TREATMENT DECISIONS FOR

MILD AND MODERATE CASES OF CLINICAL MASTITIS

by

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Dedication

I would like to dedicate my thesis especially to my husband Hernando. There is no doubt in my mind and my heart that without his continued support, patience, counsel and love I could not have completed this process. Thank you for helping make this dream come true.

To my parents, especially my mom Margarita, my daily inspiration to be a better person and better professional who taught me that education never stops and that even the largest goal can be accomplished with perseverance.

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INTRODUCTION

Mastitis is an inflammatory response of the mammary gland caused by bacterial infection and is the most common and costly health disorder of dairy cows. Clinical mastitis (CM) is often classified according to severity of the symptoms as mild (milk looks abnormal), moderate (milk looks abnormal and in addition the udder or quarter is swollen) or severe (the cow exhibits systemic signs). Many modern dairy farms have adopted best management practices that have reduced the rate of mastitis caused by contagious pathogens (such as *Staphylococcus aureus* and *Streptococcus agalactiae*), while a concurrent increase in the amount of mastitis caused by environmental pathogens (such as *E. coli, Klebsiella spp., Serratia spp.,* Coagulase-negative staphylococc*i* and environmental streptococci) has been observed.

While prevalence of pathogens has changed, the treatments of CM cases on many dairy farms have not changed. Most cows with cases of CM are treated with intramammary (IMM) antimicrobials but antimicrobial therapy is not necessary for successful treatment of clinical mastitis of all etiologies. Effective treatment of clinical mastitis depends on different factors related to the cow, the pathogen and the drug used for treatment. Understanding of factors associated with successful therapeutic outcomes would help producers make better treatment decisions and select CM cases that are more likely to respond to treatment.

Mastitis has a negative economic impact on dairy farms in terms of discarded milk, lost production, reduced milk quality and treatment costs. Development of a decision making

system that include biological and economic factors is required to help dairy farmers shape mastitis treatment policies and thus improve profitability. The objective of this thesis is to help farmers improve decision making for treatment of mild and moderate cases of clinical mastitis.

TABLE OF CONTENTS

CHAPTER 1

LITERATURE REVIEW

1.1 CLINICAL MASTITIS IN MODERN DAIRY HERDS

Mastitis is an inflammatory response of the mammary gland caused by bacterial infection and is the most costly health disorder of dairy cows. The rate of mastitis on a farm is dependent on the interaction among microorganisms, cows and the environment. Depending on the primary reservoir of the pathogen, mastitis can be classified as either environmental or contagious. Environmental mastitis is caused by pathogens that live in the cow's habitat. Exposure to these pathogens usually occurs between milkings and outside of the milking facilities. Contagious mastitis is caused by pathogens that live within the infected mammary glands and is predominantly transmitted among cows during milking time. Mastitis can remain as a subclinical infection or progress to produce clinical signs. Clinical mastitis is often classified according to severity of the symptoms. A common classification system has three levels: 1) mild - milk looks abnormal; 2) moderate - milk looks abnormal and in addition the udder or quarter is swollen; and 3) severe - the cow exhibits systemic signs.

The predominant mastitis causing pathogens are as diverse as the different control strategies adopted by dairy farmers. Prevalence of mastitis pathogens in dairy herds has changed in the last decades (Smith et al., 1985; Todhunter et al., 1995; Jayarao et al., 1999; Makovec and Ruegg, 2003; Milne et al., 2005). Different countries and regions of the world have demonstrated that is possible to reduce the number of contagious clinical mastitis cases by practicing the "Five Point Plan" developed more than 40 years ago. The five basic principles are: 1) post-dipping, 2) dry cow therapy, 3) clinical mastitis treatment, 4) culling of chronically infected cows and 5) milking machine maintenance (Dodd et al. 1969). As more herds have implemented standard control measures, pathogen prevalence has shifted, resulting in a decrease in the occurrence of mastitis caused by *Streptococcus agalactiae* and *Staphylococcus aureus* and an increase in the relative

importance of mastitis caused by *Streptococcus uberis* and *Escherichia coli.* Studies in the US and Europe have consistently reported that the prevalence of environmental mastitis pathogens has increased as the prevalence of contagious mastitis pathogens has decreased (Smith et al., 1985; Todhunter et al., 1995; Jayarao et al., 1999; Bradley and Green 2001; Makovec and Ruegg, 2003; Milne et al., 2005). In some countries, *Klebsiella spp*. and *Streptococcus dysgalactiae* are important causes of mastitis (Zadoks and Fitzpatrick, 2009).

The udders of cows are constantly exposed to bacteria that occupy many environmental niches. Consequently, mastitis caused by environmental pathogens has become a major problem in many apparently well managed dairy herds. Herds with low bulk tank SCC (<150,000 cell/mL) have usually controlled contagious pathogens, and the majority of bacteria isolated in cases of clinical mastitis can be linked to an environmental reservoir (Erskine et al., 1988; Dopfer, 1999; Bradley and Green, 2001; Hogan & Smith, 2003). Guterbock et al. (1993) reported all cases of clinical mastitis that occurred on 3 well managed dairies were caused by environmental pathogens including coliforms (37%), environmental streptococci (26%), and other environmental bacteria (13%), while 24% were culture negative. Similarly, in a study where researchers sampled cows from six well-managed, low SCC (SCC<250,000) dairy herds in England *E. coli* was the most common cause of clinical mastitis (Bradley and Green, 2001).

In the U. S., clinical mastitis has been reported to be the most common disease in dairy cattle and occurred in nearly all herds, regardless of the size (Hill et al., 2009). Several studies have shown the increasing relevance of clinical mastitis caused by environmental pathogens in modern U.S. dairy farms (Makovec and Ruegg, 2003; Hoe and Ruegg, 2005). Researchers in Wisconsin reported a decrease in the proportion of mastitis pathogens identified as *S. aureus* and *S.*

agalactiae, and an increase in the proportion of coagulase-negative staphylococci (CNS), environmental streptococci and *E. coli* obtained from milk samples, submitted to a state diagnostic Laboratory between 1994 and 2001(Makovec and Ruegg, 2003). Similar results were found in four commercial herds in Wisconsin, where the most commonly recovered bacterial pathogens causing mild and moderate cases of clinical mastitis were identified as environmental streptococci and CNS (Hoe and Ruegg, 2005).

1.2. ENVIRONMENTAL PATHOGENS CAUSING MASTITIS

Both Gram-positive and Gram-negative pathogens can cause environmental mastitis. Gram-positive pathogens include coagulase negative *Staphylococcus* (CNS) and several species of *Streptococus*. Gram-negative pathogens include coliforms (*E. coli, Klebsiella spp.* and *Enterobacter spp*.), *Serratia spp*. and others.

1.2.1 Gram-positive environmental pathogens causing mastitis

Coagulase-negative Staphylococci (CNS)

Staphylococci are often classified diagnostically based on their ability to coagulate plasma. One practical scheme classifies mastitis pathogens as either *Staphylococcus aureus* (coagulase-positive) or coagulase-negative staphylococci (CNS) (National Mastitis Council, 1999). *Staphylococcus aureus* is the best known coagulase-positive Staphylococcus species and is considered a contagious pathogen. Other coagulase positive staphylococci include *S. hyicus* and *S. intermedius*. The term coagulase-negative staphylococci (CNS) includes most staphylococci isolated from bovine milk other than *Staphylococcus aureus*. CNS are part of the normal flora of the teat skin, often colonize the streak canal and have traditionally been considered opportunistic pathogens (Pyörälä and Taponen, 2009).

The incidence of mastitis caused by CNS is usually greatest immediately after calving, declines in mid-lactation and increases again in late lactation until the cow receives dry cow therapy (Ruegg, 2001b). Lago et al., (2007) reported that CNS were pathogens most commonly isolated from subclinical infected quarters of fresh cows during the first 3 days postpartum (accounting for 51% of isolates). Taponen et al. (2006) reported that 60% of cases of clinical and subclinical mastitis caused by CNS occurred within 30 days postpartum. The incidence of mastitis caused by CNS has been reported to be greater in primiparous cows as compared to older cows (Pyörälä and Taponen, 2009). In one study, multiparous cows experienced mastitis caused by CNS more frequently during late lactation whereas primiparous cows developed infections during the first 30 days postpartum (Taponen et al., 2006).

Mastitis caused by CNS usually remains as a subclinical infection (Taponen et al., 2006; Pyörälä and Taponen, 2009). The proportion of clinical mastitis caused by CNS are usually minimal, and typically varies from 3-10% of all clinical mastitis cases (Hogan et al., 1989b; Smith et al., 1985; Todhunter et al., 1993; Oliver and Jayarao, 1997; Gillespie et al., 2009). Several researchers have reported that clinical mastitis cases caused by CNS have mild to moderate symptoms (Taponen et al., 2006; Taponen and Pyörälä, 2009).

Environmental Streptococcus Species

Environmental streptococci include species of *Enterococcus* and species of *Streptococcus* other than *S. agalactiae* (Todhunter et al., 1995). Several species of environmental streptococci have been isolated from mammary glands of cows with mastitis. Among environmental streptococci, *S. uberis* and *S. dysgalactiae* have been reported to be the most prevalent (Jayarao et al., 1999; McDougall, 1998; McDougal et al., 2007b).

Exposure to environmental streptococci can occur at anytime, but new infections are more common during prepartum period. Rates of clinical mastitis caused by environmental streptococci are greater during the first month of lactation compared to the rest of lactation (Todhunter et al., 1995; Jayarao et al., 1999). Todhunter et al. (1995) reported that the rate of clinical mastitis caused by streptococci during lactation was similar among lactation groups and only during late lactation was it greater for older cows as compared to first and second lactation cows. Infections caused by environmental streptococci can vary greatly in duration (Zadoks et al., 2003). Average duration of infection has been reported variously as short as 30 days (Smith et al., 1985; Todhunter et al., 1995; Smith and Hogan, 1993) or as long as 309 days (Zadoks et al., 2003)

Environmental streptococci can cause both clinical and subclinical mastitis (Zadoks et al., 2003; Jayarao et al., 1999; Oliver et al., 1998). It has been reported that the risk of an infected quarter becoming clinical decreases with stage of lactation (Oliver et al, 1998). Oliver et al. (1998) reported that the ratio of subclinical to clinical infections increased from 10 subclinical per 1 clinical case in early lactation to 24 subclinical per 1 clinical case in late lactation. Usually, cows presenting clinical cases of mastitis caused by environmental streptococci show mild and moderate symptoms. Todhunter et al. (1995) reported that 84% of the cows had mild signs, 13% had moderate signs and only 3% had severe symptoms when experiencing clinical mastitis caused by environmental streptococci.

1.2.2 Gram-Negative Environmental Pathogens causing Mastitis

Coliforms (Escherichia coli, Klebsiella spp. and Enterobacter spp.)

Coliforms are opportunistic bacteria that live in the cow's environment. Genera classified as coliforms include *Escherichia, Klebsiella,* and *Enterobacter*. *Escherichia coli* is a normal inhabitant of the gastrointestinal tract of warm blooded animals, *Klebsiella spp*. and *Enterobacter spp*. populate soils, grains, water, and intestinal tracts of animals. The mammary gland has two characteristics that make it ideal for growth of coliform bacteria. Coliforms have the ability to utilize lactose (the primary carbohydrate found in milk) as an energy source and they can survive anaerobic conditions inside the gland (Hogan and Smith, 2003). Gram-negative bacteria can release endotoxins at the time of cell death and start an inflammatory response that can cause decreased milk production during clinical cases (Hogan and Smith, 2003).

Prevalence of subclinical intramammary infections caused by Gram-negative bacteria seldom exceeds 5% of quarters in a herd, however greater than 25% of cows in well-managed herds may be annually diagnosed with clinical mastitis caused by coliforms (Hogan and Smith, 2003). Intramammary infections caused by coliforms occur most often at calving and during early lactation (Burvenich et al., 2003; Gröhn et al., 2005) and decrease as days in milk increase (Hogan and Smith, 2003). While risk factors vary among herds, the dry period is often the period of greatest susceptibility for acquisition of an infection caused by coliforms, especially during the first or last two weeks (Hogan & Smith, 2003). Smith et al. (1985) reported that approximately 65% of clinical cases caused by coliforms that occurred in the first two months of lactation, originated during the dry period. Coliform bacteria need iron to survive inside the mammary gland. During mammary involution high levels of lactoferrin present in mammary secretions bind to iron and iron becomes a limiting nutritional factor for bacteria growth.

Klebsiella pneumonia tends to overcome the inhibitory effect of lactoferrin and can infect the involuted mammary gland more successfully than most strains of *E. coli* (Hogan & Smith, 2003).

Older cows usually have a greater rate of clinical mastitis caused by coliform bacteria compared to primiparous cows (Hogan & Smith, 2003). Mastitis caused by coliform bacteria tends to have a relatively short duration. Todhunter et al. (1991) reported duration of IMI of less than 10 days for *E. coli* and an average of 21 days for *Klebsiella*. Short peaks of increased somatic cells in milk are observed after clinical mastitis caused by *E. coli,* and somatic cells usually return to pre-infection levels about 3-4 weeks after infection (Pyörälä et al., 1994; Haas et al., 2002). Sometimes the microorganism is eliminated before or shortly after the onset of clinical symptoms (Dopfer et al, 1999).

Most IMI caused by Gram-negative pathogens result in clinical mastitis (Smith and Hogan, 1993; Hogan and Smith, 2003). The severity of the clinical cases can range from mild local signs to severe systemic involvement (Hogan and Smith, 2003). About 10-13% of clinical mastitis cases caused by coliforms are estimated to result in severe clinical signs (Bradley and Green, 2001; Burvenich et al., 2003; Hogan and Smith, 2003). The severity of clinical disease has been positively correlated with peak number of coliform bacteria in mammary secretions (Hogan and Smith, 2003). Recurrent cases of clinical mastitis caused by coliforms have been reported as a result of reinfection from the environment or persistence of the organism within the mammary gland. Bradley and Green (2001) found that the proportion of cows with mild symptoms (67%) was greater for cows experiencing recurrent clinical cases as compared to cows experiencing their first case of coliform mastitis (43%), and concluded that recurrent cases of mastitis due to persistent infection with the same genotype tended to be less clinically severe. The repeated

isolation of the same genotype can indicate that transmission of *E. coli* strains from one cow to another is possible (Dopfer et al., 1999).

Other Gram-negative bacteria

Various species of *Serratia, Pseudomonas,* and *Proteus* are commonly found in soil, plants, feed and water and have been implicated in a number of outbreaks of mastitis in dairy cows. *Pseudomonas spp*. and *Proteus spp.* are known to commonly contaminate water hoses that are often used to wash udders before milking (Hogan and Smith, 2003).

Intramammary infections caused by *Serratia spp*. and *Pseudomonas spp*. may become chronic infections that persist through multiple lactations (Hogan and Smith, 2003). *Serratia spp.* has been isolated from mastitic milk of cows of all ages, but older cows are more susceptible to infection (Todhunter et al., 1991). Todhunter et al., (1991) reported that intramammary infections caused by *Serratia spp.* were of long duration, highly associated with the dry period, and clinical cases were generally mild (Todhunter et al., 1991). In an investigation of a single herd outbreak associated with *Serratia* marcescens, Ruegg et al., (1992) reported that few cows infected with *Serratia marcescens* had evidence of clinical mastitis and infection was independent of days in milk, production string, and daily milk production.

1.3 CONTROL OF CLINICAL MASTITIS CAUSED BY ENVIRONMENTAL PATHOGENS

The incidence of mastitis in herds can be reduced by implementation of sound preventive practices. As compared to control of contagious mastitis pathogens, there has been less progress on control of mastitis caused by environmental pathogens (Smith and Hogan, 2001). The fundamental principle of mastitis control is to decrease exposure of teat ends to potential

pathogens and to increase the resistance of dairy cows to infection. The National Mastitis Council (2000) recommends a ten point program to control mastitis including: 1) Establishment of goals for udder health, 2) Maintenance of a clean, dry, comfortable environment, 3) Proper milking procedures, 4) Proper maintenance and use of milking equipment, 5) Good record keeping, 6) Appropriate management of clinical mastitis during lactation, 7) Effective dry cow management, 8) Maintenance of biosecurity for contagious pathogens and culling of chronically infected cows, 9) Regular monitoring of udder health status, and 10) Periodic review of mastitis control program. In summary, excellent management is the foundation of a successful mastitis control program. While all ten points are important, several aspects of the 10-Point plan are more relevant for the control of environmental pathogens.

1.3.1 Managing the environment

An effective control program for mastitis caused by environmental pathogens should focus on management practices that reduce exposure of teat skin to these pathogens. Teat ends are exposed to pathogens during the milking process and in the cow's environment outside the milking parlor (housing areas, pastures, etc). Management practices such as maintenance of stalls and pastures are critical to avoid exposure of teat ends to fecal matter or mud that can result in udder infection. Hot and humid weather conditions favor growth of bacteria in the environment. Rates of clinical mastitis are often greater during summer for cows in confinement and during rainy months for cows that are housed outside on pasture or dry lots (Hogan and Smith, 2003).

Teat ends and udders are in direct contact with bedding materials, which can be a source of environmental pathogens. Bedding materials are categorized as organic or inorganic. Common

organic bedding materials used in dairy farms include: long straw, chopped straw, sawdust (both green and kiln dried), wood shavings, manure solids, pelleted corn cobs, corn fodder, old grass hay, peanut hulls and chopped newspaper. Common inorganic bedding materials include sand and crushed limestone (Smith and Hogan, 2006). Hogan et al., (1989a) reported that rates of clinical mastitis were related to bacterial counts in bedding, especially Gram-negative bacteria, and that bacterial populations were less in inorganic bedding materials compared to organic bedding materials (Hogan et al., 1989a). Environmental pathogens in organic bedding materials vary with the type of bedding. Gram-negative and coliform bacteria can be found more frequently in sawdust and wood products, whereas environmental streptococci are often prevalent in straw bedding (Bramley, 1982). Herds that experience problems with environmental pathogens may need to consider non-organic bedding materials such as sand (Hogan and Smith, 2003).

1.3.2 Antimicrobial therapy during Dry period

Dry cow therapy refers to the administration of intramammary long-acting antimicrobials at the beginning of the non-lactating period. The dry period of a cow is one of the most sensitive periods for the occurrence of new intramammary infections (IMI), especially shortly after dry-off and before calving (Robert et al., 2006). The purpose of dry cow therapy is to cure existing intramammary infections and prevent new infections (Dingwell et al., 2003).

Several studies have reported high cure rates for CNS and environmental streptococci during the dry period after administration of intramammary antimicrobial therapy at dry off (Todhunter et al. 1993; Todhunter et al., 1995; Whist et al., 2007). Robert et al. (2006) performed an extensive meta-analysis and reported that cows receiving dry cow antimicrobial therapy had fewer new

IMI due to streptococci after calving compared to untreated animals. The same meta-analysis reported limited or non-existent effect of dry cow therapy against new infection due to CNS probably because these IMI occurred very late in the dry period or during early lactation. Little efficacy of dry cow therapy against coliforms was also reported because most of the antimicrobials used in the studies have efficacy only against Gram-positive bacteria (Robert et al., 2006). However, Bradley and Green (2001) demonstrated clinical efficacy of a dry cow intramammary antimicrobial preparation with significant Gram-negative spectrum, as measured by a 50% reduction in clinical mastitis caused by Gram-negative pathogens in the subsequent lactation when compared with a product with no Gram-negative efficacy.

1.3.3 General Hygiene

Exposure to environmental pathogens often occurs between milkings. However, strict attention to milking hygiene is essential to control mastitis caused by environmental pathogens and the goal must be to apply milking units onto clean and dry teats. Milking procedures should include use of gloves, application of forestripping and effective teat disinfection both pre and post milking. It is critical to maintain good hygiene in the milking parlor, including cleanliness of milking units, platforms and teat cups. Several authors have reported outbreaks of Serratia spp. that were associated with contaminated teat cups or teat dips (Damme, 1982; Wilson et al., 2009). The effectiveness of teat disinfection has been questioned when controlling environmental mastitis (Jayarao et al., 1999). The National Mastitis Council (2009) has published a summary of peer-reviewed publications about the efficacy of pre and post-milking teat disinfectants since 1980. Almost all post-dips are effective against contagious pathogens but just a few are effective against environmental pathogens (NMC, 2009).

Special consideration has to be given to the environment of the most susceptible animals such as dry cow and periparturient heifers and cows (Todhunter et al., 1995; Pyörälä and Taponen, 2009). Herd management procedures that prioritize hygiene are critical to establish effective mastitis control programs. Green et al., (2007) reported that good hygiene measures associated with the administration of dry-cow treatments, management of the early and late dry-period facilities, and the calving area were associated with a decreased rate of clinical mastitis after calving.

1.3.4 Immunization

Vaccination using Gram-negative core antigen is a management practice that does not prevent intramammary infections but reduces the severity and duration of clinical signs associated with mastitis caused by Gram-negative bacteria (Hogan and Smith, 2003). Most commercially available Gram-negative core antigen vaccines specify efficacy against only *Escherichia coli* but data from field trials suggest that these vaccines also reduce clinical cases of mastitis caused by species in the genera *Klebsiella, Pseudomonas, Serratia,* and *Proteus* (Hogan and Smith, 2003).

1.4 TREATMENT OF CLINICAL MASTITIS CAUSED BY ENVIRONMENTAL PATHOGENS

Effective treatment for clinical mastitis depends on different factors related to the cow, the pathogen and the drug used for treatment. Cow factors associated with treatment efficacy include age, stage of lactation, effectiveness of the cow's immune response, somatic cell count (SCC), number of infected quarters, and chronicity and severity of the case (Morin, 2004; Constable and Morin, 2003; Bradley and Green., 2009). Pathogen factors include inherent characteristics of the pathogen, duration of the infection, and pathogen response to antimicrobial therapy (Morin, 2004; Constable and Morin, 2003; Bradley and Green, 2009). Drug factors include timing of

treatment in the lactation cycle, route of administration, concentration of the drug that can be maintained at the site of infection, and duration of treatment (Constable and Morin, 2003; Bradley and Green, 2009).

1.4.1 Indicators of therapy efficacy

The short-term aim of most dairy producers who treat mild or moderate clinical mastitis is to return the appearance of the milk to normal so that it can be legally sold. Different indicators of therapeutic efficacy have been used for research and on commercial farms. Short term indicators of efficacy include: clinical cure, bacteriological cure, and number of days milk is not saleable (days out of tank) (Guterbock et al., 1993;Milne et al., 2005; Hoe and Ruegg, 2005; McDougall et al., 2007b, Lago et al., 2009). Long-term outcomes have been evaluated in previous studies, including risk and days to a clinical mastitis recurrence, post-treatment somatic cell count (SCC), milk production and days to removal from herd expressed as culling and death rates (Erskine, 2004; Wenz et al., 2005; Bar et al., 2007; Apparao et al., 2009; Schukken et al., 2009; Lago et al., 2009;).

Clinical cure

Clinical cure is defined as the disappearance of clinical signs of mastitis and return to normal appearance of milk, and is one simple way of assessing treatment outcome. This could be perceived as a treatment success by the farmer, but it may reflect the reversion of a clinical case to a subclinical state (Ruegg, 2004). Lago et al. (2009) reported an average of 3 days to clinical cure (DCC) in a study assessing therapy decision for mild and moderate cases of clinical mastitis based on culture results, where mastitis caused by Gram-positive bacteria were treated and mastitis caused by Gram-negative bacteria or when no bacteria were recovered were not treated.

They also found no difference in cows treated with antimicrobials immediately after detection and those treated 24h later based on culture results. Similarly, Hoe and Ruegg (2005) reported an average of 4 days to clinical cure for mild and moderate cases of clinical mastitis, and found no differences in DCC between CM caused by Gram-positive or Gram-negative organisms.

Bacteriological cure

Bacteriological cure is estimated using a series of microbiological examinations of quarter milk samples collected before and after administration of treatment. Bacteriological cure is a more objective way to assess mastitis therapy efficacy as compared to observation of clinical cure. The advantage of using serial microbiological examinations is that the agent causing the mastitis can be identified and then, in subsequent examinations after the treatment, the absence of the same pathogen (bacteriological cure), the presence of the same pathogen (failure of treatment) or the presence of a different pathogen (new infection) can be determined. Various intervals ranging from 14 to 42 days after treatment have been used to define bacteriological cure (Milne et al., 2005; Guterbock et al., 1993;McDougall et al., 2007b). Wide variability in sampling strategies and laboratory methods employed in therapeutic trials make it difficult to compare outcomes of bacteriological cures (Ruegg, 2004). One significant disadvantage of assessing bacteriological cure is that when pre-treatment culture result is negative, it precludes the ability to assess bacteriological cure. Additionally, bacteriological cure is not practical to assess on commercial farms because it requires multiple milk cultures that can be costly and labor intensive.

Days out of tank

Days out of tank (DOOT) refers to the number of days that milk is not saleable and has to be discarded rather than sent to the bulk tank. It may include the number of days milk has an

abnormal appearance but has not been treated with antimicrobial, and the number of days the cow was treated with antimicrobials, plus the withholding period of the specific drug. Producers determine when the milk can go back to the bulk tank based on clinical cure, duration of the treatment, withholding time of the drug used and, sometimes using drug residue tests. Rodrigues et al., (2005) reported that farms discarded milk after treatment for mastitis for 6.1 days but found that smaller herds discarded milk for fewer days (5.2 d) as compared to larger herds (6.8 days). Huijps et al. (2008) assumed that the average milk discarded under Dutch circumstances to be about 6 days.

Recurrence

Recurrence is defined as the return of a sign, symptom or disease after remission. Different definitions have been used to describe the recurrence of a clinical mastitis case. Recurrence has been described by different researchers as another case of clinical mastitis in the same cow, in the same quarter, or by the same pathogen (Wenz et al., 2005; Apparao et al., 2009; Schukken et al., 2009; Bar et al., 2007). For practical purposes, producers often define recurrence as another case of clinical mastitis in the same cow, independently of quarter or pathogen. The interval used to define a new case (rather than a recurrence) varies among studies ranging from 8 to 90 days or longer (Wenz et al., 2005; Apparao et al., 2009; Schukken et al., 2009; Bar et al., 2007). Researchers also differ in defining when the interval begins. It may be counted from the day of the clinical mastitis diagnosis, from the last day of treatment or from the last day of the withholding period (Wenz et al., 2005; Apparao et al., 2009; Schukken et al., 2009; Bar et al., 2007). Recurrence can result from new infections or due to a failure to eliminate infection as a result or either insufficient treatment or treatment failure.

Post-treatment SCC

Return to low somatic cell count in cow's milk is another desired outcome after mastitis treatment. Somatic cell counts of milk are a reliable indirect measure of subclinical mastitis. Increased SCC is of economic importance to the dairy producer because milk with fewer somatic cells is more valuable to many processors. The SCC in a healthy quarter of the cow should be less than 100,000 cells/mL and >200,000 cells/mL is often used to define subclinical mastitis (Hillerton and Berry, 2005). Increased SCC are related to decreased milk production. Each doubling of SCC above 50,000 cells/ml results in a loss of 0.4 kg and 0.6 kg of milk per day in first lactation and older cows, respectively (Hortet and Seegers, 1998)

Milk yield

Cows that experience clinical mastitis rarely recover their potential milk yield (Bar et al. 2008). The effect of clinical mastitis on milk loss varies depending on the severity of the case, the number of cases in the previous and current lactation, the age of the cow, the stage of lactation when the disease occurred and the causative pathogen (Gröhn et al. 2004; Hagnestam et al., 2007; Bar et al., 2008). The reported average loss in milk yield over a 305 day lactation due to clinical mastitis ranged from 0 and 11% (Hortet and Seegers, 1998; Seegers et al., 2003; Hagnestam et al., 2007). Milk yield loss is associated with parity, and is generally greater for multiparous as compared to primiparous cows (Gröhn , 2004; Hagnestam et al., 2007).

Milk yield loss is also associated with the stage of lactation when the clinical mastitis occurs. Hagnestam et al. (2007) reported that yield losses were greater when the clinical mastitis case occurred before peak yield as compared with occurrence during late lactation. Milk yield loss is has been reported to be slighter greater for the first case of clinical mastitis compared with

repeated cases (Bar et al., 2008). The extent of milk yield loss can be associated with the causative pathogen. Gröhn et al. (2004) reported that cows experiencing clinical mastitis caused by coliforms had the greatest milk losses (6.7 to 13.1 kg/day) during the first week after diagnosis compared to cows with clinical mastitis caused by CNS or *Streptococcus spp.* (2.5 and 5.3 kg/day respectively) for the same period. The same authors reported that cows infected with environmental streptococci recovered fully in terms of milk production after clinical mastitis whereas those that experienced clinical mastitis caused by *E. coli* and *Klebsiella* never recovered full milk production, (especially primiparous cows)(Gröhn et al., 2004).

Days to removal from herd

Voluntary culling occurs when the farmer chooses to remove a healthy, fertile cow from the herd due to inadequate milk production , whereas involuntary culling occurs when the farmer is forced to remove a productive, profitable cow due to diseases (mastitis, lameness, etc), injury, infertility, or death (Weigel et al., 2003; Hagnestam-Nielsen and Østergaard, 2008). Involuntary culling decisions are based on comparison of economic benefit among various options such as treatment, culling or prevention (Bar et al., 2007). The occurrence of clinical and subclinical mastitis is well known to increase the likelihood of culling (DeGraves and Fetrow, 1993; Gröhn et al., 2005; Hadley et al., 2006). For example, Gröhn et al. (2005) estimated that cows diagnosed with clinical mastitis caused by environmental pathogens were between 2.2 to 5.3 times more likely to be culled as compared to non infected cows.

1.4.2 Antimicrobial therapy

Antimicrobial therapy is necessary for successful outcome of certain clinical mastitis cases. In the US more than 90% of cows affected with mastitis are treated with antimicrobials (Hill, 2009)

and treatment of mastitis accounts for most antimicrobial usage on dairy farms (Pol and Ruegg, 2007). Judicious use of antimicrobials should be stressed to reduce concerns about costs, efficacy, drug residues and potential development of antimicrobial resistance (Morin, 2004).

Antimicrobial therapy has been quite successful for treating mastitis caused by noninvasive bacteria such as *Streptococcus agalactiae* and coagulase-negative staphylococci but has been less effective against bacteria such as *Staphylococcus aureus*, *Streptococcus uberis,* and some coliform bacteria, which are capable of invading deeper into the udder (Smith, 2009). However, some pathogens have characteristics that make them unlikely to respond to therapy. Environmental pathogens which are unlikely to respond to antimicrobial therapy include *Arcanobacterium pyogenes*, *Bacillus*, *Mycobacterium*, *Nocardia*, *Pasteurella, Proteus, Prototheca (algae), Pseudomonas, Serratia* and Yeast (Wagner and Erskine, 2009).

A variety of antimicrobial compounds have been used in clinical trials that have assessed bacteriological cure rates for clinical mastitis caused by various environmental pathogens (Table 1.1). Treatments have included parenteral administration of Penethamate hydriodide (SQ or IM) and Penicillin G (IM), or intramammary administration of Amoxicillin, Cephapirin, Penicillin, Dihydrostreptomycin, Lincomycin, Neomycin, Ampicillin, Cloxacillin, Ceftiofur, Pirlimycin, Cefuroxime sodium, Cefalexin, Kanamycin, Cefquinome and Cefoperazone. The treatments have been administered for 2 to 8 times, 12 or 24 hours apart depending on compound. Follow up periods for determination of bacteriological cure have ranged from 5 to 42 days after diagnosis of clinical mastitis and take into account 1 to 4 post-treatment samplings. The wide degree of variation complicates comparisons of bacteriological cure rate among studies.

Equivalency studies test the null hypothesis that treatments did not differ in the proportion of bacteriological cures. Assuming an 80% proportion of bacteriological cure, approximately 200 cases per treatment group are required to demonstrate with 95% confidence and 80% power (i.e., $\alpha = 0.05$, $\beta = 0.2$) that 2 treatments are equivalent (proportion of cure within 10% of each other) (Schukken and Deluyker, 1995). Of cited studies, McDougall et al. (2007a) comes close to meeting this requirement. Bacteriological cure rates are not reliable when results come from studies using a small sample size. Overall, bacteriological cure rates vary by pathogen (Table 1.1) and have been reported as 28-100% (CNS); 46-100% (environmental streptococci); 38-100% (coliforms); and 29-67% (Klebsiella)..

Treatment of clinical mastitis caused by CNS

Although spontaneous cure of clinical and subclinical mastitis caused by CNS have been reported to be about 60-70% (McDougall, 1998; Wilson et al., 1999 Taponen et al., 2006), antimicrobial therapy is regularly used to treat mastitis caused by CNS and treatment is often perceived to be highly successful (Wilson et al., 1999).

Sawant et al. (2009) reported that the majority of CNS species from clinical and subclinical cases, (except *S. epidermidis),* were susceptible to antimicrobials commonly used for mastitis treatment (ampicillin, oxacillin, cephalothin, ceftiofur, erythromycin and pirlimycin). Resistance to ampicillin, erythromycin, methicillin and pirlimycin was observed for S. epidermidis.

Bacteriological cure rates ranging from 76 to 100% have been reported for clinical mastitis caused by CNS after treatment using intramammary antimicrobials (McDougall, 1998; McDougall, 2003; Taponen et al., 2006; McDougall, 2007b; Apparao et al. 2009). Lower bacteriological cure rates have been reported when clinical mastitis caused by CNS have been

treated with parenteral (subcutaneous or intramuscular) antimicrobials compared to antimicrobials infused intramammary (McDougall, 1998; Serieys et al., 2005).

Treatment of clinical mastitis caused by Environmental Streptococci

Low spontaneous cure rates (ranging from 0 to 48%) have been reported for clinical mastitis caused by environmental streptococci (Todhunter et al., 1995; Guterbock et al., 1993; Hillerton and Kleim, 2002; Roberson et al., 2004). Bacteriological cure rates after treatment for clinical mastitis caused by Streptococcus spp. vary widely among studies, ranging from 46 to 100% (Table 1.1). The use of antimicrobials for treatment of clinical mastitis caused by environmental streptococci is recommended. The failure to use antimicrobials to treat clinical cases of *S. uberis*, has been reported to result in frequent relapses (Morin et al., 1998; Eenennaam et al., 1995). Prolonged intramammary therapy for treatment of streptococci has been evaluated. Oliver et al., (2004) reported greater bacteriological cure rates for cows with clinical mastitis caused by induced *S. uberis* infection that received ceftiofur for 5 (88%) or 8 (100%) days as compared to 2 (44%) days of treatment.

Treatment of clinical mastitis caused by Coliforms

The immune status of the cow is a significant predictor of outcomes of clinical mastitis caused by *E. coli.* An effective cellular response by neutrophils usually successfully eliminates intramammary infection caused by *E. coli* (Dopfer et al., 1999; Burvenich et al., 2003).

Contradictory findings have been reported regarding the benefit of intramammary antimicrobial therapy for clinical mastitis caused by coliforms. Researchers have reported a wide range (39 to 100%) in bacteriological cure rates for clinical mastitis caused by coliforms (Table 1.1). When intramammary antimicrobials were not used for treatment of clinical mastitis caused by

coliforms, Guterbock et al. (1993) and Roberson et al. (2004) reported spontaneous cure rates of 58 and 78%, respectively. Intramammary use of antimicrobials appeared to have little efficacy against coliform pathogens, as greater bacteriological cure rates were observed in the untreated groups as compared to treated groups (Guterbock et al., 1993, Robertson et al. 2004). However, others have noted that cows with clinical mastitis caused by Gram-negative pathogens were less likely to develop severe symptoms or to recur when treated with antimicrobials and supportive treatment as compared to cows that received only supportive therapy (Eenennaam et al., 1995; Morin et al., 1998).

Antimicrobial treatment of clinical mastitis caused by *Klebsiella spp.* has been reported to be of little benefit. Researchers have reported bacteriological cure rates for mastitis caused by *Klebsiella spp.* of around 50% (Robertson et al., 2004; Hoe and Ruegg, 2005).

Currently available antimicrobials have minimal effect on shortening the duration of intramammary infections caused by coliform bacteria. (Hogan and Smith, 2003). The use of antimicrobials administered by intramammary or systemic routes for treating clinical mastitis caused by *E. coli* does not appreciably incrase outcomes because of the naturally short duration of these infections and the high spontaneous cure rate (Smith et al., 1985). For cows that become systemically ill, supportive therapy including oral or intravenous fluids and anti-inflammatory agents are recommended.

1.4.3 Making treatment decisions

The most valuable mastitis treatment will minimize the amount of milk discarded while maximizing efficacy against pathogens. Producers need to use all the information available about the CM case and the cow's history of clinical and subclinical mastitis to address the problem in

the most efficient way. Antimicrobials should be avoided in cows with low probability of recovery, such as cows with repeated episodes of clinical mastitis (chronic infections) where abscesses and fibrin may interfere with drug distribution in the mammary gland (Erskine et al., 2003). Treatment of clinical mastitis should be based on severity of the case. For example, immediate supportive treatment should be provided for severe cases. Other alternative management options, such as culling, drying, or "killing" a mammary quarter, may be better choices for poor candidates for antimicrobial treatment.

Knowledge of etiology and prevalence of mastitis pathogens within a herd can help farmers make better treatment decisions (Bradley & Green, 2009).Antimicrobial usage should be avoided when clinical mastitis is caused by non-responsive pathogens. Intramammary antimicrobial treatments should be selected based on a diagnosis of the causative pathogen. Diagnosis of causative pathogen could be done by sending aseptic milk samples to commercial laboratories but results take usually more than 48h to be back in the farm, delaying the treatment decision. Rapid on-farm culture systems allow producers to make strategic mastitis treatment decisions in 24h. One approach to on farm culturing is to use the Minnesota Easy Culture System II (University of Minnesota, St. Paul, MN), a commercial on-farm culture system, that offers two different types of selective culture media systems: bi-plates and tri-plates. The Bi-plate system allows the producer to identify grow of Gram-negative and Gram-positive pathogens. It has MacConkey agar on one half that selectively grows Gram-negative organisms, and Factor agar on the other half that selectively grows Gram-positive organisms. The Tri-plate system has in addition MTKT (thallium sulfate-crystal violet-B toxin blood agar) agar that selectively grows streptococci. The technique is simple and easy to use after training. Producers dip a sterile cotton swab into the milk sample and apply it over the media surface, and then the plate is incubated in

an on-farm incubator at 37 °C and is read at 24 hours. If no growth is observed, plates are rechecked after 48 hours then discarded. When using this on-farm culture system, it is recommended to freezing the samples and having the bacteria identified by sending them to a qualified laboratory on a regular basis. Antimicrobial treatment is recommended for mastitis caused by environmental Gram-positive pathogens such as environmental Streptoccoci and CNS (Hillerton and Kliem, 2002). Antimicrobial treatment is not recommended for treatment of mastitis caused by Gram-negative pathogens or when no pathogen is recovered from milk samples.

When antimicrobials are necessary, the key to success is often based on early diagnosis and administration of an effective concentration of drug for a sufficient period to produce both bacteriological and clinical cures. In the past, when milk was bought largely for volume, the primary aim of treatment was to restore milk production and the failure to eliminate infection was not a major priority. Now milk price is more dependent on measurement of quality, thus treatment may be more oriented to bacterial elimination rather than to clinical resolution (Hillerton and Berry, 2005). It is of great importance to always keep records on the farm regarding diagnosis and treatment of clinical mastitis to evaluate results and determine which treatments have worked and which have not.

1.5 ECONOMIC CONSEQUENCES OF MASTITIS

Economic impact of treatment of mastitis needs to be addressed at the farm level. Published efficacy data must be used carefully and generalizations should be avoided. Evaluations of the economic consequences of clinical mastitis based on the effects of the disease on production should be conducted for a specific herd and in a specific economic context (Seegers et al., 2003).

Losses are defined as revenue not earned, while the costs of control are real expenditures. Both have to be addressed to obtain the total economic impact of clinical mastitis (Seegers et al., 2003). Short term costs include treatment, veterinarian assistance, extra-labor, milk discarded during and after antimicrobial treatment, and loss of premiums due to increased somatic cell count of the bulk tank milk (DeGraves and Fetrow, 1993; Huijps et al., 2008). Another potential loss is due to the risk of contamination of a load of milk if treated milk is accidentally poured in the bulk tank (Seegers et al, 2003). Economic consequences of mastitis that have not received much study include short term mortality, reductions in feed intake, effect of disease on body weight and reproduction (Seegers et al., 2003). Middle and long term costs include decreased milk production, and increased risk of premature culling and fatality (Seegers et al., 2003).

The economic impact of clinical mastitis is a result of the interplay of many complex factors and no individual model will include all the factors and costs associated with every case of clinical mastitis on each farm. A model has been developed to help Dutch dairy producers estimate the economic impact of clinical mastitis (Figure1; Huijps et al. 2007) . Producers enter herd specific data or use default values. The total economic losses are divided between clinical and subclinical mastitis. The production loss due to subclinical mastitis is calculated based on the number of lactating cows and the bulk tank SCC (BTSCC). The BTSCC is used to estimate the distribution of cows with different levels of SCC and the production lost is calculated based on the assumption that every doubling of the SCC above 50,000 cells/ml results in a milk production loss of 0,4 kg milk/ day for multiparous cows. The economic impact of clinical mastitis is estimated using milk production losses, withholding days, veterinarian cost, cost of labor, cost of drugs, cost of culling and penalties. The production losses due to clinical mastitis were calculated according to the days in milk when CM occurs and the pathogen involved. The average

production loss set as default was 5% (ranging for 9% at month 1 and 1% at month 9). The rest of the categories were calculated using the number of clinical cases and the number of treatments. The number of days the milk was discarded was assumed as 6 days. It was assumed that the veterinarian would be consulted in only 5% of the clinical mastitis cases. The duration of one treatment was assumed to be 45 minutes. The expected rate of culling a cow with clinical mastitis was 15%. The penalties were due to high BTSCC. Using this model, Huijps et al (2008) concluded that most farmer underestimate the economic loss of mastitis on their farm. One disadvantage of the model is that assumes that a cow gets only one clinical mastitis case, so repeated cases are not taken into account.

Several studies in United States have reported that the average cost of a case of clinical mastitis ranges between $91-$179. (Hoblet et al., 1991; Miller et al., 1993; Rodrigues et al., 2005; Bar et al., 2008). A large proportion of the cost of clinical mastitis is associated with milk discarded after treatment. The number of days the milk is discarded depends on the severity of the case, the treatment protocol and the withhold time of the product used for treatment. The proportion of total cost of mastitis treatment associated with milk loss has been reported to be 50% and 64% by Rodrigues et al.(2005) and Bar (2008), respectively. Another important component of treatment cost is related to the choice of drug.

Researchers in Illinois compared the cost of two treatments for clinical mastitis: Supportive treatment only “N”(oxytocin and flunixin meglumine) Vs. Supportive therapy plus an Antimicrobial “A” (Cefalak (IMM) for mild cases, Cefalak (IMM) plus Oxytetracycline (IV) for moderate and Oxytetracycline (IV) plus supportive therapy for severe cases). Shim et al., (2004) reported that even though the treatment cost for “N” was lower ($28 Vs $49), the economic

losses were greater for group “N” in terms of unproduced milk and unmarketable milk as compared to group “A” ($201 Vs. $295).

Clinical mastitis caused by pathogens of environmental origin can cause substantial losses to producers in terms of milk quality, milk production and survival of dairy cattle (Hoblet et al., 1991; Miller et al., 1993; Rodrigues et al., 2005; Bar et al., 2008). The cost of clinical mastitis varies greatly for individual cows, depending on the milk yield, the lactation number, the stage of lactation, pregnancy status, current price of milk and supplies, and breeding and replacement options (Bar et al., 2008). The cost of clinical mastitis caused by environmental pathogens differs between farms. Records describing all mastitis cases and all treatments administered should be maintained to help identifying management practices that could reduce economic losses (Erskine and Barlett, 1995).

1.6 USE OF DECISION TREE ANALYSIS FOR TREATMENT OF CLINICAL MASTITIS CAUSED BY ENVIRONMENTAL PATHOGENS

Treatment decisions can be taken at the quarter level (e.g., treating or drying off a mammary quarter) or the cow level (e.g., culling a cow) (Hogeveen and Osteras , 2005). Treatment decisions for mild and moderate cases of clinical mastitis caused by environmental pathogens can be complex. Decision analysis is one strategy for simplifying complex decisions. Decision analysis is the best approach when there are multiple possible outcomes and chance is an important factor determining which outcome will occur (Dijkhuizen et al., 1997). Decision-tree analysis is probably the most frequent technique for decision analysis (Dijkhuizen et al., 1997). Decision tree analysis is a graphic representation of decisions, probabilities and events, displayed in a logical and time-sequenced manner. Decision trees are simple to understand and interpret. Decision tree analyses have been successfully used to make economic decisions about several

treatment strategies including left displaced abomasums, dry cow strategies, paratuberculosis and bovine viral diarrhea (Ruegg and Carpenter, 1989; Berry et al., 2004; Dorshorst et al., 2006; Reichel et al., 2008).

A decision tree model can be developed to study the economic outcomes of treating or not treating clinical mastitis caused by environmental pathogens. As described by Haimes (2004), decision tree includes:

1. Decision nodes, usually represented by squares, are the different choices. Branches emanating from a decision node represent decisions or actions to be investigated
2. Chance nodes, usually represented by circles, are the chance events. Branches emanating from chance nodes represent actions, chance events or states of nature i.e response to treatments coming from trial data or literature.
3. Consequences: Usually expected economic outcomes

Creation of a decision tree begins by developing a map with the actions and consequences. Next, probabilities are assigned to each of the chance node branches. Third, economic utilities are assigned to each potential outcome. Fourth, probabilities and economic utilities are combined. These steps provide a visual guide for selecting the decision that leads to the greatest expected utility and for testing it with different changes in probabilities and utilities (Sackett and Haynes, 1991). In the case of treatment of clinical mastitis caused by environmental pathogens, the choice of preferred action should be based on the greatest expected monetary value.

1.7 CONCLUSIONS

Environmental mastitis pathogens are always present in the cow's surroundings. Clinical mastitis caused by environmental pathogens affects all herds and is a major problem on well managed farms. A better understanding of the epidemiology and dynamics of clinical mastitis caused by these pathogens is needed. Prevention and use of best management practices aimed to reduce cow's exposure to pathogens are recommended to avoid detrimental economic effects of clinical mastitis on milk quality, milk production and early culling. Treatment of clinical mastitis should be done for specific pathogens and accordingly to expected cure rate. Decision trees analysis can be used to choose the most profitable approach for treatment of clinical mastitis caused by environmental pathogens.

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Table 1.1 Summary of literature of Bacteriological cure rates for mild and moderate cases of clinical mastitis caused by Environmental Pathogens treated with different antimicrobials.

Author(s) Location (# farms)	Treatment	Route	Duration	Follow up	Coagulase-negative Staphylococci	Environmental streptococci	Coliforms	Conclusions
Guterbock et al., 1993 California (3 herds) Clinical trial	Amoxicillin	IMM	3 times 12 h apart	21 days		*Strep spp.* 46% (6/13)	Coliforms 38% (8/21)	No significant differences among treatments
	Cephapirin	IMM	2 times 12 h apart			*Strep spp.* 73% (11/15)	Coliforms 50% (8/16)	
	Oxytocin (control group)	IM	2-3 times 12 h apart			*Strep spp.* 48% (10/21)	Coliforms 58% (15/26)	
McDougall, 1998 New Zealand (38 herds) Clinical trial	Penethamate hydriodide	SQ	2 times 24 h apart	12 and 21 days	CNS 53.3% (8/15)	Strep uberis 81.7% (103/126) Strep. dysgalactiae 50%(1/2)	Coliforms 100%(5/5)	No significant differences among treatments
	Penicillin-dihydrosptreptomycin	IMM	3 times 12 h apart		CNS 91.7% (11/12)	Strep uberis 84%(127/151) Strep. dysgalactiae 83.3%(5/6)	Coliforms 100%(4/4)	
Deluyker et al., 1999 France, Germany and Belgium (56 herds) Clinical trial	Lincomycin/ Neomycin	IMM	One syringe for 3 consecutive milkings	5, 14, 21 days	CNS. 50% (3/6)	*Strep. spp.* 68% (15/22)	Coliforms 68% (11/16)	Lincomycin/ Neomicyn significant better than Ampicillin/Cloxacillin.
	Ampicillin/ Cloxacillin	IMM	One syringe for 3 consecutive milkings		CNS 33.3% (1/3)	*Strep. spp.* 59% (10/17)	Coliforms 75% (9/12)	

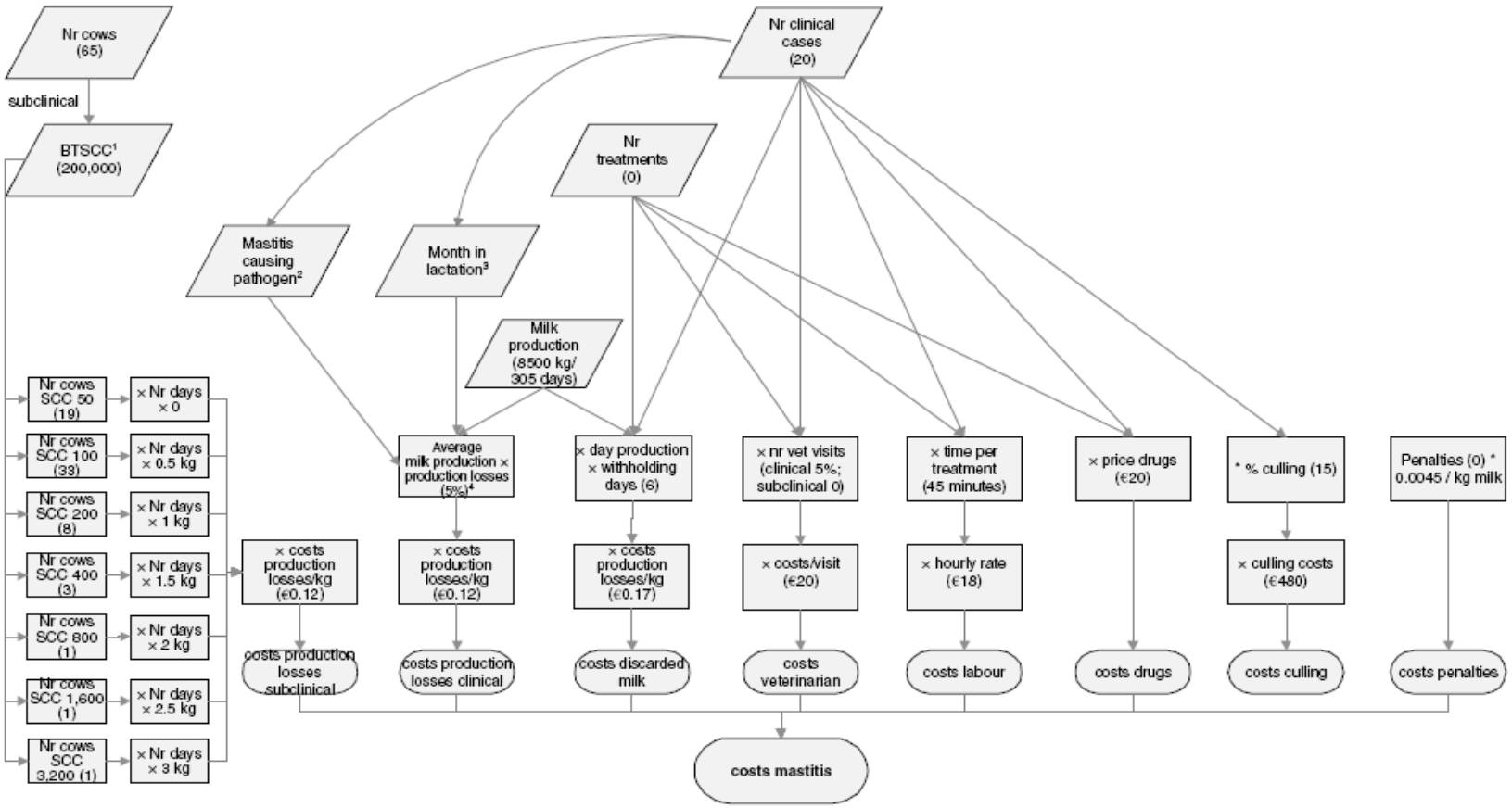
Author(s) Location (# farms)	Treatment	Route	Duration	Follow up	Coagulase-negative Staphylococci	Environmental streptococci	Coliforms	Conclusions
McDougall, 2003 New Zealand (4 herds) Clinical trial	Lincomycin and neomycin	IMM	3 times 12 h apart	21 days	CNS 100% (14/14)	Strep. uberis 75% (35/47)	E. coli 100% (2/2)	No significant differences among treatments (p>0.8).
	Penicillin and dihydrostreptomycin	IMM	3 times 12 h apart		CNS 80% (12/15)	Strep. uberis 71% (29/39)	E. coli 50% (1/2)	
Wraight, 2003 Australia (36 herds) Clinical trial	Cefuroxime	IMM	3 times 12 h apart	7 days after the end of the milk with-holding period		*Strep. uberis 81.8% (18/22)*	*E.coli 100% (10/10)*	No significant differences among treatments (p=0.27) in
	Cloxacillin	IMM	3 times 12 h apart			*Strep. uberis 75% (21/28)*	*E.coli 100% (2/2)*	
Oliver et al, 2004 Tennessee, US (1 herd) Experimentally challenged	Ceftiofur 2 days	IMM	2 times 24 h apart	7, 14, 21, and 28 d after the last treatment		*Strep. uberis* 43% (3/7)		Both of the extended therapies (5 and 8 d) had higher cure rates than the standard 2-d (P = 0.014 for the 2-d treatment regimen vs. the 5-d treatment regimen and P = 0.001 for the 2-d treatment regimen vs. the 8-d treatment regimen).
	Ceftiofur 5 days		5 times 24 h apart			*Strep. uberis* 88% (14/16)		
	Ceftiofur 8 days		8 times 24 h apart			*Strep. uberis* 100% (14/14)		
Serieys et al. , 2005 France (171 farms) Clinical trial	Penethamate hydriodide	IM	3 times 24 h apart	17 and 22 days	CNS 61% (11/18)	Strep. uberis 74% (17/23)	Coliforms 62% (18/29)	No significant differences among treatments

Author(s) Location (# farms)	Treatment	Route	Duration	Follow up	Coagulase-negative Staphylococci	Environmental streptococci	Coliforms	Conclusions
	ampicillin/ cloxacillin	IMM	3 times 24 h apart		CNS 53% (9/17)	Strep uberis 71% (12/17)	Coliforms 75% (12/16)	
Roberson et al., 2004 Virginia (1 herd) Clinical trial	Amoxicillin (IMMA)	IMM	3 times 12 h apart	36 days		Strep spp. 75% (3/4)	E. coli 89% (8/9) Klebsiella 29% (2/7)	No significant differences among treatments ($P > 0.2$)
	No treatment (Control group)					Strep spp. 29% (2/7)	E. coli 100% (4/4) Klebsiella 60% (3/5)	
	Frequently milk out (FMO) and oxytocin	IM	6 times/d			Strep spp. 22% (2/9)	E. coli 100% (3/3) Klebsiella 67% (2/3)	
	FMO + IMMA and oxytocin	IMM	3 times 12 h apart			Strep spp. 18% (2/11)	E. coli 100% (4/4) Klebsiella 50% (2/4)	
Hoe and Ruegg, 2005 Wisconsin (4 herds) Observational study	Pirlimycin (50 mg)	IMM	2 times 24 h apart	21 days	CNS 28% (5/18)	Strep. spp. 41% (17/41)	E. coli 47% (14/30) Klebsiella 36% (4/11)	

Author(s) Location (# farms)	Treatment	Route	Duration	Follow up	Coagulase-negative Staphylococci	Environmental streptococci	Coliforms	Conclusions
Taponen et al., 2006 Finland 59 herds) Observational study	Penicillin G , Cloxacillin or the combination ampicillin–cloxacillinfor	IM or IMM	3–5 days	30 days	CNS 84.3% (43/51)			The bacterial cure rate for quarters treated with antimicrobials was higher compared with untreated.
	Not treated (Control group)				CNS 58.8% (10/17)			
McDougall et al. ,2007b New Zealand (28 herds) Clinical trial	Procaine penicillin	IMM	3 times 12 h apart	21-42 days	CNS 92.9% (13/14)	Strep. uberis 90.6% (106/117) Other Streps. 73.7% (14/19)	Gram-negative rods 100% (8/8)	No significant differences among treatments (p=0.4).
	Cefuroxime sodium	IMM	3 times 12 h apart		CNS 76% (19/25)	Strep. uberis 94.6% (87/92) Other Streps. 100% (17/17)	Gram-negative rods 100% (6/6)	
	Procaine penicillin plus DHS	IMM	3 times 12 h apart		CNS 93.8% (15/16)	Strep. uberis 95.7% (110/115) Other Streps. 84% (21/25)	Gram-negative rods 100% (6/6)	
McDougal et al. ,2007a New Zealand (30 herds) Clinical trial	penethamate hydriodide	IM	3 times 24 h apart	14 and 21 days	CNS 75.9% (22/29)	Strep. uberis 87.7% (222/253) Strep. dysgalactiae 64.7% (11/17)		No significant differences among treatments
	tylosin	IM	3 times 24 h apart		CNS 90.5% (38/42)	Strep. uberis 89.8% (211/235) Strep. dysgalactiae 73.3%(11/15)		

Author(s) Location (# farms)	Treatment	Route	Duration	Follow up	Coagulase-negative Staphylococci	Environmental streptococci	Coliforms	Conclusions
Aparao et al., 2009 Wisconsin, Minnesota and Ontario (8 herds) clinical trial	cephapirin sodium	IMM	2 times 12 h apart	14 and 21 days after last treatment	CNS 100% (15/15)	Streptococci 74% (14/19)		
Bradley and Green, 2009 UK, Germany and France (192 farms) clinical trial	cefalexin and kanamycin	IMM	2 times 24 h apart	Day 16 and 25	CNS 51.5% (17/33)	Strep. uberis 64.3% (45/70) Strep. dysgalactiae 69% (20/29)	E.coli 93.3% (42/45)	cefalexin + kanamycin and cefquinome treatment groups were not significantly different from each other, but were both significantly more likely to be pathogen free posttreatment than quarters in the cefoperazone group.
	cefquinome	IMM	2 times 24 h apart		CNS 50% (12/24)	Strep. uberis 70.4% (38/54) Strep. dysgalactiae 100% (6/6)	E.coli 100% (38/38)	
	cefoperazone	IMM	3 times 12 h apart		CNS 26.1% (6/23)	Strep. uberis 66.7% (24/36) Strep. dysgalactiae 77.8% (7/9)	E. coli 80% (16/20)	

Notes: Follow up is expressed in days after diagnosis of clinical mastitis, otherwise noted.



[1] Depending on the BTSCC the number of animals are divided over the different SCC groups (SCC is given as 1,000 cells/ml)
[2] *Staphylococcus aureus* (20%), *Coliforms* (30%), *Streptococcus dysgalactiae* (15%), *Streptococcus uberis* (20%), or other (15%)
[3] 30% of the cases occur in month 1, 15% in month 2, 13% in month 3, 10% in month 4, 9% in month 5, 8% in month 6, 6% in month 7, 5% in month 8, and 4% in month 9.
[4] When *Staphylococcus aureus* is higher as default, production losses are +1%, when lower production losses are -1%. Depending on the month in lactation production losses vary between 1% (month 9) and 8% (month1).

Fig. 1. Flow chart of the calculated economic losses of mastitis where default values are given between brackets and with farm situation input (▱), calculations (▭), and output (⬭).

CHAPTER 2

RISK FACTORS ASSOCIATED WITH SHORT-TERM POST-TREATMENT OUTCOMES OF CLINICAL MASTITIS ON COMMERCIAL DAIRY FARMS

2.1 INTRODUCTION

Antimicrobial therapy is necessary for successful treatment of many cases of clinical mastitis (**CM**). In the US more than 90% of cows affected with mastitis are treated with antimicrobials (Hill et al., 2009) and treatment of mastitis accounts for the majority of antimicrobials used to treat adult dairy cows (Pol and Ruegg, 2007). Effective treatment of clinical mastitis depends on different factors related to the cow, the pathogen and the drug used for treatment. Cow factors associated with treatment efficacy include age, stage of lactation, effectiveness of the cow's immune response, somatic cell count (**SCC**), number of infected quarters, chronicity and severity of the case (Constable and Morin, 2003; Delyuker et al., 2005; Bradley and Green, 2009; Sol et al., 2000). Pathogen factors include pathogenicity and virulence, duration of the infection and response to antimicrobial therapy (Pyörälä and Pyörälä, 1998; Sol et al., 2000; Constable and Morin, 2003; Barkema et al., 2006; Bradley and Green, 2009). Drug factors include spectrum of activity, route of administration, concentration of the drug that can be maintained at the site of infection and duration of treatment (Constable and Morin, 2003; Bradley and Green, 2009). While many factors have been studied, understanding of factors associated with successful therapeutic outcomes would help producers make better treatment decisions and select CM cases that are more likely to respond to treatment.

Farmers usually evaluate treatments over the short term rather than throughout the entire lactation. While the short-term aim of most dairy producers who treat mild or moderate clinical mastitis is to return the appearance of the milk to normal, other outcomes have been used as indicators of therapy efficacy. Clinical cure, bacteriological cure, number of days milk is not

saleable (days of milk discarded), recurrence of clinical mastitis, days to removal of the cow from the herd, somatic cell count and milk production have all been used to indicate if treatment for CM was successful (Guterbock et al., 1993; Milne et al., 2005; Hoe and Ruegg, 2005; McDougall et al., 2007; Wenz et al., 2005; Bar et al., 2007; Apparao et al., 2009; Schukken et al., 2009; Lago et al., 2009). Most of published literature is about factors affecting outcomes of treating subclinical mastitis (Owens et al., 1988; Sol et al., 1997; Deluyker et al., 2005) and few papers have reported risk factors affecting short-term outcomes of clinical mastitis.

Determination of successful treatment of clinical mastitis is often difficult to establish. Nevertheless, identification of post-treatment outcomes that producers use to determine success of treatments needs further research. Outcomes such as clearance of pathogens after treatment as assessed by bacteriological cure, the immunological response to "healthy" levels of SCC (<200,000 cell/mL), the reduction in recurrent cases of CM, and the retention of the cow in the herd are all potentially important indicators of treatment success. Treatment strategies such as use of antimicrobials, discard of milk until return to normal appearance, culling, drying off the affected mammary gland quarter or the cow may be recommended depending on the probability of reaching a successful outcome. To appropriately treat cases of CM more efficiently, and reduce unnecessary use of antimicrobials, information about risk factors that influence important post-treatment outcomes is needed. A better understanding of risk factors associated with successful therapeutic outcomes would help producers make better treatment decisions. The objectives of this study were to describe selected post-treatment outcomes of cases of mild and moderate clinical mastitis and to determine risk factors associated with these outcomes.

2.2 MATERIALS AND METHODS

2.2.1 Herd and Cow Enrollment Criteria

Wisconsin dairy herds (n = 4) participated in the study between November 2008 and August 2009. Herds were required to keep mastitis records using standard computer software (Dairy Comp 305. Valley Ag Software, Tulare, CA), participate in monthly DHIA testing, actively use an on-farm culture (**OFC**) program, and use a complete milking routine that included fore-stripping for detection of mastitis.

Clinical mastitis was classified according to severity of the symptoms as mild (the only symptom was abnormal appearance of milk), moderate (abnormal appearance of milk accompanied by swelling or redness of mammary gland) or severe (cow exhibited systemic signs of illness). All cases of CM were recorded, but only lactating dairy cows presenting mild or moderate cases of clinical mastitis were eligible for enrollment. Each cow was eligible for enrolment only once. Each herd enrolled cases until about 50 eligible cases were obtained. Cases involving multiple quarters, cows presenting severe symptoms, and cows that had been treated with antimicrobials during the 14 days preceding detection of the case were excluded.

2.2.2 Sampling and Data Collection

Farm personnel were trained by researchers to classify severity of CM and to collect aseptic milk samples. After detection of an eligible case, farm personnel collected aseptic duplicate quarter milk samples before initiating treatment (**PRE**) and then treated the cow according to individual farm protocols. One duplicate milk sample was used to inoculate media for the on-farm-culture laboratory and then both duplicate milk samples were frozen. Farm personnel collected another

set of duplicate quarter milk samples approximately 21 days (14 - 44 d) after each case was enrolled (**POST**).

Study personnel collected standardized data for each case including: cow demographic information, date CM was detected, affected quarter, severity score, dates and treatment information of previous cases of CM, drugs used for treatment of current case, number of days treated, date when milk returned to normal appearance ("clinical cure") and date when milk was returned to the bulk tank. During a 60 days follow-up period information about milk yield and somatic cell count (**SCC**) at DHIA testing, and events such as culling, occurrence of new cases of CM, dry off or death were recorded.

2.2.3 Microbiological Analysis

Frozen milk samples were picked up by study personnel during weekly or biweekly farm visits and transported to the Milk Quality laboratory at the University of Wisconsin -Madison- for subsequent microbiological examination. Milk samples were thawed at room temperature and 100 μL of milk from each duplicate sample was inoculated onto each half of a blood agar plate. MacConkey agar plates were divided into quarters and 10 μL of milk from each duplicate quarter sample were streaked onto each quarter. Plates were incubated at 37^{o}C for 24 to 48 hours. Weekly samples from each farm were pooled and 100 μL were inoculated on mycoplasma culture medium (Media Laboratory -School of Veterinary Medicine at University of California. Davis, CA) and incubated in 6% CO2 at 37^{o}C for up to 14 days. Microbiological analysis was performed according to National Mastitis Council guidelines (NMC, 1999).

Isolates that grew on MacConkey agar underwent further identification using Gram stain, triple sugar iron slants, motility, indole and ornithine medias, and sodium citrate slants. Based on the

results of these tests, Gram-negative bacteria were classified as coliforms, *Serratia spp*. or other Gram-negative bacteria. Isolates that did not grow on MacConkey agar were Gram stained and underwent catalase testing. Speciation of *Streptococcus* and *Staphylococcus* species was done using commercial API system tests (BioMerieux-Vitek Inc. Durham, NC). When "no growth" was observed after 24 hours of incubation on either agar, refrigerated milk samples were incubated for 6 hours at 37°C and 1 mL of milk was inoculated on Petrifilm Staph Express (3M, St. Paul, MN) to look specifically for *S. aureus*. Confirmation of *S. aureus* was performed based on the production of a distinct pink zone around a deoxyribonuclease disk that was applied to colonies presumed to be staphylococci (Silva et al., 2005).

An intramammary infection was defined as the isolation of at least 3 colonies of the same type of bacteria from milk samples. Mixed infection was defined as the isolation of at least 3 colonies of two different types of bacteria from milk samples. Contaminated sample was defined as the isolation of 3 or more different colony types from milk samples. Results of each duplicate quarter milk sample were compared to arrive at a final case diagnosis (Table 2.1).When one duplicate milk sample was contaminated but no pathogen was recovered from paired duplicate sample the quarter was coded as "no growth". When a pathogen causing mastitis was identified in one duplicate sample, but the other duplicate sample was contaminated or no organisms were recovered, the result was coded as the pathogen. When no duplicate sample was collected, the result from the single sample was used (Table 2.1).

2.2.4 Evaluation of on-farm culture

Biplates and triplates (Minnesota Easy Culture System II, University of Minnesota, St. Paul, MN) were used by all farms for OFC. Disposable cotton swabs were used for inoculation of milk

on culture plates. Plates were incubated for 24 to 48 hours. Farmers reported "growth" on plates regardless of the number of colonies observed. Farms that used bi-plates reported results as Gram-positive (growth on Factor media), Gram-negative (Growth on MacConkey media) or "no growth." Farms that used tri-plates reported results as *Staphylococcus spp*. (growth on factor agar media only), coliforms (growth on MacConkey media agar only), *Streptococcus spp*. (Growth on Factor media agar and TKT(Thallium sulfate crystal violet B toxin blood agar)) media agar) or "no growth." Results of OFC performed using triplates were re-classified as Gram-positive (*Staphylococcus spp*., *Streptococcus spp*.), Gram-negative (coliforms) and "no growth" for comparison purposes. Microbiological results from PRE milk samples determined by the university laboratory were used as a "gold standard" for comparison with the results from OFC.

2.2.5 Definitions

Treatment protocols. Cows with CM were grouped in 3 categories based on treatment protocols used by farmers: 1) animals treated only with intramammary (**IMM**) infusion of a product containing 125mg ceftiofur hydrochloride (Spectramast. Pfizer Animal Health, Kalamazoo, MI); 2) animals treated with a variety of antimicrobial compounds which included intramammary infusion of one or two commercial products used alone or in combination with others systemic treatments; and 3) animals that did not receive any intramammary or systemic antimicrobial treatment.

Days until clinical cure. Days until clinical cure (**DCC**) were defined as the number of days until the milk returned to normal appearance.

Days of milk discarded. Days of milk discarded was defined as the number of days the milk was not eligible for sale including days of treatment and withholding period of the drug.

Microbiological Diagnosis. Microbiological outcomes of pre-treatment milk sample were categorized as Gram-positive, Gram-negative or "no growth." Gram-positive pathogens included *Streptococcus spp. Staphylococcus spp*., yeast, *Arcanobacterium pyogenes, Bacillus spp.* and *Lactobacillus spp.* Gram-negative pathogens included *Escherichia coli, Enterobacter spp., Klebsiella spp., Serratia spp*., *Citrobacter spp., Pasteurella spp.* and *Pseudomonas spp*. Quarters with non-significant growth were combined with "no growth" for analysis.

Bacteriological cure. Bacteriological cure was assessed by comparing microbiological results of PRE and POST milk samples (Table 2.2). Bacteriological cure was defined as absence of pathogens in the POST milk sample, regardless of recovery of a causative pathogen isolated in PRE milk sample. When a pathogen was recovered in PRE milk sample but the POST milk sample was culture negative, the outcome was defined as a "treatment cure", whereas when no pathogens were recovered from either the PRE or POST milk samples, the outcome was defined as a "spontaneous cure." Cows with either 'treatment cure" or "spontaneous cure" were classified as experiencing bacteriological cure (Table 2.2). A cow was classified as not experiencing bacteriological cure when any pathogen (or mixed infection) was present in the POST milk sample. A "new infection" was defined when a different pathogen (as compared to PRE milk sample) was obtained in POST milk sample or when no pathogen was recovered in PRE but a pathogen was recovered in POST milk sample. "Treatment failure" was defined when the same pathogen was present in both the PRE and POST milk samples. Cows with either "new

infection" or "treatment failure" were classified as not experiencing bacteriological cure (Table 2.2).

Recurrence. Recurrence of clinical mastitis during the 60 d follow-up period was defined as the occurrence of a case of clinical mastitis in any quarter of the same cow, after the end of the milk withholding period for the enrolled case.

Retention. Retention within the herd during the 60 d follow-up period was defined as cows remaining in the herd (lactating or dry) as opposed to leaving the herd because of sale or death.

Somatic Cell Response. Somatic cell response (**SCR**) was defined as SCC below 200,000 cells / mL at the DHIA test day occurring between 21 to 55 days post-treatment.

2.2.6 Statistical Analysis.

Descriptive statistics were used to verify data accuracy, detect missing data and observe frequency distribution of variables. Statistical analyses were carried out using SAS 9.2 (SAS Institute. Cary, North Carolina). The cow was the unit of analysis. Post-treatment outcomes were evaluated only for animals treated with IMM ceftiofur and cases with microbiologic diagnosis as Gram-positive, Gram-negative or "no growth."

The effect of selected risk factors (explanatory variables) on post-treatment outcomes (response variables) were tested using logistic regression. The outcomes evaluated were: bacteriological cure (yes, no), recurrence (yes, no), retention within the herd (yes, no), and SCR below 200,000 cells / mL (yes, no). The explanatory categorical variables used in statistical models were: farm (A, B, C, D), parity group (1, 2, 3, > 3), severity (mild, moderate), previous occurrence of CM (yes, no), and microbiological diagnosis at PRE (Gram-positive, Gram-negative, "no growth").

The explanatory continuous variables used in statistical models were: DIM at occurrence of CM case, linear somatic cell score (**LSCS**) at previous DHIA test, milk production (kg/cow per day) at previous DHIA test, duration of antimicrobial treatment (days), days until clinical cure, and days of milk discarded.

Logistic regression with generalized estimated equations was used to assess a potential clustering effect of individual observations of outcome variables within farm (Palta, 2003) using the GENMOD procedure. Individual variables from the same farm were poorly correlated, and as a result, farm was included as a fixed effect.

All explanatory variables were subjected to univariate analyses by means of Chi-square or ANOVA tests using the PROC FREQ, PROC ANOVA and PROC GLM procedures. Variables, as well as their interaction terms, with a P-value <0.25 in a univariate analysis were offered into the multivariate models. Multivariate analyses were assessed using PROC LOGISTICS.

Six separated logistic regression models were built to assess the effect of selected risk factors on post-treatment outcomes. Four models (one model for each post-treatment outcome) (models #1), included information that is commonly available for producers. Two additional models (one for Recurrence and one for SCR) (models #2) included information about bacteriological cure. The effect of farm was forced in the models for Recurrence and Retention because detection of clinical mastitis and culling policies vary among farms. Final models were selected based on biological significance and comparison of model fit statistics after using forward selection and backward elimination procedures. Goodness of fit was assessed using the Hosmer and Lemeshow test of PROC LOGISTIC

Agreement between microbiological results of PRE milk samples obtained from OFC and results obtained from the university lab was assessed by calculation of Cohen's Kappa coefficients. Kappa was determined using the PROC FREQ.The interpretation of kappa was according to Dohoo et al., (2003).

2.3 RESULTS

2.3.1 Herd Characteristics

Participating herds ranged in size from 640 to 1250 cows and contained almost all Holstein cattle (Table 2.3). Milk production was 40.2 Kg per cow per day and bulk tank SCC was 218,000 cells / mL for three months previous to the beginning of study (Table 2.3). Herds were asked to enroll the next 50 cases of mild or moderate CM and the time required to acquire those cases ranged from 31 to 101 d (Table 2.3). All cows were milked 3 times a day using a complete milking routine consisting of stripping of foremilk, pre and post dipping disinfection and drying of udders. All milking systems included automatic take-offs and all milking personnel wore gloves for milk harvesting. All lactating cows were housed in free stall barns bedded with sand (n = 3) or recycled manure (n = 1) and were fed a total mixed ration. Use of core-antigen coliform vaccination (3 to 4 times during the lactation) as part of herd health program was reported by all herds.

2.3.2 Characteristics of Clinical Mastitis

All cases of CM (n = 266), including those with severe symptoms, that occurred during the sampling period were recorded (Figure 2.1). The proportion of CM cases with mild, moderate and severe symptoms were 65%, 27% and 8%, respectively (Table 2.3). A total of 233 mild and moderate cases were eligible for enrollment in the study (Table 2.3; Figure 2.1). Cases were

excluded because the pre-treatment sample was contaminated (n = 21), the case was caused by a mixed infection (n = 5), samples were missing (n = 3), cow did not received antimicrobial treatment (n = 30) or cow was treated with protocols other than IMM ceftiofur (n = 31; Figure 1). All farms enrolled in the study used IMM ceftiofur for treatment of mild and moderate cases of CM. Farms A, B and C used ceftiofur for treatment of most cases (75%, 100% and 70%, respectively) while farm D used it in only 43% of the cases. Only data obtained from 143 cases of CM that occurred in cows who received IMM treatment with ceftiofur and had microbiological diagnosis of Gram-positive, Gram-negative or "no growth" were used for the rest of the statistical analysis (Table 2.3; Figure 2.1).

2.3.3 Population Characteristics

Of cases included in statistical analysis (n = 143) most occurred in multiparous cows (85%) compared to primiparous cows(15%), and a greater proportion exhibited mild as compared to moderate symptoms (Table 2.3). Almost 60% of cases occurred in front quarters. Seventy percent of cows did not have a history of previous cases of CM within the studied lactation (Table 2.3). Occurrence of previous cases was associated with farm ($P = 0.029$), DIM at occurrence of CM ($P < 0.001$), milk production ($P < 0.001$) and LSCS at the previous DHIA test ($P < 0.001$). Average days in milk at enrollment was 152 d and was not associated with farm ($P = 0.621$) or severity of the case ($P = 0.393$) (Table 2.4). Average LSCS at the DHIA test previous to the case was 3.6 and did not differ among farms ($P = 0.503$). However, LSCS at previous DHIA test from cows that experienced mild cases of CM was greater than those that experienced moderated cases (4.3 vs. 2.4; $P <0.001$) (Table 2.4). Milk production of enrolled cows at the DHIA test previous to the case was 45.4 kg per cow per day and differed among farms ($P < 0.001$; Table 2.4).

2.3.4 Characteristics of Treatment

Duration of treatment was 4.8 d, ranged from 1 to 15 days per case, and varied among farms ($P < 0.001$; Table 2.4). Only one cow was treated for 1 day because she died before a second treatment could be administered. Duration of treatment was about 2 d less for farm A as compared to the other farms (Table 2. 4). It was observed that producers tended to treat moderate cases for about one more day as compared to treatment of mild cases (5.4 vs. 4.6; $P = 0.049$; Table 2. 4). Most cows (94.1%) received IMM treatment according to label specifications (one IMM treatment every 24 hours for 2 to 8 consecutive days). Days to clinical cure was 5.4 d (2 to 15 d), and did not vary among farms ($P = 0.484$; Table 2. 4). Accordingly, milk was discarded for 7.7 d and did not differ among farms ($P=0.252$; Table 2. 4).

2.3.5 Agreement of on farm culture

Culture media used by farms was bi-plates (n = 1) or tri-plates (n = 3). Of all cases, only those with mild and moderate severity (73%; n = 244) had complete data from OFC and microbiological results from the university laboratory. Observed agreement between OFC results and results from the university laboratory was 74% (132/179). Observed agreement among farms was 63% (farm A), 73% (farm b), 91% (farm C) and 75% (farm D) (Table 2. 3). Farmers correctly identified 73%, 67% and 71% of Gram-negative, Gram-positive and “no growth”, respectively. The Kappa coefficient was 0.59 and indicated moderate agreement. Large variation in agreement was observed among farms. Level of agreement was fair for farm A (0.37), moderate for farms B (0.54) and D (0.57) and almost perfect agreement for farm C (0.85).

2.3.6 Microbiological Results

Most results of duplicate milk samples collected before treatment (PRE) were identical (127/143), therefore, the criteria for non-matching duplicate samples was used for only 11% of samples (Table 2.1). Most cases of CM were caused by environmental pathogens. The most prevalent pathogens isolated in PRE milk samples were environmental *Streptococci* (17%) followed by *E. coli* (10%) and *Klebsiella spp*. (8%) (Table 2.5). Less than 2% of the cases were caused by *S. aureus* and *S. agalactiae*. The most common species of pathogens classified as environmental *Streptococci* were *S. dysgalactiae, Aerococcus viridians* and *Lactococcus lactis* (Table 2.5). No *Mycoplasma* spp. were detected in pooled milk samples.

Microbiological diagnosis of the PRE samples was distributed as Gram-negative (30%), Gram-positive (28%) and "no growth" (42%) and varied among farms (P = 0.001; Table 2.3). Farm D had the greatest proportion of Gram-negative pathogens (63.1%) compared to results of Farm A (28%), B (24 %) or C (22%)(Table 2.3). Farm C had the greatest proportion of Gram-positives (50%) as compared to Farm A(17%), B (29%) or D (16%). Farm A had the greatest proportion of samples with no recovery of pathogen (55.3%) compared to farm B (16.6%), C (28.13%) or D (21.05%) (Table 2.3).

Fewer duplicate POST milk samples were identical (103/143) compared to PRE milk samples, therefore, the criteria for non-identical duplicate samples was used for 28% of the milk samples (Table 2.1). The number of cases with usable POST milk sample was reduced to 101 because milk samples were not collected (n = 5), were contaminated (n = 26), or because the cows were sold (n = 6), dried (n = 3) or died (n = 2) before the sample could be collected (Figure 1). Two POST samples diagnosed as mixed infection were included and considered as a treatment failure in the analysis of bacteriological cure. Most of the POST milk samples resulted in no bacterial

growth. The most prevalent pathogens post-treatment were environmental *Streptococcus* and *Serratia spp*.

2.3.7 Bacteriological Cure.

The overall proportion of bacteriological cure was 77.2% (78/101). The proportion of cases that were classified as bacteriological cures was not unconditionally associated with farm, parity, severity or microbiological diagnosis at PRE milk sample. While not statistically significant, only 60% of the cases occurring in cows with more than 4 lactations resulted in bacteriological cures as compared to 83.3% (first lactation), 81% (second lactation) and 89.5% (third lactation) (P = 0.087; Table 2.6). Bacteriological cure by microbiological diagnosis at PRE was 75% Gram-negative (n = 28), 62.5% Gram-positive (n = 24) and 85.7% "no growth" (n = 49) and did not vary among diagnoses (P = 0.08; Table 2.6). However, when microbiological diagnosis was categorized as culture positive (either Gram-positive or Gram-negative), or culture negative ("no growth"), there was a tendency for bacteriological cure to be less for culture positive (69.2%) as compared to culture negative (85.7%) (P = 0.059). Proportion of bacteriological cures was greater than 80% for coliforms (E. coli, Klebsiella spp. and Enterobacter spp.) as compared to 61% for environmental Streptococcus. The pathogen with the least proportion of bacteriological cure was Serratia spp. (16.7%; n = 6; Table 2.7).

Previous occurrence of CM in the studied lactation was unconditionally associated with bacteriological cure (P < 0.001***;*** Table 2.6). The proportion of cases in cows that resulted in bacteriological cure was greater for the first case of CM (86.5%) as compared to cases that were preceded by previous cases of CM (51.9%) during the studied lactation. Cases that resulted in bacteriological cures had lower LSCS at the DHIA test previous to the case as compared with

those that did not result in bacteriological cures (3.1 vs. 4.9; P = 0.004; Table 2.6). Days in milk at occurrence of the CM, duration of treatment and milk production at the DHIA test previous to the case were not unconditionally associated with bacteriological cure.

The final multivariate logistic model selected for bacteriological cure included only microbiological diagnosis pre-treatment and occurrence of previous cases of CM during studied lactation (Table 2.8). Cows that experienced CM for the first time in studied lactation were 7 times more likely to result in bacteriological cure compared with cows that had previous cases of CM in studied lactation (Table 2.8). Cases that were culture positive (either Gram-positive or Gram-negative), were 3 to 5 times less likely to result in bacteriological cure compared to cases from which pathogens were not recovered (Table 2.8).

2.3.8 Recurrence

The overall proportion of recurrence of CM was 18.2% (26/143). Of the recurrent cases, 41% occurred in a different quarter and 59% in the same quarter. Farm, parity, severity of the case, occurrence of previous cases of CM, microbiological diagnosis pre-treatment, treatment duration and previous LSCS and milk production were not unconditionally associated with recurrence (P > 0.069). However, the proportion of recurrent cases tended to increase with parity (P = 0.175; Table 2.9).

Recurrence of CM was unconditionally associated with days in milk at occurrence of the CM case (P = 0.005). Cows that experienced recurrent cases were earlier in lactation (105 d) as compared to cows that not experience a recurrent case (162 d; P = 0.006). There was a tendency for shorter duration of treatment (4.2 d) for cows who experienced a recurrent case as compared to treatment duration (5.1 d) of cows who did not experienced recurrence (P = 0.069).

Recurrence of CM was unconditionally associated with bacteriological cure (P = 0.004; Table 2.9). Most of the cases that experienced bacteriological cure did not recur (88.5%; n = 78), as compared to recurrence in cases that did not experience bacteriological cure (60.8%; n = 23; Table 2.9).

Multivariate logistic model #1 for recurrence included farm as a forced variable and DIM at the time the CM case occurred (Table 2.10). Although not statistically significant, recurrence occurred less frequently for cows on farm C as compared to recurrence in cows at the other farms (P = 0.246). A small but significant effect of DIM on recurrence of CM was observed (P = 0.006). When interpreted in terms of "months in milk" (instead of DIM), for every month after calving, the cows were 1.3 times less likely to have a recurrence of CM (Table 2.10; Figure 2).

Multivariate logistic model #2 for recurrence included bacteriological cure, farm (forced), and previous occurrence of CM (Table 2.10). In model #2, cows experiencing the first case of CM during the studied lactation were 11 times more likely to have recurrent cases of CM, compared to those cows with previous cases of CM during the studied lactation (P = 0.013; Table 2.10). Cows that did not have bacteriological cure were 16 times more likely to have a recurrence compared to those that experience bacteriological cure (P = 0.001; Table 2.10).

2.3.9 Retention within the Herd

During the 60 day follow up period, 87.4% of cows (n = 143) were retained in the herd (Table 2.11). Cows remained in the herd either milking (n = 115) or were dried off at the end of their lactation (n = 10). Most animals that left the herd were sold (n = 16), but some died (n = 2). Both cows that died were recently fresh. Reasons reported for culling were "mastitis" (n = 10), "low milk production" (n = 2) and "other" reasons (n = 4).

Parity, previous occurrence of CM, days in milk at CM and previous milk production were unconditionally associated with retention, while farm, severity of the case, microbiology diagnosis of the CM case, duration of treatment and previous LSCS were not unconditionally associated with retention ($P > 0.38$). Younger cows were more likely to stay in the herd compared to older cows ($P = 0.04$). More than 90% of the animals with 3 or less parities remained in the herd while only 75% of the animals with 4 or more parities stayed in the herd (Table 2.11). A greater proportion of animals that experienced CM for the first time (92%) remained in the herd compared to animals that had previous cases of CM during the studied lactation (76%) ($P = 0.024$). Cows that left the herd experienced CM later in lactation (194 DIM) as compared to those that remained in the herd (145 DIM) ($P = 0.048$). Cows that remained in the herd produced considerably more milk per day (46.4 kg) at the test date previous to the case as compared to cows that left the herd (37.7 kg) ($P = 0.003$).

The final multivariate logistic model for Retention within the herd included only farm (forced) and previous milk production. The effect of farm was not significant for retention ($P = 0.54$) but it was forced in the model because culling decisions are usually farm dependant. Previous milk production had a positive effect on retention. Every 1 kilogram increase of milk yield at the DHIA test before occurrence of CM, increased the odds of the cow remaining in the herd by 9% (Table 2.12). Cows milking more than 50 kg of milk per day in the DHIA test previous to the CM case had more than 90% probability to remain in the herd (Figure 3).

2.3.10 Somatic Cell Response

The proportion of cows that experienced SCR was 58.2% (71/122) and was not unconditionally associated with parity, severity of the case, microbiological diagnosis at PRE, DIM at occurrence

of CM, duration of the treatment or milk production at the DHIA test before occurrence of CM. Proportion of cows that experienced SCR varied among farms (P = 0.04), and was greater for Farm A (70%) and least for Farm D (31%) (Table 2.13). SCR was observed in more animals that experienced the first case of CM in studied lactation (66.7%) as compared to those that had experienced previous cases of CM (34.4%). Cows that experienced SCR after treatment had less previous LSCS (2.9) as compared to cows that did not have SCR (4.3; P = 0.002). SCR was unconditionally associated with bacteriological cure (P < 0.001; Table 2.13).

Multivariate logistic model #1 for SCR included LSCS at previous DHIA test, occurrence of previous cases of CM in studied lactation and the interaction between these two variables. The probability of experiencing SCR was associated with the previous LSCS and an interaction was observed with occurrence of a previous CM during studied lactation (P = 0.008; Table 2.14). For the first case of CM the probability of having SCR was around 65% regardless of the LSCS in the previous DHIA test, while for recurrent cases the probability of having SCR was greater when the LSCS in the previous DHIA test was below 4 and decreased steeply as the LSCS increased (Figure 4).

Multivariate logistic model #2 for SCR included farm and bacteriological cure, and both were significantly associated with the probability of experiencing SCR (P < 0.02; Table 2.14). Bacteriological cure had a strong effect on SCR, cows that did not experience bacteriological cure were 71.4 times less likely to be classified as having SCR (Table 2.14). Farm B, C and D were 4 to 43 times less likely to have SCR when compared to Farm A (Table 2.14).

2.4 DISCUSSION

The focus of this research was on the short-term outcomes after treatment of mild or moderate cases of CM because farmers usually evaluate treatments over the short term rather than the entire lactation. Post-treatment outcomes considered "successful" in this study were the clearance of pathogens after treatment as assessed by bacteriological cure, the response to "healthy" levels of SCC (<200,000 cell/mL), the reduction in recurrent cases of CM, and the retention of the cow in the herd. Several associations between risk factors and post-treatment outcomes identified in this study could help farmers to recognize cows that may respond to appropriate therapy, guide treatment decisions, and improve milk quality.

While farms that participated in this study were volunteers, characteristics and management practices were typical of larger modern free-stalls dairy farms operating in Wisconsin. Daily milk yield, and bulk tank SCC were similar to like sized herds participating in DHIA programs in this region (www.AgSource.com) and it is likely that these results can be extrapolated to similar herds. Most of the mild and moderate cases of CM in this study were caused by environmental pathogens such as *E. coli, Klebsiella spp., Serratia spp.,* coagulase-negative staphylococci and environmental *streptococci*; less than 2% of the cases were caused by contagious pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae;* and no Mycoplasma spp. were diagnosed. The distribution of pathogens observed in this study is typical of modern US dairy farms that have controlled mastitis caused by contagious pathogens (Smith et al., 1985; Todhunter et al., 1995; Jayarao et al., 1999; Makovec and Ruegg, 2003; Hoe and Ruegg, 2005; Milne et al., 2005).

Specific treatment protocols were not used as a criterion to enroll herds or cases in this study, because the objective was to observe post-treatment outcomes from protocols currently used on

commercial dairy herds. Although, all treatments were recorded, only cases treated with IMM ceftiofur were used for statistical analysis because this treatment was extremely common (74%) and the number of treatments with other compounds was not sufficient for analysis. Ceftiofur is a broad-spectrum third-generation cephalosporin antimicrobial that inhibits bacterial cell wall synthesis by interfering with enzymes essential for peptidoglycan synthesis. Commercial IMM ceftiofur tubes are labeled for treatment of clinical mastitis caused by coagulase-negative *staphylococci, Streptococcus dysgalactiae,* and *E. coli.*

Absence of pathogens in pre-treatment milk samples was the most common bacteriological diagnosis in this study (42%) and is in agreement with other published studies (Roberson et al., 2004; Hoe and Ruegg, 2005; Lago, 2009). Although it has been previously reported that unfavorable conditions during storage in farm freezers, may decrease viability of bacteria (Dinsmore et al., 1992), in current study the proportion of culture negative samples from university laboratory results was similar to results obtained using OFC (plated directly from fresh milk samples) (45%). Absence of pathogens in milk samples from cases of CM could be the result of spontaneous clearance of pathogens (Smith et al., 1985) or the relatively short duration of infections caused by Gram-negative bacteria (Sears et al. 1993).

Longer days to clinical cure (5.4 d) were observed in current study compared to 3 to 4 days reported by other mastitis researchers (Hoe and Ruegg, 2005; Lago, 2009). The number of days until clinical cure was apparent was not included in statistical models because most farmers administered IMM antimicrobials until clinical cure was observed; thus days to clinical cure was highly correlated with duration of treatment. Farmer may have perceived clinical cure as a

treatment success, but many of the cases reverted to a subclinical state as observed by SCC remaining increased after treatment or the presence of bacteria in the POST milk samples.

Bacteriological cure is a more objective way to assess efficacy of mastitis therapy compared to observation of clinical cure, however is not practical and is not typically evaluated on farms. This study was not designed as a clinical trial and untreated control animals were not included, thus outcomes observed in this study are a result of both treatment and spontaneous cures. Previous researchers have used a variety of sampling strategies to define bacteriological cure (Oliver et al., 2004ab; Milne et al., 2005; Guterbock et al., 1993; McDougall et al., 2007). Despite the difficultly in comparing bacteriological cure among studies, the proportion of bacteriological cure observed in this study was reasonably consistent with previous research.

Culture negative milk samples are typically excluded from evaluations of treatment outcomes, especially when the purpose of a trial is to evaluate antimicrobial therapy. Present study included a unique definition of "spontaneous cure" as a classification of bacteriological cure (when pathogens were absent in PRE and POST samples), while most previous studies excluded the cases where no pathogen was isolated from pre-treatment samples. Few researchers have reported treatment outcomes for culture negative cases (Guterbock et al. 1993; Roberson et al., 2004). In current study, bacteriological cure for cases where pathogen was not recovered in PRE sample was 85% and was similar to those reported by Guterbock et al. (1993) (82%) and Roberson et al. (2004) (100%). Unless a farm is utilizing an OFC system, they do not typically have microbiological diagnosis before initiating treatment and thus treat many microbiologically negative cases using IMM antimicrobials. Therefore the perception of treatment outcomes includes both culture negative and culture positive cases. The inclusion of culture negative cases

in this study was an attempt to better evaluate outcomes from the complete spectrum of CM cases.

The overall proportion of bacteriological cure (77.2%) observed in the current study was greater than previously reported by Bradley and Green (2009) (65%) who combined data from 3 separate clinical trials of cephalosporin antimicrobials. A potential reason for the difference could be the use of a single sample to define bacteriological cure in current study. Regardless, the absence of a control group makes it impossible to establish if successful outcomes were a result of antimicrobial treatment or a result of the cow's immune response before the antimicrobial was administered. Most mastitis research is conducted on commercial dairy farms and because of producer resistance few studies include negative control groups. The inclusion of negative control groups in future studies would enhance our understanding of the benefits of antimicrobial therapy for various pathogen groups.

The proportion of bacteriological cure observed in this study for Gram-positive pathogens (62.5%), was similar to that reported by Oliver et al (2004) (53.7%) for subclinical cases caused by Gram-positive pathogens, and treated using the same antimicrobial (ceftiofur). Oliver et al., (2004b) reported that extended IMM therapy using ceftiofur to treat mastitis experimentally induced using *Streptococcus uberis* resulted increased proportion of bacteriological cure (88% for 5 d and 100% for 8 d). The failure to use antimicrobials to treat clinical cases of mastitis caused by *S. uberis*, has been reported to result in frequent relapses (Morin et al., 1998; Eenennaam et al., 1995) and most researchers recommend IMM antimicrobial therapy for these cases.

Researchers have reported a wide range of bacteriological cure (38 to 100%) for clinical mastitis caused by Gram-negative pathogens (Guterbock et al., 1993; McDougall, 1998; Hoe and Ruegg, 2005; Bradley and Green, 2009). Overall bacteriological cure observed for Gram-negative pathogens in this study (75%) was within the ranges reported previously. Contradictory findings have been reported regarding the benefit of IMM antimicrobial therapy for clinical mastitis caused by Gram-negative pathogens. Most IMM antimicrobials commercially available in the U.S. are not labeled for treatment of Gram-negative pathogens. IMM use of antimicrobials appeared to have little efficacy against coliform pathogens, as greater bacteriological cures were observed in untreated groups as compared to treated groups (Guterbock et al., 1993; Robertson et al. 2004). However most antimicrobials used in previous studies were not effective against Gram-negative pathogens. Gram-negative pathogens are considered to be diverse in pathogenicity, duration of infection, and response to therapy (Hogan and Smith, 2003). The least bacteriological cure in current study was observed for CM caused by *Serratia spp.* and agrees with previous studies indicating poor responses to antimicrobial therapy (Bowman et al., 1986; Isaksson and Holmberg. 1984;). It is generally recommended to avoid the use of antimicrobials when clinical mastitis is caused by non-responsive pathogens (National Mastitis Council, 1999; Erskine et al., 2003).When *Serratia spp* was excluded, 91% of Gram-negative pathogens included in this study resulted in bacteriological cure after treatment, however in the absence of a control group is impossible to distinguish between spontaneous or treatment cures.

In agreement with previous research (Owens et al., 1988; Sol et al., 2000; Bradley and Green, 2009; Borne et al., 2010) cases that resulted in bacteriological cures had lower LSCS at the DHIA test previous to the case as compared with those that did not result in bacteriological cures. Increased SCC may indicate that cows were chronically infected with subclinical mastitis

before the development of the clinical case and others have reported that chronically infected cows have poorer response to therapy (Deluyker et al., 1999; Melchior et al., 2006). Similarly, cows that experienced previous cases of CM were less likely to experience bacteriological cure of the enrolled case. This finding is in agreement other studies that have reported an association between previous cases of CM and the probability of reinfection (Houben et al., 1993; Steeneveld et al., 2008). Likewise, cows that did not experience bacteriological cure were more likely to have recurrent cases. Examination of the cow's history of clinical and subclinical mastitis (i.e. individual SCC from monthly test) before making a treatment decision should be recommended to direct mastitis therapy

Recurrence (or relapse) of CM has been described by different researchers as another case of clinical mastitis in the same cow, in the same quarter, or by the same pathogen (Wenz et al., 2005; Apparao et al., 2009; Schukken et al., 2009). The interval used to define a new case (rather than a recurrence) varies among studies ranging from 8 to 90 days or longer (Wenz et al., 2005; Apparao et al., 2009; Schukken et al., 2009; Bar et al., 2007). Researchers also differ in defining when the interval begins. It may be counted from the day of the diagnosis of clinical mastitis, from the last day of treatment or from the last day of the withholding period (Wenz et al., 2005; Apparao et al., 2009; Schukken et al., 2009). Based on the economic importance to producers, we used a cow level definition of recurrence (another case of clinical mastitis in the same cow, independently of quarter or pathogen). Producers often focus on economic losses due to discarded milk regardless of the quarter affected. However, recording quarter and pathogen is useful for future decisions. In some instances, drying off a chronically infected mammary gland quarter could be a more optimal treatment as compared to repetitive treatments of glands that are unlikely to cure. The recognition of recurrence of CM is dependent upon the detection level of

the herd and is especially impacted by the use of forestripping during pre-milking cow preparation. All herds included in this study practiced forestripping and the proportion of recurrence observed (18% within 60 days post treatment) was similar to previous reports (17% within 30 days reported by Hoe and Ruegg (2005) and 23% within 90 days reported by Wenz et al. (2005)).

Similar to Wenz et al., (2005), cows that did not experience bacteriological cure were more likely to experience a recurrent case of CM. It is interesting to note that approximately 40% of the recurrent cases occurred in a different quarter yet the failure to experience bacteriological cure of the initial case was a strong predictor of recurrence at the cow level. It is likely that the relationship between bacteriological cure and recurrence at the quarter level is even greater than indicated in this study. Cows that were enrolled with a case of CM earlier in lactation were more likely to experience recurrence. Others have noted greater incidence of CM in higher producer animals during periods of peak milk production (Bar et al. 2007) which may reflect reduced immunological capabilities. The observation of greater risk of recurrence for cases occurring in earlier lactation may indicate that treatments should be more aggressive during these periods to reduce recurrences throughout the remainder of lactation.

Most of the animals that left the herd were culled. Culling decisions are directly affected by diseases (such as clinical mastitis) that result in marked decreases in milk production (DeGraves and Fetrow, 1993; Gröhn et al., 1998; Gröhn et al., 2005; Hadley et al., 2006). In this instance milk production was the primary risk factor associated with retention of a cow within the herd. Cows who produced less than 20 kg of milk per day at the previous DHIA test had less than 50% probability of remaining in the herd. Herds included in this study were large commercial farms

with a ready supply of replacement heifers. Other researchers have previously reported that greater milk yield was protective against culling (Gröhn et al., 1998).

Somatic cell response below 200,000 cells / mL is another desired outcome after treating mild and moderate cases of clinical mastitis. Increased SCC is of economic importance to the dairy producer because milk with fewer somatic cells is more valuable to many processors. Somatic cell counts over 200,000 cells / mL are often used to define subclinical mastitis (Hillerton and Berry, 2005). Since characterization of short term outcomes was the objective of this study, only one test after 21 days had elapsed between treatment and the DHIA test was used. This test could have been either the first or the second DHIA test after enrollment of the case. Although no association was found between SCR and microbiological diagnosis at PRE milk sample in this study, Haas et al (2004) reported that the reduction of SCC after treatment varies by pathogen and could vary from 3 to 7 weeks. One experiment using induced CM with *E. coli* showed that SCC peaked 2 days after inoculation, and pre-infection SCC values returned within 3-4 weeks after challenge (Pyörälä et al. 1994). In contrast, experimental infection with *S. aureus*, increased SCC 24 hours after infection and remained increased for at least 48 days (Shoshani et al., 2000).

In present study, the SCR after treatment of CM caused by Gram-negative pathogens was very similar to cases where no pathogen was recovered. While 63% of cases caused by Gram-negative pathogens or “no growth” had somatic cell response below 200,000 cell/mL., only 44% of cases caused by Gram-positive bacteria reached this level. Others have previously reported similarities between Gram-negative pathogens and “no growth”, in terms of SCC patterns and milk production losses after CM case (Haas et al., 2002; Gröhn et al., 2004)

The interaction between level of SCC before the case and occurrence of previous cases of CM on the SCR observed in this study was very interesting. For the first case of CM, the probability of SCR was around 65% regardless of the previous SCC, while for cases preceded by CM cases the probability of SCR was greater when previous SCC was below 200,000 cells / mL and decreased steeply as the SCC increased. Similar to Nyman et al. (2010), low SCC at the test day before CM lowered risk of SCC <200,000 cells / mL after the case probably indicating the absence of subclinical mastitis. The duration of subclinical infection after treatment of CM has not been well reported for the variety of pathogens observed in this study.

Etiology of the CM case plays an important role in determining the probability of having bacteriological cure and therefore determining the appropriate treatment. The use of OFC is an important diagnostic tool used to identify the pathogen and direct therapy (Neeser et al., 2006; Lago, 2009). Use of selective culture media to identify growth of Gram-negative and Gram-positive pathogens is technically easy. However, as observed in this study, and according to Lago (2009), the accuracy in identifying pathogen growth using OFC varied among farms. The use of OFC needs to be accompanied by oversight of personnel who are adequately trained and supervised.

2.5 CONCLUSIONS

Characterization of selected post-treatment outcomes of cases of mild and moderate clinical mastitis was performed and risk factors associated with these outcomes were identified. The results demonstrated that cows are more likely to have bacteriological cure when experiencing CM for the first time in the lactation and when no pathogen is recovered from the pre-treatment sample. When the cow experienced bacteriological cure, she was less likely to experience

recurrent cases and was more likely to have SCR below 200,000 cells / mL post-treatment. When SCC before CM was > 200,000 cells / mL the probability of having SCR after treatment was diminished. Assessment of bacteriological cure on farm is not feasible for many farms, however post-treatment outcomes such as recurrence and SCR, are strongly associated with bacteriological cure and when monitored can be used to help determine if a treatment has been successful. Information about etiology of CM, history of clinical and subclinical (SCC) mastitis and parity are useful to review when making strategic treatment decision.

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Table 2.1. Criteria used to define diagnosis of cases based on microbiological results from duplicate milk samples (A and B) for pre-treatment (PRE) and post-treatment (POST) milk samples obtained from mild and moderate cases of clinical mastitis.

Microbiological diagnosis of A	Microbiological diagnosis of B	Diagnosis of Case	PRE		POST	
			N	(%)	N	(%)
Identical to B[1]	Identical to A[1]	As identified	127	(89.0)	103	(72.0)
Pathogen[2]	"No growth"	Pathogen	2	(1.4)	1	(0.7)
Pathogen[2]	Contaminated[3]	Pathogen	1	(0.7)	2	(1.4)
"No growth"	Contaminated[3]	"No growth"	11	(8.0)	14	(10.0)
Pathogen[2]	missing	Pathogen	2	(1.4)	7	(5.0)
No sample	No sample	missing	0	(0.0)	16	(11.0)
Total			143	(100.0)	143	(100.0)

[1]Pathogen, "no growth" or contamination

[2]Isolation of at least 3 colonies of the same type of bacteria

[3]Isolation of 3 or more different colony types

Table 2.2 Criteria used to define bacteriological cure comparing microbiological diagnosis of pre-treatment (PRE) and post-treatment (POST) milk samples.

PRE	POST	Outcome Categories	N	(%)	Bacteriological cure
Pathogen	"No growth"	Treatment Cure	36	(35.6)	Yes
"No growth"	"No growth"	Spontaneous cure	42	(41.6)	Yes
Pathogen	Different pathogen	New infection	3	(3.0)	No
"No growth"	Pathogen or mixed infection	New infection	7	(7.0)	No
Pathogen	Same pathogen	Treatment Failure	13	(12.8)	No
Total			101	(100.0)	

Table 2.3. Characteristics of herd, cows, cases of clinical mastitis (CM).

	Farms											
	A		B		C		D		P-value	All farms		
Variable	N	(%)	N	(%)	N	(%)	N	(%)		N	(%)	mean
Number of milking cows	1250[a]		1250[a]		800[b]		640[c]		<0.001			985
Milk production[1](kg/cow/day)	36[a]		39[a]		44[b]		42[b]		<0.001			40
Bulk tank SCC[1] (x1000 cells / mL)	240[a]		250[a]		168[b]		213[a]		<0.001			218
Duration of sampling period (d)	62		31		101		67					65
All cases of clinical mastitis	85		64		53		64			266		
Mild	57	(67)	45	(70)	29	(55)	41	(62)		172	(65)	
Moderate	21	(25)	16	(25)	21	(40)	14	(22)		72	(27)	
Severe	7	(8)	3	(5)	3	(6)	9	(14)		22	(8)	
Cases eligible for enrollment	73		61		48		51		0.086	233		
Cases treated with imm ceftiofur	55		61		34		22		<0.001	172		
Cases used in statistical analysis[2]	47		45		32		19		0.003	143		
Severity									0.349			
Mild	34	(72)	32	(71)	18	(56)	11	(58)		95	(66)	
Moderate	13	(28)	13	(29)	14	(44)	8	(42)		48	(34)	
pre-treatment diagnosis									0.001			
Gram-positive	8	(17)	13	(29)	16	(50)	3	(16)		40	(28)	
Gram-negative	13	(28)	11	(24)	7	(22)	12	(63)		43	(30)	
“no growth”	26	(55)	21	(47)	9	(28)	4	(21)		60	(42)	
Parity									0.438			
First parity	7	(15)	5	(11)	4	(12)	5	(26)		21	(15)	
Second parity	20	(42)	15	(33)	10	(31)	9	(47)		54	(38)	
Third parity	7	(15)	13	(29)	6	(19)	2	(10)		28	(19)	
+Third parity	13	(28)	12	(27)	12	(38)	3	(16)		40	(28)	
Previous occurrence of CM[3]									0.029			
Yes	11	(23)	18	(40)	5	(16)	9	(47)		43	(30)	
No	36	(77)	27	(60)	27	(84)	10	(53)		100	(70)	
Cases used on-farm culture (OFC)	56		43		44		36			179		
Correctly identified by OFC	35	(63)	30	(70)	40	(91)	27	(75)		132	(74)	

[ab] Means with the same superscript are not significantly different ($P < 0.05$)

[1] Herd average for the last three months; [2]Mild and moderate cases treated with intramammary ceftiofur and diagnosed as Gram-positive, Gram-negative or “no growth” at pre-treatment milk sample; [3]During studied lactation

Table 2.4 Characteristics of cows and treatments of mild and moderate cases of clinical mastitis treated with intramammary ceftiofur.

	Farm									Severity					All Cases	
	A		B		C		D			Mild		Moderate				
Variables	N	Mean	N	Mean	N	Mean	N	Mean	P-val	N	Mean	N	Mean	P-val	N	Mean
DIM	47	167.0	45	143.0	32	149.0	19	141.0	0.621	95	157.0	48	142.0	0.393	143	152
Individual LSCS[1, 2]	42	3.6	45	3.7	28	3.2	18	4.4	0.503	88	4.3	45	2.4	<0.001	133	3.6
Milk yield[2] (kg/day)	43	40.8 [a]	45	44.3 [a]	28	53.8 [b]	18	45.7 [a]	<0.001	89	44.5	45	47.1	0.212	134	45.4
Duration of treatment (d)	46	3.5 [a]	42	5.6 [b]	32	5.6 [b]	19	5.2 [b]	<0.001	95	4.6	44	5.4	0.049	139	4.8
Days to clinical cure	46	5.1	40	5.6	31	5.8	19	5.3	0.484	93	5.3	43	5.8	0.143	136	5.4
Days of milk discard	46	7.1	42	7.7	32	8.1	19	7.8	0.252	95	7.5	44	8.1	0.122	139	7.7

[ab] Means with the same superscript are not significantly different ($P < 0.05$)

[1]Linear Somatic Cell Score

[2]Value from monthly DHIA test previous to the enrolled CM case

Table 2.5. Microbiological diagnosis of milk samples obtained from mild and moderate cases of clinical mastitis collected pre-treatment (PRE) or post-treatment (POST).

Microbiological diagnosis	PRE		POST	
	N	(%)	N	(%)
Gram-negative	43	(30.1)	7	(4.9)
Escherichia coli	14	(9.8)	0	
Klebsiella spp.	11	(7.7)	1	(0.7)
Enterobacter spp.	8	(5.6)	0	
Serratia spp.	7	(4.9)	5	(3.5)
Other Gram-negative[1]	3	(2.1)	1	(0.7)
Gram-positive	40	(28.0)	14	(9.8)
Environmental *Streptococci*[2]	25	(17.5)	9	(6.3)
Streptococcus dysgalactiae	10	(7.0)		
Aerococcus viridians	6	(4.2)		
Lactococcus lactis	3	(2.1)		
Streptococcus equines	2	(1.4)		
Streptococcus mitis	2	(1.4)		
Streptococcus suis	1	(0.7)		
Streptococcus salivarus	1	(0.7)		
Other Gram-positive[3]	8	(5.6)	5	(3.5)
Coagulase negative staphylococci	4	(2.8)	0	
Staphylococcus chromogenes	2	(1.4)	0	
Staphylococcus. Simulans	2	(1.4)	0	
Staphylococcus aureus	1	(0.7)	0	
Streptococcus agalactiae	1	(0.7)	0	
Yeast	1	(0.7)	0	
"No growth"	60	(42.0)	78	(54.5)
Contaminated samples[4]	0	0	26	(18.2)
Missing samples	0	0	16	(11.2)
Mixed infection[4]	0	0	2	(1.4)
Total	143	(100.0)	143	(100.0)

[1] *Citrobacter spp.*, *Pasteurella spp.*, and *Pseudomonas spp*. were coded as other Gram-negative.
[2] Environmental Streptococci for POST milk samples were not diagnosed as specie level
[3] *A. pyogenes*, *Bacillus* and *Lactobacillus* were coded as other Gram-positives.
[4] Pre-treatment milk samples that were contaminated or had mixed infections were excluded.

Table 2.6. Unconditional associations (P < 0.25) between bacteriological cure and selected risk factors that were included in the initial logistic regression model from 101 cases of mild or moderate clinical mastitis after treatment with intramammary ceftiofur.

		Bacteriological cure					
		Yes		No			
Variables	Levels	N	%	N	%	Total	P-value
Overall		78	(77.2)	23	(22.8)	101	<0.001
Parity	1	10	(83.3)	2	(16.7)	12	0.087
	2	34	(81.0)	8	(19.0)	42	
	3	17	(89.5)	2	(10.5)	19	
	>3	17	(60.7)	11	(39.3)	28	
Occurrence of Previous CM	Yes	14	(51.9)	13	(48.1)	27	<0.001
	No	64	(86.5)	10	(13.5)	74	
Diagnosis pre-treatment	Gram-positive	15	(62.5)	9	(37.5)	24	0.080
	Gram-negative	21	(75.0)	7	(25.0)	28	
	“No growth”	42	(85.7)	7	(14.3)	49	

Table 2.7.

Proportion of bacteriological cure for 101 cases of mild or moderate clinical mastitis by microbiological diagnosis pre-treatment.

Pre-treatment diagnosis	Bacteriological cure	n (%)	Total
Gram-negative	21	(75.0)	28
Escherichia coli	9	(90.0)	10
Klebsiella spp.	4	(80.0)	5
Enterobacter spp.	5	(100.0)	5
Serratia spp.	1	(16.7)	6
Other Gram-negative[1]	2	(100.0)	2
Gram-positive	15	(62.5)	24
Environmental *Streptococci*	11	(61.1)	18
Other Gram-positive[2]	4	(80.0)	5
Coagulase-negative staphylococci	0	(0.0)	1
"No growth"[3]	42	(85.7)	49
Total	78	(77.2)	101

[1]*Citrobacter spp., Pasteurella spp.,* and *Pseudomonas spp*. were coded as other Gram-negative.
[2] *A. pyogenes, Bacillus* and *Lactobacillus* were coded as other Gram-positives.
[3]Clasified as spontaneous cure

Table 2.8

Final logistic regression model of risk factors for bacteriological cure for 101 cases of mild and moderate clinical mastitis (CM) after treatment with intramammary ceftiofur. Estimated coeficients (β), standard error (SE) for the coefficient, odds ratio (OR), 95% confidence interval (CI) for OR, and significance level are given for each variable.

					95% CI	
Predictors	β	SE	P-value	OR	Lower	Upper
Intercept	0.83	0.27				
Diagnosis pre-treatment			0.063			
Gram-negative	-0.15	0.38		0.4	0.10	1.34
Gram-positive	-0.67	0.38		0.2	0.06	0.79
“no growth”	Ref.					
Occurrence of previous CM			<0.001			
No	0.97	0.27		7.1	2.39	20.93
Yes	Ref.					

Table 2.9

Unconditional associations (P < 0.25) between recurrence of clinical mastitis (CM) and selected risk factors that were included in the initial logistic regression model from 143 cases of mild or moderate clinical mastitis after treatment with intramammary ceftiofur.

		Recurrence of CM					
		Yes		No			
Variable	Levels	N	%	N	%	Total	P-value
Overall		26	(18.2)	117	(81.8)	143	
Farm	A	9	(19.1)	38	(80.9)	47	0.223
	B	11	(24.4)	34	(75.6)	45	
	C	2	(6.3)	30	(93.8)	32	
	D	4	(21.1)	15	(78.9)	19	
Parity	1	1	(4.8)	20	(95.2)	21	0.175
	2	9	(16.7)	45	(83.3)	54	
	3	5	(17.9)	23	(82.1)	28	
	>3	11	(27.5)	29	(72.5)	40	
Previous occurrence of CM	Yes	5	(11.6)	38	(88.4)	43	0.183
	No	21	(21.0)	79	(79.0)	100	
Bacteriological cure[1]	Yes	9	(11.5)	69	(88.5)	78	0.004
	No	9	(39.1)	14	(60.9)	23	

[1] Bacteriological cure was not able to be assessed in 42 cases.

Table 2.10

Final logistic regression model of risk factors for recurrence of clinical mastitis (CM) for 143 cases of mild and moderate CM after treatment with intramammary ceftiofur (Model #1). Model #2 included bacteriological cure as a predictor (N = 101). Estimated coeficients (β), standard error (SE) for the coefficient, odds ratio (OR), 95% confidence interval (CI) for OR, and significance level are given for each variable.

					95% CI	
Predictors	β	SE	P-val	OR	Lower	Upper
Model # 1						
Intercept	-0.568	0.431				
Farm (forced)			0.246			
A	0.315	0.386	0.413	4.3	0.883	22.35
B	0.537	0.371	0.147	5.3	1.058	27.186
C	ref.					
D	0.228	0.491	0.557	4.1	0.658	26.565
Months in milk at CM	-0.2552	0.093	0.006	0.8	0.645	0.930
Model # 2						
Intercept	-1.885	0.443				
Farm			0.1			
A	0.252	0.517	0.625	9.8	0.847	114.142
B	1.032	0.525	0.049	21.4	1.816	253.152
C	ref.					
D	0.747	0.659	0.257	16.1	1.071	242.728
Bacteriological cure			0.001			
No	1.397	0.489	0.001	16.3	3.05	87.616
Yes	ref.					
Previous CM			0.013			
No	1.207	0.428	0.013	11.2	1.644	76.086
Yes	ref.					

Table 2.11

Unconditional associations between retention within the herd and selected risk factors that were included in the initial logistic regression model from 143 cases of mild or moderate clinical mastitis after treatment with intramammary ceftiofur.

		Retention within the herd					
		Yes		No			
	Levels	N	%	N	%	Total	P-value
Overall		125	(87.4)	18	(12.6)	143	
Farm	A	43	(91.5)	4	(8.5)	47	0.741
	B	38	(84.4)	7	(15.6)	45	
	C	28	(87.5)	4	(12.5)	32	
	D	16	(84.2)	3	(15.8)	19	
Parity	1	19	(90.5)	2	(9.5)	21	0.04
	2	51	(94.4)	3	(5.6)	54	
	3	25	(89.3)	3	(10.7)	28	
	>3	30	(75.0)	10	(25.0)	40	
Previous occurrence of CM	Yes	33	(76.7)	10	(23.3)	43	0.024
	No	92	(92.0)	8	(8.0)	100	

Table 2.12

Final logistic regression model of risk factors for retention within the herd for 134 cases of mild and moderate clinical mastitis (CM) after treatment with intramammary ceftiofur. Estimated coeficients (β), standard error (SE) for the coefficient, odds ratio (OR), 95% confidence interval (CI) for OR, and significance level are given for each variable.

					95% CI	
Predictors	β	SE	P-val	OR	Lower	Upper
Intercept	-1.697	1.241				
Farm (Forced)			0.542			
A	ref.					
B	-0.229	0.434	0.597	0.4	0.11	1.62
C	0.041	0.637	0.948	0.5	0.08	3.64
D	-0.461	0.566	0.415	0.3	0.06	1.82
Previous milk yield (kg)	0.087	0.03	0.004	1.1	1.03	1.16

Table 2.13

Unconditional associations (P < 0.25) between recurrence somatic cell response and selected risk factors that were included in the initial logistic regression model from 122 cases of mild or moderate clinical mastitis after treatment with intramammary ceftiofur.

		Somatic Cell Response					
		< 200,000 cell/mL		> 200,000 cell/mL			P-value
Variables	Levels	N	%	N	%	Total	
Overall		71	(58.2)	51	(41.8)	122	
Farm	A	28	(70.0)	12	(30.0)	40	0.040
	B	19	(51.4)	18	(48.6)	37	
	C	19	(65.5)	10	(34.5)	29	
	D	5	(31.3)	11	(68.8)	16	
Parity	1	9	(52.9)	8	(47.1)	17	0.193
	2	31	(64.6)	17	(35.4)	48	
	3	17	(68.0)	8	(32.0)	25	
	>3	14	(43.8)	18	(56.3)	32	
Occurrence of previous CM	Yes	11	(34.4)	21	(65.6)	32	<0.001
	No	60	(66.7)	30	(33.3)	90	
Diagnosis pre-treatment	Gram-positive	14	(43.8)	18	(56.3)	32	0.163
	Gram-negative	23	(63.9)	13	(36.1)	36	
	“no growth”	34	(63.0)	20	(37.0)	54	
Bacteriological cure[1]	Yes	19	(95.0)	1	(5.0)	20	<0.001
	No	20	(25.9)	57	(74.1)	77	

[1]Bacteriological cure was not assessed in 23 cases

Table 2.14

Final logistic regression model of risk factors for somatic cell response for 115 cases of mild and moderate clinical mastitis (CM) after treatment with intramammary ceftiofur (Model #1). Model #2 (n = 97) included bacteriological cure as a predictor. Estimated coeficients (β), standard error (SE) for the coefficient, odds ratio (OR), 95% confidence interval (CI) for OR, and significance level are given for each variable.

					95% CI	
Predictors	β	SE	P-val	OR	Lower	Upper
Model 1						
Intercept	1.95	0.69				
LSCS[1]	-0.46	0.16	0.003			
PrevCM[2]						
No	-1.23	0.69	0.077			
Yes	ref.					
Interaction LSCS[1] *PrevCM[2]	0.41	0.16	0.008			
PrevCM[2] : yes				0.6	-0.61	-0.30
PrevCM[2] : no				0.9	-0.14	0.05
Model 2						
Intercept	-1.22	0.55				
Farm			0.02			
A	ref.					
B	-0.41	0.44		0.3	0.08	1.13
C	0.96	0.53		1.2	0.24	5.60
D	-1.34	0.54		0.1	0.02	0.57
Bacteriological cure			<0.001			
No	-2.15	0.55		0.01	0.002	0.12
Yes	ref.					

[1]Linear somatic cell score (LSCS) at previous DHIA test

[2] Occurrence of previous clinical mastitis in studied lactation

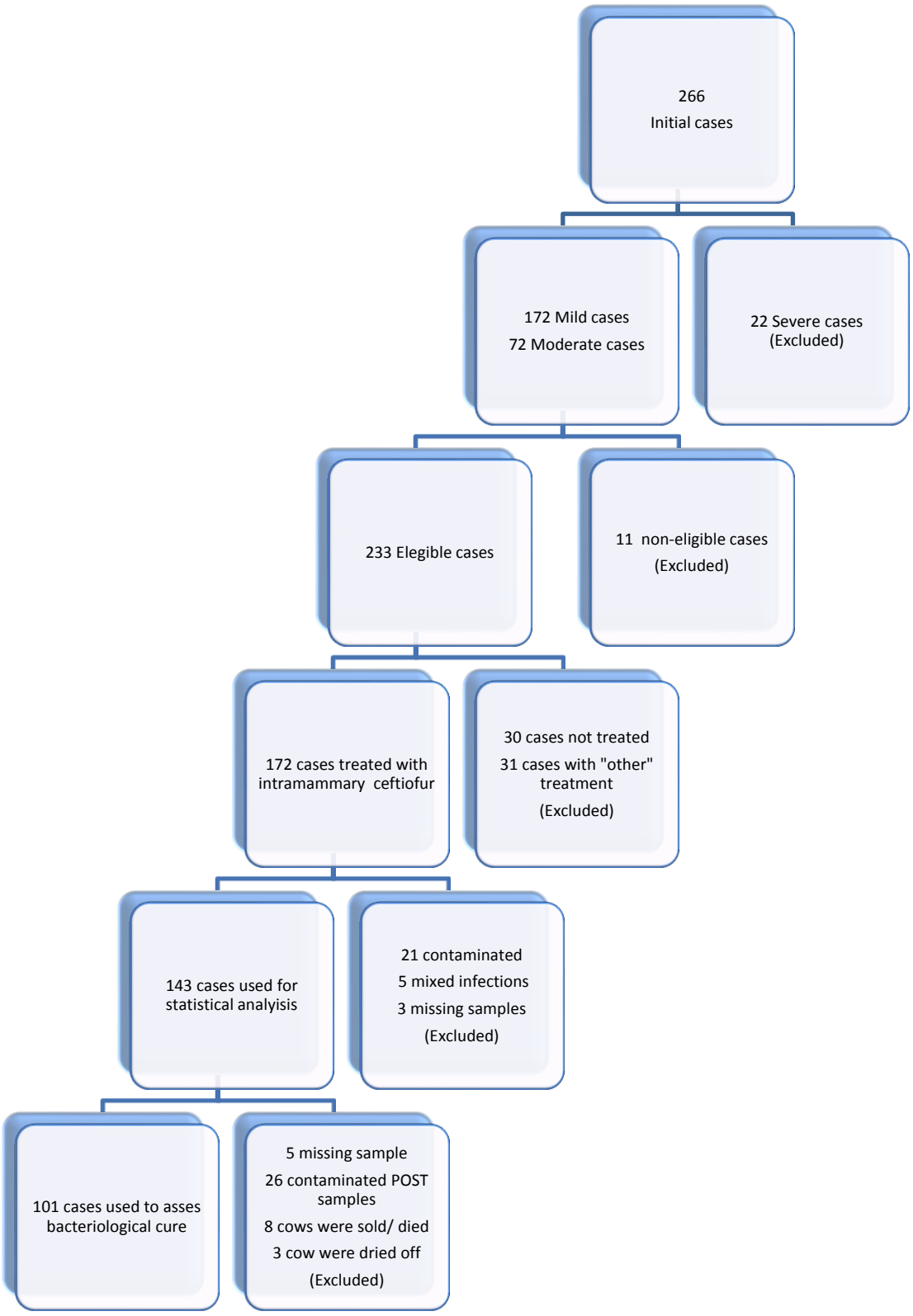

Figure 2.1. Flow diagram with explanation of cases and exclusions

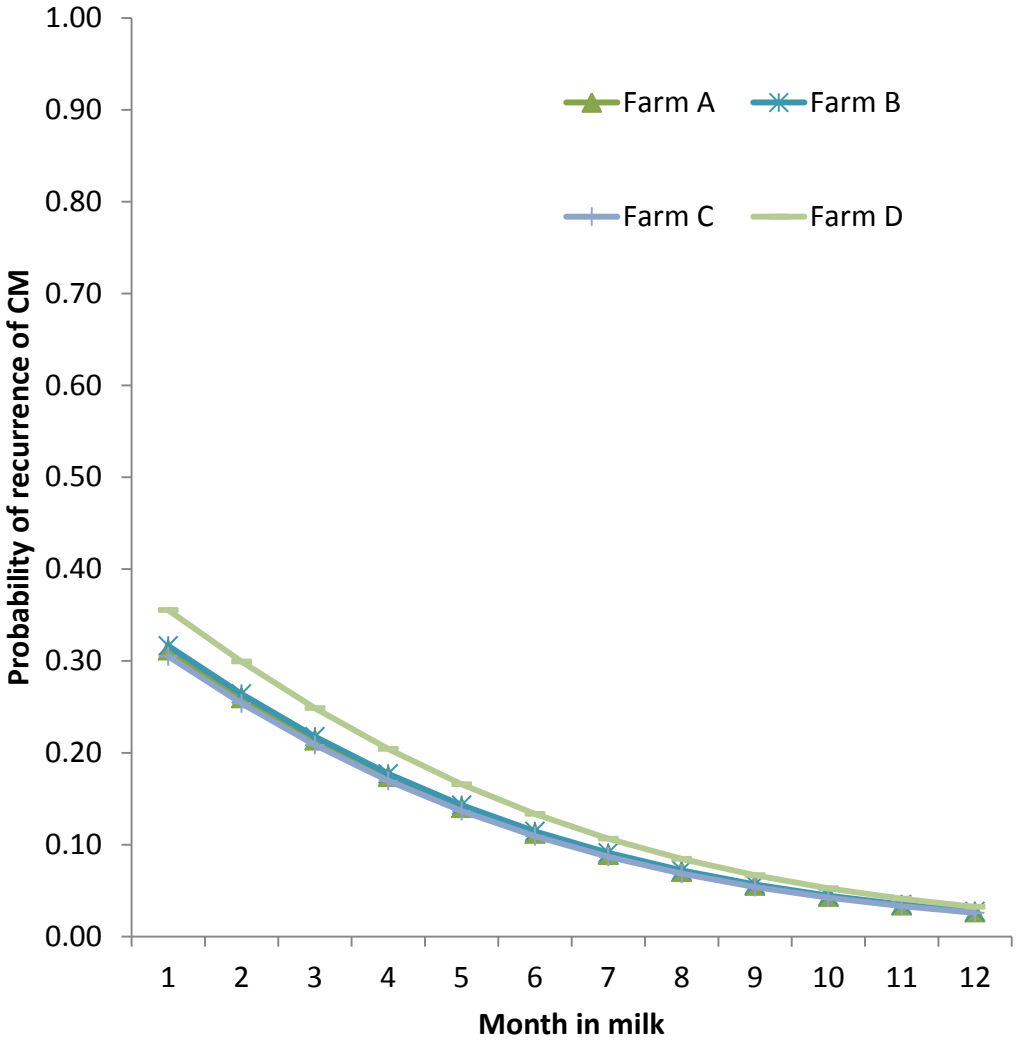

Figure 2.2. Probability of Recurrence of clinical mastitis by cow's month in milk.

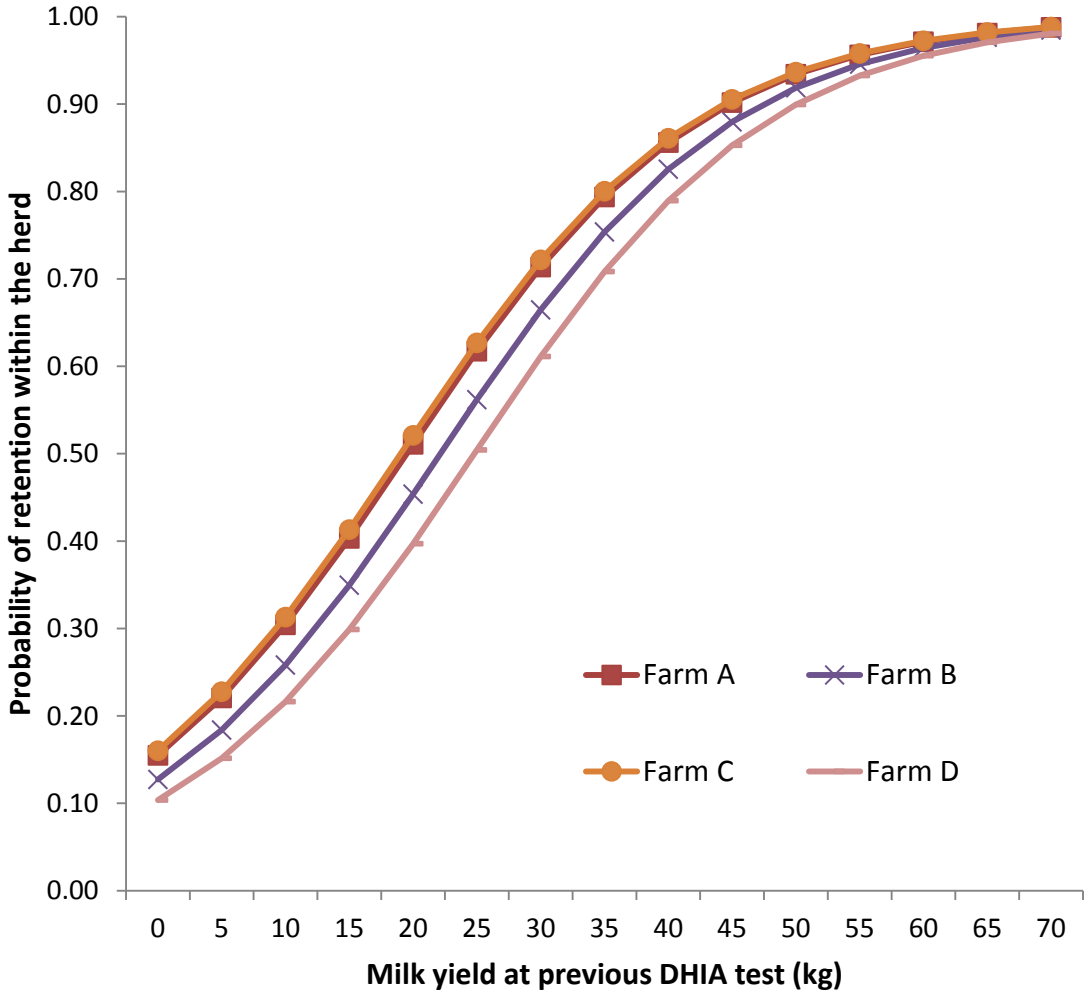

Figure 2.3. Probability of Retention of the cow within the herd for various milk yield (kg) in previous DHIA test by farms.

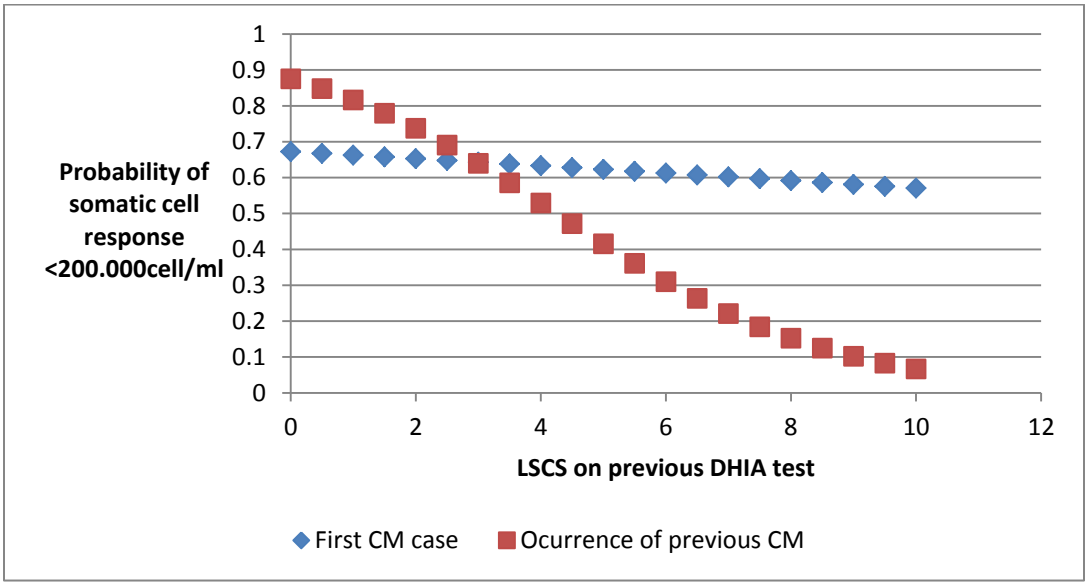

Figure 2.4. Probability of somatic cell response below 200,000 cell/mL was associated with the previous linear somatic cell score (LSCS) from last DHIA test and it was different depending on weather the CM case was the first one during studied lactation, or if it was a recurrent case (P = 0.008)

CHAPTER 3

DECISION TREE ANALYSIS OF TREATMENT STRATEGIES FOR MILD AND MODERATE CASES OF CLINICAL MASTITIS

3.1 INTRODUCTION

Mastitis is an inflammatory response of the mammary gland caused by bacterial infection and is the most common and costly health disorder of dairy cows (Ruegg, 2003). Mastitis has a negative economic impact on dairy farms in terms of discarded milk, lost production, reduced milk quality and treatment costs (Seegers et al., 2003; Gröhn et al. 2004). While antimicrobial therapy is not necessary for successful treatment of clinical mastitis (**CM**) caused by all pathogens, most cows with cases of CM are treated with intramammary antimicrobials (Pol and Ruegg, 2007; Hill et al., 2009).

Clinical mastitis is often classified according to severity as mild (milk looks abnormal), moderate (milk looks abnormal and in addition the udder or quarter is swollen) or severe (the cow exhibits systemic signs). While immediate action using systemic treatment is generally recommended for severe cases of CM, selective treatment based on causative pathogen is often recommended for mild and moderate cases. On-farm culture (**OFC**) programs are one approach used to help farmers rapidly diagnose the pathogen responsible for CM (Neeser et al., 2006; Lago, 2009). On-farm culture programs generally use selective medias to differentiate among Gram-positive or Gram-negative pathogens and vary treatments according to etiology (Neeser et al., 2006; Lago, 2009). Using OFC, microbiological results can be obtained within 24 hours, as opposed to waiting at least 48h to receive results from a diagnostic laboratory. Short term clinical and bacteriological outcomes have been reported for cows that received selective treatment of CM based on OFC results (Neeser et al., 2006; Lago, 2009). However, economic outcomes of selective treatment based on OFC have not been estimated.

The evaluation of treatment strategies for CM should be at the cow level and based on biological and economic factors. Biological outcomes from clinical trials using various treatments for CM have been described (Roberson et al. 2004; Hoe and Ruegg, 2005; Suojala et al., 2010) but the economic impact of mastitis treatment protocols has received less attention. In recent years, the use of extended duration therapy has been recommended and some studies support the concept that extended therapy significantly increases treatment efficacy for some mastitis pathogens (Deluyker et al., 2002; Gillespie et al., 2002; Oliver et al., 2003, 2004ab). However, the economic impact of mastitis treatments that are administered for extended durations has not been evaluated.

Decision tree analyses have been successfully used to evaluate economic decision making for treatment of various diseases of dairy cows (Ruegg and Carpenter, 1989; Berry et al., 2004; Dorshorst et al., 2006). Decision tree analysis is a graphic representation of decisions, probabilities and events, displayed in a logical and time-sequenced manner (Berry et al., 2004). However, the use of decision tree analysis to evaluate treatments used for mild and moderate cases of CM at the individual cow level has not been previously reported.

Development of a decision making system that includes biological and economic factors is required to avoid making decisions based solely on intuition, help dairy farmers make mastitis treatment policies and improve profitability. The objective of this study was to use decision tree analysis to evaluate various treatments for the first case of mild or moderate clinical mastitis under a variety of realistic farm scenarios.

3.2 MATERIALS AND METHODS

3.2.1 Overview of the Decision Tree Model

Decision tree analysis was carried out using TreePlan (Decision Toolworks, San Francisco, CA). Decision tree analyses were determined at the cow level for either primiparous or multiparous cows who were experiencing a mild or moderate case of clinical mastitis, in a single mammary gland quarter. Cases were assumed to be the first case of CM occurring in the current lactation at 30 DIM. Economic calculations were based on consequences of CM until the end of a 305d lactation. Decisions were ordered to reflect the sequence of decisions made by dairy producers. Economic values and probabilities were derived from research literature, chapter 2 of this thesis and expert knowledge (in a few instances where research data was not available).

The decision tree (Figure 1) was constructed using:

1) Decision nodes (represented by squares) with branches that represented strategies to be investigated and that were controllable (e.g., use of various OFC systems and use of various treatments strategies). Estimated costs were assigned to each decision branch.

2) Probability nodes (represented by circles) with branches that represented uncontrollable events (e.g., distribution of pathogens causing CM, probability of bacteriological cure and probability of recurrence). Estimated probabilities and costs were assigned to each probability branch and summed to 100%.

3) Terminal nodes (represented by triangles) which summed the partial cash flows along a unique path leading to each terminal node.

3.2.2 Producer decisions

Use of On-farm culture. After detection of CM, the initial decision was related to use of OFC (Figure 1). Three initial decisions were evaluated: 1) Use OFC, wait 24 hours before initiating

treatment (**OFCW**). After detection of CM, an aseptic milk sample was collected and OFC is set up. No treatment was initiated during the first 24h but milk was discarded. After 24 h, treatment was initiated based on results of OFC; 2) Use OFC, begin treatment before results were known (**OFCT**). After detection of CM, an aseptic milk sample was collected and OFC was set up. Intramammary (**IMM**) antimicrobial treatment was initiated immediately but after 24 hours, the treatment was readjusted based on results of OFC; and 3) Treat without OFC (**NOOFC**). Treatment was performed without diagnosis of causative pathogen.

Treatment strategies. The secondary decision was related to the treatment of the CM case. Four different treatment strategies were evaluated (Figure 1). The strategies that included the use of IMM antimicrobial consisted on the same generic drug but different durations. Milk withholding period was assumed to be 3 days after the last treatment. When the initial decision was OFCW or NOOFC the treatment decisions were: 1) Do not treat the cow with antimicrobials (**NOT**); 2) Use IMM antimicrobial treatment for 2 days (**2DT**); 3) Use IMM antimicrobial treatment for 5 days (**5DT**); and 4) Use IMM antimicrobial treatment for 8 days (**8DT**). When the initial decision was OFCT, similar treatment decisions were used but in this case, since the IMM antimicrobial treatment had already been initiated, the options were to stop treatment (**STOP**) or to continue treatment for 1 (**C1DT**), 4 (**C4DT**) or 7 (**C7DT**) days. Strategies 5DT, 8DT, C4DT and C7DT were considered extended treatment.

3.2.3 Probability Events

Distribution of Etiologies. The baseline distribution of pathogens (“Scenario A”) was based on data from chapter 2 and represented the distribution of pathogens observed on typical large commercial dairy herds located in Wisconsin. Pathogens for Scenario A were distributed as 2%

(*S. aureus)*, 19% (Environmental *Streptococci)*, 14% (coagulase-negative *Staphylococci (***CNS**)), 24% (*E. coli),* 6% (*Klebsiella* spp.) and 35% ("no growth") (Table 3.1). The etiology of CM was categorized as Gram-positive pathogens, Gram-negative pathogens or "no growth" (no pathogen recovered) to represent outcomes of OFC . Gram-positive pathogens included *Staphylococcus aureus*, environmental *Streptococci* and CNS. Gram-negative pathogens included *Escherichia coli* and *Klebsiella spp.* It was assumed that the diagnosis obtained by using OFC was 100% accurate. For OFCW and OFCT, each decision node was followed by a probability node with 3 branches (Gram-positive, Gram-negative or "no growth") (Figure 1). NOOFC was followed directly by the treatment strategies decision (Figure 1), in this case the same distribution of pathogens was modeled but treatment was not based on the diagnosis of causative pathogen.

Probability of Bacteriological Cure. Probabilities of bacteriological cure (Table 3.2) were estimated based on previous research (Smith et al., 1985; Morin et al. 1998; Pyörälä and Pyörälä, 1998; Wilson et al., 1999; Gillespie et al., 2002; Oliver et al., 2004; Roberson et al. 2004; Deluyker et al., 2005; Hoe and Ruegg, 2005; McDougall et al., 2007; Bradley and Green, 2009; Suojala et al. 2010; Borne et al., 2010). The probability of bacteriological cure was estimated for primiparous cows by treatment strategy and etiology, and it was assumed that the probability of bacteriological cure for multiparous cows was 5% less (Table 3.2). Since no difference in post treatment outcomes were reported by Lago (2009) the same probabilities of bacteriological cure were used for cows treated with antimicrobial immediately after detection of CM and those treated 24 hours later. The probability of bacteriological cure was estimated by pathogen, thus weighted averages were calculated to determine the overall bacteriological cure for Gram-positives and Gram-negative pathogens based on the distribution of pathogens (Table 3.2).

Probability of recurrence. The probability of experiencing recurrent cases of CM was estimated based on the occurrence of bacteriological cure as described in chapter 2. For primiparous cows, the probability of recurrence was assumed to be 2% for cases that resulted in bacteriological cure or 25% for cows that experienced persistent infection (no bacteriological cure). For multiparous cows, the probability of recurrence was assumed to be 12% for cases that resulted in bacteriological cure or 35% for cows that experienced persistent infection (no bacteriological cure). Recurrent cases were assumed to have same etiology and same severity as first case. All recurrences were assumed to be treated for 5 days and milk was discarded for 8 days. The first recurrent case (second case of CM) was assumed to occur 30 days (60 DIM) after the occurrence of the initial CM case. For the first recurrent cases the probability of bacteriological cure and probability of recurrence were assumed to be the same as those assumed for the first case of CM. The second recurrence (third case of CM) was assumed to occur 30 days (90 DIM) after the first recurrent case.

3.2.4 Economic consequences of mastitis

The economic consequences of mastitis in the analysis included the costs of diagnosis (OFC), treatment , labor , discarded milk, milk production losses (due to clinical and subclinical mastitis), culling and transmission of infection to other cows (only for CM caused by *Staphylococcus aureus*). Milk production losses included milk loss due to CM, discarded milk, and milk loss due to subclinical mastitis (Figure 2). To allocate milk production losses after occurrence of CM(30 to 305 DIM), the daily potential milk production of a cow (primiparous or multiparous cows) was calculated based on typical lactation curves for WI dairy cows (http://dairymgt.info/tools.php#1). Total potential milk yield from 30 to 305 DIM was estimated to be 9,670 kg and 11,188 kg for primiparous and multiparous cows, respectively. The average

U.S. milk price between 2008 and 2009 of $0.33/kg was used as the baseline (http://future.aae.wisc.edu/data/monthly_values/by_area/6?area=US) (Table 3.4). Thus, potential income from milk production for studied period was $3,191.10 and $3,692.04 for primiparous and multiparous cows, respectively. Farm labor was valued at $13 per hour (Table 3.4). No costs were included for veterinary labor because treatment of mild and moderate cases of CM are routinely performed by farm personnel rather than veterinarians.

Milk production losses due to clinical mastitis. After occurrence of CM and for the remainder of the lactation, pathogen specific milk production losses were estimated for primiparous and multiparous cows based on Gröhn et al. (2004). Gröhn et al., (2004), estimated daily pathogen specific milk loss for 71 days after occurrence of CM, after which production losses at day 71 were repeated for the remainder of the lactation (305d). Because milk losses were estimated by pathogen, weighted averages of milk yield losses were calculated for Gram-positives and Gram-negative pathogens, based on the distribution of pathogens (Table 3.5). For primiparous cows estimated milk loss from 30 to 305 DIM was 288 kg (Gram-positives), 823 kg (Gram-negatives) and 1017 kg ("no growth") (Table 3.5). For multiparous cows estimated milk loss from 30 to 305 DIM was 325 kg (Gram-positives), 427 kg (Gram-negatives) and 166 kg ("no growth") (Table 3.5).

Milk Discarded. To avoid double counting milk losses, a "corrected" daily milk production was calculated. Daily milk losses due to CM by etiology category were subtracted from potential daily milk production to obtain the "corrected" daily milk production (Figure 2).

$$\text{"Corrected" milk production} = \text{Potential milk production} - \text{Milk losses due to Clinical mastitis}$$

Daily "corrected" milk production was the amount of milk assumed to be discarded per day. When IMM antimicrobials were used, days of discarded milk were calculated as duration of the treatment plus withholding period (3d). For cows not treated with IMM antimicrobials, losses due to discarded milk were assumed for four days (Lago, 2009; Chapter 2). Days of discarded milk ranged from 4 to 11 days. Cost associated with days of discarded milk varied by duration of treatment, etiology category and DIM.

Milk production losses due to subclinical mastitis. When cows experienced bacteriological cure no additional losses attributable to subclinical mastitis were assumed. When cows did not experience bacteriological cure, reduced milk production was assumed due to the effects of subclinical mastitis. Milk loss was estimated as 0.4 kg /day for primiparous cows and 0.6 kg /day for multiparous cows for every two-fold increase of SCC greater than 50,000 cells / mL (Seegers et al., 2003). The somatic cell count of cows that did not experience bacteriological cure was assumed to be 800,000 cells / mL as described in chapter 2. Therefore, it was assumed that milk production was decreased by 1.6 and 2.4 kg per cow per day for primiparous and multiparous cows, respectively. For CM caused by Gram-positive bacteria, milk production losses were assumed to persist for the remainder of the lactation while for CM caused by Gram-negative bacteria or when no pathogen was recovered ("no growth"), milk production losses occurred for only 2 months after occurrence of the case (Haas et al., 2004). To avoid double counting milk loss, the reduction in milk production began after the end of the milk withholding period (Table 3.6). For primiparous cows that did not experience bacteriological cure, the average cost of milk loss due subclinical mastitis was $141.47 (Gram-positives), $27.85 (Gram-negatives) and $27.85 ("no growth") (Table 3.6). For multiparous cows that did not experience bacteriological cure, the average cost of milk loss due subclinical mastitis was $212.06 (Gram-positives), $41.78 (Gram-

negatives) and $41.78 (“no growth”) (Table 3.6). Since no additive effect is known, losses due to subclinical mastitis were assumed only for the first case of CM (no additional losses were assessed for recurrent cases).

Diagnostic costs. The cost of performing OFC was estimated to be $6.00 and included microbiological media ($2.25), disposable materials ($0.50) such as swabs and gloves, and 15 min of labor ($3.25) (Table 3.4). The fixed cost of purchasing an incubator was not included in cost of OFC because it was assumed that the OFC program was already established on the farm. Costs for OFCW included cost of OFC and one day of discarded milk, and were $14.95 and $18.85 for primiparous and multiparous cows, respectively. Costs for OFCT included cost of OFC, one day of discarded milk and one day of IMM treatment and were $21.70 and $25.60 for primiparous and multiparous cows, respectively. Cost for NOOFC included only the cost associated with one day of discarded milk and was $8.95 and $12.85 for primiparous and multiparous cows, respectively.

Treatment Costs. The cost of one day of IMM treatment was assumed as $6.75 and included one antimicrobial tube ($3.50) and 15 min of labor ($3.25) (Table 3.4). The total cost of each treatment strategy was calculated by adding the cost of treatment strategy (one day of IMM treatment times the duration on the treatment) and the cost of milk discarded. The cost of not treating the cow with antimicrobials (or stopping the treatment after obtaining a pathogen diagnosis the next day) ranged from $25.39 to $29.36 for primiparous cows and from $32.89 to $42.13 for multiparous cows (Table 3.7). The cost of treating the cow with antimicrobials for two days ranged from $40.60 to $62.43 for primiparous cows and from $50.60 to $83.71 for multiparous cows (Table 3.7). The cost of treating the cow with antimicrobials for five days

ranged from $86.79 to $113.32 for primiparous cows and from $104.01 to 146.92 for multiparous cows (Table 3.7). The cost of treating the cow with antimicrobials for eight days ranged from $133.96 to $166.41 for primiparous cows and from $164.70to $212.02 for multiparous cows (Table 3.7). For OFCW and OFCT, pathogen specific costs of discarded milk were estimated using Gröhn et al. (2004). For NOOFC the cost of milk discard was based on a weighted average depending on the distribution of pathogens included in each scenario.

Cost of Potential transmission of Staphylococcus aureus. Cows infected with *S. aureus* that did not experience bacteriological cure were assumed to remain subclinically infected, and the potential transmission of contagious pathogens to herdmates was estimated. Similar to Swinkles et al. (2005b), non-bacteriologically cured cows were assumed to remain infected for the remainder of the lactation (275d) and each infected cow was assumed to infect 0.25 additional cows. To calculate the cost of transmission, the cost of a treating a CM case for a standard 5d treatment was multiplied by 0.25 and by the prevalence of *S. aureus*. The cost of trasmition was then added to the total cost of recurrences.

Cost of premature culling. The cost of premature culling was based on Dorshorst et al. (2006). It was assumed that the culled animal was immediately replaced by a pregnant heifer with the same production level as the culled animal. The cost of a pregnant replacement heifer was $1500. The probability that the replacement heifer delivered a female calf was 53% (47% probability of delivering a male calf). The value of a male calf was $50 versus $250 for a female calf. Thus the weighted average value of a calf was $144. The estimated salvage value when the cow was culled was $600. The total cost of culling (TCC) was calculated by subtracting the estimated salvage value and the value of the calf from the value of the replacement heifer (i.e. $1500 –

$144 – $600 = $756). Discounting was used to calculate the cost of culling relative to the expected productive life of a cow. The assumed culling rate in the herd was 30%. The expected number of months for a cow in the herd was calculated as 1 divided by cull rate, and multiplied by 12. Thus, the expected life (ELM) of a cow was 40 months. Using monthly interest rate (IR) of 0.05% (5% annual discount rate) the monthly cost of voluntary culling was estimated using the following equation:

$$\text{Monthly cost of culling} = \frac{\text{TCC}}{\frac{1-(1+\text{IR})^{-\text{ELM}}}{\text{IR}}}$$

The monthly cost of culling was $20.90, This value was charged to the month of early culling. For example, if a primiparous cow was culled at 60 DIM (2 months in milk), 38 months would be considered lost, resulting in premature culling cost of $794 (38 x $20.90). The premature cost of culling a multiparous cow was assumed for a second parity cow. The average calving interval was assumed to be 14 months. For example if a multiparous cow was culled at 90 DIM (3 months in milk), 23 months would be considered lost (i.e. 40 – 14 – 3 = 23), resulting in premature cost of culling of $480.70. In summary, the cost of culling was calculated by the difference in the value of replacement heifer by the value of salvage and offspring, discounted by month of early culling. The cost of culling was then the pro-rated monthly value multiplied by the number of months of early culling. For primiparous cows, cost of premature culling was $794.20 and $773.30 for animals culled at 60 and 90 DIM, respectively. For multiparous cows, cost of premature culling was $501.60 and $480.70 for animals culled at 60 and 90 DIM, respectively.

Cost of losing a mammary gland quarter. It was assumed that 10% of cows experiencing a recurrent case resulted in drying off of the infected mammary gland and a subsequent 15% reduction in milk yield for the remainder of the lactation. Milk production loss due to drying off chronically infected mammary gland was adjusted by DIM.

Costs due to recurrence of mastitis: The costs due to recurrent cases included the total cost of 5d IMM treatment, potential loss of a mammary gland quarter and potential transmission (for cases caused by *S. aureus*). For primiparous cows, assuming that for the first recurrence 95% of the cases were treated and 5% were culled, the average cost of the first recurrence was $192.22. For multiparous cows, assuming that for the first recurrence 90% of the cases were treated and 10% were culled, the average cost of the first recurrence was $231.91. For primiparous cows, assuming that for the second recurrence 10% of the cases were treated and 90% were be culled, the average cost of the second recurrence was $44.86. For multiparous cows, assuming that for the second recurrence 5% of the cases were treated and 90% were culled, the average cost of the second recurrence was $74.75.

3.2.5 Analysis of model outcomes

Economic losses. The decision tree had 144 terminal values that represented the sum of the partial cash flow (total costs) of each possible outcome. The proportional impact of CM on milk income was estimated by dividing each terminal value by the estimated total milk income that would have been generated if the cow did not experience CM.

Expected Monetary Values. The economically optimal path in the decision tree was calculated by comparison of expected monetary values (**EMV**). Expected monetary values were calculated using a process of “averaging out and folding back” and were the sums of the products of the

monetary value of each outcome and the probability of that outcome occurring. The optimal treatment strategy was the option with the least negative EMV (i.e. minimum losses).In this model, EMV are negative and represent reduction in milk income, thus an EMV of -$5 would be more optimal than -$10.

Sensitivity Analyses. Sensitivity analyses were carried out using "what if" application included in Microsoft Excel. Sensitivity analyses were performed using the minimum and maximum values of milk price, cost of farm labor, cost of antimicrobials and cost of OFC under the baseline prevalence (Scenario A) (Table 3.4). Additional sensitivity analyses were performed by creating two additional scenarios with two realistic pathogen distributions. Scenario B was characterized by a greater prevalence of CM caused by contagious pathogens (*S. aureus*) and Scenario C was characterized by a greater prevalence of CM caused by coliforms (Table 3.1).

3.3 RESULTS

3.3.1 Economical losses.

Four situations were possible after treatment: 1) Cow experienced bacteriological cure and CM did not recur, 2) Cow experienced bacteriological cure but the CM did recur, 3) Cow did not experience bacteriological cure and CM did not recur or 4) Cow did not experience bacteriological cure but the CM did recur. Proportionally, the least economical losses were observed for cows that experienced bacteriological cure and did not have recurrent cases of CM (best case scenario) for primiparous cows (4-15% of potential milk income was decreased) and for multiparous cows (3-9% of potential milk income was decreased) (Table 3.8). The greatest proportion of losses was observed for cows that did not experience bacteriological cure and had recurrent cases of CM (worst case scenario) for primiparous cows (17-23% of potential milk

income was decreased) and multiparous cows (12-23% of potential milk income was decreased) (Table 3.8). The greatest difference between the best and worst case scenario was for CM caused by Gram-positive pathogens (13-15%) compared to CM caused by Gram-negative pathogens and "no growth" (7-9%) (Table 3.8).

3.3.2 Expected Monetary Values for Scenario A (baseline distribution of pathogens).

For primiparous cows, the least negative EMV was for NOOFC (-$323.10), but the difference on EMV among strategies was less than $2.26 per case of CM (Table 3.9). For multiparous cows, the least negative EMV was for OFCW (-$263.79), and the differences with the EMV from the other two strategies were less than $2.83 (Table 3.9).

When the OFCW system was used, and the etiology of CM was Gram-positive, the treatment strategy with the least negative EMV was 2DT for primiparous cows (-$251.32) and multiparous cows (-$366.97) (Table 3.10). When the etiology of CM was Gram-negative, the treatment strategy with the least negative EMV was NOT for both primiparous cows (-$340.12) and multiparous cows (-$266.35) cows (Table 3.10). Similarly, when the etiology of CM was "no growth", the treatment strategy with the least negative EMV was NOT for primiparous cows (-$383.80) and multiparous cows (-$159.60) cows (Table 3.10).

When the OFCT system was used, and the etiology of CM was Gram-positive, the treatment strategy with the least negative EMV was C1DT for primiparous cows (-$241.73) and multiparous cows (-$353.25) cows (Table 3.10). When the etiology of CM was Gram-negative, the treatment strategy with the least negative EMV was STOP for primiparous cows (-$346.87) and multiparous cows (-$273.10) (Table 3.10). Similarly, when the etiology of CM was "no

growth", the treatment strategy with the least negative EMV was STOP for primiparous cows (-$390.55) and multiparous cows (-$166.35) (Table 3.10).

When the NOOFC system was used, the etiology of CM was unknown, and the treatment strategy with the least negative EMV was NOT for primiparous cows (-$323.10) and 2DT for multiparous cows (-$266.62) (Table 3.10). However, for primiparous cows, the EMV for the strategy 2DT was only $3.65 greater than the EMV for NOT.

For all OFC systems and all etiologies, a large difference was observed in EMV of extended treatments (5DT, 8DT, C4DT and C7DT) compared to the least negative EMV (Table 3.10). For primiparous and multiparous cows the difference in EMV ranged from $33.50 to $163.28 greater for extended treatments (Table 3.10). When OFCW and OFCT were used, the greatest difference was observed for treating "gram-negative" and "no growth" for 8 days compared to not treating (Table 3.10).

3.3.3 Expected Monetary Values for Scenario B (greater prevalence of Staphylococcus aureus).

For primiparous cows, the least negative EMV was for NOOFC (-$361.44), and the differences with the EMV from the other two strategies were less than $6.66 per case of CM (Table 3.9). For multiparous cows, the least EMV was for NOOFC (-$420.57), and the differences with the EMV from the other two strategies were less than $9.09 per case of CM (Table 3.9).

When the OFCW system was used, and the etiology of CM was Gram-positive, the treatment strategy with the least negative EMV was 2DT for primiparous cows (-$362.53) and multiparous cows (-$517.49) (Table 3.10). When the etiology of CM was Gram-negative, the treatment strategy with the least negative EMV was NOT for primiparous cows (-$378.58) and multiparous

cows (-$289.61) (Table 3.10). Similarly, when the etiology of CM was “no growth”, the treatment strategy with the least negative EMV was NOT for primiparous cows (-$383.80) and multiparous cows (-$159.60) (Table 3.10).

When the OFCT system was used, and the etiology of CM was Gram-positive, the treatment strategy with the least negative EMV was C1DT for primiparous cows (-$354.00) and multiparous cows (-$504.59) (Table 3.10). When the etiology of CM was Gram-negative, the treatment strategy with the least negative EMV was STOP for primiparous cows (-$385.33) and multiparous cows (-$96.36) (Table 3.10). Similarly, when the etiology of CM was “no growth”, the treatment strategy with the least negative EMV was STOP for primiparous cows (-$390.35) and multiparous cows (-$166.57) (Table 3.10).

When the NOOFC system was used, the etiology of CM was unknown, and the treatment strategy with the least negative EMV was 2DT for primiparous cows (-$361.44) and multiparous cows (-$420.57) (Table 3.10).

For all OFC systems and all etiologies a large difference was observed for EMV of extended treatments (5DT, 8DT, C4DT and C7DT) compared to the least negative EMV (Table 3.10). For primiparous cows the difference ranges from $26.58 to $124.15 greater for extended treatments (Table 3.10). The largest difference was observed for treating “no growth” for 8 days when using OFCW system (EMV = -$507.75) (Table 3.10).

3.3.4 Expected Monetary Values for Scenario C (greater prevalence of coliforms).

For primiparous cows, the least negative EMV was for NOOFC (-$313.89), and the differences with the EMV from the other two strategies were less than $7.68 per CM case (Table 3.9). For

multiparous cows, the least EMV was for NOOFC (-$261.28), and the differences with the EMV from the other two strategies were less than $5.11 per CM case (Table 3.9).

When the OFCW system was used, and the etiology of CM was Gram-positive, the treatment strategy with the least negative EMV was 2DT for primiparous cows (-$222.40) and multiparous cows (-$401.01) (Table 3.10). When the etiology of CM was Gram-negative, the treatment strategy with the least negative EMV was NOT for primiparous cows (-$323.39) and multiparous cows (-$255.35) (Table 3.10). Similarly, when the etiology of CM was "no growth", the treatment strategy with the least negative EMV was NOT for primiparous cows (-$383.59) and multiparous cows (-$158.44) (Table 3.10).

When the OFCT system was used, and the etiology of CM was Gram-positive, the treatment strategy with the least negative EMV was C1DT for primiparous cows (-$212.81) and multiparous cows (-$387.56) (Table 3.10). When the etiology of CM was Gram-negative, the treatment strategy with the least negative EMV was STOP for primiparous cows (-$330.14) and multiparous cows (-$262.10) (Table 3.10). Similarly, when the etiology of CM was "no growth", the treatment strategy with the least negative EMV was STOP for primiparous cows (-$390.34) and multiparous cows (-$165.19) (Table 3.10).

When the NOOFC system was used, the etiology of CM was unknown, and the treatment strategy with the least negative EMV was NOT for primiparous cows (-$313.89) and multiparous cows (-$261.28) (Table 3.10). However, for primiparous cows, the strategy 2DT had only $3.65 difference with the NOT strategy.

For all OFC systems and all etiologies a large difference was observed for EMV of extended treatments (5DT, 8DT, C4DT and C7DT) compared to the least negative EMV (Table 3.10). For primiparous cows the difference ranges from $32.91 to $124.15 greater for extended treatments (Table 3.10). The largest difference was observed for treating "no growth" for 8 days when using OFCW system (EMV = -$507.75) (Table 3.10).

3.3.5 Sensitivity Analysis

For primiparous and multiparous cows, milk price had the greatest effect on the model outcomes (EMV) (Table 3.11 and 3.12). The model was not very sensitive to changes in labor, treatment or cost of OFC, since minimal differences in EMV were observed for the extremes of these values when compared to EMV from the baseline scenario (Table 3.11 and 3.12).

For primiparous and multiparous cows when OFCW and OFCT systems were used, the treatment strategies with the least negative EMV was consistently 2DT or C1DT for Gram-positives, and NOT or STOP for Gram-negatives and "no growth," regardless of pathogen distribution.

For primiparous cows when NOOFC system was used, the treatment strategy with the least negative EMV was NOT for most of situations, except when the cost of treatment was minimal (in this situation, the decision with least negative EMV was 2DT), however the difference in EMV was less than $1.00.

For multiparous cows when NOOFC system was used, the treatment strategy with the least negative EMV was 2DT for most situations, except when the treatment cost was maximum or when the milk price was minimal. In this situation, the decision with least negative EMV was NOT, however the difference in EMV was $3.00 greater than NOT.

3.4 DISCUSSION

Decision tree analysis is an approach to decision making based on combining scientific knowledge with economic considerations. Rather than simply evaluating clinical or bacteriological cure rates, the use of decision tree analysis at the cow level allowed the comparison of the economic impact of a variety of mastitis treatment strategies that are commonly used by dairy farmers in WI. The model is an attempt to define the economically optimal treatment strategy for generic treatment of CM balancing the benefits and cost of treatment. While OFC systems were included in this study, the objective was not to determine if the use of OFC was economically optimal, but to determined the most economically efficient treatment strategy under a variety of potential management situations. The tree included best possible assumptions of the costs and biological outcomes based on published field trial data and in some cases where reliable data was not available, assumptions were based on conservative estimates of the authors. The decisions used in the tree were ordered to reflect the sequence of decisions made by dairy producers.

Clinical mastitis is a complex disease that involves different biological factors. Factors related to the cow such as parity, stage of lactation, number of mammary gland quarters infected and previous history of clinical and subclinical mastitis are known to be risk factors for treatment outcomes (Constable and Morin, 2003; Delyuker et al., 2005; Bradley and Green, 2009; Sol et al., 2000). The analysis was done separately for primiparous and multiparous cows because of the different shapes of their lactation curves and because parity is an important factor that is usually considered when making treatment decisions. The characteristics of the hypothetical cow modeled in this analysis were typical of cows that experience mild and moderate cases of clinical mastitis that are expected to result in relatively successful post-treatment outcomes. The

modeled cow was relatively early in lactation, with a single mammary gland quarter affected and without previous cases of CM. Cows that were experiencing severe cases of mastitis, were in the early or late stages of lactation, were affected with concurrent disease or were affected with pathogens that are isolated only infrequently from cases of CM were not included in this model.

Most mastitis research has focused on outcomes of treatment of mastitis caused by contagious pathogens such as *Staphylococcus aureus*. However, many modern dairy farms have successfully controlled mastitis caused by contagious pathogens and the distribution of pathogens causing mastitis is often dominated by environmental pathogens (Makovec and Ruegg, 2003; Milne et al., 2005). Additionally, 20-40% of CM cases have been reported to yield no growth (Roberson et al., 2004; Hoe and Ruegg, 2005; Lago, 2009), probably because the cow's immune system has successfully eliminated the infection (Smith et al., 1986; Sears et al., 1993). The distribution of pathogens modeled in this study was typical of modern US dairy farms. The greater diversity of mastitis pathogens occurring on modern dairy farms has resulted in many farms adopting the use of OFC systems to better target treatments for specific diagnoses (Neeser, et al., 2006). In some instances, (such as recovery of no pathogens from CM cases) antimicrobial treatments are not administered and in other instances the duration of treatment may be varied based on diagnosis. Most dairy farms that use OFC limit their diagnoses to categories such as Gram-positive, Gram-negative or "No growth"; however the decision tree included the underlying pathogen distribution within these categories to estimate bacteriological cure and production losses. The inclusion of this distribution enhanced the accuracy of the model, taking advantage of recent research describing pathogen specific bacteriological cure and milk losses (Gröhn et al., 2004; Hass et al. 2004; Oliver et al., 2004).

Use of OFC programs is a simple and easy technique that, when correctly utilized, allows producers to identify the possible pathogen causing CM (Neeser et al., 2006; Lago, 2009). Many progressive dairy producers use OFC to determine etiology of CM case and develop selective treatments accordingly. When OFC is used, IMM antimicrobials are often administered to cows experiencing CM caused by Gram-positive pathogens and in some instances IMM antimicrobials are not used for CM caused by Gram-negative pathogens or when no pathogen is isolated (Lago, 2009). At least two different OFC schemes are used by farmers (Neeser et al., 2006). The first scheme is to postpone initiation of treatment for 24h until microbiological results from OFC are available (OFCW), which has been reported not to have adverse effects on outcomes of mild and moderate cases of CM (Lago, 2009). The second scheme is to start IMM antimicrobial treatment right after CM detection and re-adjust treatment based on microbiological results obtained from OFC after 24 h of incubation (OFCW).While OFC is often used on larger farms, many farmers have not yet implemented the use of OFC and treatment of CM cases is done without knowledge of causative pathogen. For this reason NOOFC was included in the tree to reflect all possible options.

Using the assumptions that were included in this model, only small differences in EMV were observed among all OFC systems (OFCW, OFCT, NOOFC) (Tables 3.9-3.12). Greater differences in EMV were observed based on duration of treatment and the overall differences among OFC systems were primarily a result of the model selecting shorter duration treatments (or no treatment) as the optimal economic pathway used to calculate overall EMV. In reality, the cost savings that occurs when OFC is used is generally associated with reduced milk discard due to fewer IMM antimicrobial treatments. In this model, those savings were not apparent because the model generally recommended no treatment or short duration therapy. If a farm was using

short duration therapy (or no treatment) as the primary mastitis treatment strategy, this model indicates that OFC is not likely to result in additional economic benefits. In contrast, herds that routinely use extended duration therapy without regard for pathogen diagnosis could incur considerable savings by adopting OFC. For example, a 1000 cow dairy with a 40% incidence of CM and a distribution of pathogens similar to scenario A (baseline) would experience 400 first cases of mastitis per year. If the treatment strategy was 5 d of IMM antimicrobial without regard to diagnosis (NOOFC) the EMV (loss) for each case occurring in primiparous cows would be approximately $369 (from Table 3.10) or $147,600 per year (for 400 cases). In contrast, the overall EMV for each case treated using a strategy of OFCW would be $325 or $130,000 per year. In this instance, the use of OFC would result in approximately $18,000 in annual savings.

The treatment strategies used on the model reflect the reality of treatments used on many modern dairy farms. Varying durations of treatment and the inclusion "no IMM treatment" were based on common practices used in the U.S. Although, most IMM antimicrobials commercially available in US are not labeled for treatment of Gram-negative pathogens, the generic drug used for the model was assumed to be effective against both Gram-negative and Gram positive pathogens, allowed for use for extended duration therapy and required 72 hours of milk discard. These characteristics are similar to at least one popular IMM antimicrobial marketed in the U.S.

Although bacteriological cures are not typically assessed on farms, the inclusion of this outcome in the model allowed us to estimate the economical consequences of CM. Greater probability of bacteriological cure was assumed for primiparous cows as compared to multiparous cows because researchers consistently report that greater parities are associated with a reduced probabilities of cure (Sol et al., 2000; Barkema et al., 2006). Several clinical trials have

addressed bacteriological cure after treatment of CM using different compounds and differing treatment durations. The wide variability in research methodologies used in therapeutic trials make it difficult to compare bacteriological cure among studies. Research data describing bacteriological cure using similar antimicrobial compounds was not available for all the pathogens and all treatment durations included in the model. For this reason assumptions of bacteriological cure were based on a logical combination of relevant clinical trials that used different active compounds, different durations and, in some instances, were used to assess bacteriological cure after treatment of subclinical mastitis cases (when data from appropriate studies of CM was not available).

With the exception of bacteriologically negative cases ("no growth"), cows receiving IMM antimicrobials were assumed to have greater bacteriological cure than cows not receiving antimicrobials (Oliver et al., 2004b; Borne et al., 2010). Most research of extended therapy used for treatment of CM described outcomes for mastitis caused by Gram-positive pathogens (Gillespie et al., 2002; Oliver et al., 2003; Oliver et al., 2004ab; Roy et al., 2009). For CM caused by Gram-positive pathogens, the probability of bacteriological cure was assumed to increase with increased duration of treatment (Deluyker et al., 2005; Gillespie et al., 2002; Oliver et al., 2004). Most IMM antimicrobials commercially available in U.S. are not labeled for treatment of Gram-negative pathogens, and for CM caused by Gram-negative pathogens, the probability of bacteriological cure was not influenced by treatment duration. Very little research has described outcomes for cases of CM which have not yielded bacterial growth (Roberson et al., 2004; Chapter 2), and bacteriological cure was not increased with increased duration of treatment for this category of etiology. Similar or greater proportion of bacteriological cure has been reported for CM caused by *E. coli* treated without use of antimicrobials (Guterbock et al.,

1993; Robertson et al., 2004).The greatest bacteriological cure was assumed for cows infected with *E. coli* (Borne et al., 2010) and when no pathogen was recovered (Roberson et al. 2004). The least bacteriological cure was assumed for cows infected with *S. aureus* (Oliver et al., 2004; Gillespie et al., 2002).

The probability of recurrence have been reported to be around 20% (Hoe and Ruegg, 2005; Wenz et al., 2005; Chapter 2) and is known to vary with parity. In this model, the overall probability of recurrence was estimated as 13% and 23% for primiparous and multiparous cows, respectively. Some previous models used to estimate economic losses of CM did not assume recurrence of CM (Huijps et al., 2007), this model included the potential occurrence of two additional cases of CM. As described previously, cows that did not experience bacteriological cure were more likely to experience recurrent cases (Wenz et al., 2003, chapter 2). Almost no data was found to estimate the probability of recurrence of CM by pathogen, so probability of recurrence was assumed to be equal for all pathogens. However, recurrence was driven by the probability of bacteriological cure and bacteriological cure was estimated based on etiology. The cost of recurrences (treatment, discarded milk, potential loss of a mammary gland quarter and potential transmission of contagious pathogens) were similar for first and second recurrence. The overall cost of recurrence appears to be larger for the first recurrence (as compared to the second) because the cost of the second recurrence is multiplied by a succession of probabilities (probabilities of cure, recurrence, and treatment). Some possible effects of mastitis were difficult to estimate because research literature is insufficient. For example, no research was available to document the potential reduction in milk yield when a mammary gland quarter is selectively dried off. Our estimate of a 15% reduction in milk yield may be an overestimate. However, the impact of this assumption on the model was very small because the milk yield losses were

approximately $40 to $50, occurred only in 10% of recurrent cases, and thus, the impact of the reduction of milk yield due to drying a mammary gland quarter was minimal.

Culling decisions are directly affected by diseases (such as clinical mastitis) that result in marked decreases in milk production (DeGraves and Fetrow, 1993; Gröhn et al., 2005; Hadley et al., 2006). Occurrence of previous cases of CM is a risk factor associated with less probability of cure. Some larger U.S. farms have an abundance of replacement animals and elect to aggressively cull cows that experience recurrent cases of mastitis. This policy is often referred to as the "three strikes and out" rule. This aggressive culling policy consists of culling most of the cows that experience the third case of CM during current lactation, since the probability of the cow to have a successful outcome is reduced every time a new case is experienced. This policy was included in this model to reflect current management practices.

Information about pathogen specific losses attributable to CM is sparse and the estimates used in this model were the best available information to estimate milk loss for cases of CM occurring on modern dairy farms. Pathogen specific milk production losses were estimated based on research conducted by Gröhn et al. (2004). However, these estimated included CM cases of all severities and in various stages of lactation, in contrast to the mild and moderate cases occurring at 30 DIM evaluated in this model. Gröhn et al (2004) reported milk yield losses for cases of treated mastitis in absence of reporting bacteriological cure so the impact of additional losses caused by subclinical mastitis are not differentiated. The largest estimated milk loss was for CM caused by *Klebsiella spp.* with losses of 1435 kg for primiparous cows and 711 kg for multiparous cows. Estimated milk losses when CM was caused by *S. aureus* were 718 kg and 558 kg for primiparous and multiparous cows, respectively. Interestingly, Gröhn et al., (2004)

reported that primiparous cows affected with CM caused by environmental *Streptococci* produced an additional 90 kg of milk and multiparous cows affected with CM caused by CNS produced an additional 76 kg of milk. While these estimates are unusual and may reflect characteristics of the underlying herds included in that study, these estimates were used in the decision tree model. Based on the data provided by Gröhn et al., (2004) milk production losses due to CM were greater for primiparous cows as compared to losses of multiparous cows. The primary reason for this outcome was the large difference (850kg) in estimated losses for CM cases where no pathogen was recovered. Gröhn et al. (2004) reported that primipaours cows affected with CM that were diagnosed as "no growth" resulted in production losses of 1017 kg in contrast to 166 kg of milk yield loss for multiparous cows. As explained by Gröhn et al. (2004) while losses for multiparous cows became smaller by day 43 after diagnosis, losses for primiparous cows remained substantial for the remainder of the studied period. Other researchers have suggested that CM cases that yield no bacteria have similar characteristics as Gram-negative bacterial infections (Morin and Constable, 1998).

Somatic cell counts >200,000 cell/ml are an indicator of subclinical infection, and subclinical infection is known to reduce milk production. Subclinical mastitis subsequent to unresolved CM can cause long term negative effects on milk production (Hortet and Seegers, 1998). The effects of subclinical mastitis were included in the calculations for this model for cases of CM that did not result in bacteriological cure. The effects of CM on lactation curves for SCC differ among the pathogens isolated (Haas et al., 2002; Haas et al., 2004). As reported by Haas et al., (2002, 2004), after a case of CM caused by *E. coli* or for culture negative samples SCC rapidly decreased. In contrast, for cases of CM caused by *S. aureus* and environmental streptococci, SCC remained increased after the occurrence. Based on this information, the assumption of two

months of milk losses due to subclinical mastitis were assumed for Gram-negative pathogens and "no growth" results; and losses due to subclinical mastitis caused by Gram-positive pathogens were assumed to persist for the remainder of the lactation.

The great economic impact of CM is well known and has been previously described (Seegers et al., 2003; Halasa et al., 2007; Bar et al., 2008). The largest proportion of economic losses caused by mastitis (reduction of milk production) is generally not evident for farmers. Economical losses caused by CM are usually underestimated by farmers (Huijps et al., 2008). When assessing direct economic impact of mastitis, costs (i.e. extra resource use) and losses (i.e. reduced revenues) have to be aggregated (Seegers et al., 2003). There is a large variation among studies in the calculation of the economic impact of CM. This decision tree model used similar components to calculate the economic losses attributable to mastitis as compared to models of developed by Huijps et al., (2008) at the farm level and Swinkles et al., (2005ab) at the cow level. While both of those models were developed for specific European situations, this model is specific for U.S. conditions. Inclusion of pathogen specific estimations to calculate costs of CM is unique and likely improves the precision of the estimates of economic damage caused by CM as compared to previous models. Although other studies have reported losses including milk loss due to CM, discarded milk, and due to subclinical mastitis (Shim et al.,2004; Huijps et al., 2008), this decision tree uniquely includes pathogen specific milk losses (Gröhn et al., 2004; Haas et al., 2002; Haas et al., 2004). The decision tree included milk production losses due to clinical and subclinical mastitis, discarded milk, cost of drugs, diagnostic, labor, culling and recurrences; this components are similar to previous studies (Seegers et al., 2003; Huijps et al., 2008).

The cost per case of clinical mastitis varies widely among studies due to the inclusion of different costs and diverse objectives and populations studied. The total cost of CM in our model ranged from $106 to $867 and included costs of drugs, labor,discarded milk, milk losses due to clinical and subclinical mastitis, culling and recurrences,. For example, a CM case caused by a Gram positive pathogen treated for 2d, and assuming that the cow did not experience bacteriological cure and recur, would cost $743 distributed as diagnostic costs (2%), milk loss due to CM (14%), treatment cost (11%), milk loss due to subclinical mastitis (29%) and cost of recurrence (44%). Bar et al. (2008) estimated that average cost of a case of CM was $179 and was distributed as due to drugs (11%), discarded milk (11%), labor (5%), milk yield losses (64%) and mortality (7%), however cost of recurrences were not included. .Rodrigues et al. (2005) calculated the partial cost of a case of CM for Wisconsin dairy herds participating in a milk quality program and reported that the average cost per case of CM was $91, distributed as discarded milk (60%), cost of treatments (21%) and cost of labor (19%). To make this data comparable to our model and including only the cost included by Rodrigues et a.,(2005), the partial cost per case of mild and moderate CM in our model ranged from $25 (no IMM antimicrobial) to $212 (8d extended treatment) per case depending on the treatment strategy. For example, a 2d treatment, when NOOFC was used, was $50 per case for primiparous cows and included treatment cost (27%) and milk discarded (73%); and $60 per case for multiparous and included treatment cost (20%) and milk discarded (80%). It is important to note that milk discarded corresponds to what we called “corrected” daily milk yield, thus milk losses due to CM were already discounted. Estimates from this model are greater compared with Rodrigues et al. (2005), especially in terms of cost of milk discarded. The reason could be that our estimations are for high producing cows and milk discarded was given the same value of regular milk.

Discarded milk usually accounts for a large proportion of economic losses attributable to CM (Seegers et al., 2003; Halasa et al., 2007; Rodrigues et al., 2005). The assumption of discarding milk for 4d, when no antimicrobial was administered was based on the duration of days until the disappearance of clinical signs previously reported (Hoe and Ruegg, 2005; Lago, 2009; Study Chapter 2). Our model assumed 1 d less of discarded milk when a cow was not treated with antimicrobials (4d milk discarded) compared to treatment with antimicrobial for 2d (5d milk discarded). When CM was caused by a Gram-negative pathogen or "no growth', the best treatment strategy was not to treat with antimicrobials. To reduce the loss from discarded milk in this case the use of "quarter milker" may be recommended, and as soon as milk appears normal, it can send directly on to the bulk tank.

Extended duration IMM therapy has been shown to result in increased bacteriological cures for mastitis caused by *Staphylococcus aureus* and some environmental Streptococci but the routine use of extended duration therapy was not economically optimal under any circumstance evaluated in this study. Previous researchers have used partial budgeting to evaluate the economic impact of different treatment strategies for subclinical IMM infection caused by environmental *Streptococci* or *Staphylococcus aureus* (Swinkels et al., 2005ab). Similar to the results reported herein, Swinkels et al. (2005a) concluded that extended treatment is not economically feasible, due to increased cost of antimicrobials and increased losses due to milk discard. The same authors (Swinkels et al., 2005b) reported that extended duration treatment of subclinical mastitis caused by *Staphylococcus aureus* was economically justified only in circumstances when the risk of transmission to other cows was great.

When CM is treated without knowledge of etiology, it is difficult to justify the routine use of extended duration therapy for treatment of the first case of CM. While the least economic loss was typically associated with either a no treatment option or a 2d course of therapy, the difference in EMV between no treatment and 2d treatments were generally very small. Based on existing research, bacteriological cures were only marginally improved by 5d of therapy relative to 2 d of therapy. These small increases (5-10%) in bacteriological cure were not sufficient to offset the larger losses attributable to more days of discarded milk. In light of the limited amount of pathogen specific research and the uncertainty inherent in models, it is not prudent to conclude that no treatment is preferred but care should be taken to recommend extended duration therapy only in circumstances where etiologies and clinical experience suggest that a beneficial economic impact will result.

Sensitivity analyses were performed to explore the impact of changes in selected inputs on important model outputs (e.i. EMV) and to identify input variables with a strong impact on the model outputs. Milk price was the only input variable that influenced the model. While for most variables (labor, treatment and OFC costs) the EMV did not change when using extreme values, EMV changed when milk price was set at minimum and maximum values. When milk price was set low, the EMV were less negative indicating that the reduction in milk income was lesser compared to the base line. Similarly, when milk price was set high, the EMV were more negative indicating that the reduction in milk income was greater compared to the base line. This can be explained by the fact that milk production losses (kg) remained the same but the dollar value assigned to them affected the EMV.

Decision tree is an effective method for determining the most economical treatment strategy for commercial dairy herds and an useful instructional tool to understand the complex interactions affecting the economics of CM treatment. The biological assumptions of this model could be strengthened by field studies designed to better characterize post-treatment outcomes in dairy cows. Further study to extrapolate the model on cows with different DIM, previous history of clinical and subclinical mastitis is needed.

3.5 CONCLUSION

A decision tree was developed to evaluate at the cow level the economic impact of selected mastitis treatment strategies Culture based therapy strategies allowed for the most judicious use of antibiotics. For most scenarios used in this study, the results of the model suggested that the best strategy was to treat Gram-positives for 2 days and avoided antimicrobials for CM cases caused by Gram-negative pathogens or when no pathogen was recovered ("no growth"). Use of extended therapy (5 or 8 days) resulted in the lowest EMV. The tree could be a useful instructional tool, helping farmers and veterinarians understand the interactions between biological and economical factors when a cow experiences a mild or moderate case of CM.

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Table 3.1. Distribution of pathogen for scenarios A (Baseline), B (Greater prevalence of *S. aureus*) and C (Greater prevalence of Coliforms).

	Scenario (Characteristic)		
Etiology of CM (%)	A (Baseline scenario)	B (Greater contagious)	C (Greater Coliforms)
Gram-positive	0.35	0.70	0.15
Staphylococcus aureus	0.02	0.40	0.01
Environmental *Streptococci*	0.19	0.24	0.10
Coagulase-negative *Staphylococci*	0.14	0.06	0.04
Gram-negative	0.30	0.15	0.70
Escherichia coli	0.24	0.10	0.60
Klebsiella spp.	0.06	0.05	0.10
"No growth"	0.35	0.15	0.15

Table 3.2. Estimated probabilities of bacteriological cure by pathogen and duration of intramammary treatment used for treatment of clinical mastitis occurring in primiparous and multiparous cows.

Etiology of clinical mastitis	Treatment duration (days)	Bacteriological Cure		Sources
		Primiparous cows (%)	Multiparous cows (%)	
Staphylococcus aureus	0	0.05	0.00	Gillespie et al., 2002; Deluyker et al., 2002; Oliver et al., 2004b
	2	0.15	0.10	
	5	0.25	0.20	
	8	0.40	0.35	
Environmental *Streptococci*	0	0.30	0.25	Morin et al. 1998; Deluyker et al., 2000 and 2001; Hoe and Ruegg, 2005; McDougall et al., 2007
	2	0.60	0.55	
	5	0.70	0.65	
	8	0.80	0.75	
Coagulase-negative *Staphylococci*	0	0.60	0.55	Oliver et al., 2004b; Hoe and Ruegg, 2005; McDougall et al., 2007; Borne et al., 2010
	2	0.75	0.70	
	5	0.80	0.75	
	8	0.85	0.80	
Escherichia coli	0	0.80	0.75	Wilson et al., 1999; McDougall et al., 2007; Bradley and Green, 2009; Borne et al., 2010; Suojala et al. 2010.
	2	0.90	0.85	
	5	0.90	0.85	
	8	0.90	0.85	
Klebsiella spp.	0	0.40	0.35	Smith et al., 1985; Pyörälä and Pyörälä, 1998; Roberson et al., 2004; Hoe and Ruegg, 2005
	2	0.50	0.45	
	5	0.50	0.45	
	8	0.50	0.45	
"No growth"	0	0.95	0.90	Roberson et al. 2004; Chapter 2, 2010.
	2	0.95	0.90	
	5	0.95	0.90	
	8	0.95	0.90	

Table 3.3. Weighted average of estimated bacteriological cure for Gram-positive, Gram-negative and "no growth", based on distribution of pathogens used in Scenario A

		Bacteriological Cure	
Etiology of clinical mastitis	Treatment duration (days)	Primiparous cows (%)	Multiparous cows (%)
Gram-positive	0	0.41	0.36
	2	0.63	0.58
	5	0.71	0.66
	8	0.80	0.75
Gram-negative	0	0.72	0.67
	2	0.82	0.77
	5	0.82	0.77
	8	0.82	0.77
"No growth"	0	0.95	0.90
	2	0.95	0.90
	5	0.95	0.90
	8	0.95	0.90

Table 3.4. Description of assumed costs (baseline), minimum and maximum scenarios for the initial diagnostic or treatment decision.

Description of costs	Baseline ($)	Minimum ($)	Maximum ($)
Milk price per kg	0.33	0.22	0.44
Farm Labor per hour	13.00	8.00	18.00
Intramammary antimicrobial per tube	3.50	2.50	4.50
Total cost of intramammary treatment per day[1]	6.75	4.50	9.00
Culture plates	2.25	1.25	3.25
Disposable material[2]	0.50	0.50	0.50
Total cost of OFC per culture[3]	6.00	3.75	8.25

[1]Include one intramammary antimicrobial tube and labor (15 min), does not include discarded milk.
[2]Cost of disposable material was not changed for minimum and maximum scenarios.
[3]Include microbiological media, disposable materials and labor (15 min)

Table 3.5. Estimated effects of the first occurrence of pathogen specific clinical mastitis on milk yield (kg) from 30DIM to 305 DIM by etiology of CM under baseline pathogen prevalence using data from Gröhn et al., (2004). Positive values indicate milk gain.

	Difference in Milk Yield (kg)	
Etiology of clinical mastitis	Primiparous cows	Multiparous cows
Gram-positive [1]	-288.42	-325.21
Staphylococcus. aureus	-718.43	-558.53
Environmental. Streptococci	90.30	-596.80
Coagulase-negative Staphylococci	-740.96	76.72
Gram-negative [1]	-823.11	-427.36
Escherichia. coli	-670.09	-356.38
Klebsiella spp	-1435.19	-711.29
“No growth”	-1016.78	-166.12

[1]Weighted average based on baseline pathogen distribution

Table 3.6. Estimated cost of production loss ($) due to subclinical mastitis when cows did not experience bacteriological cure (SCC = 800,000 cells/mL) assumed that milk production was decreased by 1.6 and 2.4 kg per cow per day for primiparous and multiparous cows, respectively (Seegers et al., 2003). For CM caused by Gram-positive bacteria, milk production losses were assumed to persist for the remainder of the lactation while for CM caused by Gram-negative bacteria or when no pathogen was recovered ("no growth") milk production losses occurred for only 2 months after occurrence of the case (Haas et al., 2004). Reduction in milk production began after the end of the milk withholding period

	Cost of Production Losses due to Subclinical Mastitis					
	Primiparous cows ($)			Multiparous cows ($)		
	Gram-positive	Gram-negative	"no growth"	Gram-positive	Gram-negative	"no growth"
OFCW[1]						
Do not treat, discard 3d	143.09	29.57	29.57	214.63	44.35	44.35
Treat 2d, discard 5d	142.03	28.51	28.51	213.05	42.77	42.77
Treat 5d, discard 8d	140.45	26.93	26.93	210.67	40.39	40.39
Treat 8d, discard 11d	138.86	25.34	25.34	208.30	38.02	38.02
OFCT[2]						
Stop treat, discard 3d	143.09	29.57	29.57	214.63	44.35	44.35
Continue 1d, discard 4d	142.56	29.04	29.04	213.84	43.56	43.56
Continue 4d, discard 7d	140.98	27.46	27.46	211.46	41.18	41.18
Continue 7d, discard 10d	139.39	25.87	25.87	209.09	38.81	38.81
NOOFC[3]						
Do not treat, discard 3d	143.09	29.57	29.57	214.63	44.35	44.35
Treat 2d, discard 4d	142.56	29.04	29.04	213.84	43.56	43.56
Treat 5d, discard 7d	140.98	27.46	27.46	211.46	41.18	41.18
Treat 8d, discard 10d	139.39	25.87	25.87	209.09	38.81	38.81
Average Milk loss d	141.47	27.85	27.85	212.06	41.78	41.78

[1] Use OFC and wait 24 hours for microbiology results to base treatment on diagnostic
[2]Use OFC and treat immediate then after 24 hours, the treatment is readjusted based on diagnostic
[3] Do not to use OFC

Table 3.7. Estimated partial cost ($) of treating a clinical mastitis case including labor, intramammary antimicrobial treatment and discarded milk.

	Partial cost of Mastitis Treatment					
	Primiparous Cows ($)			Multiparous Cows ($)		
	Gram-positive	Gram-negative	"no growth"	Gram-positive	Gram-negative	"no growth"
OFCW[1]						
Do not treat, discard 3d	29.36	25.68	25.39	42.13	32.89	39.79
Treat 2d, discard 5d	62.43	56.29	55.82	83.71	68.31	79.82
Treat 5d, discard 8d	113.32	103.75	102.32	146.92	123.55	140.75
Treat 8d, discard 11d	166.41	154.11	149.75	212.02	184.48	203.71
OFCT[2]						
Stop treat, discard 3d	29.36	25.68	25.39	42.13	32.89	39.79
Continue 1d, discard 4d	45.89	40.98	40.60	62.92	50.60	59.81
Continue 4d, discard 7d	96.04	87.46	86.79	125.57	104.01	120.13
Continue 7d, discard 10d	148.87	137.56	133.96	190.42	164.70	182.84
NOOFC[3]	Weighted average[4]			Weighted average[4]		
Do not treat, discard 3d	26.86			38.54		
Treat 2d, discard 4d	49.32			64.88		
Treat 5d, discard 7d	96.98			123.95		
Treat 8d, discard 10d	147.01			186.80		

[1] Use on-farm culture and wait 24 hours for microbiology results to base treatment on diagnostic
[2]Useon-farm culture and treat immediate then after 24 hours, the treatment is readjusted based on diagnostic
[3] Do not to use OFC
[4]pathogen specific milk yield loss weighted by the distribution of pathogens

Table 3.8. Proportion of milk income lost due to CM for remainder of lactation, (30-305 DIM) relative to potential income of $3191 (primiparous cows) and $3692 (multiparous cows) by various potential case outcomes

On-farm culture system	Etiology	Treatment Strategy	Proportion of Milk Income Lost due to CM: Primiparous cows (%) BCNR[1]	BCR[2]	NBCNR[3]	NBCR[4]	Multiparous cows (%) BCNR[1]	BCR[2]	NBCNR[3]	NBCR[4]
OFCW[5]	Gram-	Do not treat	4.4	8.9	12.3	17.0	4.6	10.4	13.1	19.0
		Treat 2d	5.41	9.9	13.4	18.0	5.7	11.5	14.2	20.1
		Treat 5d	7.0	11.4	15.0	19.5	7.4	13.1	15.9	21.8
		Treat 8d	8.7	13.0	16.6	21.1	9.2	14.8	17.7	23.5
	Gram-	Do not treat	9.8	10.7	17.1	18.0	5.2	6.4	13.4	14.6
		Treat 2d	10.8	11.6	18.0	18.9	6.2	7.3	14.3	15.5
		Treat 5d	12.2	13.1	19.5	20.4	7.7	8.8	15.8	16.9
		Treat 8d	13.8	14.6	21.1	21.9	9.3	10.4	17.5	18.5
	No growth	Do not treat	11.8	12.7	18.2	19.1	3.1	4.3	11.0	12.2
		Treat 2d	12.7	13.6	19.1	20.0	4.2	5.3	12.1	13.2
		Treat 5d	14.2	15.0	20.6	21.5	5.8	6.9	13.7	14.8
		Treat 8d	15.7	16.5	22.1	22.9	7.5	8.5	15.4	16.4
OFCT[6]	Gram-	Do not treat	4.6	9.1	12.5	17.2	4.7	10.6	13.3	19.2
		Treat 2d	5.1	9.6	13.1	17.7	5.3	11.1	13.8	19.7
		Treat 5d	6.7	11.1	14.6	19.2	7.0	12.7	15.5	21.4
		Treat 8d	8.3	12.7	16.3	20.8	8.8	14.4	17.3	23.1
	Gram-	Do not treat	10.0	10.9	17.3	18.2	5.4	6.6	13.6	14.8
		Treat 2d	10.5	11.4	17.8	18.7	5.9	7.1	14.0	15.2
		Treat 5d	11.9	12.8	19.2	20.1	7.3	8.5	15.5	16.6
		Treat 8d	13.5	14.3	20.8	21.6	9.0	10.0	17.1	18.2
	No growth	Do not treat	12.0	12.9	18.4	19.3	3.3	4.5	11.2	12.4
		Treat 2d	12.5	13.4	18.9	19.8	3.8	5.0	11.7	12.9
		Treat 5d	13.9	14.8	20.3	21.2	5.4	6.6	13.3	14.5
		Treat 8d	15.4	16.2	21.8	22.6	7.1	8.2	15.0	16.1
NOOFC[7]	Gram-	Do not treat	4.1	8.6	12.1	16.7	4.3	10.1	12.8	18.8
	Gram-		9.6	10.6	16.9	17.8	5.2	6.4	13.4	14.6
	No growth		11.6	12.6	18.1	19.0	2.9	4.1	10.8	12.0
	Gram-	Treat 2d	4.8	9.3	12.8	17.4	5.0	10.8	13.5	19.5
	Gram-		10.3	11.3	17.6	18.5	5.9	7.1	14.1	15.3
	No growth		12.3	13.3	18.8	19.7	3.6	4.8	11.5	12.7
	Gram-	Treat 5d	6.3	10.7	14.3	18.8	6.6	12.3	15.1	21.0
	Gram-		11.8	12.7	19.1	20.0	7.5	8.6	15.7	16.8
	No growth		13.8	14.7	20.3	21.1	5.2	6.3	13.1	14.2
	Gram-	Treat 8d	7.9	12.2	15.8	20.3	8.3	14.0	16.8	22.6
	Gram-		13.4	14.2	20.7	21.5	9.2	10.3	17.4	18.4
	No growth		15.4	16.2	21.8	22.6	6.9	7.9	14.8	15.8

[1]BCNR=Cow experienced bacteriological cure and did not have recurrences
[2]BCR = Cow experienced bacteriological cure but had recurrences
[3]NBCNR = Cow did not experience bacteriological cure and did not have recurrences
[4]NBCR = Cow did not experience bacteriological cure and had recurrences
5 Use on-farm culture and wait 24 hours for microbiology results to base treatment on diagnostic
[6]Useon-farm culture and treat immediate then after 24 hours, the treatment is readjusted based on diagnostic
[7] Do not to use OFC

Table 3.9. Expected monetary values (EMV) of the first decision node in the decision tree (regarding the use of on-farm culture systems) for baseline prevalence (Scenario A), greater prevalence of contagious pathogens (Scenario B) and greater prevalence of coliforms (Scenario C). Least negative EMV is underlined.

	Expected Monetary Values ($)					
	Primiparous Cows			Multiparous Cows		
Scenario:	A	B	C	A	B	C
OFCW[1]	-324.33	-368.10	-317.27	-264.20	-429.66	-262.67
OFCT[2]	-325.36	-364.15	-321.57	<u>-263.79</u>	-422.65	-266.39
No OFC[3]	<u>-323.10</u>	<u>-361.44</u>	<u>-313.89</u>	-266.62	<u>-420.57</u>	<u>-261.28</u>
Difference[4]	1.23	2.72	3.38	0.41	2.08	1.39

[4]Difference between first and second least EMV.

Table 3.10. Expected monetary values of different treatment strategies under different OFC systems for baseline prevalence (Scenario A), high prevalence of contagious pathogens (Scenario B) and high prevalence of coliforms (Scenario C). Least negativeEMV is underlined.

			Expected Monetary Values ($)					
			Primiparous cows			Multiparous cows		
On-farm		Scenario	A	B	C	A	B	C
Culture System	OFC Results	Treatment						
OFCW[1]	Gram-positive	Do not treat	-264.94	-369.16	-239.86	-392.01	-529.95	-431.76
		Treat 2d	-251.32	-362.53	-222.40	-366.97	-517.49	-401.01
		Treat 5d	-285.65	-389.11	-255.31	-406.42	-549.08	-437.79
		Treat 8d	-321.85	-411.59	-289.98	-447.33	-574.95	-475.95
	Gram-negative	Do not treat	-340.12	-378.58	-323.39	-266.35	-289.61	-255.35
		Treat 2d	-362.25	-400.41	-345.65	-290.05	-313.41	-279.02
		Treat 5d	-409.42	-447.41	-392.89	-344.75	-368.22	-333.66
		Treat 8d	-459.50	-497.44	-442.99	-405.13	-428.25	-394.20
	"no growth"	Do not treat	-383.80	-383.60	-383.59	-159.60	-159.82	-158.44
		Treat 2d	-414.18	-413.98	-413.97	-199.47	-199.69	-198.31
		Treat 5d	-460.60	-460.40	-460.39	-260.16	-260.38	-259.00
		Treat 8d	-507.95	-507.75	-507.74	-322.88	-323.10	-321.72
OFCT[2]	Gram-positive	Stop treat	-271.69	-375.91	-246.61	-398.76	-536.70	-438.51
		Continue 1d	-241.73	-354.00	-212.81	-353.25	-504.59	-387.56
		Continue 4d	-275.27	-379.99	-244.94	-392.08	-535.45	-423.71
		Continue 7d	-311.17	-402.15	-279.31	-432.68	-560.98	-461.55
	Gram-negative	Stop treat	-346.87	-385.33	-330.14	-273.10	-296.36	-262.10
		Continue 1d	-353.79	-392.02	-337.16	-279.27	-302.53	-268.28
		Continue 4d	-399.97	-437.99	-383.44	-332.14	-355.68	-321.02
		Continue 7d	-449.80	-487.77	-433.28	-392.28	-415.46	-381.32
	"no growth"	Stop treat	-390.55	-390.35	-390.34	-166.35	-166.57	-165.19
		Continue 1d	-405.74	-405.54	-405.53	-186.28	-186.50	-185.13
		Continue 4d	-451.85	-451.65	-451.64	-246.37	-246.59	-245.21
		Continue 7d	-498.94	-498.74	-498.73	-308.84	-309.06	-307.69
NO OFC[3]		Do not treat	-323.10	-366.74	-313.89	-266.97	-432.38	-261.28
		Treat 2d	-326.75	-361.44	-322.76	-266.62	-420.57	-267.70
		Treat 5d	-368.48	-393.44	-366.89	-317.10	-459.16	-319.05
		Treat 8d	-412.48	-423.48	-414.00	-371.22	-495.36	-376.31

[1] Use on-farm culture and wait 24 hours for microbiology results to base treatment on diagnostic
[2]Useon-farm culture and treat immediate then after 24 hours, the treatment is readjusted based on diagnostic
[3] Do not to use OFC

Table 3.11. Sensitivity analyses for primiparous cows. Least negative EMV is underlined.

				Milk Price/kg ($)		Labor cost/hour ($)		Treatment cost($)		OFC cost ($)	
OFC System	Etiology	Treatment Strategy	Baseline	0.22	0.44	8.00	18.00	4.50	9.00	3.75	8.25
			Expected Monetary values for primiparous cows ($)								
OFCW			-324.33	-222.65	-426.01	-324.33	-324.33	-321.97	-326.69	-322.08	-326.58
	Gram positive	Do not treat	-264.94	-185.36	-344.52	-264.94	-264.94	-263.24	-266.64	-262.69	-267.19
		Treat 2d	-251.32	-178.52	-324.13	-251.32	-251.32	-245.70	-256.95	-249.07	-253.57
		Treat 5d	-285.65	-207.36	-363.93	-285.65	-285.65	-273.47	-297.82	-283.40	-287.90
		Treat 8d	-321.85	-237.43	-406.26	-321.85	-321.85	-303.12	-340.57	-319.60	-324.10
	Gram negative	Do not treat	-340.12	-231.91	-448.32	-340.12	-340.12	-339.21	-341.02	-337.87	-342.37
		Treat 2d	-362.25	-250.30	-474.20	-362.25	-362.25	-357.09	-367.41	-360.00	-364.50
		Treat 5d	-409.42	-288.50	-530.34	-409.42	-409.42	-397.51	-421.33	-407.17	-411.67
		Treat 8d	-459.50	-328.64	-590.36	-459.50	-459.50	-440.84	-478.16	-457.25	-461.75
	No growth	Do not treat	-383.80	-258.84	-508.77	-383.80	-383.80	-383.47	-384.14	-381.55	-386.05
		Treat 2d	-414.18	-283.59	-544.77	-414.18	-414.18	-409.34	-419.02	-411.93	-416.43
		Treat 5d	-460.60	-321.29	-599.92	-460.60	-460.60	-449.02	-472.19	-458.35	-462.85
		Treat 8d	-507.95	-359.61	-656.29	-507.95	-507.95	-489.61	-526.29	-505.70	-510.20
OFCT			-325.36	-224.80	-425.92	-325.36	-325.36	-321.54	-329.18	-323.11	-327.61
	Gram positive	Stop treat	-271.69	-192.11	-351.27	-271.69	-271.69	-267.74	-275.64	-269.44	-273.94
		Continue 1d	-241.73	-172.13	-311.34	-241.73	-241.73	-236.10	-247.36	-239.48	-243.98
		Continue 4d	-275.27	-200.44	-350.09	-275.27	-275.27	-263.09	-287.45	-273.02	-277.52
		Continue 7d	-311.17	-230.31	-392.03	-311.17	-311.17	-292.45	-329.89	-308.92	-313.42
	Gram negative	Stop treat	-346.87	-238.66	-455.07	-346.87	-346.87	-343.71	-350.02	-344.62	-349.12
		Continue 1d	-353.79	-244.66	-462.91	-353.79	-353.79	-348.63	-358.95	-351.54	-356.04
		Continue 4d	-399.97	-282.20	-517.74	-399.97	-399.97	-388.06	-411.88	-397.72	-402.22
		Continue 7d	-449.80	-322.17	-577.42	-449.80	-449.80	-431.14	-468.46	-447.55	-452.05
	No growth	Stop treat	-390.55	-265.59	-515.52	-390.55	-390.55	-387.97	-393.14	-388.30	-392.80
		Continue 1d	-405.74	-277.97	-533.52	-405.74	-405.74	-400.90	-410.58	-403.49	-407.99
		Continue 4d	-451.85	-315.46	-588.24	-451.85	-451.85	-440.26	-463.44	-449.60	-454.10
		Continue 7d	-498.94	-353.60	-644.28	-498.94	-498.94	-480.60	-517.28	-496.69	-501.19
NOOFC			-323.10	-219.04	-427.15	-323.10	-323.10	-321.54	-324.08	-323.10	-323.10
		Do not treat	-323.10	-219.04	-427.15	-323.10	-323.10	-322.11	-324.08	-323.10	-323.10
		Treat 2d	-326.75	-224.93	-428.57	-326.75	-326.75	-321.54	-331.96	-326.75	-326.75
		Treat 5d	-368.48	-259.23	-477.74	-368.48	-368.48	-356.59	-380.37	-368.48	-368.48
		Treat 8d	-412.48	-295.02	-529.94	-412.48	-412.48	-393.91	-431.05	-412.48	-412.48

[1] Use on-farm culture and wait 24 hours for microbiology results to base treatment on diagnostic. [2]Use on-farm culture and treat immediate then after 24 hours, the treatment is readjusted based on diagnostic. [3] Do not to use OFC

Table 3.12. Sensitivity analyses for multiparous cows. Least negative EMV is underlined

				Milk Price/kg ($)		Labor cost/hour ($)		Treatment cost($)		OFC cost ($)	
OFC System	Etiology	Treatment Strategy	Baseline	0.22	0.44	8.00	18.00	4.50	9.00	3.75	8.25
			Expected Monetary values for multiparous cows ($)								
OFCW			-264.20	-189.09	-339.32	-264.20	-264.20	-260.74	-267.66	-261.95	-266.45
	Gram positive	Do not treat	-392.01	-277.82	-506.20	-392.01	-392.01	-389.26	-394.77	-389.76	-394.26
		Treat 2d	-366.97	-262.79	-471.15	-366.97	-366.97	-360.26	-373.68	-364.72	-369.22
		Treat 5d	-406.42	-294.84	-517.99	-406.42	-406.42	-393.14	-419.69	-404.17	-408.67
		Treat 8d	-447.33	-327.84	-566.82	-447.33	-447.33	-427.50	-467.15	-445.08	-449.58
	Gram negative	Do not treat	-266.35	-189.48	-343.22	-266.35	-266.35	-264.35	-268.35	-264.10	-268.60
		Treat 2d	-290.05	-208.62	-371.49	-290.05	-290.05	-283.79	-296.32	-287.80	-292.30
		Treat 5d	-344.75	-251.83	-437.66	-344.75	-344.75	-331.73	-357.76	-342.50	-347.00
		Treat 8d	-405.13	-298.84	-511.42	-405.13	-405.13	-385.36	-424.90	-402.88	-407.38
	No growth	Do not treat	-159.60	-115.05	-204.15	-159.60	-159.60	-158.14	-161.06	-157.35	-161.85
		Treat 2d	-199.47	-146.13	-252.81	-199.47	-199.47	-193.51	-205.43	-197.22	-201.72
		Treat 5d	-260.16	-193.34	-326.98	-260.16	-260.16	-247.45	-272.87	-257.91	-262.41
		Treat 8d	-322.88	-241.90	-403.85	-322.88	-322.88	-303.42	-342.34	-320.63	-325.13
OFCT			-263.79	-190.28	-337.31	-263.79	-263.79	-258.87	-268.71	-261.54	-266.04
	Gram positive	Stop treat	-398.76	-284.57	-512.95	-398.76	-398.76	-393.76	-403.77	-396.51	-401.01
		Continue 1d	-353.25	-253.65	-452.86	-353.25	-353.25	-346.54	-359.97	-351.00	-355.50
		Continue 4d	-392.08	-285.29	-498.88	-392.08	-392.08	-378.81	-405.35	-389.83	-394.33
		Continue 7d	-432.68	-318.07	-547.29	-432.68	-432.68	-412.86	-452.51	-430.43	-434.93
	Gram negative	Stop treat	-273.10	-196.23	-349.97	-273.10	-273.10	-268.85	-277.35	-270.85	-275.35
		Continue 1d	-279.27	-201.43	-357.11	-279.27	-279.27	-273.01	-285.54	-277.02	-281.52
		Continue 4d	-332.14	-243.43	-420.85	-332.14	-332.14	-319.12	-345.15	-329.89	-334.39
		Continue 7d	-392.28	-290.27	-494.29	-392.28	-392.28	-372.51	-412.05	-390.03	-394.53
	No growth	Stop treat	-166.35	-121.80	-210.90	-166.35	-166.35	-162.64	-170.06	-164.10	-168.60
		Continue 1d	-186.28	-137.34	-235.23	-186.28	-186.28	-180.33	-192.24	-184.03	-188.53
		Continue 4d	-246.37	-184.14	-308.59	-246.37	-246.37	-233.66	-259.07	-244.12	-248.62
		Continue 7d	-308.84	-232.55	-385.14	-308.84	-308.84	-289.38	-328.30	-306.59	-311.09
NOOFC			-266.62	-188.35	-341.97	-266.62	-266.62	-260.31	-269.04	-266.62	-266.62
		Do not treat	-266.97	-188.35	-345.59	-266.97	-266.97	-264.89	-269.04	-266.97	-266.97
		Treat 2d	-266.62	-191.28	-341.97	-266.62	-266.62	-260.31	-272.93	-266.62	-266.62
		Treat 5d	-317.10	-231.33	-402.87	-317.10	-317.10	-304.10	-330.10	-317.10	-317.10
		Treat 8d	-371.22	-273.80	-468.64	-371.22	-371.22	-351.54	-390.90	-371.22	-371.22

[1] Use on-farm culture and wait 24 hours for microbiology results to base treatment on diagnostic. [2]Use on-farm culture and treat immediate then after 24 hours, the treatment is readjusted based on diagnostic. [3] Do not to use OFC

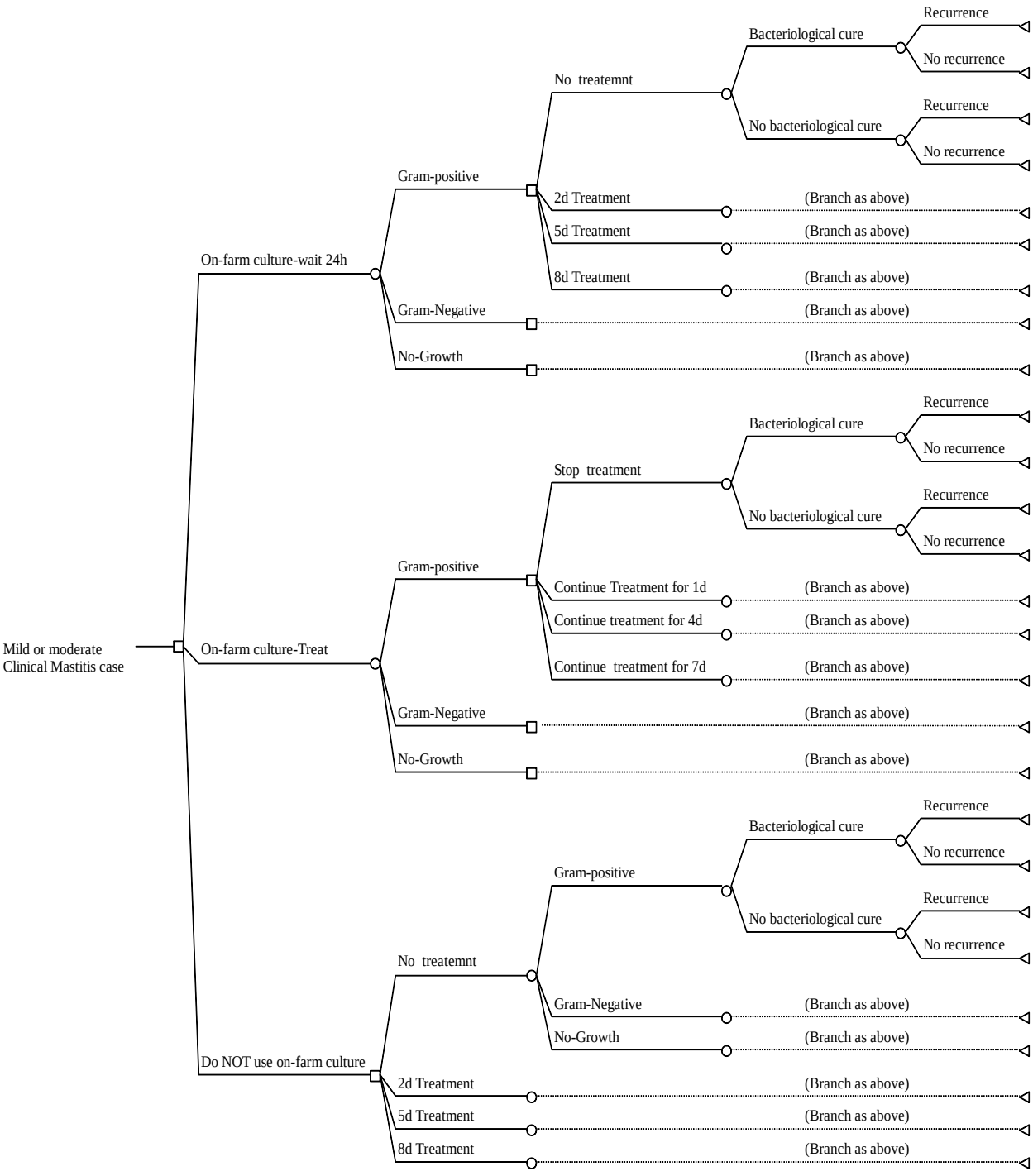

Figure 3.1. Simplified structure of the decision tree. Decision nodes are represented by squares with branches that represented strategies. Probabilities nodes are represented by circles with branches that represented probability events. Terminal nodes represented by triangles

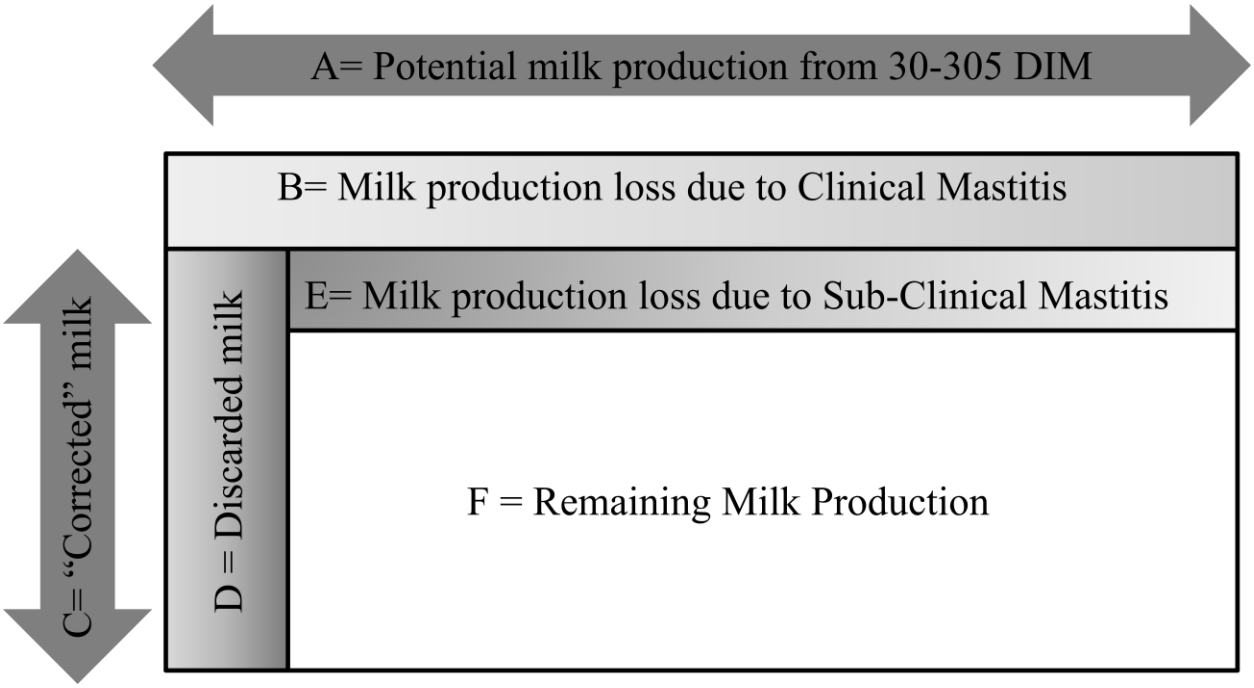

Figure 3. 2. Graphical representation of allocation of milk losses after occurrence of clinical mastitis from 30 to 305 DIM. Portion B corresponds to the pathogen specific milks losses due to a case of CM and varies by pathogen and DIM, as estimated by Gröhn et al. (2004). Portion D correspond to the milk discarded when milk is abnormal, when the cow is receiving intramammary antimicrobial treatment or during the withholding period. Portion E correspond to the milk loss due to subclinical mastitis after not experiencing bacteriological cure, for this example the loss remains for the rest of the lactation (Gram-positive) instead of lasting 2 months (Gram-negative or "no growth'). Portion F correspond to the milk produced after allocation all milk production losses.

SUMMARY AND CONCLUSION

The objectives of this thesis were to help farmers improve decision making for treatment of mild and moderate cases of clinical mastitis, to determine risk factors associated with selected post-treatment outcomes and to use decision tree analysis to evaluate various treatments under a variety of realistic farm scenarios.

Microbiological analysis of pre and post milk samples and cow's demographic information were evaluated from cases of mild and moderate severity. The effect of selected risk factors (explanatory variables) on post-treatment outcomes (response variables) were tested using logistic regression. The results demonstrated that cows are more likely to have bacteriological cure when experiencing CM for the first time in the lactation and when no pathogen is recovered from the pre-treatment sample. When the cow experienced bacteriological cure, she was less likely to experience recurrent cases and was more likely to have SCR below 200,000 cells / mL post-treatment. When SCC before CM was > 200,000 cells / mL the probability of having SCR after treatment was diminished. Assessment of bacteriological cure on farm is not feasible for many farms, however post-treatment outcomes such as recurrence and SCR, are strongly associated with bacteriological cure and when monitored can be used to help determine if a treatment has been successful. Information about etiology of CM, history of clinical and subclinical (SCC) mastitis and parity are useful to review when making strategic treatment decision.

A decision tree was developed to evaluate at the cow level the economic impact of selected mastitis treatment strategies for mild or moderate cases of clinical mastitis. The

tree included two decision and three probability events. First decision was regarding the use of on-farm culture (Two schemes of using OFC and not using OFC) and the second decision was regarding the treatment strategy (no antimicrobials o antimicrobials for 2, 5 or 8d). The tree probabilities modeled were: distribution of etiologies (Gram-positive, Gram-negative or "no growth"); probability of bacteriological cure. (yes or no) and probability of recurrence (yes or no). The economic consequences of mastitis in the analysis included the costs of diagnosis (OFC), treatment , labor , discarded milk, milk production losses (due to clinical and subclinical mastitis), culling and transmission of infection to other cows (only for CM caused by *Staphylococcus aureus*).All milk losses were pathogen specific. For most scenarios used in this study, the results of the model suggested that the best strategy was to treat Gram-positives for 2 days and avoided antimicrobials for CM cases caused by Gram-negative pathogens or when no pathogen was recovered ("no growth"). Use of extended therapy (5 or 8 days) resulted in the lowest EMV.

The tree could be a useful instructional tool, helping farmers and veterinarians understand the interactions between biological and economical factors when a cow experiences a mild or moderate case of CM. The biological assumptions of this model could be strengthened by field studies designed to better characterize post-treatment outcomes in dairy cows.