hertl, D. Bar, and Y. T. Gröhn. 2010. The cost of different types of lameness in dairy cows calculated by dynamic programming. Prev. Vet. Med. 97:

1-8.

Cha, E., D. Bar, J. A. Hertl, L. W. Tauer, G. Bennett, R. N. González, Y. H. Schukken, F. L. Welcome, Y. T. Gröhn. 2011. The cost and management of different types of clinical mastitis in dairy cows estimated by dynamic programming. J. Dairy Sci. 94: 4476-4487.

Cha, E., J. A. Hertl, Y. H. Schukken, F. L. Welcome, and Y. T. Gröhn. 2012. Risk of a first case and recurrent cases of bacteria specific clinical mastitis in dairy cows. *Under review*.

Cha, E., Hertl JA, Schukken YH, Welcome FL, Gröhn YT. 2012b. The effect of repeated episodes of bacteria specific clinical mastitis on mortality and culling in Holstein dairy cows. *Under review*.

Dairy Health USA, 2012 http://www.pbsanimalhealth.com/category/Dairy/Mastitis-Treatments/D80200.html#cat_top (accessed 6 Jan 2012)

Dechow, C. D., and R. C. Goodling. 2008. Mortality, culling, by sixty days in milk, and production profiles in high- and low-survival Pennsylvania herds. J. Dairy Sci. 91: 4630-4639.

Demeter, R.M., Kristensen, A.R., Dijkstra, J., Oude Lansink, A.G., Meuwissen, M.P. and van Arendonk, J.A. 2011. A multi-level hierarchic Markov process with Bayesian updating for herd optimization and simulation in dairy cattle. J. Dairy Sci. 94: 5938-62

De Vries, A. 2006. Economic value of pregnancy in dairy cattle. J. Dairy Sci. 89:3876-3885.

Dogan, B., Klaessig, S., Rishniw, M., Almeida, R.A., Oliver, S.P., Simpson, K. and Schukken,

Y.H. 2006. Adherent and invasive *Escherichia coli* are associated with persistent bovine mastitis. Veterinary Microbiology. 116:270-282

Döpfer, D., Barkema, H.W., Lam, T.J.G.M., Schukken, Y.H., and Gaastra, W. 1999. Recurrent clinical mastitis caused by *Escherichia coli* in dairy cows. J. Dairy Sci. 82:80-85

Erskine, R. J., S. Wagner, and F. J. DeGraves. 2003. Mastitis therapy and pharmacology. Vet. Clin. Food Anim. 19:109-138.

Esslemont, R. J., and M. A. Kossaibati. 1997. Culling in 50 dairy herds in England. Veterinary Record. 140: 36-39.

Federal Reserve Bank of Kansas City. Agricultural Credit Survey 'Interest rate data – agricultural interest rates: Operating loans, Federal Reserve Bank of Kansas City-Tenth Federal Reserve District, Quarterly Agricultural Credit Survey' http://www.kansascityfed.org/research/indicatorsdata/agcredit/ (accessed 23 Jan 2011).

Giaever, H. B., 1966. Optimal dairy cow replacement policies. Ph.D. thesis, University of California, Berkeley, University Microfilms, Ann Arbor, Michigan.

González, R. N., J. S. Cullor, D. E. Jasper, T. B. Farver, R. B. Bushnell, and M. N. Oliver. 1989. Prevention of clinical coliform mastitis in dairy cows by a mutant *Escherichia coli* vaccine. Can. J. Vet. Res. 53:301–305.

Green, M.J., Green, L.E., Medley, G.F., Schukken, Y.H. and Bradley, A.J. 2002. Influence of dry period bacterial intramammary infection on clinical mastitis in dairy cows. J. Dairy Sci. 85:2589-2599

Gröhn, Y. T., S. W. Eicker, V. Ducrocq, and J. A. Hertl. 1998. Effect of diseases on the culling of Holstein dairy cows in New York State. J. Dairy. Sci. 81: 966-978.

Gröhn, Y. T., S.W. Eicker, and J.A. Hertl. 1995. The association between previous 305-day milk yield and disease in New York State dairy cows. J. Dairy Sci. 78:1693-1702.

Gröhn, Y.T., Erb, H.N., McCulloch, C.E., Saloniemi, H.S. 1990. Epidemiology of mammary gland disorders in multiparous Finnish Ayrshire cows. Preventive Veterinary Medicine, 8, 241-252.

Gröhn, Y. T., D. J. Wilson, R. N. González, J. A. Hertl, H. Schulte, G. Bennett, Y. H. Schukken. 2004. Effect of pathogen-specific clinical mastitis on milk yield in dairy cows. J. Dairy Sci. 87: 3358-3374.

Gröhn, Y.T., R. N. González, D. J. Wilson, J. A. Hertl, G. Bennett, H. Schulte, and Y. H. Schukken. 2005. Effect of pathogen-specific clinical mastitis on herd life in two New York State dairy herds. Preventive Veterinary Medicine. 71: 105-125.

Halasa, T., Nielen, M., Huirne, R.B.M. and Hogeveen, H. 2009. Stochastic bio-economic model of bovine intramammary infection. Livestock Science. 124:295-305.

Hazlett, M. J., P. B. Little, M. G. Maxie, and D. A. Barnum. 1984. Fatal mastitis of dairy cows: a retrospective study. Can. J. Comp Med. 48: 125-129.

Hertl, J. A., Y. T. Gröhn, J. D. G. Leach, D. Bar, G. J. Bennett, R. N. González, B. Rauch, F. L. Welcome, L. W. Tauer, Y. H. Schukken. 2010. Effects of clinical mastitis caused by

gram-positive and gram-negative bacteria and other organisms on the probability of conception in New York State Holstein dairy cows. J. Dairy Sci. 93: 1551-1560.

Hertl, J. A., Y. H. Schukken, D. Bar, G. J. Bennett, R. N. González , B. J. Rauch, F. L. Welcome, L. W. Tauer, Y. T. Gröhn. 2011. The effect of recurrent episodes of clinical mastitis caused by gram-positive and gram-negative bacteria and other organisms on mortality and culling in Holstein dairy cows. J. Dairy Sci. 94: 4863-4877.

Hertl, J. A., Y. H. Schukken, F. L. Welcome, L. W. Tauer and Y. T. Gröhn. Effects of repeated pathogen-specific clinical mastitis episodes on milk yield in Holstein dairy cows. *In preparation*

Hoards Dairyman, 2011 'A 400,000 SCC milk limit after all' http://www.hoards.com/blog_SCC-milk-limit <accessed 2 Jan 2012>

Hogan, J. S., V. L. Bogacz, M. Aslam, and K. L. Smith. 1999. Efficacy of an *Escherichia coli* J5 bacterin administered to primigravid heifers. J. Dairy Sci. 82: 939-943.

Hogan, J. S., W. P. Weiss, D. A. Todhunter, K. L. Smith, and P. S. Schoenberger. 1992. Efficacy of an *Escherichia coli* J5 mastitis vaccine in an experimental challenge trial. J. Dairy Sci. 75: 415–422.

Houben, E.H.P, Dijkhuizen, A.A., van Arendonk, J.A.M. and Huirne, R.B.M. 1993. Short and long-term production losses and repeatability of clinical mastitis in dairy cattle. Journal of Dairy Science, 76, 2561-2578

Houben, E. H. P., R. B. M. Huirne, A. A. Dijkhuizen, and A. R. Kristensen. 1994. Optimal replacement of mastitic cows determined by a hierarchical Markov process. J. Dairy Sci. 77:2975–2993.

Howard, R., 1960. Dynamic programming and Markov processes. The M.I.T. Press, Cambridge, MA.

Jenkins, K. B., Halter, A. N., 1963. A multistage stochastic decision model: Application to

replacement of dairy cows. Technical Bulletin 67, Agricultural Experimental Station, Oregon State University.

Kristensen, A. R., Ostergaard, V., 1982. Optimalt udskiftningstidspunkt for malkekoen fastlagt ved en stokastisk model. Beretning fra Statens Husdyrbrugsforsog 533, National Institute of Animal Science.

Kristensen, A. R. 1988. Hierarchic Markov processes and their applications in replacement models. Eur. J. Oper. Res. 35:207-215.

Kristensen, A. R., 1989. Optimal replacement and ranking of dairy cows under milk quotas. Acta Agricultura Scandinavica. 39:311–318.

Kristensen, A. R. 1991. Maximization of net revenue per unit of physical output in Markov decision processes. Europ. Rev. Agr. Econ. 18:231-244.

Kristensen, A. R. 1996. Textbook notes of herd management: Dynamic programming and Markov decision processes. Dina Notat No. 49, Dept. of Animal Science and Animal Health, Royal Veterinary and Agricultural University, Denmark.

Kristensen, A. R. 2003. A general software system for Markov decision processes in herd management applications. Comput. Electron. Agric. 38:199-215.

Kristensen, A. R and E. Jørgensen. 2000..Multi-level hierarchic Markov processes as a framework for herd management support. Annals of Operations Research, 94: 69–89

Kristensen, A.R., Jørgensen, E. and Toft, N. 2010. Herd Management Science II. Advanced

topics. University of Copenhagen, Faculty of Life Sciences. Academic Books, Copenhagen.

Lehtolainen, T., S. Suominen, T. Kutila, and S. Pyörälä. 2003. Effect of intramammary *Escherichia coli* endotoxin in early vs. late-lactation dairy cows. J. Dairy Sci. 86:2327-2333.

Leitner, G. E. Lubashevsky, Z. and Trainin. 2003. *Staphylococcus aureus* vaccine against mastitis in dairy cows, composition and evaluation of its immunogenicity in a mouse model. Vet. Immunol. Immunopathol. 93: 159-167.

Ma, Y., Ryan, C., Barbano, D.M., Galton, D.M., Rudan, M.A. and Boor, K.J. 2000. Effects of somatic cell count on quality and shelf-life of pasteurized fluid milk. J. Dairy Sci. 83: 264-74

Milne, M.H., Biggs, A.M., Barrett, D.C., Young, F.J., Doherty, S., Innocent, G.T. and Fitzpatrick, J.L. 2005. Treatment of persistent intramammary infections with *Streptococcus uberis* in dairy cows. Veterinary Record. 157: 245-250

Neerhof, H. J., P. Madsen, V. P. Ducrocq, A. R. Vollema, J. Jensen, and I. R. Korsgaard. 2000. Relationships between mastitis and functional longevity in Danish black and white dairy cattle estimated using survival analysis. J. Dairy Sci. 83: 1064-1071.

Neeser, N. L., W. D. Hueston, S. M. Godden, and R. F. Bey. 2006. Evaluation of the use of an on-farm system for bacteriologic culture of milk from cows with low-grade mastitis. JAVMA, 228:254-260.

Nielsen, L.R., Jørgensen, E., Kristensen, A.R. and Østergaard, S. (2010). Optimal replacement policies for dairy cows based on daily yield measurements. Journal of Dairy Science. 93:75-92

Østerås, O, Edge, V.L. and Martin, S.W. (1999). Determinants of success or failure in the elimination of major mastitis pathogens in selective dry cow therapy. Journal of Dairy Science. 82:1221-1231

Østergaard, S., Chagunda, M.G.G., Friggens, N.C., Bennedsgaard, T.W. and Klaas, I.C. 2005. A stochastic model simulating pathogen-specific mastitis control in a dairy herd. J. Dairy Sci. 88:4243-4257.

Perrin, R.K. 1972. Asset Replacement Principles. American Journal of Agricultural Economics. 54: 60-68

Petrovski, K.R., Heuer, C., Parkinson, T.J. and Williamson, N.B. 2009. The incidence and aetiology of clinical bovine mastitis on 14 farms in Northland, New Zealand. New Zealand Veterinary Journal. 57:109-115.

Rajala, P.J. and Gröhn, Y.T. 1998. Disease occurrence and risk factor analysis in Finnish Ayrshire cows. Acta Vet Scand. 39:1-13.

Rajala-Schultz, P. J., and Y. T. Gröhn. 1999. Culling of dairy cows. Part III. Effects of diseases, pregnancy status and milk yield on culling in Finnish Ayrshire cows. Prev. Vet. Med. 41, 295-309.

Reksen, O, Sølverød, L., Branscum, A.J. and Østerås, O. 2006. Relationships between milk culture results and treatment for clinical mastitis or culling in Norwegian dairy cattle. J. Dairy Science. 89:2928-2937

Riekerink,R.G.M.O., Barkema, H.W., Kelton, D.F. and Scholl, D.T. 2008. Incidence risk of clinical mastitis on Canadian dairy farms. J. Dairy Sci. 91:1366-1377.

Rothman, K.J. 1986. Modern Epidemiology. First Ed. Little, Brown and Company. Boston/Toronto

Sampimon, O.C., Barkema, H.W., Berends, I.M.G.A., Sol, J. and Lam, T.J.G.M. 2009. Prevalence and herd-level risk factors for intramammary infection with coagulase-negative staphylococci in Dutch dairy herds. Veterinary Microbiology. 134:37-44.

Sargeant, J.M., Scott, H.M., Leslie, K.E., Ireland, M.J. and Bashiri, A. 1998. Clinical mastitis in dairy cattle in Ontario: Frequency of occurrence and bacteriological isolates. Can Vet J. 39:33-38

SAS Institute. 2006. SAS OnlineDoc 9.1.3. SAS Institute Inc., Cary, NC.

SAS Institute Inc., version 9.2. 2008. Cary, NC, USA

Schukken, Y. H., J. Hertl, D. Bar, G. J. Bennett, R. N. González, B. J. Rauch, C. Santisteban, H. F. Schulte, L. Tauer, F. L. Welcome, Y. T. Gröhn. 2009. Effects of repeated gram-positive and gram-negative clinical mastitis episodes on milk yield loss in Holstein dairy cows. J. Dairy Sci. 92: 3091-3105

Schukken, Y.H., G.J. Bennett, M.J. Zurakowski, H.L. Sharkey, B.J. Rauch, M.J. Thomas, B. Ceglowski, R.L. Saltman, N. Belomestnykh and R.N. Zadoks. 2011. Randomized clinical trial to evaluate the efficacy of a 5-day ceftiofur hydrocholoride intramammary treatment on nonsevere gram-negative clinical mastitis. J. Dairy Sci. 94: 6203-6215

Silva, B. O., D. Z. Caraviello, A. C. Rodrigues, and P. L. Ruegg. 2005. Evaluation of petrifilm for the isolation of *Staphylococcus aureus* from milk samples. J. Dairy Sci. 88:3000-3008.

Smith, B. J., 1971. The dairy cow replacement problem. an application of dynamic programming. Bulletin 745, Florida Agricultural Experiment Station, Gainesville, Florida.

Sørensen, L. P., T. Mark, M. K. Sørensen, and S. Østergaard. 2010. Economic values and expected effect of selection index for pathogen-specific mastitis under Danish conditions. J. Dairy Sci. 93:358-369.

Steeneveld, W., Hogeveen, H., Barkema, H.W., van den Broek, J. and Huirne, R.B.M. 2008. The influence of cow factors on the incidence of clinical mastitis in dairy cows. J. Dairy Sci. 91:1391-1402

Thomsen, P. T., A. M. Kjeldsen, J. T. Sørensen, and H. Houe. 2004. Mortality (including euthanasia) among Danish dairy cows (1990-2001). Preventive Veterinary Medicine, 62: 19-33.

USDA – Economic Research Service. 2010. Monthly milk costs of production. Accessed Jan. 17, 2011. http://www.ers.usda.gov/Data/CostsAndReturns/TestPick.htm#milkproduction.

USDA – Economic Research Service. 2012. Accessed Mar. 1, 2012. http://www.ers.usda.gov/data/costsandreturns/testpick.htm#milkproduction.

USDA – National Agricultural Statistics Service, 2012. Accessed Mar. 1, 2012. http://quickstats.nass.usda.gov.

Vaarst, M. and Enevoldsen, C. 1997. Patterns of clinical mastitis manifestations in Danish

organic dairy herds. Journal of Dairy Research. 64: 23-37

van Arendonk, J. A. M., 1985. Studies on the replacement policies in dairy cattle. ii.optimum policy and influence of changes in production and prices. Livestock Production Science 13:101–121.

van Arendonk, J. A. M., Dijkhuizen, A. A., 1985. Studies on the replacement policies in dairy cattle. iii. optimum policy and influence of changes in production and prices. Livestock Production Science 13:333–349.

van Arendonk, J. A. M., 1986. Studies on the replacement policies in dairy cattle. iv. influence of seasonal variation in performance and prices. Livestock Production Science 14:15–28.

Waage, S., P. Jonsson, and A. Franklin. 1994. Evaluation of a cow-side test for detection of gram-negative bacteria in milk from cows with mastitis. Acta vet. scand. 35:207-212.

Wallemacq, H., D. Bedoret, J. Pujol, C. Desmet, P.-V. Drion, F. Farnir, J. Mainil, P. Lekeux, F. Bureau, and L. Fiévez. 2012. CD40 triggering induces strong cytotoxic T lymphocyte responses to heat-killed *Staphylococcus aureus* immunization in mice: A new vaccine strategy for staphylococcal mastitis. Vaccines 30: 2116-2124.

Western Canadian Dairy Herd Improvement Services. DHI options - Somatic Cell Counts. http://www.agromedia.ca/ADM_Articles/content/dhi_scc.pdf (accessed 9 Nov 2012)

White, L.J., Schukken, Y.H., Dogan, B., Green, L., Döpfer, D., Chappell, M.J. and Medley, G.F. 2010. Modelling the dynamics of intramammary *E. coli* infections in dairy cows: understanding mechanisms that distinguish transient from persistent infections. Vet Res. 41:13-28

Wilson, D.J., R. N. González and H. H. Das. 1997. Bovine mastitis pathogens in New York and

Pennsylvania: Prevalence and effects on somatic cell count and milk production. J Dairy Sci 80:2592–2598

Wilson, D. J., B. A. Mallard, J. L. Burton, Y. H. Schukken, and Y.T. Gröhn. 2007. Milk and serum J5-specific antibody responses, milk production change, and clinical effects following intramammary *Escherichia coli* challenge for J5 vaccinate and control cows. Clin Vaccine Immunol. 14:693–699.

Yalcin, C., and A. W. Stott. 2000. Dynamic programming to investigate financial impacts of mastitis control decisions in milk production systems. J. Dairy Res. 67:515–528.

Yazdankhah, S. P., H. Sørum, H. J. S. Larsen, and G. Gogstad. 2001. Rapid method for detection of gram-positive and –negative bacteria in milk from cows with moderate or severe clinical mastitis. J. Clin. Microbiol. 39:3228-3233.

Zadoks, R.N., Allore, H.G., Barkema, H.W., Sampimon, O.C., Wellenberg, G.J., Gröhn, Y.T. and Schukken, Y.H. 2001a. Cow- and Quarter-Level Risk Factors for *Streptococcus uberis* and *Staphylococcus aureus* Mastitis. J. Dairy Sci. 84:2649-2663

Zadoks, R.N., Allore, H.G., Barkema, H.W., Sampimon, O.C., Gröhn, Y.T. and Schukken, Y.H. 2001b. Analysis of an outbreak of *Streptococcus uberis* mastitis. J. Dairy Sci. 84:590-599

Zadoks, R.N. Gillespie, B.E., Barkema, H.W., Sampimon, O.C., Oliver, S.P. and Schukken, Y.N. 2003. Clinical, epidemiological and molecular characteristics of *Streptococcus uberis* infections in dairy herds. Epidemiol. Infect. 130:335-3