EFFECT OF SELECTIVE DRY COW THERAPY ON UDDER HEALTH OF US DAIRY HERDS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
The Degree Doctor of Philosophy in the Graduate School of The Ohio State University

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2007

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ABSTRACT

Loss of milk production and decreased quality of milk due to the occurrence of bovine mastitis is a worldwide problem. Efficient control of mastitis in dairy herds includes, among other management practices, the use of antimicrobials at the end of lactation to improve profitability of dairy herds and welfare of dairy cows. Antimicrobials used at the end of lactation have a therapeutic effect when there is an intramammary infection, and a prophylactic effect to prevent the development of new infections during the dry period. The research herein evaluates the effect that antimicrobials used at the end of lactation have on udder health in the subsequent lactation. The first section of this dissertation discusses the importance of the dry period for the mammary gland and its relevance for production of high quality milk in the following lactation. The second section addresses the importance of sampling strategy and interpretation of microbiological culture results in the diagnoses of intramammary infections. The third section evaluates the utility of farm records and quality milk indicators in the identification of infected cows at the end of lactation. The fourth section evaluates the effect of the selective use of antimicrobials at the end of lactation on occurrence of clinical mastitis and on milk yield in the lactation after treatment.

Cost-effective methods to identify infected cows at the end of lactation are needed when mastitis control programs based on treatment of only infected cows are
implemented. Different combinations of somatic cell counts with clinical mastitis records to identify infected cows at the end of lactation are evaluated. It is shown that the ability of the selection criteria to identify infected cows depends on the prevalence of infection and type of pathogens present in the herd. Farms with high prevalence of mastitis due to contagious pathogens might benefit from the treatment of all cows at the end of lactation.

The present study evaluated the effect of somatic cell count levels at the end of lactation on the occurrence of clinical mastitis and milk yield. Cows with an elevated somatic cell count at the end of lactation had an increased hazard of contracting clinical mastitis and they had reduced milk yield in the following lactation even though they received antimicrobial treatment. For cows with low somatic cell counts at the end of lactation, the effect of antimicrobial dry cow therapy on the occurrence of clinical mastitis depended on the number of lactations. Increased hazard of contracting clinical mastitis for uninfected second lactation cows treated at dry-off was observed. The effect of dry cow therapy on the milk yield of uninfected cows was shown to be variable from farm to farm, with either no effect, an increase, or a decrease in milk yield, depending on the farm.

Results indicate the importance of careful consideration of farm characteristics and level of somatic cell counts at the end of lactation before advising the use of antimicrobials.
Dedicated to my grandmother Eusebia, to the memory of my grandfather, Francisco, and especially to my husband Jorge, and to my daughter Andrea, for their unconditional love and support.
ACKNOWLEDGMENTS

I am grateful to Universidad Centroccidental “Lisandro Alvarado” for sponsor
my graduate program and to my entire department mates that are taken care of my
course load, without their commitment it would not be here.

I wish to thank my adviser, Dr. Rajala-Schultz, for her support,
encouragement, friendship, and constructive discussion about the research project and
results. I am grateful to her and her husband, J.J. for the love that my daughter and I
have received during all this time here in Columbus.

I thank Dr. Hoblet for having confidence in me and invite me to come to OSU
to pursue a PhD in veterinary preventive medicine, for his love and support all along
this five years, I will be grateful for ever to him and his family.

I wish to thank Dr. DeGraves for all of his feedback during the writing process
and for his confidence on my work and encouragement.

I am especially grateful to Dolores Fisher, my American mother, for taking
care of me and my family, since the day of my arrival to the department, making me
feel at home.

I thank Dr. Cheyney Meadows for his sincere friendship. He sets the example
to follow not only during my graduate program but also as a professional and as a
person.
I thank Yayoi Fukuhara for all of her priceless help, Jennifer Holguin for her help at the lab, Rick Howard, Dr. Silveira, and Dr. Meiring for helping me to find the farms to conduct this research.

I thank Dr. Jennifer Walker for helping me so dearly with the sampling of cows always that I need help, and for her sincere friendship and support, I will always be grateful and thesaurus her friendship. I am indebted to Dr. Lindsey Long for her help sampling cows when I have the accident ….Thanks!

I thank Dr. Pamela Dennis for her friendship, Dr. Alecia Nougle for her reception and help when I started my graduate program, Alison Hurwitch for helping me with my English when I arrived to this country, Dr. Silvia De Camps for being such a good friend.

I wish to thank all my fellow graduate students, for their support and kindness making the department a rich environment to learn and to work together, and to all staff and faculty for their help during this phase of my live. I appreciate all the help received from the office of fees and deposit and account receivable, Tina and Sam thanks!

I am especially grateful to COBA/Select Sires for the endowment given to the College of Veterinary Medicine at OSU, which allowed me to begin my research. This research was supported by USDA Animal Health Formula Funds through the Council for Research at OSU College of Veterinary Medicine, and by USDA/NRI-CGP 2005-35204-15587.
I wish to thank to Rocio and Armando Hoet for all of their help and loving care, and also for their tender love to my daughter.

I am deeply grateful to my grandparents for given me the best gifts of all, love and education. To my sister Carmen for being my best friend and to my mother Ada for her loved and support when I need it most. Above all I thank my husband Jorge for believe in me, for his love, and endless support that have make possible to complete this goal.
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<td>AIC</td>
<td>Akaike’s information criterion</td>
</tr>
<tr>
<td>AMP305PL</td>
<td>Actual 305-d milk yield from previous lactation</td>
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<tr>
<td>BIC</td>
<td>Schuarz’ Bayesian criterion</td>
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<tr>
<td>cfu</td>
<td>Colony forming units</td>
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<td>CI</td>
<td>Confidence intervals</td>
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<td>CM</td>
<td>Clinical mastitis</td>
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<tr>
<td>CMT</td>
<td>California mastitis test</td>
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<tr>
<td>CNS</td>
<td>Coagulase negative staphylococci</td>
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<td>CS</td>
<td>Consecutive samples</td>
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<td>DCT</td>
<td>Dry cow therapy</td>
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<td>DHI</td>
<td>Dairy Herd Improvement</td>
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<td>DS</td>
<td>Duplicate samples</td>
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<td>DIM</td>
<td>Days in milk</td>
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<td>IMI</td>
<td>Intramammary infections</td>
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<td>LRT</td>
<td>Likelihood ratio test</td>
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<tr>
<td>NMC</td>
<td>National Mastitis Council</td>
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<td>NPV</td>
<td>Negative predictive value</td>
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<td>PPV</td>
<td>Positive predictive value</td>
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<td>PRVDD</td>
<td>Previous days dry</td>
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<td>ROC</td>
<td>Receiver operating characteristics</td>
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<td>SDCT</td>
<td>Selective dry cow therapy</td>
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<td>TDCT</td>
<td>Total dry cow therapy</td>
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CHAPTER 1

INTRODUCTION

Mastitis, inflammation of the mammary gland, is the most important and costly disease of dairy cows worldwide [4, 7]. Mastitis losses are due to loss of milk production, discarded milk due to antimicrobials withhold periods and physical alterations, costs of treatment and care veterinary, increased labor costs, and culling of cows [1, 3]. Due to the negative economic consequences of the disease, mastitis control measures are particularly important. One of these control measures is the treatment of cows at dry-off with antimicrobials to eliminate existing and to prevent new intramammary infections at the end of lactation (i.e., dry cow therapy, DCT) [2, 6, 8].

Treatment of all quarters of all cows at the end of lactation is a cornerstone of many mastitis control programs (i.e., total dry cow therapy, TDCT). However, there are countries that only use DCT in infected cows or quarters at the end of lactation, leaving uninfected healthy cows or quarters without treatment (i.e., selective dry cow therapy, SDCT). In those countries that effectively implement SDCT, production of high quality milk is achieved, with somatic cell counts in bulk tanks lower than 200,000 cells/ml on a national level [5]. Selectively treating infected cows offers an
opportunity to reduce the use of antimicrobials in dairy operations and the likelihood of transfer and maintenance of resistance genes in bacterial populations.

At the moment, it is strongly believed that SDCT would be detrimental to udder health of cows under intensive production systems, thus not economically justified. For a switch from total to selective DCT to be economically feasible, this alternative needs to maintain similar udder health as the traditional practice of treating all cows at dry-off. Implementation of SDCT requires identification of infected and uninfected cows at the end of lactation. The method used to select cows for treatment must be easy to use, economical, and effective to ensure treatment of infected cows only.

Most of the studies designed to evaluate the effect of SDCT in US dairy herds were conducted during the seventies and eighties, thus reassessment of the selection criteria used to identify infected cows and the effect of SDCT on udder health needs to be evaluated under current conditions in modern US dairy herds.

The Central Hypothesis inducing this research project was that SDCT could be used as an effective mastitis control procedure in modern US dairy herds.

The specific objectives of this project were:

1. To determine agreement between results from single samples and results from paired duplicate, successive and consecutive quarter milk samples, using different definitions of intramammary infections, based on the epidemiology of the pathogens.
2. To evaluate the use of clinical mastitis history and SCC from monthly Dairy Herd Improvement records in identification of infected and uninfected cows at dry-off.
3. To evaluate the effect of SDCT on occurrence of clinical mastitis during early lactation.
4. To evaluate the effect of SDCT in milk yield during the subsequent lactation.

1.1 Literature Cited


CHAPTER 2

LITERATURE REVIEW

2.1. Bovine Mastitis

Mastitis is defined as inflammation of the mammary gland and is primarily caused by invasion of pathogenic bacteria [15, 39, 74]. The disease can be classified as subclinical and clinical based on manifestation of clinical signs. Subclinical mastitis is characterized for the absence of clinical symptoms and milk of normal appearance. Clinical mastitis (CM) is characterized by the presence of abnormal milk and/or udder inflammation (redness, heat, swelling, and pain) with or without systemic symptoms. Based on the source of infection and reservoir of microorganism, mastitis can be classified as contagious and environmental [25, 74, 92, 104]. Contagious mastitis is transmitted from cow to cow during milking and the mammary gland is the reservoir for the pathogens (Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma spp., and Corynebacterium bovis). In environmental mastitis, the environment that surrounds the cows is the source of infection and reservoir of microorganisms. All the rest of microorganisms belong to this group (e.g., Escherichia coli, Klebsiella spp., Enterobacter spp., Pseudomonas spp., yeast, and algae). The group of coagulase negative staphylococci is part of normal skin flora and is considered opportunistic [25, 74].
Mastitis is the most common and costly disease in dairy cattle worldwide [33, 75, 102]. Economic losses are due to reduction in milk production, discarded milk, treatment costs, veterinary costs, increased labor, and premature culling [15, 26]. A reduction of 2.3 billion kg in milk production, due to sub-clinical mastitis, during 1996 was reported by Losinger (2005) in the US. Overall economic losses in the US are estimated to be between $140 and $300/cow/year [57, 70]. Using these estimated losses per cow and the number of cows in the US for 2006 (9.1 million of cows), the total aggregate losses for the US dairy industry during 2006 can be estimated at $1.3 to $2.7 billion. In another study, costs due to cases of CM were estimated to be $107/case, with more than 80% of the losses due to reduction in milk production and discarded milk [40].

Due to the negative economic consequences of the disease, mastitis control measures are particularly important to prevent and to reduce exposure to pathogenic bacteria and to reduce the duration of IMI [17, 31]. The National Mastitis Council recommends a comprehensive mastitis control program with 10-points [58]. The program includes: 1) establishment of goals for udder health (average of somatic cell counts (SCC) and CM); 2) maintenance of a clean, dry, and comfortable environment; 3) use of proper milking procedures; 4) maintenance of milking equipment; 5) adequate record keeping (CM, SCC, prevalence and incidence of sub-clinical mastitis); 6) proper management of CM in lactating cows; 7) effective dry cow management; 8) biosecurity for contagious pathogens and culling of chronically infected cows; 9) frequent monitoring of udder health; 10) regular review of mastitis control program in the herd.
2.2. Diagnosis of Intramammary Infection

The gold standard for identifying intramammary infection (IMI) is the microbiological culture of aseptically collected milk samples [44, 48, 64, 81, 82]. Collection of two or more samples and interpretation of culture results in series is usually recommended for research purposes [38, 64]. When duplicate samples are collected, interpretation in series implies that the same pathogen must be isolated from both samples to declare an IMI [38, 64]. If three or more samples are collected, the same pathogen must be recovered from two out of three samples before labeling a sample positive [38, 64]. The goal of the current standard of collecting at least two samples and interpreting them in series is to minimize false positive results. Thus, isolation of microorganisms commonly found in the teat skin and environment are considered as etiological agents of IMI if they are isolated from both samples, reducing false positives. Even though some strains of *S. aureus* can be found in the skin, most of the mastitis cases related to this pathogen in dairy herds are caused by strains highly adapted to the mammary gland [104]. Thus, isolation of this pathogen from at least one sample might be indicative of a true IMI and labeling the sample as negative based on the current requirement of minimum of two positive samples will most likely increase the percentage of false negatives.

The number of colony forming units (cfu/ml) of specific pathogens can be considered during interpretation of culture results to diagnose IMI. Currently, there are no consistent guidelines regarding how many cfu/ml of milk are required to indicate a true infection and several different cut-off values have been reported in the literature for declaring a sample bacteriologically positive: ≥ 100 cfu/ml [3, 34, 83, 96], ≥ 200 cfu/ml [3, 21], ≥ 300 cfu/ml [84], ≥ 500 cfu/ml [4, 71, 79, 80, 83]; or ≥
1000 cfu/ml [3, 96]. However, most often investigators and/or published literature do not provide any information about the details on how infections were diagnosed.

Consideration of the epidemiology of mastitis pathogens (i.e., cfu/ml and potential source of the organism) might improve accuracy of the diagnosis of IMI. Furthermore, assessment of the effect of number of samples collected per quarter on the proportion of false positives and false negatives are needed to improve the accuracy of the diagnosis of IMI.

2.3. Dry Period

The non-secretory stage of the mammary gland between consecutive calvings is known as the dry period, with dairy cows being dried off usually 60 days before the next expected calving date. The average number of days dry for Holstein cows in the US have been reported to be 61 days (±6 days), with few herds with less than 45 days [51]. Traditionally the dry period has been divided into three phases: active involution, steady stage, and lactogenesis-colostrogenesis [46, 90]. Capuco et al (1997) found that there is no loss of tissue and epithelial structures of the mammary gland of dairy cows, and alveolar structure is preserved during the dry period. Changes in the mammary gland of dairy cows are due to modification in secretory state of the gland instead of tissue regression or involution. The authors observed that there was absorption of milk components during early dry period followed by initiation of synthesis and secretion activity with accumulation of mammary secretion during the final phase of lactogenesis. Pregnancy in dairy cows inhibits mammary involution and induces preservation of alveolar structure during the dry period [23].

The dry period allows for the repair or replacement of injured or senescent mammary cells before the next lactation [22]. This has been confirmed by a recent
study [95], that found an increased cellular proliferation during the dry period as compared with a lower and constant cellular proliferation during lactation. In that study, cellular proliferation reached the highest level close to parturition. Cellular turnover during the dry period is essential for maximal milk production during the following lactation [63].

There is recent interest to reevaluate the length of the dry period, based on the possibility of increased income from milk production, easier management of dry cows, reduction of incidence of metabolic diseases, and efficient management of dry cows facilities [36]. However, short dry periods increase the level of SCC in the following lactation as compared with the standard period of 60-days [53]. Moreover, a decrease in lifetime milk yield can be expected when the period is shorter than 30 days [52].

2.3.1 Susceptibility to Intramammary Infections

Importance of the dry period in susceptibility of the mammary gland to IMI has been extensively studied [30, 60-63, 90]. Those studies have clearly demonstrated that the mammary gland has an increased susceptibility to IMI when secretory changes are taking place, which is during the early dry period and close to parturition. Infections acquired during the dry period that persist until the next lactation are frequently associated with cases of CM [60, 62], increased SCC in milk during early lactation [93], and extensive economic losses [62, 63].

Understanding of the epidemiology of pathogens present at the teat end is important to prevent IMI. At dry-off, exposure of the mammary gland to pathogens transmitted from cow to cow (i.e., contagious pathogens) is reduced, while exposure to pathogens commonly found in the environment (i.e., environmental pathogens)
remains constant throughout the dry period [30, 62]. The most common pathogens found during the dry period are environmental streptococci, coliforms, and coagulase negative staphylococci (CNS) [60, 61, 91, 93]. Smith et al (1985) observed an increase in the rate of coliform IMI during the first and last 25% of the dry period. They also reported that the rate of streptococci IMI followed the same distribution as coliform IMI, but was higher during the last half of the dry period. The overall rate of new IMI due to environmental pathogens increased 3-fold from late lactation to early dry period, with 3.6 times more quarters infected at calving than at dry-off [61]. Recently, Bradley and Green (2000) reported that the risk of the mammary gland to acquire IMI during the dry period is highest before the onset of colostrogenesis, with no increase in the prevalence of Enterobacteriaceae between three and one week pre-partum. They also suggested that even though the mammary gland is highly susceptible to acquire new enterobacteria IMI during the early dry period, it is resistant to clinical mastitis caused by these organisms during the dry period, possibly due to the high concentration of lactoferrin.

2.4. Antimicrobial treatment at dry-off

Administration of antimicrobial preparations in the mammary gland at the end of lactation, also known as dry cow therapy (DCT) is considered the most effective measure to eliminate existing IMI and to prevent new IMI during the dry period [16, 27, 30, 43, 54, 59, 73, 77, 89]. Antimicrobial treatment at the end of lactation offers the advantages of uniform concentration of antibiotics that remains in the mammary gland once milking is stopped, higher doses of antimicrobials than during lactational treatment, and no losses due to milk discard [35]. Most of DCT preparations are oil-
based suspensions and persist in the mammary gland only for 2-3 weeks during early and mid dry period [35].

The highest prevalence of contagious pathogens occurs at the end of lactation and early dry period and DCT has been shown to be highly effective to control these pathogens, thus lowering the prevalence by the start of the next lactation [93]. Prevalence of pathogens commonly found in the environment or skin flora increases throughout the dry period with constant exposure of the mammary gland to this group of pathogens over the dry period [13, 60-62, 91, 93]. Dry cow therapy effectively controls Gram-positive environmental pathogens during early dry period, but offers no protection against IMI at the end of the dry period because there is not an adequate concentration of antimicrobials left in the mammary gland close to parturition [14, 91, 93]. Additionally, most of the commercially approved products for DCT use in the US are not effective against Gram-negative pathogens, thus limiting the efficacy of DCT to control mastitis due to environmental pathogens [14, 41, 62, 90, 91, 93].

There are two commonly used approaches to implement DCT, so called total and selective DCT. Total dry cow therapy (TDCT) implies treatment of all quarters of all cows with antimicrobials at the end of lactation. The use of antimicrobials only for cows confirmed or suspected to be to be infected at the end of lactation, is known as selective dry cow therapy (SDCT). Thus, the main difference between these methods is treatment of healthy cows. While most US dairy herds (75%) use TDCT as part of their mastitis control program [98], some member countries of the International Dairy Federation used SDCT as an effective measure to promote production of high quality milk (e.g., Finland, New Zealand, Norway, Sweden, and Switzerland) [49].
The use of antimicrobials in animal production for prophylaxis, chemotherapy, and growth promotion can induce development of antimicrobial resistance [103], and may select for resistance among bacteria in animals [1], and potentially cross boundaries between animals and humans [103]. Dry cow therapy creates selection pressure for the acquisition and maintenance of resistance genes because it is designed to persist in the mammary gland for several weeks in prophylactic concentrations [6]. Total dry cow therapy is the only blanket use of antimicrobials in dairy herds, thus mastitis control programs based on TDCT are conducive to overuse of antimicrobials [86, 100]. Due to growing concern over the use of antimicrobials in animal production and the possible development of antimicrobial resistance in bacterial populations and the potential threat that this represents for public health [8, 45, 101], SDCT has recently received more attention. With the most prudent approach for using antimicrobials with respect to DCT, only infected cows expected to respond to treatment receive DCT, those with chronic infections are slaughtered and uninfected cows are left without treatment [16, 20, 68, 78]. This could be expected to reduce antimicrobial use and consequently selection pressure from antimicrobials.

2.4.1 Efficacy of Dry Cow Therapy

According with Hogan (1994) the effectiveness of DCT is defined as reduction of prevalence of IMI at calving. Thus, cure rate of infected cows treated at dry-off as well as prophylactic effect of DCT during the dry period on the rate of new IMI has been used as a measure of efficacy of DCT. The occurrence of clinical mastitis, level of SCC, and milk production in the following lactation should also be considered as a measure of DCT efficiency.
Cure rates for IMI after DCT have ranged from 35 to approximately 90% [11, 12, 19, 27, 32, 37, 42, 47, 59, 73, 89, 94]. The prophylactic effect of DCT in uninfected cows is approximately 5 to 15%, thus between 85 to 95% of cows will not acquire a new IMI during the dry period [9, 19, 56, 86]. However, cure rate after DCT is not a random event, and it is related to cow, pathogen, and herd factors [5, 16, 27, 69, 86]. Among those risk factors are age of a cow, number of infected quarters, level of SCC at dry-off, occurrence of CM during lactation, IMI with \emph{S. aureus} resistant to penicillin, and prevalence of mastitis in the herd [5, 27, 68, 69, 86, 94]. Older cows with more than one quarter infected are less likely to cure than younger cows [5, 20, 86, 94]. Increased SCC before dry-off and several positive culture results reduce the likelihood of cure in cows infected with \emph{S. aureus} [5, 27, 68, 69, 94]. Moreover, cows that had CM during lactation are less likely to cure than cows without CM [69, 86]. Infections with penicillin-resistant strains of \emph{S. aureus} reduced success of DCT [5, 68, 69, 94]. Cows from herds with low prevalence of mastitis have a tendency to respond successfully to DCT [20, 68, 69].

\textbf{2.4.1. Efficacy of Total Dry Cow Therapy}

Numerous studies have been conducted to evaluate the efficacy of DCT. Most commonly this has been done by randomly allocating cows to either receive or not to receive intramammary antibiotic infusions at the end of lactation, regardless of their infection status [10, 37, 41, 54, 56, 59, 85, 89]. In these studies, treated cows had lower prevalence of IMI at calving, acquired fewer IMI during the dry period, and had fewer persistent infections than untreated cows. Based on these results, treatment of all cows at the end of lactation has been recommended. However, no conclusions about the effect of DCT on uninfected cows can be drawn from these studies.
2.4.1.2. Efficacy of Selective Dry Cow Therapy

Evaluation of the effect of SDCT on new IMI and occurrence of clinical mastitis was recently published [7] and, according to the authors, cows enrolled in the study were either free of IMI or infected only with minor pathogens (i.e., CNS and Corynebacterium bovis). In this study cows were randomly allocated either to receive DCT or not to receive DCT, using a random number table. No attempts were made to classify cows as infected or suspected to be infected in order to assure treatment of all infected cows at dry-off, as is recommended whenever SDCT is implemented. Without confirmation of infection status at dry-off, it is likely that infected cows were assigned to the untreated group. Even though infected cows were most likely assigned to both experimental groups through randomization, the presence of major contagious pathogens at dry-off likely increased the likelihood of infections at calving and CM during the following lactation in untreated cows as compared with those that received treatment. Therefore, even though the investigators claimed their study evaluated the effects of SDCT and they concluded that treated cows had a significantly reduced incidence of IMI and clinical mastitis during the following lactation, this study was not appropriately designed to assess the effect of selectively treating infected cows at dry-off, thus no conclusions about the effect of SDCT can be drawn from this study.

On the other hand, from studies that truly have used selection criteria to identify uninfected and infected cows/quarters, three different study designs have been used to evaluate the efficacy of SDCT. The first approach is treatment of only those cows identified as infected, leaving uninfected quarters/cows without DCT [18, 76, 78, 88]. Using this approach, rate of new IMI can be measured in uninfected cows, while therapeutic and prophylactic effect of DCT can be measured in the
treatment group. The second approach is treatment of infected cows and random allocation of uninfected cows either to receive DCT or not to receive DCT [19, 20]. Using the second approach, a fair comparison between untreated and treated uninfected cows can be made and the effect of DCT in udder health of uninfected cows can be assessed. The third approach is random allocation of infected cows to either receive or not to receive DCT [65-67, 69]. Using the third approach the effect of DCT in infected udders can be evaluated as well as spontaneous cures in untreated cows. This approach has mainly been used in studies conducted in Norway, where DCT is not routinely recommended even for infected cows.

Two main conclusions can be made from studies that used the first approach in regards to IMI: 1) uninfected cows left without DCT had a lower or similar rate of new IMI than infected cows that received DCT [18, 76, 78, 88], and 2) prevalence of IMI at calving was similar between infected cows that received DCT and uninfected cows left without treatment [76, 78, 88]. Thus, selective use of DCT (i.e., leaving uninfected healthy cows untreated) had no detrimental effect on the udder health in these studies.

Four conclusions can be drawn from the study of Browning et al (1990) using the second approach: 1) cows uninfected and infected at dry-off had similar rates of new IMI over the dry period; 2) treated and untreated uninfected cows had similar rates of new IMI over the dry period, regardless of their treatment status; 3) cows infected at dry-off had a higher incidence of CM than uninfected cows; and 4) uninfected treated cows had a higher incidence of CM than uninfected untreated cows. It appears from this study that leaving uninfected cows without DCT did not
affect negatively udder health, but additionally, treatment of uninfected cows increased the likelihood of contracting clinical mastitis during early lactation.

Finally, three conclusions can be made from studies using the third approach, where infected cows were randomly assigned to receive or not to receive DCT and uninfected cows received no treatment: 1) treatment of infected cows reduced the prevalence of IMI at calving; 2) treatment reduced the incidence rate of new IMI; and 3) treatment reduced the incidence rate of CM [65-67, 69]. Thus, studies using this approach confirmed the need to treat infected cows at dry-off.

2.5. Identification of Infected Cows

Since the main concept of SDCT is to treat only infected cows and to leave healthy cows untreated, an obvious challenge with the implementation of SDCT is efficient and accurate identification of infected cows. As previously mentioned, the gold standard for identifying IMI is the microbiological culture of aseptically collected milk samples [44, 48, 64, 81, 82], and this method has been used by several researchers as a criterion for identification of infected quarters and/or cows for allocation to SDCT at dry-off [18, 20, 79]. However, in commercial herds, collection of milk samples for culturing prior to dry off to identify infected cows would be impractical and expensive [10, 30]. Thus, selection of cows for treatment requires practical and inexpensive methods [20, 30, 72] that use information readily available on farms [72].

One possible method is selection of cows based on SCC [50]. Due to the significant difference in SCC between healthy and infected udders, classification of cows as infected or uninfected is possible [28, 55]. The threshold of 200,000 cells/ml has frequently been used for classification purposes [24, 28, 87]. Use of farm records
regarding the occurrence of CM is also important when making decisions about SDCT [67, 69, 99]. Information on SCC can be combined with CM history to improve the accuracy of identifying cows for treatment.

Identification of infected cows based on confirmed IMI combined with occurrence of CM during the current lactation [88] and with SCC over 100,000 cells/ml at the end of lactation have also been used as criteria to identify cows for SDCT [65-67, 100]. Rindsig et al. (1978) classified cows as ‘suspected to be infected’ and ‘uninfected’, based on the presence of SCC higher than 500,000 cells/ml one month before dry-off, California Mastitis Test (CMT) positive score 2 or 3 at dry-off, and history of clinical mastitis [76]. Similarly, Poutrel and Rainard (1981) classified cows as ‘infected’ based on positive results of the CMT one month before dry-off. Hassan et al (1999) selected quarters with high N-acetylo-beta-D-glucosaminidase values for selective treatment of infected quarters. Recently Robert et al. (2006) reported on selection criteria currently used by farmers in France to allocate cows to SDCT. These criteria were based on SCC at dry-off or during previous lactation, alone or combined either with CM history or milk yield at dry-off.

**2.5.1. Assessment of the Selection Criteria**

Evaluation of the diagnostic characteristics of the selection criteria can be performed using sensitivity and specificity analysis. In this case, sensitivity is the proportion of cows with IMI that are classified as infected, while specificity is the proportion of cows without IMI that are classified as uninfected, based on the selection criteria [29, 97]. The use of CMT results obtained one month prior to dry-off to identify infected cows at the end of lactation resulted in sensitivity and specificity of 57.1 and 80.7%, respectively [72]. When SCC one month prior to dry-off and CM
history were used in combination with CMT scores by Rindsig et al. (1978), sensitivity and specificity of 80.6 and 51.9%, respectively, can be calculated from their published data. Finally, using the results from quarter level data published by Robert et al (2006), a sensitivity of 42.9% and specificity of 69.3% can be calculated for these criteria.

Selection criteria with high sensitivity will have few false negatives, ensuring that infected cows will be identified and treated, while selection criteria with high specificity will have few false positives [29]. When cows are selected for SDCT it is almost unavoidable to treat some uninfected cows as well as to miss some infected cows, because there are no perfect selection criteria. Thus, the possible cost of misclassifications (i.e., false positives and false negatives) [2] and the type of pathogens present on the farm need to be considered before implementation of SDCT. The ability of SCC and CM history to identify infected cows in modern US dairy herds is unknown. This information is needed before SDCT can be effectively implemented and the effect of SCDT on udder health in the following lactation under US production conditions needs to be determined.

2.6. Summary

The dry period is essential for the cellular turnover of mammary gland and optimization of milk production. Although the use of dry cow therapy is highly recommended for the control of bovine mastitis in cows infected at the end of lactation, the effect of therapy on occurrence of clinical mastitis and milk yield needs to be determined.
2.7. Literature Cited


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CHAPTER 3

DIAGNOSIS OF INTRAMAMMARY INFECTIONS BASED ON SAMPLING STRATEGY, EPIDEMIOLOGY OF PATHOGENS AND AGREEMENT BEYOND CHANCE

3.1. Abstract

Isolation of pathogens from duplicate or multiple milk samples is traditionally used to determine the cause of IMI. The objective of the present study was to determine how well results from single samples agree with results obtained from pairs of duplicate, successive and consecutive quarter milk samples, considering epidemiology of mastitis pathogens and using different interpretation strategies to diagnose IMI. Four definitions of IMI were evaluated, based on the number of colony forming units (cfu) and the type of pathogens isolated. Agreement between microbiological results from single and paired samples was assessed by calculating percentage of agreement and kappa coefficient for each sampling strategy; sensitivity and specificity for single samples were also calculated using the microbiological results from paired samples as the gold standard. A total of 1216 Holstein cows from four Ohio dairy herds were sampled. The highest percentage of agreement was observed when determination of IMI was based on major pathogens, regardless of the sampling strategy used: diagnosing IMI based on the presence of 100 or more cfu/ml of major pathogens in single milk samples had 93 to 98% of agreement with
respective paired samples. However, when IMI was diagnosed based on the presence of 100 or more cfu/ml of contagious pathogens or at least 500 cfu/ml of all other pathogens, agreement beyond chance was almost perfect between single and duplicate samples (kappa 0.86). Thus, the same result was obtained from single and paired duplicate samples in most cases. As the time between sample collections increased, a reduction in kappa coefficient and in percentage of agreement was observed. The highest agreement for single samples was observed with duplicate pairs and the lowest with consecutive samples. Our results suggest that diagnosing IMI based on 100 or more cfu/ml of contagious pathogens or at least 500 cfu/ml of all other pathogens (i.e., considering the epidemiology and the number of microorganisms isolated) from a single, carefully collected aseptic quarter milk sample will maximize the efficiency of detecting true IMI infections.

3.2. Introduction

Mastitis is defined as inflammation of the mammary gland and is primarily caused by invasion of pathogenic bacteria [13, 24]. Determination of the cause of intramammary infection (IMI) is typically accomplished by microbiological culture of aseptically collected milk samples and interpretation of the culture results [15]. Molecular techniques can also be used to identify species and strains of pathogens using methods such as polymerase chain reaction and DNA fingerprinting. These newer techniques can be successfully used in epidemiologic research to identify a source of infection [18, 29]. Milk samples for microbiological culture are commonly collected at a single milking or during separate milkings. Sampling strategies for a single milking include collection of single, duplicate [17], or successive samples (i.e.,
before and after milking). Consecutive sampling implies collection of milk samples during different milking times [17].

Interpretation of culture results can be seen as a two step process. The first step involves an analysis of the number of specific pathogens to declare the sample positive [3]. Currently, there are no consistent guidelines regarding how many colony forming units (cfu)/ml of milk are required to indicate a true infection and several different cut-off values have been reported in the literature to declare a sample bacteriologically positive: ≥ 100 cfu/ml [25], ≥200 cfu/ml [6], ≥ 300 cfu/ml [26], or ≥ 500 cfu/ml [2, 22]. However, most of the published literature has not indicated how many cfu/ml were used to diagnose IMI. The second step of the process concerns the interpretation of the results when more than one sample is available (i.e., series or parallel). In this regard, the National Mastitis Council (NMC) [12, 20] recommends series interpretation of duplicate samples (i.e., the same pathogen must be isolated from both samples to declare IMI). Similarly, when consecutive samples are obtained, it has been recommended that the same pathogen be recovered from two out of three samples before labeling a sample positive [12, 20].

Several studies have measured the level of agreement between samples taken in pairs as either duplicate [10, 17, 23] or consecutive samples [7, 11]. These analyses have measured the agreement of bacteriological results between the first and the second sample of the pair. Duplicate or consecutive samples interpreted in series are currently considered the gold standard in IMI diagnosis [20]. However, the requirement of multiple samples to diagnose IMI increases the cost of sampling and processing of milk samples [10]. It is important to quantify the agreement beyond chance between results from single quarter milk samples compared to results from
paired samples interpreted in series (i.e., the current gold standard). This information would be particularly useful for large field trials, where a single milk sample collection strategy is often the most practical option.

The objective of the present study was to determine agreement between results from single samples and results from paired duplicate, successive and consecutive quarter milk samples, using different definitions of IMI, based on the epidemiology of the pathogens.

3.3. Materials and methods

3.3.1 Study population

Milk samples were collected from 1216 Holstein cows in two commercial and two State institutional Ohio dairy herds enrolled in an ongoing dry cow therapy investigation. Quarter milk samples were collected according to NMC guidelines [20]. After collection, samples were immediately placed on ice, transported to the laboratory, and stored at -20° C before microbiology procedures.

3.3.2. Microbiology procedures

Samples were examined microbiologically according to NMC guidelines [20]. Briefly, milk samples were examined by plating 0.01 ml of milk on trypticase soy agar with 5% sheep blood and on MacConkey agar plates. Plates were incubated for 48 hours at 37° C and bacterial growth was recorded at 24 and 48 hours of incubation.

Colonies were first identified based on colony morphology, hemolytic pattern, and Gram stain result. Staphylococci were differentiated to presumptive *Staphylococcus aureus* and coagulase negative staphylococci (CNS), based on the catalase test and coagulase tube test. Streptococci and enterococci species were differentiated to *Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus*...
uberris, and Enterococcus spp. based on the catalase test, esculin hydrolysis, growth in 6.5% NaCl broth, hippurate hydrolysis and the Christie, Atkins, Munch-Petersen test (CAMP). Corynebacterium spp. and Arcanobacterium pyogenes were differentiated based on the catalase test. Gram-negative microorganisms were differentiated to Escherichia coli, Enterobacter spp., Klebsiella spp., Pseudomonas spp., and other Gram-negatives based on lactose fermentation, Motility-Indol-Ornithine test (MIO), Simmons citrate agar test, reactions in triple sugar iron agar test and oxidase test. Species of yeast, Nocardia spp. and Bacillus spp. were differentiated based on Gram-stain morphology. Samples were not inoculated in selective media for isolation of Mycoplasma spp. Colonies present in the solid media were counted and recorded as cfu/ml of milk. Samples were considered contaminated whenever more than two types of colonies were present in the sample [20].

3.3.3. Sampling strategies

Three different sampling strategies were evaluated: 1) duplicate samples (DS), foremilk samples taken one immediately after the other at the last milking at dry-off; 2) successive samples (SS), pre- and post-milking samples collected at the last milking at dry-off; and 3) consecutive samples (CS), foremilk samples taken at the second and third day post-partum.

3.3.4. Definitions of IMI

Based on microbiological results, four definitions of IMI were evaluated: 1) Isolation of $\geq 100$ cfu/mL of any pathogen (INF1); 2) Isolation of $\geq 100$ cfu/mL of major pathogens (INF2) (S. aureus, coliforms, streptococci, enterococci, A. pyogenes, Nocardia spp., and other Gram-negatives); 3) Isolation of $\geq 100$ cfu/mL of contagious pathogens (S. aureus and Str. agalactiae) or $\geq 500$ cfu/mL of all other pathogens (i.e.,
non-contagious) (INF3); and 4) Isolation of $\geq 100$ cfu/ml of major pathogens or $\geq 500$ cfu/ml of minor pathogens (CNS and *Corynebacterium* spp.) (INF4).

### 3.3.5. Interpretation of results

Microbiological results obtained from the DS, SS, and CS pairs were interpreted in series: isolation of the same species of a pathogen from both samples from the same quarter was considered a positive result [20].

### 3.3.6. Statistical analysis

The level of agreement between the microbiological results from single samples (i.e., the first sample of each pair) obtained from a quarter and the results from paired samples from the same quarter were assessed in each of the sampling strategies (DS, SS, and CS). Percentage of agreement was calculated as the number of times the same rating was assigned to the results obtained from single and paired samples. The degree of agreement beyond chance between the two ratings for single and paired samples based on a dichotomous response (infected=1, non-infected=0) was expressed as a kappa coefficient and its 95% confidence interval (CI) for each of the sampling strategies [4].

Sensitivity and specificity, with exact 95% CI, for the single samples were also calculated for each sampling strategy and IMI definition, using the microbiological culture results of the paired samples interpreted in series as the “gold standard”.

The analyses were performed at quarter level in STATA 9.2 (STATA, College Station, Texas).
3.4. Results

3.4.1 Pathogens isolated

A total of 12,096 quarter milk samples were processed from 1216 cows. Of the milk samples collected, there were 2032 duplicate, 1184 successive samples, and 2832 consecutive sample pairs. A total of 548 quarter milk samples were contaminated (4.5%); pairs with one or two contaminated samples were omitted from analysis.

Bacterial growth was found in 34.3% of milk samples. Minor pathogens represented the highest percentage of microorganisms isolated from all samples (Figure 3.1). CNS was the most common group of pathogens for all samples, followed by Enterococcus spp. and S. aureus in duplicate samples and Corynebacterium spp. and Enterococcus spp. in successive samples. For consecutive samples, Enterococcus spp., and Str. uberis were the most frequently isolated species after CNS (Figure 3.2). No Str. agalactiae was isolated from any of the samples processed.

Summary statistics for cfu/ml for the most common pathogens isolated from single quarter milk samples are presented in Table 3.1. For minor pathogens, a median of 700 cfu/ml was observed for CNS and 100 cfu/ml for Corynebacterium spp. The most common major pathogens isolated were Enterococcus spp. (median of 500 cfu/ml), Str. uberis (median of 2100 cfu/ml), and S. aureus (median of 5100 cfu/ml). Results from the second sample of the pair were very similar to those observed in the first sample (data not shown).

3.4.2 Percentage of agreement and kappa statistic

The percentage of agreement between single samples and paired samples interpreted in series was evaluated for the four definitions of IMI in each of the sampling strategies (Table 3.2). The highest agreement was observed with INF2
(97.8%; i.e., $\geq 100$ cfu of major pathogens) and INF3 (97.4%; i.e., $\geq 100$ cfu of contagious pathogens or $\geq 500$ cfu of all other pathogens) when single samples were compared with the results from paired duplicate samples. In the case of single samples compared with the results from paired successive samples, the highest agreement was also observed with INF2 (94.8%) followed by INF3 (93.6%). Agreement between results from single samples and paired consecutive samples was higher for INF2 (92.7%) than any other IMI definition.

The level of agreement beyond chance for each sampling strategy, measured by kappa statistic, is also shown in Table 3.2. Contrary to the simple percentage of agreement, the highest agreement between single and paired samples was observed when infection was defined based on isolating $\geq 100$ cfu of contagious pathogens or $\geq 500$ cfu/ml of all other pathogens (INF3) regardless of the sampling strategy used (kappa 0.62 to 0.86).

3.4.3. Sensitivity and specificity

When series interpretation of the results from paired samples was used as the gold standard, the point estimate for sensitivity for single samples was, by definition, 100% for each of the sampling strategies evaluated. However, the precision of the point estimate varies based on sample size and can be expressed with the 95% CI. Depending on the definition of IMI used and the number of samples analyzed for each sampling strategy some variation in the minimum sensitivity of single samples, given as the lower limit of the 95% CI, was observed (Table 3.3).

Defining IMI as the presence of 100 or more cfu/ml of major pathogens (INF2) resulted in the highest specificity across all sampling strategies (Table 3). Single samples had specificity (and 95% CI) of 97.7% (96.9 to 98.3%), 94.7% (93.2
to 95.9%), and 92.2% (90.9 to 93.4%) when compared with the results of paired samples from duplicate, successive, and consecutive samples, respectively. The second highest specificity for all sampling strategies was observed when IMI was defined as 100 or more cfu/ml of contagious pathogens or at least 500 cfu/ml of non-contagious pathogens (INF3) (Table 3.3). Single samples from duplicate pairs had a lower percentage of false positives than those in successive and consecutive samples with both of these IMI definitions (INF2: 2.3%, INF3: 2.8%), while single samples in consecutive sampling resulted in the highest percentage of false positives (INF2: 7.8%, INF3: 13.1%).

3.5. Discussion

3.5.1. Sampling strategy

Implementing an appropriate milk sampling scheme will depend on the nature and overall objective of the study [16]. Traditionally, milk sampling schemes have involved obtaining multiple samples per quarter. This practice is based on the guidelines of the International Dairy Federation for determination of prevalence and incidence of infection, and cure rates in experimental herds [16]. However, in field trials, determination of prevalence of infection can be based on a single, aseptically collected milk sample [16]. Generally, in large field studies, single milk samples are collected under the assumption that the presence of misclassification bias will be equally distributed among the experimental groups [7].

Requiring results from repeated samples (e.g., duplicate or consecutive) to diagnose IMI is time consuming and the cost of sample collection and processing may be prohibitive in large scale field trials [10]. However, an even more important consideration relates to the required sample size for any given study design. The
desired difference in frequency of disease to be detected between the experimental groups has a direct impact on the sample size needed to conduct the research. For example, if the expected frequency of IMI at quarter level is 5% in the treatment group and 10% in the control group, 476 quarter samples from 119 cows would be required to detect a difference with significance level of 0.05 and power of 80% (Epi Info™ 3.3.2). If the total number of samples collected during a study could not be increased, and repeated samples/quarter are required, a loss in statistical power is unavoidable. Power to detect a significant difference of the same magnitude on a quarter level reduces to 47% with duplicate or successive samples and to 31% with three consecutive samples (STATA, College Station, Texas). In other words, there is only 31% and 47% probability that we would be able to detect the expected difference due to the smaller number of quarters sampled. Thus, the benefit of a repeated sampling strategy needs to be weighed against the consequent reduction in statistical power due to a smaller sample size. If results obtained from single quarter milk samples are equivalent to results obtained from repeated samples, then the increase in power enables detecting smaller differences between treatment groups and achieving more precise estimates of IMI in the population under study.

3.5.2. Assessment of agreement between samples

Agreement between microbiological results was assessed by calculation of percentage of agreement and kappa coefficients. Using the kappa statistic to assess the level of agreement between two tests offers the advantage of considering the agreement beyond chance [9], in contrast with the simple percentage of agreement between the results from two tests. Previous studies have reported percentage of agreement between the first and second sample of a pair, using different sampling
schemes [10, 17, 23]. In the present study, the percentage of agreement was mainly calculated to enable comparison between our results with those published in the literature.

Diagnosing IMI based on 100 or more cfu/ml of contagious pathogens (i.e., *S. aureus* or *St. agalactiae*) or at least 500 cfu/ml of all other pathogens (INF3) had the highest true agreement (i.e., kappa coefficient) between single and paired samples (Table 3.2). Using INF3 definition takes into account both major and minor pathogens and the epidemiology of these pathogens by considering the number of microorganisms in the diagnosis. On the other hand, diagnosis of IMI with INF2 had the highest percentage of agreement between single and paired samples, suggesting that when only major pathogens are considered a high percentage of single samples can yield the same results as paired milk samples just by chance. Our results with INF2 are similar to those reported by Jasper et al. (1974) for duplicate samples (96.2%), while the percentage of agreement observed between single and paired consecutive samples was higher in our study (91.1%) than the agreement reported by Griffin et al. (1977) for any major pathogen from samples collected one week apart (87.4%).

Using the reference values for kappa statistics from Dohoo et al. (2003), diagnosing IMI using INF3 had almost perfect agreement (defined as kappa >0.8) between single and paired duplicate samples. In other words, the same outcome was achieved with one sample as with paired samples in almost all cases. Quarters declared infected by the current, widely accepted gold standard of isolating the same pathogen from duplicate samples were always declared positive using a single sample. As expected, single samples identified more infections than duplicate samples. The
true agreement observed with INF3 in this study was considered almost perfect, whereas Dingwell et al. (2005) reported substantial agreement based on kappa statistics for *S. aureus*, between the first and the second sample of duplicate pairs.

### 3.5.3. Differences among sampling strategies.

Based on our results, the differences observed between sampling strategies in the level of agreement can be partly explained by pathogen distribution in milk samples and the lag-time between samplings. Even though the overall distribution of pathogens obtained from the first and the second sample of the pairs were quite similar for all of the sampling strategies (Figure 3.1), pairs of duplicate samples were most alike. It is expected that samples collected at the same point in time (i.e., duplicates) are most likely to yield the same pathogens. When successive samples are collected, it has been proposed that milk samples collected after milk removal (i.e., post-milking samples) will have less contamination [28]. Some pathogens that colonize the streak canal without causing intramammary infection will be removed by milking [20]. Therefore, lower agreement is expected when the results from single samples are compared with the results obtained from pairs of successive samples than from pairs of duplicate samples.

As the time between samplings increased, a reduction in the agreement beyond chance was observed, regardless of the definition of IMI used. Single samples had the highest kappa coefficients with results from duplicate pairs and the lowest kappa coefficients with pairs of consecutive samples. The same trend was observed for percentage of agreement (Table 3.2). It has been suggested that results from quarter milk samples collected during three consecutive weeks are needed to diagnose IMI, and the quarter is considered infected when major pathogens are isolated from at least
two out of the three samples [11]. If the trend observed in the present study were to be extrapolated, it can be expected that for samples obtained consecutively with more than one day between sample collections, the agreement will be even lower than the one observed with consecutive samples in the present study. Organisms isolated from milk samples collected one or two weeks apart can not automatically be considered causal agents of the same IMI.

3.5.4. Interpretation of culture results and IMI definitions

When paired duplicate samples were interpreted in series, using either INF1 or INF3, 8.7% of the samples which were declared positive for *S. aureus* with single samples were negative with duplicates samples. This is highly relevant considering contagious pathogens, where identification of infected cows is important to control and prevent spread of infection. In the case of CNS when INF1 was used (i.e., one colony of any pathogens enough to declare an infection), 37.4% of the samples that were declared negative with paired samples were classified as infected with a single sample. However, when culture results from a single quarter milk sample were interpreted using INF3, only 8.6% of the negative samples based on paired samples were considered positive, because this definition required at least 500 cfu/ml of non-contagious pathogens to declare a sample positive. It is impossible to say based on the current data whether these were true infections missed with the series interpretation of paired samples or whether they were false positives based on the single samples.

3.5.5. Interpretation of results through sensitivity and specificity

Sensitivity is the proportion of infected animals that are test positive, while specificity is the proportion of non-infected animals that are test negative [9]. Microbiological culture is traditionally considered the gold standard (or the ‘truth’).
when diagnosing IMI. In the present study, the gold standard was the bacteriological result obtained from sample pairs interpreted in series following recommendations of NMC [20] and using three different sampling strategies.

Some researchers have recommended evaluating sensitivity and specificity of culture results, instead of percentage of agreement, when results from two tests are compared to diagnose IMI [8, 27]. The disadvantage of this approach is that arbitrarily one of the tests needs to be considered ‘the truth’, even though the ‘truth’ is, in fact, unknown. When the interest is to determine how well two tests with dichotomous outcomes truly agree, without looking for a gold standard, determination of kappa coefficients is more appropriate than determination of the test characteristics [9].

The highest specificity, the ability to correctly identify non-infected cows, was observed when IMI was defined by major pathogens (INF2), regardless of the sampling strategy used (Table 3.3). These results are similar to those reported for major pathogens by Dinsmore et al. (1991), Sears et al. (1991) and Buelow et al. (1996). A test with high specificity will have few false positives, while a test with high sensitivity will have few false negatives. Calling a sample positive due to the presence of 100 or more cfu/ml of minor or non-contagious pathogens (e.g., INF1) ignores the fact that these organisms are present as part of the normal skin flora and/or are ubiquitous in the environment, thus increasing the possibility of false positives due to contamination. The goal of the current standard of collecting at least two samples and interpreting them in series is to minimize false positive results. This goal can be achieved more effectively and with less expense by using single quarter milk
samples and adjusting for the number of organisms isolated from the samples based on the epidemiology of the infection.

There is no single definition of IMI that can consider the epidemiology of all the different pathogens that produce infection, therefore using a definition of IMI that increases our ability to identify truly infected cows (i.e., high sensitivity) while reducing false positives (i.e., high specificity) has practical implications: fewer samples will be called positive due to contaminants or considered falsely negative, and the estimation of disease frequency (e.g., incidence or prevalence) from the data will be closer to the true value in the population. This was achieved with our third IMI definition, with 100% of sensitivity and 97.2% specificity. When the objective is “to rule out a disease” a test with high sensitivity is preferred to reduce false negatives [9]. This is very important when the interest is to identify cows infected with contagious pathogens that shed intermittently (i.e., *S. aureus*) [27]. The probability of detecting positive cows can be increased with parallel interpretation of multiple samples. In parallel interpretation, isolation of a pathogen in one (or more) of the samples is adequate to call it positive. Thus, parallel interpretation of two diagnostic tests increases the proportion of animals that test positive, and decreases the number of false negatives [9]. In the case of non-contagious pathogens interpreting samples in series is preferable, because using parallel interpretation would result in more samples being called positive due to contamination. However, the most efficient approach would be to base the diagnosis on a single sample, with consideration of cfu/ml and potential source of the organism.
3.5.6. Number of cfu/ml for specific pathogens

In a low percentage of samples, isolation of less than 100 cfu/ml of staphylococci and streptococci from infected quarters is possible [5]. Even though considering the number of colonies to diagnose IMI is not a practice universally accepted, the NMC Research Committee (1987) in a publication from 20 years ago stated that for *Str. agalactiae* and group G streptococci, the isolation of even one colony is highly significant (i.e., 100 cfu/ml). Although no *Str. agalactiae* was isolated in the present study, it can be expected that isolation of at least one colony (i.e., minimum level of detection using 0.01 ml of milk) will suffice to make a diagnosis. In addition, for *S. aureus* even though the presence of 100 cfu/ml can be indicative of infection, the presence of 200 cfu/ml or more is more relevant [19]. That is in agreement with the findings of the present study, where the minimum number of *S. aureus* isolated was 100 cfu/ml and 75% of the single samples had at least 1100 cfu/ml (Table 3.1).

On the other hand, some of the common mastitis pathogens can colonize teat skin and/or the teat canal without causing IMI [20], and therefore isolation of at least 500 cfu/ml of milk is generally required as an indication of IMI with these types of pathogens [5]. In the present study, 25% of the samples had a maximum of 100 cfu/ml and 50% had at the most 700 cfu/ml of CNS (Table 3.1).

With regard to streptococci other than *Str. agalactiae*, Gram-negatives and other microorganisms, the Research Committee of NMC (1987) considered the isolation of more than 1000 cfu/ml in a pure culture highly significant. We found that 25% of the samples had a maximum of 200 cfu/ml of *Enterococcus* spp., 700 cfu/ml of *Str. uberis*, and at the most 400 cfu/ml of *E. coli* (Table 3.1). As these groups of
pathogens is commonly found in the environment [15, 20, 24], they can be present in samples either as a result of contamination during sample collection or as true infectious agents. Interpreting culture results with our third definition of IMI (INF3) requires high numbers of these pathogens to declare a sample positive, thus, accounting for the possibility of contamination.

As in the current study, isolation of 100 or more cfu/ml of contagious pathogens has been used to diagnose IMI from quarter milk samples collected from cows with clinical [1, 14] and sub-clinical mastitis [21, 27]. However, when non-contagious major pathogens are considered, a higher cut-off for cfu/ml has been suggested as a requirement to diagnose IMI, e.g., \( \geq 200 \) cfu/ml [1] or 400 cfu/ml [21]. For minor pathogens, much higher thresholds have been used (1000 to 4000 cfu/ml) to declare an IMI [1, 21] than what was used in the current study. However, with as high of a threshold as 1000 or 4000 cfu/ml, some true infections may be called falsely negative.

3.5.7. Epidemiology of mastitis pathogens

The epidemiology of mastitis pathogens is an important factor that must be taken into account when diagnosing IMI. Etiology of bovine mastitis has changed in the last 40 years [13], evolving from mastitis mainly caused by contagious pathogens to mastitis caused by microorganisms that are found mostly in the environment or as part of the normal skin flora. Consideration of this evolving epidemiology of mastitis is important when deciding the best way to define and diagnose IMI and the role of pathogens which so far have been considered to play a minor role should not be overlooked.
In the present study, the most common pathogens isolated were minor pathogens (i.e., CNS and Corynebacterium spp.), Enterococcus spp., Str. uberis and S. aureus. Percentage of contagious pathogens (i.e., S. aureus) recovered from samples collected at dry-off or at calving was low (less than 2% of all quarter samples were positive), and minor pathogens represented the majority of isolates both at dry-off and at calving. Samples collected at calving had a higher percentage of pathogens that are ubiquitous in the environment, the skin, or the teat canal, than samples collected at dry-off.

3.6. Conclusions

Diagnosis of IMI requires consideration of multiple factors, from the sampling strategy and interpretation of the culture results to the epidemiology of the causal organisms. Our results suggest that requiring only 100 or more cfu/ml of contagious pathogens from a single sample minimizes the number of false negative results with less expense than duplicate or successive samples and thus increases our ability to correctly identify and diagnose quarters infected with these pathogens. Also, declaring single samples positive due to the presence of a relatively high number of non-contagious pathogens (≥500 cfu/ml) will correctly identify actual infections rather than contaminants. Requiring isolation of the same pathogen from two consecutive quarter milk samples results in missed diagnosis of contagious infections.

3.7. References


<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Mean(SD)</th>
<th>Min</th>
<th>10p</th>
<th>25p</th>
<th>Median</th>
<th>75p</th>
<th>90p</th>
<th>Max</th>
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<tr>
<td>CNS(^5)</td>
<td>3147.7 (5049.1)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>700</td>
<td>3900</td>
<td>10,000</td>
<td>30,000</td>
</tr>
<tr>
<td>CORYN(^6)</td>
<td>949.3 (2337.2)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>400</td>
<td>1600</td>
<td>10,000</td>
</tr>
<tr>
<td>E. coli</td>
<td>4022.4 (8341.3)</td>
<td>100</td>
<td>100</td>
<td>400</td>
<td>1500</td>
<td>4200</td>
<td>10,000</td>
<td>50,000</td>
</tr>
<tr>
<td>ENTC(^7)</td>
<td>1925.6 (3933.3)</td>
<td>100</td>
<td>100</td>
<td>200</td>
<td>500</td>
<td>1600</td>
<td>5000</td>
<td>22,800</td>
</tr>
<tr>
<td>KLEB(^8)</td>
<td>1781.3 (3144.4)</td>
<td>100</td>
<td>100</td>
<td>200</td>
<td>800</td>
<td>1600</td>
<td>5000</td>
<td>12,500</td>
</tr>
<tr>
<td>S. aureus</td>
<td>7592.3 (7139.2)</td>
<td>100</td>
<td>200</td>
<td>1100</td>
<td>5100</td>
<td>10,200</td>
<td>20,000</td>
<td>20,000</td>
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<tr>
<td>DYSG(^9)</td>
<td>5505.3 (6568.1)</td>
<td>100</td>
<td>100</td>
<td>200</td>
<td>2500</td>
<td>10,000</td>
<td>20,000</td>
<td>20,000</td>
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<tr>
<td>S. uberis</td>
<td>4356.9 (5092.6)</td>
<td>100</td>
<td>100</td>
<td>700</td>
<td>2100</td>
<td>6900</td>
<td>11,000</td>
<td>20,000</td>
</tr>
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</table>

\(^1\)Standard Deviation  
\(^2\)Minimum  
\(^3\)Percentile  
\(^4\)Maximum  
\(^5\)Coagulase negative staphylococci  
\(^6\)Corynebacterium spp.  
\(^7\)Enterococcus spp.  
\(^8\)Klebsiella spp.  
\(^9\)S. dysgalactiae

Table 3.1: Mean, standard deviation, percentiles, minimum, and maximum of colony forming units for main pathogens isolated from single milk samples
<table>
<thead>
<tr>
<th>IMI$^1$</th>
<th>Duplicate Samples</th>
<th></th>
<th></th>
<th>Consecutive Samples</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>% Agreement</td>
<td>Kappa</td>
<td>% Agreement</td>
<td>Kappa</td>
<td>% Agreement</td>
</tr>
<tr>
<td>INF1$^2$</td>
<td>91.6</td>
<td>0.69 (0.23-0.73)</td>
<td>81.9</td>
<td>0.49 (-0.003,0.53)</td>
<td>66.8</td>
</tr>
<tr>
<td>INF2$^3$</td>
<td>97.8</td>
<td>0.71 (0.23-0.78)</td>
<td>94.8</td>
<td>0.45 (-0.01,0.55)</td>
<td>91.1</td>
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<tr>
<td>INF3$^4$</td>
<td>97.4</td>
<td>0.86 (0.45-0.89)</td>
<td>93.6</td>
<td>0.69 (0.23,0.75)</td>
<td>89.7</td>
</tr>
<tr>
<td>INF4$^5$</td>
<td>96.3</td>
<td>0.82 (0.40-0.85)</td>
<td>90.6</td>
<td>0.61 (0.11,0.66)</td>
<td>85.4</td>
</tr>
</tbody>
</table>

$^1$Definition of Intramammary Infection

$^2$INF1: Isolation of ≥ 100 cfu/ ml of milk of any pathogen

$^3$INF2: Isolation of ≥ 100 cfu/ ml of milk of major pathogens (S. aureus, Coliforms, streptococci, enterococci, A. pyogenes, Nocardia spp., and other Gram-negatives)

$^4$INF3: Isolation of ≥ 100 cfu/ ml of milk of contagious pathogens (S. aureus and Str. agalactiae) or

≥ 500 cfu/ ml of the other pathogens (i.e., non-contagious)

$^5$INF4: Isolation of ≥ 100 cfu/ ml of milk of major pathogens or ≥ 500 cfu/ ml of milk of minor pathogens (CNS and Corynebacterium spp.)

$^6$95% Confidence Interval

Table 3.2: Percentage of agreement and kappa statistics between the first sample and the respective pair of duplicate, successive, and consecutive samples for every IMI definition

68
<table>
<thead>
<tr>
<th>IMI¹</th>
<th>Duplicate Samples</th>
<th>Successive Samples</th>
<th>Consecutive Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>Sensitivity</td>
</tr>
<tr>
<td>INF1²</td>
<td>100 (98.4,100)</td>
<td>90.5 (89.1,91.8)</td>
<td>100 (97.6,100)</td>
</tr>
<tr>
<td>INF2³</td>
<td>100 (93.9,100)</td>
<td>97.7 (96.9,98.3)</td>
<td>100 (87.7,100)</td>
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<tr>
<td>INF3⁴</td>
<td>100 (97.9,100)</td>
<td>97.2 (96.3,97.9)</td>
<td>100 (96.5,100)</td>
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<tr>
<td>INF4⁵</td>
<td>100 (98.1,100)</td>
<td>95.9 (94.9,96.8)</td>
<td>100 (96.4,100)</td>
</tr>
</tbody>
</table>

¹Definition of Intramammary Infection
²INF1: Isolation of ≥ 100 cfu/ml of milk of any pathogen
³INF2: Isolation of ≥ 100 cfu/ml of milk of major pathogens (S. aureus, Coliforms, streptococci, enterococci, A. pyogenes, Nocardia spp., and other Gram-negatives)
⁴INF3: Isolation of ≥ 100 cfu/ml of milk of contagious pathogens (S. aureus and Str. agalactiae) or ≥ 500 cfu/ml of the other pathogens (i.e., non-contagious)
⁵INF4: Isolation of ≥ 100 cfu/ml of milk of major pathogens or ≥ 500 cfu/ml of milk of minor pathogens (CNS and Corynebacterium spp.)
⁶95% Confidence Interval

Table 3.3: Sensitivity, specificity and their 95% confidence intervals for the first sample and pairs of duplicate, successive, and consecutive samples for every IMI definition
Figure 3.1: Distribution of major and minor pathogens in the first (1\textsuperscript{st}) and second (2\textsuperscript{nd}) sample of duplicate samples (DS), successive samples (SS), and consecutive samples (CS)
Figure 3.2: Distribution of *Str. uberis* (SUB), *S. aureus* (SA), *E. coli* (EC), *Enterococcus* spp. (ENTC), other pathogens (Others), coagulase negative staphylococci (CNS), and no growth (NG), in the first (1<sup>st</sup>) and second sample (2<sup>nd</sup>) of duplicate samples (DS), successive samples (SS), and consecutive samples (CS)
CHAPTER 4

USING DAIRY HERD IMPROVEMENT RECORDS AND CLINICAL MASTITIS HISTORY TO IDENTIFY SUBCLINICAL MASTITIS INFECTIONS AT DRY-OFF

4.1. Abstract

The objective of the present study was to evaluate the use of clinical mastitis history (CM) and SCC from monthly Dairy Herd Improvement records in identification of infected and uninfected cows at dry-off. A total of 647 Holstein cows were classified as uninfected or infected at dry-off based on somatic cell counts (SCC) during the last three months of lactation and clinical mastitis history (CM). Cows with SCC above the SCC-threshold and/or with CM were considered to be potentially infected; otherwise they were considered to be uninfected. Overall prevalence of intramammary infections (IMI) when diagnosis was based on single samples was 34.3% (95% Confidence intervals 30.7-38.1). The most common pathogens isolated were coagulase negative staphylococci. Identifying potentially infected cows based on a threshold of 100,000 cells per ml and CM history had sensitivity and specificity of 87.4 and 32.5%, when IMI were diagnosed from single quarter milk samples. Positive and negative predictive values were 40.3 and 83.1%, respectively, for 34.3% prevalence of IMI. Similar test characteristics were observed
when diagnosis of IMI was based on isolation of the same pathogen from paired 
samples.

4.2. Introduction

Mastitis is the most common and costly disease in dairy cattle worldwide [15, 34]. Due to the negative economic consequences of the disease, mastitis control 
measures are particularly important. One of these control measures is the treatment of 
cows at dry-off with antimicrobials to eliminate existing and to prevent new 
intramammary infections at the end of lactation (i.e., dry cow therapy, DCT) [6, 24, 37].

According with the National Animal Health Monitoring System Dairy 2002 
study, 75% of US dairy herds treat all quarters of all cows with antibiotics at the end 
of lactation as part of their mastitis control program (i.e., total dry cow therapy, 
TDCT) [46]. However, selective dry cow therapy (SDCT) has recently received more 
attention due to growing concern over the use of antimicrobials in animal production 
and the possible development of antimicrobial resistance in bacterial populations and 
the potential threat that this represents for public health [3, 19, 48]. When SDCT is 
implemented, only those cows suspected to be infected are treated, while uninfected 
cows are left without treatment [6, 38], thus reducing antimicrobial use and 
consequently selection pressure from antimicrobials. The general concern about 
SDCT, however, is that leaving cows without treatment is detrimental for the health 
of the udder, once the prophylactic effect of DCT is lost. However, member countries 
of the International Dairy Federation (IDF) that use SDCT (e.g., Finland, New 
Zealand, Norway, Sweden, and Switzerland), produce very high quality milk with 
national averages of somatic cell counts (SCC) less than 250,000 cells/ml [22] or even
lower than 140,000 cells/ml [30, 31]. This is a clear indication that SDCT can be used successfully to maintain udder health.

A challenge with the implementation of SDCT is efficient and accurate identification of infected cows. Selection of cows for treatment also requires practical and inexpensive methods [8, 14, 32] that use information readily available on the farm [32]. One possible method is selection of cows based on SCC [23]. Due to the significant difference in SCC between healthy and infected udders, classification of cows as infected or uninfected is possible [11, 26]. The threshold of 200,000 cells/ml has generally been used for classification purposes [9, 11, 42]. Use of farm records regarding the occurrence of clinical mastitis is also important when making decisions about SDCT [47]. Information on SCC can be combined with clinical mastitis history to improve the effectiveness of identifying cows for treatment.

Many studies have been conducted to evaluate the efficacy of DCT. Most commonly this has been done by randomly allocating cows to either receive or not to receive intramammary antibiotic infusions at the end of lactation, regardless of their infection status [4, 5, 41]. A variety of criteria have been used to identify uninfected and infected cows/quarters in studies that evaluated the effect of SDCT. Based on these criteria cows were allocated to receive treatment or not to receive treatment, leaving uninfected cows/quarters untreated [8, 17, 48]. To the best of our knowledge assessment of a set of criteria to identify infected cows at dry-off to implement SDCT has not being evaluated in the US for over 20 years. The objective of the present study was to evaluate the use of clinical mastitis history and SCC from monthly Dairy Herd Improvement (DHI) records in identification of infected and uninfected cows at dry-off.
4.3. Materials and methods

4.3.1. Study population and sampling

Holstein cows from two commercial and two State institutional Ohio dairy herds enrolled in an ongoing dry cow therapy investigation were sampled at dry-off (i.e., milk samples collected during the last milking immediately prior to administration of DCT). Paired quarter milk samples were collected at the last milking at the end of lactation from each cow according to NMC guidelines [27]. After collection, samples were immediately placed on ice, transported to the laboratory, and stored at -20° C until microbiology procedures.

Somatic cell counts (SCC) from the current lactation of every cow in each farm were obtained electronically from PCDART (Herd Manager © 2005 Dairy Records Management Systems, Raleigh, NC). Individual clinical mastitis (CM) records were collected at each farm for two complete calendar years before the beginning of the study and data were entered into electronic spreadsheets (Microsoft Office Excel 2003). Spreadsheets were updated on a weekly basis during the study.

4.3.2. Microbiology procedures

Samples were examined microbiologically according to NMC guidelines [27]. Briefly, milk samples were examined by plating 0.01 ml of milk on trypticase soy agar with 5% sheep blood and on MacConkey agar plates. Plates were incubated for 48 hours at 37° C and bacterial growth was recorded at 24 and 48 hours of incubation.

4.3.3. Identification of infected cows

Based on monthly records for composite milk samples from DHI and history of CM during the current lactation, four selection criteria were evaluated for classification of healthy cows as uninfected or infected at dry-off: 1) Cows without
CM and SCC <100,000 cells/ml during the last three months of lactation were considered uninfected, all others were considered infected (SC1); 2) Cows without CM and with SCC < 200,000 cells/ml during the last three months of lactation were considered uninfected, otherwise cows were considered infected (SC2); 3) As criterion two, but additionally if a cow experienced a case of CM during the first three months of the lactation and her SCC was < 100,000 cells/ml for the rest of the lactation she was also considered uninfected. All others were considered infected (SC3); 4) Cows without CM and SCC< 300,000 cells/ml during the last three months of lactation were considered uninfected, all other cows were considered infected (SC4).

4.3.4. Microbiological gold standard

Two separate analyses were performed based on culture results from single and paired milk samples from the same quarter. 1) Based on microbiological results from single samples, a quarter was considered infected if ≥ 100 cfu/ml of major contagious pathogens (S. aureus and Str. agalactiae) or ≥ 500 cfu/ml of any other pathogen were isolated (i.e., coagulase negative staphylococci (CNS), Corynebacterium spp., Archanobacterium pyogenes, Bacillus spp., coliforms (Escherichia coli, Enterobacter spp., and Klebsiella spp.), yeast and other Gram-negative rods). 2) Based on microbiological results from paired samples, a quarter was considered infected if the same pathogen (≥ 100 cfu/ml) was isolated from both samples of the same quarter [27].

A cow was considered infected when at least one quarter was positive. A sample with more than two types of colonies was considered contaminated. Cows with two or more contaminated quarter samples were dropped from the analysis.
4.3.5. **Statistical analysis**

The abilities of the four selection criteria to properly identify infected and uninfected cows were evaluated separately for both data sets (i.e., IMI diagnosed based on single samples or paired samples) by calculating sensitivity and specificity with exact 95% confidence interval (CI) [13] using microbiological culture results as the gold standard. Positive predictive values (PPV), negative predictive values (NPV), and prevalence of intramammary infection (IMI) were also calculated. Non-parametric receiver operating characteristics (ROC) curves were calculated for the somatic cell scores (SCS) of the geometric mean of the last three SCC tests, geometric mean of the last two SCC test, and of the last test day SCC. Transformation of SCC to SCS was performed according with the following formula:

$$\text{SCS} = \log_2(\text{SCC}/100,000) + 3 \quad [10].$$

The analyses were performed at cow level in STATA 9.2 (STATA, College Station, Texas).

4.4. **Results**

4.4.1. **Reasons for exclusion**

A total of 2660 quarter milk samples were collected from 665 Holstein cows sampled at dry-off. A total of 18 cows (2.7%) were dropped because of contaminated samples (4 presumably uninfected and 14 presumably infected cows), with data from 647 cows used for the analyses.

4.4.2. **Prevalence of infection**

Overall prevalence of infection at dry-off from two set of samples interpreted in series was 32.2% (95% CI: 28.6-35.9). When results from single samples were
used, prevalence was 34.3% (95% CI: 30.7-38.1). Prevalence of infection for each of the study farms is shown in Table 4.1.

Microbiological results from the first and second quarter milk sample are shown in Figure 4.1. Most of the samples had no growth, with 73.7 and 77.0% from first and second samples, respectively. The percentage of contaminated samples was 2.0 and 1.3% from first and second samples, respectively. The most common pathogens isolated were CNS, followed by pathogens ubiquitous in the environment. *S. aureus* was isolated from less than 1.7% of the samples. No *Str. agalactiae* was isolated from any of the milk samples.

There were a total of 173 (25.7%) and 33 (4.5%) cows infected with CNS and *S. aureus* at dry-off, respectively. Of cows with IMI at dry-off, 77.9% (173/222) and 68.8% (143/208) were infected with CNS when diagnosis was based on single and paired samples, respectively. The percentage of *S. aureus* infected cows was 14.9% (33/222) and 13.9% (29/208), when diagnosis was based on single and paired samples, respectively.

### 4.4.3. Sensitivity and specificity of the selection criteria for single samples

Sensitivity and specificity of the four selection criteria are shown in Table 4.2. Sensitivity ranged from 64.9 to 87.4%. Sensitivity was highest with classification of cows without CM and with SCC below 100,000 cells/ml during the last three months of lactation as uninfected (SC1). Specificity ranged from 32.5 to 60.9%. The highest specificity was observed when a threshold of SCC <200,000 cells/ml for cows without CM or SCC<100,000 cells/ml for those that suffered CM early in their lactation was used (SC3). Positive predictive values ranged from 40.3 to 46.5%, with SC3 having the highest probability that a cow identified by the criteria as infected was
actually an infected cow. Negative predictive values ranged from 76.1 to 83.1%, SC1 had the highest probability that a cow identified as uninfected by the criteria did not have an IMI.

The highest percentage of cows classified as infected (74.3%) was obtained with SC1 (Table 4.2). The percentage (number missed/total infected) of *S. aureus* infected cows classified as uninfected was 0% (0/33), 18.2% (6/33), 27.3% (9/33), and 30.3% (10/33) with SC1, SC2, SC3, and SC4, respectively.

### 4.4.4. Sensitivity and specificity of the selection criteria from paired samples

Sensitivity and specificity of the four selection criteria, when the same pathogen was isolated from both paired samples of the same quarter is shown in Table 4.3. Sensitivity ranged from 67.3 to 88.5%. The highest sensitivity was obtained using SC1. Specificities ranged from 32.8 to 62.4%, with SC3 having the highest specificity. Positive predictive values ranged from 38.4 to 46.8%, the highest result was observed when cows were classified using SC3. Negative predictive values ranged from 77.3 to 85.7%, the highest NPV was observed with SC1.

Cows classified as infected, based on the four selection criteria, were as low as 47.9% (SC3) and as high as 70.0% (SC1). Percentage (number missed/ total infected) of *S. aureus* infected cows misclassified was 0% (0/29), 6.9% (2/29), 6.9% (2/29), and 13.8% (4/29) when SC1, SC2, SC3, and SC4 were used, respectively.

### 4.4.5. Performance of the selection criteria

The performance of the selection criteria with the highest sensitivity using single samples (SC1) was evaluated using four different levels of prevalence of IMI. Selecting cows without CM and SCC below 100,000 cells/ml during the last three months of lactation as uninfected had the highest NPV when prevalence of IMI was
15%. On the other hand, the highest PPV was observed when prevalence was 45% (Table 4.4).

The ability of SCC to discriminate uninfected (without IMI) from infected cows at dry-off was evaluated using ROC curves of the SCS during the last three months of lactation. Areas under the curves (95% CI) obtained from the analyses when diagnosis of IMI was based on single and paired samples, are shown in Table 4.5. Somatic cell scores of the geometric mean of SCC from the last three tests had the highest area under the curve when diagnosis of IMI was based on single or paired samples (Fig. 4.2).

4.5. Discussion

4.5.1 Prevalence of infection.

Prevalence of infected cows at dry-off, due to any pathogen, ranges from 28 to 50.4% [8, 36, 44]. Caution must be taken when comparing published results due to differences in study design and in interpretation of culture results. Generally, prevalence is reported as the presence of any pathogen, regardless of the number of microorganisms isolated from the samples [5, 17]. In the present study, prevalence of IMI was similar to previous results reported in the literature, either when single or paired samples were used to diagnose IMI [7, 8, 31, 40].

The proportion of cows classified as infected by a test (e.g., the selection criteria used in the present study) is known as apparent prevalence or post-test prevalence [13]. Poutrel and Rainard (1981) reported a 57.0% post-test prevalence after identification of cows suspected to be infected at dry-off using California Mastitis Test (CMT) results obtained one month before dry-off. A similar level of apparent prevalence at dry-off was reported by Rindsig et al. (1978), when cows were
classified as infected based on SCC, CMT, and CM history. Additionally, in a recent study by Huxley et al. (2002), 47.8% of cows were classified as uninfected using SCC and CM history as selection criteria. These results are similar to the apparent prevalence found in this study with three of the selection criteria (SC2, SC3, and SC4) in both data sets. When the cut-off value for SCC was 100,000 cells/ml (SC1), the apparent prevalence in the present study (70.0%) was higher than reported by either Rindsig et al. (1978) or Poutrel and Rainard (1981). However, it is important to point out that there are differences among theses studies in IMI definitions, sampling strategy (i.e., single, duplicate, or consecutive samplings), volume of milk plated, and type of pathogens isolated, which makes direct comparisons difficult.

The epidemiology of mastitis pathogens is an important factor that must be taken into account when diagnosing IMI. Etiology of bovine mastitis has changed in the last 40 years [18], evolving from mastitis caused mainly by contagious pathogens to mastitis caused by microorganisms that are found mostly in the environment or are part of the normal skin flora. During the seventies, contagious pathogens were responsible for approximately 80% of infected quarters at dry-off [36]. This percentage decreased to approximately 20% during the eighties when at the same time percentage of quarters infected with minor pathogens increased to almost 48% [32]. In the present study, a low percentage of contagious pathogens were found, with 5.1% of cows infected with *S. aureus* (1.7% of quarters) and no *Strep. agalactiae* isolated. The most common pathogens isolated were CNS (26.7% of cows, 15.5% of quarters infected).
4.5.2. Identifying infected cows

The gold standard for identifying IMI is the microbiological culture of milk samples [21, 27, 40]. Microbiological results from quarter milk samples have been used by several researchers as a criterion for identification of infected quarters and/or cows for allocation to SDCT at dry-off [7, 8, 28, 29, 39]. In commercial herds, collection of milk samples for culturing prior to dry off to identify infected cows would be impractical and expensive [5, 14]. Thus, if infected cows are going to be identified at the end of lactation, a simple, effective and inexpensive method is needed [8, 32]. The information used for this purpose should be readily available to the herdsman to maximize the treatment of only infected cows [32].

Some researchers have used a combination of test results (e.g., CMT, N-acetylo-beta-D-glucosaminidase test, and SCC) and history of clinical mastitis as criteria to identify infected cows/quarters for treatment at dry-off [17, 36, 43]. Among all these methods, selection of cows based on SCC is most commonly used [23]. At present, 47% of US dairy herds participate in DHI mastitis screening programs [49]. In those farms, test reports of SCC from composite samples of all four quarters from every milking cow are received on a monthly basis, which makes the use of this approach feasible.

Somatic cells in milk are represented mainly by leukocytes and a few epithelial cells [26]. During infection and inflammation, leukocytes migrate from the bloodstream to the site of infection in the mammary gland [16, 33]. The major factor influencing the increase of somatic cells in milk is the infectious status of the udder and for this reason somatic cells have been used as a proxy of IMI to monitor udder health [16, 26, 42]. It is generally accepted that SCC from uninfected quarters are
usually below 200,000 cells/ml [16, 35], thus the use of this practical threshold allows classification of cows as infected or uninfected [9, 11, 42].

Information regarding CM from individual cow records can help identification of infected cows at dry-off, improving the selection made on the basis of SCC alone [47]. Østerås et al. (1999) reported that cows treated for acute clinical mastitis during the previous lactation were more likely to be infected with major pathogens in the next lactation. Thus, it can be beneficial for those cows to be considered infected at dry-off and to receive DCT (i.e., therapeutic effect), regardless of their SCC during the last trimester of the lactation.

4.5.3. Sensitivity and specificity of SCC threshold of 200,000 cells/ml

Sensitivity is the proportion of cows with IMI that are classified as infected (i.e., test positive), while specificity is the proportion of cows without IMI that are classified as uninfected (i.e., test negative) [13]. Microbiological culture is traditionally considered the gold standard (or the ‘truth’) when diagnosing IMI [21, 27, 40]. In the present study, when paired quarter samples were used in the analyses, the gold standard was the bacteriological result obtained from paired samples interpreted in series following recommendations of NMC [27]. Generally the presence of one or more colonies of a pathogen isolated from paired quarter samples are considered indicative of IMI, so calling a sample positive due to the presence of any pathogen makes comparison feasible with previously published results. However, adjustment for the number and type of microorganisms present in a sample (i.e. major contagious and other microorganisms) allows consideration of the epidemiology of mastitis pathogens and similar definitions have been used by other researchers [2, 30, 40].
The sensitivity of SCC threshold of 200,000 cells/ml from monthly composite samples to identify infected cows has been reported to be between 72.6 to 89% [11, 25, 45]. However, Middleton et al. (2004) reported a sensitivity of 46% for a somatic cell score of four (range 141,000-282,000, mid point 200,000 cells/ml). Comparison of our results with those reported in the literature is challenging, due to differences in study designs and in definitions of infection that were used as the gold standard in previous studies. In the present study, using a cut-off value for SCC of 200,000 cells/ml and CM history to identify infected cows (i.e., either SC2 or SC3) from either single or paired quarter milk samples, the sensitivity ranged from 64.9 to 69.4%, similar to that reported by Dohoo and Leslie (1991), but lower than results reported by McDermott et al. (1982) and Timms and Schultz (1987). Specificity for the same SCC threshold had been reported to be 75 to 85.5% [11, 25, 45]. Estimates of specificity in the present study varied between 50.1 and 62.4%, depending on the number of samples used to diagnose IMI, which is lower than previously reported (Tables 4.2 and 4.3).

4.5.4. Changing SCC thresholds

The use of a reduced SCC threshold is recommended when SCC are used to identify cows for treatment at dry-off [12, 35]. Employing a low SCC threshold ensures that very few infections are missed (i.e. high sensitivity and few false negatives) and that most infected cows are treated [12, 35]. However, when prevalence of IMI is low a higher threshold may be used [12]. As expected, in our study a lower SCC threshold (i.e., 100,000 cells/ml) increased the sensitivity of the selection criteria to over 87% in both data sets (Tables 4.2 and 4.3). The fraction of cows erroneously classified as uninfected (i.e., false negatives) was reduced from
35.1% to 12.3%, in the single sample data set, and from 32.7% to 11.5% in the paired samples data set. Similarly, McDermott et al (1982) reported high sensitivity (92%) working with a SCC threshold of 100,000 cells/ml. Schepers et al. (1997) found approximately 83% sensitivity for SCC threshold of 100,000 cells/ml from individual quarter samples.

In this study, when the SCC threshold was increased to 300,000 cells/ml, sensitivities and specificities were similar to those observed with the SCC of 200,000 cells. Sensitivity results from both data sets were similar to those reported by McDermott et al. (1982) for 300,000 cells threshold, but specificities were lower than those reported by the authors [25].

4.5.5. Sensitivity and specificity of selection criteria to identify infected cows at dry-off

The use of CMT results obtained one month prior to dry-off to identify infected cows at the end of lactation resulted in sensitivity and specificity of 57.1 and 80.7%, respectively [32]. When SCC one month prior to dry-off and clinical mastitis history were added to CMT scores to have more comprehensive criteria for identification of infected cows at dry-off by Rindsig et al. (1978), sensitivity and specificity of 80.6 and 51.9%, respectively, can be calculated from their published data. Recently Robert et al. (2006) reported on selection criteria currently used by farmers in France to allocate cows to SDCT. These criteria were based on SCC at dry-off or during previous lactation, alone or combined either with CM history or milk yield at dry-off. Each farmer used his/her own criteria and no detailed information about the SCC cut-off value was published by the authors [37]. Using the results from
quarter level data published by the authors, a sensitivity of 42.9% and specificity of 69.3% can be calculated for these criteria.

All of the selection criteria based on SCC and CM history, proposed in the current study, showed a higher sensitivity than the values reported either by Poutrel and Rainard (1981) or Robert et al. (2006). However, only the criteria with a cut-off value of 100,000 cells/ml had a similar sensitivity to that reported by Rindsig et al. (1978). In regard to the specificity, our results are similar to those reported by Rindsig et al. (1978) and Robert et al. (2006), but were lower than those found by Poutrel and Rainard (1981).

4.5.6. Predictive values

Predictive values are the probabilities that the cow truly has or does not have IMI, depending on whether she was classified as infected (i.e., test positive) or uninfected (i.e., test negative). Positive and negative predictive values change with the prevalence of infection in the population and the test characteristics (i.e., sensitivity and specificity) [13]. The lower the prevalence of IMI the higher the NPV, while the higher the prevalence the higher the PPV [12, 13, 40]. In this study, the probability that a cow classified as uninfected was truly uninfected was between 76.1 and 83.1%, depending on the selection criteria used when the prevalence of IMI was 34.3%. These results were similar to the NPV reported by Rindsig et al. (1978) with 28% of prevalence and lower than the results of McDermott et al. (1982) for a prevalence of 24%. The probability that a cow classified as infected was indeed infected was between 40.3 and 46.5%, depending on the selection criteria used, for a prevalence of 34.3%. Roughly, half of the cows classified as infected and that would be treated if SDCT was used were actually not infected, thus we will treat more cows than
potentially needed. These results were similar to the 39% PPV reported by Rindsig et al. (1978) and 38 to 55% reported by McDermott et al. (1982), with prevalence of 28 and 24%, respectively.

4.5.7. Discrimination value of SCC

The ability of SCC to discriminate between cows with and without IMI was evaluated using ROC curves of the SCS from the geometric mean of the SCC from the last three, last two months, and from the last SCC test. Using ROC curve instead of sensitivity and specificity obtained from a fixed-value of a SCC threshold gave the overall ability of SCC to differentiate uninfected cows from those with IMI [13]. The probability that a random cow with IMI had a SCS higher than a random cow without mastitis during any of the last three months of lactation, was highest when SCS from the geometric mean of the last three test were considered in both data sets. These results were similar to those reported by Detilleux et al. (1999) for major contagious pathogens [10]. In the present study, an increase in SCC above any of the three thresholds analyzed during at least one of the last three months of lactation was used to classify a cow as infected. Results from ROC curves for SCS from the last test were lower than for the geometric mean of the last three tests with both IMI diagnosis definitions, suggesting a higher probability of identifying potentially infected cows when information for the last trimester of lactation is used, in agreement with Reneau (1986) and Bradley et al. (2004), who suggested using more than one test result to identify infected cows.

4.6. Practical implications

Use of any selection criteria to implement SDCT will depend on individual farm characteristics, since prevalence of IMI can differ from one farm to another.
Management styles and record keeping abilities of specific farms as well as characteristics of the selection criteria (i.e., sensitivity and specificity) need to be considered if and when planning to implement SDCT. Selection criteria with high sensitivity will have few false negatives, ensuring that infected cows will be identified and treated, while selection criteria with high specificity will have few false positives [13]. Every herdsman needs to consider the possible cost of misclassifications (i.e., false positives and false negatives) [1] and the type of pathogens present in the farm when selection criteria to identify potentially infected cows is used. When the prevalence of contagious pathogens in the herd is high, false negatives and thus leaving infected cows untreated are undesirable, and it is preferable to treat all quarters of all cows [20]. On the other hand, if the goal is reduction of the use of antimicrobials and minor pathogens or pathogens ubiquitous in the environment are the most prevalent microorganisms, selective treatment of only those cows suspected to be infected is a reasonable approach. It is unavoidable to treat some uninfected cows because there are no perfect selection criteria.

Our results suggest that the selection criteria with the highest sensitivity was based on using a cut-off value for SCC of 100,000 cells/ml and CM history. Cows were considered uninfected when their SCC was below the threshold and no cases of CM were reported for that lactation. When assessing the practical value of these selection criteria for individual farms using predictive values, it was observed that when prevalence of IMI is low, the percentage of false negatives was also low. In these types of herds, it can be expected that when the proportion of cows infected with major contagious pathogens is low, most of the infected cows will be properly identified by the selection criteria. On the other hand, herds with high prevalence of
IMI showed higher proportion of false negatives than the low prevalence farm. If major contagious pathogens are the most prevalent pathogens on these types of farms, treating all cows is the best approach to improve udder health and to produce high quality milk.

4.7. References


23. Kirk, J.H., S.L. Berry, J.P. Reynolds, J.P. Maas, and A. Ahmadi, Sensitivity and specificity analysis for somatic cell count (SCC) used to predict


<table>
<thead>
<tr>
<th></th>
<th>Herd A</th>
<th>Herd B</th>
<th>Herd C</th>
<th>Herd D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence$^1$</td>
<td>20.0 (15.4-25.3)</td>
<td>41.0 (34.5-47.3)</td>
<td>45.5 (28.1-63.6)</td>
<td>38.6 (28.4-49.6)</td>
</tr>
<tr>
<td>No. Cows</td>
<td>270</td>
<td>256</td>
<td>33</td>
<td>88</td>
</tr>
<tr>
<td>Minor$^3$</td>
<td>64.8</td>
<td>65.7</td>
<td>80.0</td>
<td>14.7</td>
</tr>
<tr>
<td>Major$^4$</td>
<td>31.5</td>
<td>24.8</td>
<td>0.0</td>
<td>11.8</td>
</tr>
<tr>
<td>Mixed Mm$^5$</td>
<td>3.7</td>
<td>9.5</td>
<td>20.0</td>
<td>14.7</td>
</tr>
</tbody>
</table>

$^1$Calculated from double samples with the same pathogen isolated from both samples
$^2$95% confidence intervals
$^3$Percentage of cows infected with minor pathogens (i.e., coagulase negative staphylococci and Corynebacterium spp.)
$^4$Percentage of cows infected with major pathogens
$^5$Percentage of cows infected with mixed infections major-minor pathogens

Table 4.1: Prevalence of intramammary infections, total number of cows, and percentage of cows infected with major pathogens, minor pathogens and mixed infections by study herd
<table>
<thead>
<tr>
<th>Uninfected Cows</th>
<th>SC1</th>
<th>SC2</th>
<th>SC3</th>
<th>SC4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SCC &lt;100,000</td>
<td>SCC &lt;200,000</td>
<td>SCC &lt;200,000</td>
<td>SCC &lt;300,000</td>
</tr>
<tr>
<td>No CM</td>
<td>No CM</td>
<td>No CM</td>
<td>CM &lt; 90 DIM²</td>
<td>No CM</td>
</tr>
<tr>
<td>Sensitivity³</td>
<td>87.4 (82.3-91.5)⁴</td>
<td>76.6 (70.4-81.9)</td>
<td>64.9 (58.2-71.1)</td>
<td>68.0 (61.5-74.1)</td>
</tr>
<tr>
<td>Specificity</td>
<td>32.5 (28.0-37.2)</td>
<td>46.8 (42.0-51.7)</td>
<td>60.9 (56.1-65.6)</td>
<td>53.2 (48.3-58.0)</td>
</tr>
<tr>
<td>PPV⁵</td>
<td>40.3 (35.9-44.9)</td>
<td>42.9 (38.0-47.9)</td>
<td>46.5 (40.8-52.2)</td>
<td>43.1 (37.9-48.5)</td>
</tr>
<tr>
<td>NPV⁶</td>
<td>83.1 (76.6-88.5)</td>
<td>79.3 (73.7-84.1)</td>
<td>76.9 (71.9-81.3)</td>
<td>76.1 (70.8-80.8)</td>
</tr>
<tr>
<td>NCCI (%)⁷</td>
<td>481 (74.3)</td>
<td>396 (61.2)</td>
<td>310 (47.9)</td>
<td>350 (54.1)</td>
</tr>
<tr>
<td>Prevalence</td>
<td>34.3 (30.7-38.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Selection criteria
²Cows that had CM during the first 90 days in milk (DIM) but SCC <100,000 cells/ml during the rest of the lactation
³Gold Standard: Isolation of ≥ 100 cfu/ml of *S. aureus* or *Str. agalactiae* or ≥ 500 cfu/ml of any other pathogen.
⁴95 % Confidence intervals
⁵Positive predictive values
⁶Negative Predictive Values
⁷Total number of Cows Classified as Infected

Table 4.2: Sensitivity, specificity, positive and negative predictive values for the selection of uninfected cows at dry-off based on somatic cell counts (SCC) during the last three month of lactation and clinical mastitis history (CM) with culture results from single samples as the gold standard
<table>
<thead>
<tr>
<th>Uninfected Cows</th>
<th>SC1</th>
<th>SC2</th>
<th>SC3</th>
<th>SC4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SCC &lt;100,000 No CM</td>
<td>SCC &lt;200,000 No CM</td>
<td>SCC &lt;200,000 CM &lt; 90 DIM</td>
<td>SCC &lt;300,000 No CM</td>
</tr>
<tr>
<td>Sensitivity³</td>
<td>8.5 (83.3-92.5)⁴</td>
<td>76.9 (70.6-82.5)</td>
<td>69.7 (62.9-75.9)</td>
<td>67.3 (60.5-73.6)</td>
</tr>
<tr>
<td>Specificity</td>
<td>32.8 (28.4-37.4)</td>
<td>46.7 (41.9-51.5)</td>
<td>62.4 (57.7-66.9)</td>
<td>52.6 (47.8-57.4)</td>
</tr>
<tr>
<td>PPV⁵</td>
<td>38.4 (34.0-42.9)</td>
<td>40.6 (35.7-45.6)</td>
<td>46.8 (41.1-52.5)</td>
<td>40.2 (35.0-45.6)</td>
</tr>
<tr>
<td>NPV⁶</td>
<td>85.7 (79.5-90.6)</td>
<td>81.0 (75.6-85.7)</td>
<td>81.3 (76.7-85.3)</td>
<td>77.3 (72.1-81.9)</td>
</tr>
<tr>
<td>NCCI (%)⁷</td>
<td>479 (74.0)</td>
<td>394 (60.9)</td>
<td>310 (47.9)</td>
<td>348 (53.8)</td>
</tr>
<tr>
<td>Prevalence</td>
<td>32.2 (28.6-35.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

³Selection criteria
²Cows that had CM during the first 90 days in milk (DIM) but SCC<100,000 cells/ml during the rest of the lactation
³Gold Standard: Isolation of the same pathogen from both samples (≥ 100 cfu/ml)
⁴95 % Confidence intervals
⁵Positive predictive values
⁶Negative Predictive Values
⁷Total number of Cows Classified as Infected

Table 4.3: Sensitivity, specificity, positive and negative predictive values for the selection of uninfected cows at dry-off based on somatic cell counts (SCC) during the last three month of lactation and clinical mastitis history (CM) with culture results from paired samples as the gold standard
Table 4.4: Positive and negative predictive values, false negatives, and false positives for classification of cows without clinical mastitis (CM) and with somatic cell counts below 100,000 cells/ml during the last three month of lactation as uninfected for different prevalence of intramammary infections at dry-off.

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>15%</th>
<th>25%</th>
<th>35%</th>
<th>45%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPV$^2$</td>
<td>18.6(10.3-29.7)$^3$</td>
<td>30.1(19.9-42.0)</td>
<td>41.3(30.1-53.3)</td>
<td>51.3(39.6-62.9)</td>
</tr>
<tr>
<td>NPV$^4$</td>
<td>93.3(77.9-99.2)</td>
<td>88.9(70.8-97.7)</td>
<td>84.0(63.9-95.5)</td>
<td>75.0(53.3-90.2)</td>
</tr>
<tr>
<td>FN$^5$</td>
<td>6.7</td>
<td>11.1</td>
<td>16.0</td>
<td>25.0</td>
</tr>
<tr>
<td>FP$^6$</td>
<td>81.4</td>
<td>69.9</td>
<td>58.7</td>
<td>48.7</td>
</tr>
</tbody>
</table>

1 Gold standard: Isolation of $\geq$ 100 cfu/ml of S. aureus or Str. agalactiae or $\geq$500 cfu/ml of any other pathogens from single samples. Sensitivity 84.2% and specificity 36.9%
2 Positive predictive values
3 95% Confidence intervals
4 Negative predictive values
5 False negatives (%)
6 False positives (%)

Table 4.4: Positive and negative predictive values, false negatives, and false positives for classification of cows without clinical mastitis (CM) and with somatic cell counts below 100,000 cells/ml during the last three month of lactation as uninfected for different prevalence of intramammary infections at dry-off.
<table>
<thead>
<tr>
<th></th>
<th>Last three tests</th>
<th>Last two tests</th>
<th>Last test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single samples¹</td>
<td>0.71 (0.67-0.75)²</td>
<td>0.69 (0.65-0.73)</td>
<td>0.66 (0.62-0.70)</td>
</tr>
<tr>
<td>Double samples³</td>
<td>0.72 (0.68-0.76)</td>
<td>0.71 (0.67-0.75)</td>
<td>0.69 (0.64-0.73)</td>
</tr>
</tbody>
</table>

¹Isolation of ≥ 100 cfu/ml of *S. aureus* or *Str. agalactiae* or ≥500 cfu/ml of any other pathogens
²95% Confidence intervals
³Isolation of the same pathogen from both samples (≥ 100 cfu/ml)

Table 4.5: Estimate of the area under ROC curves for somatic cell scores of the last three month assessing true intramammary infections at dry-off throughout culture results from single and paired quarter milk samples
Figure 4.1: Microbiological culture results from first and second quarter milk samples collected at dry-off
Figure 4.2: ROC curves to show the ability of somatic cell scores (SCS) during the last three months of lactation to discriminate cows with intramammary infections from those uninfected when diagnosis was based on culture results from single (A) and paired quarter samples (B).
CHAPTER 5

CLINICAL MASTITIS DURING EARLY LACTATION AND SELECTIVE DRY COW THERAPY

5.1. Abstract

Total dry cow therapy is a cornerstone of many mastitis control programs. However, selective dry cow therapy (SDCT) is receiving attention due to growing concern over antimicrobial resistance. The objective of the present study was to evaluate the effect of SDCT on the occurrence of clinical mastitis during early lactation. Cows with somatic cell counts (SCC) less than 200,000 cells/ml during the last 3 months of lactation and no history of clinical mastitis (CM) on the current lactation were considered uninfected (low-SCC cows) and randomly allocated either to receive or not to receive treatment at dry-off; others were considered high-SCC cows and were treated. Survival analysis was used in the data analysis.

A total of 873 Holstein cows in four Ohio dairy herds were sampled. Low-SCC cows had a lower prevalence of infection at dry-off than high-SCC cows (22.5 and 48.7%, respectively, P=0.000). Prevalence of infection at calving was 54.6 and 54.8% for low and high-SCC cows, respectively (P=1.00). The hazard of contracting CM was associated with the level of SCC during the last three months of the previous lactation, presence of intramammary infections at dry-off, parity, milk yield at dry-
off, and the interaction DCT-parity. Having high SCC at dry-off increased the hazard of CM after calving in the subsequent lactation 2.7, 2.5 times, 89% and 97% during the first 15, 30, 60, and 90 days, respectively, in second lactation cows. High-SCC cows that were infected at dry-off had an even higher hazard of CM than uninfected low-SCC cows that increased with time. Infected cows with four or more lactation had the highest hazard of CM.

Among low-SCC cows, prevalence of infections at dry off, for untreated (19.8%) and treated (25.0%) cows, was not different (P=0.206). Prevalence of infection at calving was 57.1 and 52.1% for untreated and treated cows, respectively (P=0.36). There was a significant interaction between DCT and lactation number. The hazard of contracting CM for second lactation cows was 89, 75, 44, and 43% higher for treated than for untreated cows, during the first 15, 30, 60 and 90 days, respectively, adjusted for the effect of infection and milk yield at dry-off. However, treated cows with more than two lactations had a lower hazard of contracting CM than untreated cows in the same parity groups.

5.2. Introduction

Mastitis is the most common and costly disease in dairy cattle worldwide [18, 40, 56]. Due to the negative economic consequences of the disease, mastitis control measures are particularly important. One of these control measures is treatment of cows at dry-off with antimicrobials to eliminate existing and to prevent new intramammary infections at the end of lactation (i.e., dry cow therapy, DCT) [6, 28, 42]. The use of blanket therapy, where all quarters of all cows receive antibiotic DCT is a common practice in more than 75% of U.S. herds [53]. However, selective dry cow therapy (SDCT) has recently received more attention due to growing concern
over the use of antimicrobials in animal production and the possible development of antimicrobial resistance in bacterial populations and the potential threat this represents for the public health [3, 24, 54]. The use of DCT only in cows suspected to be infected at the end of lactation offers an opportunity to reduce the use of antimicrobials in dairy operations.

Development of clinical mastitis during early lactation (first 90 days) is common and may be due to new IMI acquired during the dry period [13, 48]. The general concern about SDCT is that leaving cows without treatment is detrimental for the health of the udder once the prophylactic effect of DCT is lost. For a switch from total to selective DCT to be economically feasible, the new alternative needs to maintain similar udder health as the traditional practice.

The objectives of the present study were to: (1) evaluate the occurrence of clinical mastitis during early lactation in selectively treated cows; and (2) evaluate the occurrence of clinical mastitis during early lactation in low and high-SCC cows. We hypothesized that there is no difference in occurrence of clinical mastitis during the next lactation between treated and untreated cows with low-SCC at dry-off and that high-SCC cows have a higher incidence of clinical mastitis than low-SCC cows.

5.3. Materials and methods

5.3.1. Study population

Holstein cows from two commercial and two State institutional Ohio dairy herds were classified as uninfected or infected based on SCC during the last three months of lactation and clinical mastitis (CM) history during the current lactation. Cows without CM and with SCC < 200,000 cells/ml during the last 3 months of lactation were considered uninfected (low-SCC cows). If a cow had experienced a
case of CM during the first 90 days of the current lactation but her SCC was < 100,000 cells/ml for the rest of the lactation she was also considered uninfected. All other cows were assumed infected (high-SCC cows). Somatic cell counts from the current lactation of every cow in each farm were obtained electronically from PCDART (Herd Manager © 2005 Dairy Records Management Systems, Raleigh, NC) and individual CM records were collected at each farm for two complete calendar years before the beginning of the study and the data were entered into electronic spreadsheets (Microsoft Office Excel 2003).

5.3.2. Treatment allocation

Cows that were assumed uninfected were randomly allocated to either receive treatment or not to receive treatment at dry-off; all cows considered infected based on their SCC and CM history were treated. No control (i.e., untreated) group was assigned for the infected cows for ethical reasons. If a cow had CM on the day of dry-off she was excluded from the study. The list of treatment allocation was only available to the investigators (AT and PR-S) while the personnel at the farms were blinded with regards to the treatment status of the cows. Commercially licensed products containing benzathine cloxacillin or cephapirin benzathine were used as DCT.

5.3.3. Milk sampling

Two sets of quarter milk samples per cow were collected at dry-off and within the first five days after calving. Single samples were collected from quarters with CM before administration of antimicrobial treatment. Samples were kept at -20° C until transportation to the laboratory on weekly intervals. All milk samples were examined microbiologically according to National Mastitis Council guidelines [33]. Sampling at
dry-off was performed by the researchers, while samples at calving and from CM were collected by either the investigators or the farm personnel. Farms were visited once a week to collect milk samples, to update CM records, and to evaluate the general conditions of the dry cows. Instructions for sample collection were given before the beginning of the study and a written protocol was kept on each farm.

5.3.4. **Definition of intramammary infections (IMI)**

A quarter was considered infected if \( \geq 100 \) colony forming units (cfu)/ml of contagious major pathogens (\( S. \) aureus and \( Str. \) agalactiae) or \( \geq 500 \) cfu/ml of all other pathogens were isolated (coagulase negative staphylococci (CNS), \( Corynebacterium \) spp., \( Archanobacterium \) pyogenes, \( Bacillus \) spp., coliforms (\( Escherichia \) coli, \( Enterobacter \) spp., and \( Klebsiella \) spp.), yeast and other Gram-negative rods) based on microbiological results from single samples.

A cow was considered infected when at least one quarter was positive. If any growth was present in samples from CM the samples were considered positive regardless of the number of cfu/ml of pathogens isolated. Samples with more than two types of colonies were considered contaminated. If the first set of quarter milk samples was contaminated (at dry-off and at calving), the second set of samples was used to assess the prevalence of IMI. Cows with two or more contaminated quarter samples were dropped from the analysis.

5.3.5. **Clinical mastitis**

The presence of abnormal milk or udder inflammation with or without systemic symptoms was considered a case of clinical mastitis following the standard operating procedures of each farm.
The definition of CM was discussed with farm managers, and the importance of recording all cases and sample collection was emphasized in each farm.

5.3.6. Periods at risk

Four intervals of days at risk for CM were analyzed: from calving until 15, 30, 60, and 90 days in milk (DIM). Only the first case of CM was considered for the analysis, once a cow experienced a case she was no longer considered at risk. Cows that did not experience a case of CM were censored at the end of the respective risk period.

5.3.7. Statistical analysis

5.3.7.1. Complete data set

The main interest of the analysis was to compare development of the first case of CM among low and high-SCC cows during early lactation at cow level. The hazard of contracting CM during four early lactation periods (15, 30, 60, 90 days) was assessed with survival analysis using Cox’s proportional hazards model with a shared frailty on a herd level. The frailty (i.e., latent random effect) was assumed to follow a gamma distribution, according to the following linear model [27]:

\[
h_{jk}(t/\alpha_j) = \alpha_k h_o(t) \exp(\beta X_{jk}) [5.1]\]

For \( j=1,2,\ldots, n_k \) cows in \( K_{th} \) cluster (i.e., herd)

Where:

\( h_{jk}(t/\alpha_j) = h(t/X_{jk}) \) hazard at time ‘t’ given a set of covariates

\( \alpha_k \) : frailty for the \( k^{th} \) herd

\( \alpha_k h_o(t) \) : is the baseline hazard

\( (\beta X_{jk}) \) : coefficients of the covariates (X)
The significance of the variance of the frailty was tested using the likelihood-ratio test, under the null hypothesis that the variance is equal to zero. The frailty term was kept in the model whenever the variance was statistically significant (i.e., P-value ≤0.05) and/or if herd appeared as a confounder.

Independent variables considered during analysis were classification status of the cows based on SCC of the previous lactation and CM history (i.e., low and high-SCC cows), DCT, presence of IMI at dry-off, milk yield at dry-off (MYDO), lactation number, number of days dry (i.e., length of the dry period), and calving season. Presence of IMI at dry-off was a dichotomous variable based on microbiological culture results from single quarter milk samples collected at dry-off (Infected=1; Uninfected=0). Lactation was categorized in three groups (second, third, and fourth or higher). Milk yield at dry-off was estimated using the last two test days according to the following formula and centered at 19.8 Kg (median of the data) and scaled to reflect a 10-kg change:

\[
\Delta \text{Milk/day} = \frac{(\text{milk on second last test} - \text{milk on last test})}{30} [5.2]
\]

\[
\text{MYDO} = \text{milk on last test} - (\Delta \text{Milk/day} \times \text{days from last test to dry-off}) [5.3]
\]

To model the effect of season on occurrence of CM the day of calving was transformed into two variables, sine(calving day) and cosine(calving day), following the method used by Schukken et al. (1992) and Østerås et al. (2006):

\[
\text{Sine (calving day)} = \sin [2 \times \pi \times \text{(calving day}/365)] [5.4]
\]

\[
\text{Cosine (calving day)} = \cos [2 \times \pi \times \text{(calving day}/365)] [5.5]
\]
5.3.7.2. **Low-SCC cow data set**

The same analyses were performed using data from low-SCC cows only and the main interest was to compare the effect of treatment (i.e., DCT) on the hazard of CM in early lactation. The variable milk yield at the last test day was centered at 24.5 (median of the low-SCC data) and scaled to reflect a 10-kg change.

5.3.7.3 **Modeling procedure**

Initially, univariable proportional hazards models were built for each independent variable against the outcome. Variables with p-value from the Wald $\chi^2$ test or likelihood ratio test (LRT) $\leq 0.25$ were included in the initial multivariable model. The risk factor of interest (i.e., low and high-SCC classification for the complete data set and DCT for the low-SCC data set) was forced in the model. Backward selection procedure was used to run a sequence of models adjusted for the independent variables previously selected, dropping each variable individually. Variables with significant LRT (p$<0.05$) were kept in the model.

To evaluate whether a variable was a confounder the change in the coefficient of the risk factor of interest was calculated after the variable had been dropped from the model. Any variable that induced a change $\geq 10\%$ was kept in the model as a potential confounder. Once a model where all independent variables were significant, biologically plausible two-way interactions were tested. These were 1) classification status x presence of IMI at dry-off; 2) classification status x parity; 3) Presence of IMI at dry-off x parity; and 4) DCT x parity. Interaction terms were kept in the model if they were significant (LRT: P$<0.05$ or Akaike’s information criterion (AIC) of the model with the interaction term was lower than in the model without the interaction term).
5.3.7.4. Assumptions of models

The assumption of proportional hazards for the independent variables was assessed through the interaction of each variable with the logarithm of time [1, 26, 27]. If a variable violated this assumption, a time-varying covariate was introduced in the model as an interaction term between the variable in question and the logarithm of time. If a time-varying covariate (tvc) was included in the model, the estimated coefficients for the covariate as a fixed effect and as tvc were used to calculate the hazard ratio as follow:

\[
HR = \exp(\beta_{covariates} + (\beta_{tvc \times \log(time)}) [5.6]
\]

The non-informative censoring assumptions were evaluated through sensitivity analysis [19]. The analyses were performed at cow level in STATA 9.2 (STATA, College Station, Texas).

5.4. Results

5.4.1. Complete Data-Set

5.4.1.1. Intramammary infections at dry-off and at calving

A total of 432 low-SCC and 441 high-SCC cows were enrolled in the study. Four high-SCC cows (0.5%) were dropped from the analysis because both sets of samples collected at dry-off were contaminated. Total number of cows, average daily milk production, average SCC, and average SCC scores for each farm at enrolment into the study are shown in Table 5.1.

Forty two percent of the cows were in their second lactation, 29.1% were in their third lactation, and 28.9% had four or more lactations (up to 11). The distribution of calvings was uniform around the year with 20, 24, 34, and 22% of cows calving during December-February, March-May, June-August, and September-
November, respectively. The prevalence of IMI at dry-off for low and high-SCC cows was 22.5% (95% CI: 18.6-26.7) and 48.7% (95% CI: 43.9-53.5), respectively (Fisher’s exact P=0.000). Microbiological culture of quarter milk samples at dry-off was negative in 73.7% of the samples, with 1.3% of samples contaminated. Distributions of microorganisms isolated from quarter milk samples from low and high-SCC cows at dry-off are shown in Figure 5.1.

Prevalence of infection at calving at cow level was 54.6% (95% C.I.: 49.5-59.6) and 54.8% (95% C.I.: 49.4-60.0) for low and high-SCC cows, respectively (Fisher’s exact P=1.00). Overall, negative culture results were obtained from 54.3% of quarter milk samples with 4.8% of samples contaminated. The most common pathogens isolated at dry-off and at calving were CNS, 63.7 and 61.7%, respectively.

5.4.1.2. Clinical mastitis

The percentage of cows that had a case of CM during the first 90 DIM was 26.4%, with 73.6% of cows censored at the end of the risk period. Among the CM cases, 11.6 and 14.7% were in low and high-SCC cows, respectively. The majority of the cases occurred during the first month after calving, with 55.6% during the first 15 DIM, 10.5% between 16-30 DIM, 15.7% between 31-60 DIM, and 18.3% between 61-90 DIM.

Mean times to first case of CM for the four risk periods analyzed are shown in Table 5.2. There was no difference between low and high-SCC cows in the time to first case of CM in any of the periods at risk based on Log-rank test (P> 0.05). However, the adjusted survival and cumulative hazard curves for the first 15 days suggested that high-SCC cows experienced CM earlier in lactation than low-SCC
cows (Figure 5.2). A similar pattern was observed for the other risk periods as well (graphs not shown).

The hazard of contracting CM was affected by SCC during the last three months of previous lactation (i.e., a cow being classified as low or high-SCC cow), DCT, presence of IMI at dry-off, milk yield at dry-off, parity, and the interaction between DCT and parity. The variable IMI at dry-off violated the proportional hazard assumption, in other words, the effect of IMI at dry-off on the hazard of contracting CM in the following lactation varied between the parity groups, (the hazard did not stay proportional as time went on). Calving season and length of the previous dry period were neither statistically significant nor confounders, so they were dropped from the model. However, the majority of CM cases occurred in cows that calved during summer season (June-August). (Table 5.3).

The hazard of contracting CM during early lactation for cows that were uninfected at dry-off was higher for high-SCC cows than for low-SCC cows in their third and fourth or higher lactation. Second lactation high-SCC cows had significantly higher hazard (1.9 to 2.7 times) of contracting CM than second lactation low-SCC cows (Table 5.4). As previously mentioned, infectious status at dry-off was a time-varying covariate in all of the models. In calculating hazard ratios when there is a time-dependent covariate, a meaningful time period must be chosen to consider the effect of time for appropriate interpretation of the hazard ratios. Using the estimated coefficients from the model with 90 DIM as the time at risk, we considered every 5 days from calving to 90 DIM. The adjusted hazard of contracting CM for high-SCC cows infected at dry-off was higher than the hazard for low-SCC cows without IMI at
dry-off in each parity group. Fourth or higher lactation high-SCC cows had the highest hazard of contracting CM (Figure 5.3).

5.4.1.3. Frailty effect

The shared frailty (i.e., herd) was highly significant in all models (P<0.000) and herd was a significant confounder of the effect of SCC in the hazard of contracting CM. Modeling without the shared frailty induced a change in the estimated coefficient for high-SCC cows of 59, 50, 46, and 23% for the 15, 30, 60, and 90 DIM, respectively.

5.4.2. Low-SCC cows data set

5.4.2.1. Intramammary infections at dry-off and at calving

Among the low-SCC cows, 212 were untreated (49.07%) and 220 received DCT at dry-off (50.93%). There was no difference in the prevalence of IMI at dry-off between experimental groups (Fisher’s exact P=0.206). Untreated low-SCC cows had IMI prevalence of 19.8% at dry-off (95% CI: 14.7-25.8) and treated low-SCC cows had 25.0% (95% CI: 19.4-31.3). At dry-off most of the quarter samples were negative (81.1%), indicating that the classification based on SCC and CM history quite accurately identified uninfected cows. Of the samples collected at dry-off 1.1% were contaminated. Of the positive samples 72.0% were CNS, 7.7% enterococci, 1.6% S. aureus, 4.8% streptococci, 2.3% coliforms, and 11.6% of other pathogens.

Prevalence of infection at calving was 57.1% (95% CI: 49.9-64.1) and 52.1% (95% CI: 44.8-59.3) for untreated and treated cows, respectively (P=0.36). From quarter milk samples, 58.6% were negative and 3.7% were contaminated. CNS were the most common pathogens isolated (65.6%), followed by enterococci (9.5%), streptococci (8.2%), coliforms (7.8%), and S. aureus (3.2%).
5.4.2.2. Clinical mastitis

Mean times to the first case of CM for each time at risk are shown in Table 5.2. Overall, 101 low-SCC cows experienced a clinical case of mastitis during the first 90 days of lactation (23.4%) while 331 cows were censored (76.6%). Of the untreated and treated cows 12.3 and 11.1% had a case of CM, respectively (P=0.495). The majority of the cases occurred during the first two weeks of lactation (62.4%), and more than 90% of the cases occurred in cows that calved during the months of March throughout August (i.e., spring and summer).

Even though there was no difference among treatment groups (P=0.5770), the adjusted plots of the survival functions and cumulative hazards for 15 days (Figure 5.4) suggested that low-SCC cows that received DCT contracted CM earlier than those that did not receive DCT. The same pattern was observed for 30, 60 and 90 DIM (graphs not shown).

The effect of treatment was the main interest of the analysis when modeling the hazard of contracting CM during early lactation. The presence of an IMI at dry-off, milk yield at dry-off, parity, and the interaction between treatment and lactation number were important risk factors for the hazard of contracting CM during early lactation (Table 5.5). Calving season and length of previous dry period were neither significant nor confounders of the effect of DCT over the hazard of contracting CM during early lactation, so they were not included in the final model.

The effect of DCT on the hazard of contracting CM for low-SCC cows depended on the parity of the cow. The hazard of contracting CM was 89, 75, 44, and 43% higher for treated second lactation low-SCC cows than the hazard for untreated second lactation low-SCC during the first 15, 30, 60, and 90 DIM, respectively,
adjusted for the presence of IMI and milk yield at dry-off. Third and fourth or higher lactation low-SCC cows that received DCT had lower hazard of contracting CM than their untreated counterparts during each of the periods at risk analyzed (Table 5.6).

No violations of the assumption of proportional hazards were observed in the low-SCC data. The assumption of non-informative censoring was fulfilled in both data sets.

5.4.2.3. Shared frailty

The shared frailty on herd level was highly significant for the models of 15 and 30 DIM. For the models for 60 and 90 DIM the frailty was significant (P<0.05). Herd was an important confounder of the effect of DCT in the hazard of contracting CM during the first 15 and 30 DIM. If the effect of herd was not adjusted in the models a change in the coefficient for DCT of 12, 11, 5, and 3% was observed for the first 15, 30, 60, and 90 DIM.

5.4.2.4. Clinical mastitis culture results

Even though each farm manager was encouraged to collect quarter milk samples from all clinical cases, out of 229 cows with CM cases milk samples were collected from 134 cows. There were 12 cows with more than one quarter affected. Negative results were obtained from 25.1% of the samples and 7.2% of the samples were contaminated. Among the positive samples, 39.8% were coliforms, 15.0% streptococci, 16.0% CNS, 12.4% enterococci, 6.2% *S. aureus*, and 10.6% of other pathogens.
5.5. Discussion

5.5.1. Prevalence of infection at calving

In the present study, the most common pathogens isolated either at dry-off or at calving were CNS (>60%) in agreement with previous reports [21, 38, 52]. *S. aureus*, a major contagious pathogen, was isolated in a low proportion of the samples at both time points, however, twice as often at dry-off than at calving.

Response to treatment of *S. aureus* during lactation have been reported to be low and ranged from 9 to 75% [20, 37, 50], explaining in part the higher prevalence at dry-off than at calving. Also, cure rates after DCT ranged from 35 to 90% [4, 5, 7, 10, 16, 21, 23, 25, 30, 39, 47, 49], generally higher than those reported during lactation, but they can decrease with increasing age of the cow, number of infected quarters, duration of infection, level of SCC before treatment, presence of CM on previous lactation, and presence of *S. aureus* strains resistant to penicillin [2, 8, 10, 34, 35, 46, 49].

The goal of DCT is to cure existing IMI at the end of lactation, with contagious pathogens as the main target of the therapy, as well as to prevent the development of new IMI over the dry period [6, 28, 42]. Thus, for farms that apply total DCT, a lower prevalence of *S. aureus* during early lactation can be expected. The likelihood of infection of cows may increase toward the end of lactation, if the pathogen is present on the farm and there are failures in the milking routine or milking equipment function that allows transmission of the agent from cow to cow during milking. This is in contrast with experience from Norway, where cows had the highest prevalence of *S. aureus* during the first 74 DIM and the lowest toward the end of lactation [36], which can be due partly to differences in management practices. The
proportion of samples with coliforms was four times higher at calving than at dry-off. These findings are consistent with increased susceptibility of the mammary gland to coliform during the late dry period and during early lactation [32, 48].

A common concern about SDCT is the lack of prevention of new IMI during the dry period [14]. Results from this study found no difference in the prevalence of infection at calving among low-SCC treatment groups, suggesting that DCT did not effectively cure (and/or prevent) infections during the dry period. This may be due to new IMI’s acquired at the end of the dry period when DCT offers no protection at all against infections.

5.5.2. Clinical mastitis during early lactation

Overall incidence of CM observed during early lactation in the present study was comparable to those reported by MacMillan et al (1983), Schukken et al. (1989), Browning et al. (1990), Berry et al (1997), and Østerås (2002), but higher that those reported by Eberhart and Buckalew (1977) and Miltenburg et al. (1996). Clinical mastitis in dairy cows is generally higher during early lactation, with the majority of cases occurring during the first month of lactation [9, 15, 43] and even during the first week post-partum [9, 40, 56]. Results from South-East Queensland, the Netherlands, The United Kingdom, and Norway showed that 21, 25, 31, and 48% of CM cases occurred during the first month of lactation, respectively [9, 29, 36, 55]. Also, studies from US reported that 54.4% of the cases occurred during the first 15 days [15] and 55-67% during the first 30 DIM [13, 15]. Our results, in both data sets, are in agreement with these studies with most of the clinical cases (>65%) occurring during the first month of lactation and with the vast majority taking place during the first 15 DIM (>55%).
Hogan et al (1989) reported that herds with low SCC had the highest rate of CM during early lactation, with 20% of the cases occurring during the first week post-partum and 31% of the cases occurring during the first month of lactation. Moreover, Erskine et al (1988) found that herds with low SCC had a higher incidence of clinical mastitis than herds with high SCC, and that the highest incidence of CM in low SCC herds (16.2%) occurred during the first 30 DIM [17]. Even though direct comparison between studies is difficult due to differences in study design, both low and high-SCC cows in the present study also had the majority of CM cases during the first 30 DIM. The incidence of CM observed in low-SSC cows (11.6%) was similar to the 12.3% reported by Browning et al (1990) during the first 90 DIM and the 9% reported by Daniel et al (1982) for the same period. When only cases from the first month of lactation are considered the incidence of CM in low-SCC cows was similar to the 16.2% reported by Erskine et al (1988) during the first 30 DIM.

Rajala et al (1998) and Rajala-Schultz et al (1999) reported that the first case of CM occurred on average at 44 to 47 days after calving when data from complete lactations were analyzed. Similarly, Dohoo et al (1982) found a median time to first case of CM of 54 days for cows that required local treatment and 22 days for those cows requiring systemic therapy during early lactation. In our study, mean time to first case of CM was around 22 days in both data sets for the first 90 DIM with the treated low-SCC cows experiencing CM earlier in lactation than the rest of the cows.

Most of the previous studies on CM have reported number of incident cases per 100 cows [44], incidence rate [15, 22, 31], or lactational incidence risk or rate [11, 12, 40, 41]. Relative risk or hazard ratios of clinical mastitis from Cox’s regression analysis have been reported by Østerås and Sandvik (1996), Green et at
Survival analysis (Cox’s proportional hazard models) offers the opportunity of adjusting for the presence of potential confounders, allowing comparison of the survival times to be less biased than a simple comparison of survival times between groups [26].

With shared frailty in the Cox’s proportional hazard model, it is possible to account for the dependence among cows within a herd and to account for unmeasured or ‘latent’ herd effects [27]. In this study, herd as a random effect (i.e., shared frailty) in the Cox’s proportional hazard models was significant in all risk periods evaluated in both data sets. Latent unmeasured herd effects (e.g., hygiene in calving areas, nutrition, bedding, milking routine, and grouping of cows) were highly significant in the complete data set. From the change induced in the models once the frailty was dropped, it appeared that the effect of herd in the hazard of CM is most important during the first month for low and high-SCC cows. However, for high-SCC cows the impact of herd factors remained important confounders until the end of the third month. Improvement in herd management will have the highest impact during the first month of lactation, and it is crucial to maintain optimal management of the herd not to increase the hazard of CM in cows with high-SCC. Similarly, Whist et al (2006) reported a significant effect of herd when it was modeled as shared frailty.

Caution must be taken when interpreting the results from the models because the estimated coefficients can only be used to obtain hazard ratios conditioned on the same level of frailty [27], or farms with similar characteristics.

Diagnosis of CM was based on standard operative procedures in place on each farm and differences in detection accuracy and intensity can be expected between farms. However, we do not expect any differential reporting of cases between the
study groups because the treatment assignment of the study cows (i.e., untreated and treated) was unknown to farm employees.

The effect of DCT, presence of IMI at dry-off, parity, and milk yield at dry-off were all significant variables when modeling the hazard of contracting CM. Even though, in previous studies, season has been found to be an important risk factor for CM with an increase in cases of CM during early summer and a decrease during fall [22, 45], in the present study season was not significantly associated with the hazard of CM. A possible explanation is the lack of statistical power to detect the association between season and hazard of contracting clinical mastitis. However, most of the cases of CM in the complete data set occurred in cows that calved during the summer as has been observed previously [22, 45]. During the modeling process in data analysis, season was considered first as a categorical variable, but models were unstable due to sparse data, with most of the CM cases occurring during spring and summer. For this reason, transformation of the day of calving was performed to adjust for the effect of season as previously described by Schukken et al. (1992) and Østerås et al. (2006). The length of the dry period was not a significant risk factor or confounder of the effect of SCC or DCT over the hazard of contracting CM.

An increased occurrence of CM in older cows as compared to second lactation cows have been reported by Barkema et al (1998), Sargeant et al (1998) and Østerås (2002). Østerås and Sandvik (1996) and Whist et al (2006) also found the highest hazard of contracting CM in cows with three or more lactations using Cox’s proportional hazard models. A similar association was observed in the present study, for cows that were infected at dry-off and have high SCC. Fourth or higher lactation high-SCC cows with IMI at dry-off had a higher hazard of CM than low-SCC cows.
uninfected at dry-off with fourth or more lactations. High-SCC cows with four or more lactations uninfected at dry-off also had an increased hazard of CM, as compared to uninfected low-SCC in the same parity group but it was not statistically significant (P>0.05). Even though, we did not calculate cure rates in the present study it could be expected that old cows infected at dry-off and with high SCC at the end of lactation will be less likely to clear the infection and have an increase hazard of contracting CM after calving as have been described previously [8, 46, 49].

Hogan et al. (1989) in a field survey of CM incidence in low SCC farms found higher rates of CM cases among second lactation cows than older cows. This was similar to results from this study, where second lactation low-SCC cows that were uninfected at dry-off and received DCT had a higher hazard of contracting CM during early lactation than untreated second lactation low-SCC cows. Furthermore, second lactation high-SCC cows without IMI at dry-off had a significantly higher hazard (P<0.05) of contracting CM than uninfected second lactation low-SCC cows adjusted for treatment, in agreement with results reported by Daniel et al. (1982). Previous studies have reported an increase in the incidence of mastitis after DCT in herds with low SCC [7, 28]. The result observed for low-SCC second lactation cows agrees with results of Browning et al (1990), who found that cows that were uninfected at the end of the previous lactation and received DCT had more CM cases than untreated uninfected cows, but no information is available in that study regarding the distribution of parity in the study groups to establish a direct comparison.

In summary, cows that had elevated SCC at the end of previous lactation had an increase hazard of contracting CM than cows with low SCC in the first 90 DIM of
the following lactation. This is in agreement with the findings of Rupp et al (2000) that lower level of SCC did not increase the risk of CM.

5.5.3. Clinical mastitis culture results

In the current study, collection of milk samples from CM cases was dependent mainly on the herd managers. Even though farms were visited weekly and personnel reminded constantly about the importance of sample collection from all clinical cases and despite the fact that results were faxed or e-mailed to the farm managers within 72 hours after submission, not all cases were sampled. It is, however, important to point out that all of the farms kept good CM records and we believe most cases were recorded even if we did not receive milk samples from them.

Coliforms were the most common pathogens isolated from CM cases as has been previously reported for herds with low SCC [17, 22, 44, 51], as well as Wilesmith et al (1986) and Miltenburg et al (1996) from herds with different levels of SCC.

The presence of pathogens ubiquitous in the environment or skin flora in the majority of the samples with a low proportion of samples with major contagious pathogens is characteristic of farms where mastitis control programs with routine use of blanket DCT at the end of lactation is implemented.

5.6. Conclusions

The hazard of contracting CM in the next lactation was associated with the level of SCC observed at the end of previous lactation, DCT, presence of IMI at dry-off, parity, and milk yield at dry-off. Cows that had high SCC during the last trimester of the previous lactation were more likely to contract CM during early lactation than cows with low SCC. The hazard was higher for high-SCC cows with two or four or
more lactations without IMI at dry-off than for low-SCC cows. Presence of IMI at dry-off increased the hazard of contracting CM in four or higher lactation high-SCC cows. Treating all high-SCC cows did not guarantee a reduction in the hazard of contracting clinical mastitis in the following lactation. Parity and presence of IMI must be considered when decisions about DCT are made, older cows with high SCC and infected at dry-off may not benefit from treatment.

The presence of IMI at dry-off did not increase the hazard of CM in low-SCC cows. The effect of DCT on the hazard of contracting CM in low-SCC cows was associated with parity. Second lactation cows that had low SCC at dry-off without IMI had a higher hazard of contracting CM if they received DCT than uninfected cows left without treatment. Low-SCC cows with more than two lactations that received DCT had a lower hazard of contracting CM than untreated cows in the same parity group.

Herd was a significant confounder of the effect of SCC and DCT in the hazard of CM, being most important for cows with high-SCC. Global recommendations, either treating all cows on a herd or selectively treating only infected cows are not warranted. Treating only those cows that have a high likelihood to respond positively to treatment after consideration of farm characteristics is advised.

5.7. References


<table>
<thead>
<tr>
<th></th>
<th>Herd A</th>
<th>Herd B</th>
<th>Herd C</th>
<th>Herd D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cows (Milking cows)</td>
<td>1,139 (1007)</td>
<td>365 (321)</td>
<td>88 (77)</td>
<td>212 (189)</td>
</tr>
<tr>
<td>Daily milk production (lb/cow)</td>
<td>79.8</td>
<td>78.4</td>
<td>68.6</td>
<td>64.0</td>
</tr>
<tr>
<td>Daily bulk tank weight (lb)</td>
<td>82,320</td>
<td>27,279</td>
<td>4,828</td>
<td>11,230</td>
</tr>
<tr>
<td>Average SCC</td>
<td>145,000</td>
<td>340,000</td>
<td>449,000</td>
<td>418,000</td>
</tr>
<tr>
<td>Average SCS (% cows score&lt;4)</td>
<td>2.0 (80)</td>
<td>2.6 (68)</td>
<td>3.3 (56)</td>
<td>3.4 (49)</td>
</tr>
</tbody>
</table>

Table 5.1: Dairy Herd Improvement rolling herd averages of total number of cows, average daily milk production for milking cows, and average SCC and somatic cell score (SCS) of each farm at enrolment in the study.
<table>
<thead>
<tr>
<th>Group</th>
<th>15 DIM(^1)</th>
<th>30 DIM</th>
<th>60 DIM</th>
<th>90 DIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-SCC Cows(^2)</td>
<td>2.3 (1.7-2.9)(^3)</td>
<td>4.4 (2.8-6.1)</td>
<td>12.7 (8.8-16.5)</td>
<td>21.7 (16.2-27.2)</td>
</tr>
<tr>
<td>Untreated</td>
<td>2.6 (1.6-3.7)</td>
<td>4.7 (2.3-7.2)</td>
<td>12.3 (6.9-17.6)</td>
<td>23.4 (15.4-31.3)</td>
</tr>
<tr>
<td>Treated</td>
<td>2.0 (1.4-2.6)</td>
<td>4.1 (2.0-6.3)</td>
<td>13.1 (7.5-18.7)</td>
<td>19.8 (12.3-27.3)</td>
</tr>
<tr>
<td>Number CM</td>
<td>63</td>
<td>70</td>
<td>87</td>
<td>101</td>
</tr>
<tr>
<td>High-SCC Cows(^4)</td>
<td>2.8 (2.2-3.4)</td>
<td>7.6 (5.4-9.7)</td>
<td>15.0 (11.5-18.5)</td>
<td>27.8 (22.7-32.9)</td>
</tr>
<tr>
<td>Number CM</td>
<td>64</td>
<td>81</td>
<td>100</td>
<td>128</td>
</tr>
<tr>
<td>Total Number CM</td>
<td>127</td>
<td>151</td>
<td>187</td>
<td>229</td>
</tr>
</tbody>
</table>

\(^1\)Days in milk  
\(^2\)Cows without CM and <200,000 cells/ml during the last three mo. of previous lactation, or with CM during the first 90 DIM and <100,000 during the rest of lactation  
\(^3\)95 % Confidence Interval  
\(^4\)Cows with \(\geq\)200,000 cells/ml during any of the last three mo. of previous lactation or with CM after 90 DIM

Table 5.2: Mean time to first case of clinical mastitis (CM) and number of first cases during four periods at risk for low and high-SCC cows
<table>
<thead>
<tr>
<th>Variable</th>
<th>15 DIM(^1)</th>
<th>30 DIM</th>
<th>60 DIM</th>
<th>90 DIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-SCC at dry-off(^2)</td>
<td>0.497 (0.24)</td>
<td>0.486 (0.22)</td>
<td>0.333 (0.19)</td>
<td>0.389 (0.0.18)</td>
</tr>
<tr>
<td>Dry cow therapy (DCT)</td>
<td>0.487 (0.42)</td>
<td>0.445 (0.38)</td>
<td>0.298 (0.32)</td>
<td>0.287 (0.30)</td>
</tr>
<tr>
<td>IMI(^4) at dry-off</td>
<td>-0.748 (0.32)</td>
<td>-0.753 (0.29)</td>
<td>-0.712 (0.27)</td>
<td>-0.791 (0.26)</td>
</tr>
<tr>
<td>Milk yield at dry-off(^5)</td>
<td>-0.013 (0.01)</td>
<td>-0.006 (0.01)</td>
<td>-0.002 (0.01)</td>
<td>-0.003 (0.01)</td>
</tr>
<tr>
<td>Lactation Number (Reference: Second)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>0.993 (0.45)</td>
<td>0.854 (0.42)</td>
<td>0.758 (0.38)</td>
<td>-0.882 (0.34)</td>
</tr>
<tr>
<td>Fourth or Higher</td>
<td>1.301 (0.47)</td>
<td>1.043 (0.44)</td>
<td>1.007 (0.39)</td>
<td>1.090 (0.36)</td>
</tr>
<tr>
<td>Interaction DCT*Lactation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCT*Third</td>
<td>-1.128 (0.53)</td>
<td>-1.113 (0.49)</td>
<td>-0.737 (0.38)</td>
<td>-0.862 (0.34)</td>
</tr>
<tr>
<td>DCT*Fourth or higher</td>
<td>-0.759 (0.52)</td>
<td>-0.587 (0.48)</td>
<td>-0.411 (0.42)</td>
<td>-0.506 (0.39)</td>
</tr>
<tr>
<td>IMI at dry-off*log DIM</td>
<td>0.533 (0.27)</td>
<td>0.409 (0.16)</td>
<td>0.308 (0.11)</td>
<td>0.329 (0.09)</td>
</tr>
<tr>
<td>Theta(^6) (Standard error)</td>
<td>0.669 (0.48)</td>
<td>0.499 (0.36)</td>
<td>0.274 (0.21)</td>
<td>0.183 (0.15)</td>
</tr>
</tbody>
</table>

\(^1\)Days in milk
\(^2\)Cows with ≥200,000 cells/ml during any of the last three mo. of previous lactation or with CM after 90 DIM. The reference group were cows with <200,000 cells/ml (low-SCC cows)
\(^3\)Standard error
\(^4\)Intramammary infection
\(^5\)Milk yield was centered at 19.8 kg
\(^6\)Variance of the shared frailties (P<0.000)

Table 5.3: Estimated coefficients and standard errors for the hazard of contracting clinical mastitis (CM) during four periods at risk for high-SCC cows
<table>
<thead>
<tr>
<th>Lactation</th>
<th>15 DIM&lt;sup&gt;4&lt;/sup&gt;</th>
<th>30 DIM</th>
<th>60 DIM</th>
<th>90 DIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second</td>
<td>2.68 (1.18, 6.05)&lt;sup&gt;5&lt;/sup&gt;</td>
<td>2.54 (1.22, 5.27)</td>
<td>1.89 (1.01, 3.50)</td>
<td>1.97 (1.11, 3.49)</td>
</tr>
<tr>
<td>Third</td>
<td>0.87 (0.42, 1.81)</td>
<td>0.83 (0.41, 1.67)</td>
<td>0.90 (0.51, 1.59)</td>
<td>0.83 (0.49, 1.39)</td>
</tr>
<tr>
<td>≥ Fourth</td>
<td>1.25 (0.65, 2.43)</td>
<td>1.41 (0.74, 2.69)</td>
<td>1.25 (0.69, 2.23)</td>
<td>1.19 (0.69, 2.01)</td>
</tr>
</tbody>
</table>

<sup>1</sup>Based on the model in Table 5.3  
<sup>2</sup>Cows with ≥200,000 cells/ml during any of the last three mo. of previous lactation or with CM after 90 days in milk  
<sup>3</sup>Cows without CM and with <200,000 cells/ml or with CM during the first 90 days in milk and <100,000 cells during the rest of lactation  
<sup>4</sup>Days in milk  
<sup>5</sup>95 % Confidence Interval

Table 5.4: Estimated hazard ratios and 95% confidence intervals for the hazard of contracting clinical mastitis (CM) during four periods at risk for high-SCC<sup>2</sup> cows without intramammary infections (IMI) at dry-off as compared to low-SCC<sup>3</sup> cows without IMI at dry-off, adjusted for the effect of dry cow therapy and milk yield at dry-off.
Table 5.5: Estimated coefficients and standard errors for the risk of contracting clinical mastitis (CM) during four periods at risk for low-SCC\(^1\) cows randomly allocated either to receive or not to receive dry-cow therapy (DCT) at dry-off\(^2\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>15 DIM(^3)</th>
<th>30 DIM</th>
<th>60 DIM</th>
<th>90 DIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCT</td>
<td>0.638 (0.43)</td>
<td>0.557 (0.39)</td>
<td>0.368 (0.33)</td>
<td>0.355 (0.31)</td>
</tr>
<tr>
<td>IMI(^4) at dry-off</td>
<td>-0.512 (0.41)</td>
<td>-0.552 (0.39)</td>
<td>-0.419 (0.32)</td>
<td>-0.254 (0.27)</td>
</tr>
<tr>
<td>Milk yield at dry-off(^5)</td>
<td>-0.022 (0.01)</td>
<td>-0.018 (0.01)</td>
<td>-0.019 (0.01)</td>
<td>-0.016 (0.01)</td>
</tr>
<tr>
<td>Lactation Number (Reference: Second)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>1.003 (0.45)</td>
<td>0.877 (0.42)</td>
<td>0.818 (0.38)</td>
<td>0.934 (0.34)</td>
</tr>
<tr>
<td>Fourth or Higher</td>
<td>1.362 (0.47)</td>
<td>1.122 (0.44)</td>
<td>1.130 (0.39)</td>
<td>1.176 (0.36)</td>
</tr>
<tr>
<td>Interaction DCT*Lactation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCT*Third</td>
<td>-1.798 (0.72)</td>
<td>-1.415 (0.63)</td>
<td>-0.799 (0.38)</td>
<td>-0.914 (0.35)</td>
</tr>
<tr>
<td>DCT*Fourth or higher</td>
<td>-0.898 (0.62)</td>
<td>-0.803 (0.59)</td>
<td>-0.666 (0.52)</td>
<td>-0.744 (0.49)</td>
</tr>
<tr>
<td>Theta(^6) (Standard Error)</td>
<td>1.066 (0.98)</td>
<td>0.535 (0.46)</td>
<td>0.181 (0.18)</td>
<td>0.075 (0.09)</td>
</tr>
</tbody>
</table>

\(^1\)Cows without clinical mastitis and <200,000 cells/ml during the last three mo. of previous lactation or with CM during the first 90 days in milk and <100,000 cells/ml during the rest of lactation. Reference group were untreated low-SCC cows

\(^2\)Days in milk

\(^3\)Standard error

\(^4\)Intramammary infections

\(^5\)Milk yield was centered at 21.9 kg

\(^6\)Variance of the shared frailties (P<0.05)
<table>
<thead>
<tr>
<th>Lactation</th>
<th>15 DIM(^3)</th>
<th>30 DIM</th>
<th>60 DIM</th>
<th>90 DIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second</td>
<td>1.89 (0.81,4.44)(^4)</td>
<td>1.75 (0.80,3.79)</td>
<td>1.44 (0.75,2.77)</td>
<td>1.43 (0.78,2.62)</td>
</tr>
<tr>
<td>Third</td>
<td>0.31 (0.10,0.96)</td>
<td>0.42 (0.16,1.10)</td>
<td>0.65 (0.35,1.19)</td>
<td>0.57 (0.33,0.99)</td>
</tr>
<tr>
<td>Fourth</td>
<td>0.77 (0.33,1.81)</td>
<td>0.78 (0.33,1.84)</td>
<td>0.74 (0.34,1.61)</td>
<td>0.68 (0.33,1.40)</td>
</tr>
</tbody>
</table>

\(^1\)Based on the model in Table 5.5  
\(^2\)Cows without clinical mastitis and <200,000 cells/ml during the last three mo. of previous lactation or with CM during the first 90 days in milk and <100,000 cells/ml for the rest of the lactation. Reference group were untreated low-SCC cows in the same parity group  
\(^3\)Days in milk  
\(^4\)95 % Confidence Interval

Table 5.6: Estimated hazard ratios\(^1\) and 95% confidence intervals, for the risk of contracting clinical mastitis during four periods at risk for low-SCC\(^2\) cows randomly allocated either to receive or not to receive dry-cow therapy (DCT) at dry-off by lactation number, adjusted for the effect of intramammary infections and milk yield at dry-off
Figure 5.1: Distribution of pathogens isolated from quarter milk samples at dry-off from low and high-SCC cows
Figure 5.2: Kaplan-Meier Survival Curves (A) and Cumulative Hazards (B) for low and high-SCC cows during the first 15 days of lactation
Figure 5.3: Estimated hazard ratios (HR) for contracting clinical mastitis during the first 90 days of lactation for high-SCC cows with intramammary infections (IMI) at dry-off as compared with low-SCC cows without IMI at dry-off by lactation number. Adjusted for the effect of dry cow therapy and milk yield at dry-off
Figure 5.4: Kaplan-Meier Survival Curves (A) and Cumulative Hazards (B) for low-SCC cows randomly allocated either to receive or not to receive dry-cow therapy at dry-off during the first 15 days of lactation
CHAPTER 6

SELECTIVE DRY COW THERAPY AND MILK YIELD
DURING THE FOLLOWING LACTATION

6.1. Abstract

The objective of the present study was to evaluate the effect of SDCT on daily milk yield during the subsequent lactation after treatment. Cows with somatic cell counts (SCC) less than 200,000 cells/ml during the last 3 months of lactation and no history of clinical mastitis (CM) on the current lactation were considered uninfected (low-SCC cows) and randomly allocated either to receive or not to receive treatment at dry-off; others were considered high-SCC cows and were treated. Daily milk yields were compared using repeated measures analysis (PROC MIXED) in SAS® (SAS Institute Inc., Cary, NC). The regression analysis accounted for non-independence by using an autoregressive correlation structure for measurements obtained on the same cow over time.

Data from a total of 762 Holstein cows in four Ohio dairy herds were included in the analyses. Daily milk yield was significantly associated with somatic cell scores (SCS), parity, occurrence of CM, length of the dry period, and actual 305-d milk yield in previous lactation. There was no statistical difference in milk yield between low
and high-SCC cows, but the model suggested that having high SCC at dry-off reduced milk yield in the subsequent lactation. An increase of SCS of one point above 2.8 reduced daily milk yield by 0.6 kg/day (P<0.0001). Occurrence of CM during early lactation significantly reduced milk production (P=0.0109), and cows with three or more lactation lost more milk than second lactation cows (P<0.0001).

Among low-SCC cows, there was no association between intramammary infections at dry-off and milk yield. Increased SCS above 2.2 reduced milk yield by 0.6 kg/day. There were no statistical differences in milk yield between untreated and treated low-SCC cows, but the effect of DCT on milk yield varied with farm.

6.2. Introduction

Inflammation of the mammary gland is the most costly disease in dairy herds worldwide [12, 26, 36]. Mastitis control programs include treatment of cows at dry-off with antimicrobials to eliminate existing and to prevent new intramammary infections at the end of lactation (i.e., dry cow therapy, DCT) [4, 20, 29]. The use of blanket therapy, where all quarters of all cows receive antibiotic DCT is a common practice in more than 75% of U.S. herds [33]. However, selective dry cow therapy (SDCT) has recently received more attention due to growing concern over the use of antimicrobials in animal production and the possible development of antimicrobial resistance in bacterial populations and the potential threat this represents for public health [2, 16, 34]. The use of DCT only in cows suspected to be infected at the end of lactation offers an opportunity to reduce the use of antimicrobials in dairy operations.

The general concern about SDCT is that leaving cows without treatment is detrimental for the health of the udder once the prophylactic effect of DCT is lost and subsequent milk production losses during the following lactation. For a switch from
total to selective DCT to be economically feasible, this alternative needs to maintain similar udder health as the traditional practice and have no negative effect on milk production in the following lactation.

The objectives of the present study were to: (1) evaluate the effect of dry cow therapy in daily milk yield in selectively treated cows; and (2) compare daily milk yield of low and high-SCC cows during the subsequent lactation. We hypothesized that there is no difference in milk yield during the next lactation between treated and untreated cows with low-SCC at dry-off and that high-SCC cows have lower milk yield than low-SCC cows.

6.3. Materials and methods

6.3.1. Study Population

Holstein cows from two commercial and two institutional dairy herds in Ohio were classified as uninfected or infected based on SCC during the last three months of lactation and clinical mastitis (CM) history during the current lactation. Cows without CM and with SCC < 200,000 cells/ml during the last 3 months of lactation were considered uninfected (low-SCC cows). If a cow had experienced a case of CM during the first 90 days of the current lactation, but her SCC was < 100,000 cells/ml for the rest of the lactation, she was also considered uninfected. All other cows were assumed infected (high-SCC cows). Somatic cell counts from the current lactation of every cow in each farm were obtained electronically from PCDART (Herd Manager © 2005 Dairy Records Management Systems, Raleigh, NC) and individual CM records were collected on each farm for two complete calendar years, before the beginning of the study, and the data were entered into electronic spreadsheets
(Microsoft Office Excel 2003). After calving, cows were followed until the end of lactation (dry-off) or culling.

6.3.2. Treatment allocation

Cows that were assumed uninfected were randomly allocated to either receive treatment or not to receive treatment at dry-off; all cows considered infected based on their SCC and CM history were treated. No control (i.e., untreated) group was assigned for the infected cows for ethical reasons. If a cow had CM on the day of dry-off she was excluded from the study. The list of treatment allocation was only available to the investigators (AT and PR-S) while the personnel on the farms were blinded with regards to the treatment status of the cows. Commercially licensed products containing benzathine cloxacillin or cephapirin benzathine were used as DCT.

6.3.3. Milk sampling

Two sets of quarter milk samples per cow were collected at dry-off and within the first five days after calving. Single samples were collected from quarters with CM before administration of antimicrobial treatment. Samples were kept at -20°C until transportation to the laboratory at weekly intervals. All milk samples were examined microbiologically according to National Mastitis Council guidelines [22]. Sampling at dry-off was performed by the researchers, while samples at calving and from CM were collected by either the investigators or the farm personnel. Farms were visited once a week to collect milk samples, to update CM records, and to evaluate the general conditions of the dry cows. Instructions for sample collection were given before the beginning of the study and a written protocol was kept on each farm.
6.3.4. Definition of intramammary infections (IMI)

A quarter was considered infected if $\geq 100$ colony forming units (cfu)/ml of contagious major pathogens ($S.\ aureus$ and $Str.\ agalactiae$) or $\geq 500$ cfu/ml of all other pathogens were isolated (coagulase negative staphylococci (CNS), Corynebacterium spp., Archanobacterium pyogenes, Bacillus spp., coliforms ($Escherichia\ coli$, Enterobacter spp., and Klebsiella spp.), yeast and other Gram-negative rods) based on microbiological results from single samples. A cow was considered infected when at least one quarter was positive. Samples with more than two types of colonies were considered contaminated. If the first set of quarter milk samples was contaminated (at dry-off and at calving), the second set of samples was used to assess the prevalence of IMI. Cows with two or more contaminated quarter samples were dropped from the analysis.

6.3.5. Clinical mastitis

The presence of abnormal milk or udder inflammation, with or without systemic symptoms, was considered a case of CM following the standard operating procedures of each farm. Only the first case of CM during the first 90 DIM was considered for analysis.

6.3.6. Milk yield

Monthly milk yield, SCC, somatic cell scores (SCS), and actual 305-d milk yield from previous lactation (AMP305PL) for every cow on each farm were obtained electronically from PCDART (Herd Manager © 2005 Dairy Records Management Systems, Raleigh, NC) approximately every 30 days. The lactation was divided into stages (19 stages) as previously described by Rajala-Schultz et al (1999), with the following modification: milk records taken from 61 DIM until 320 DIM were
grouped into 20-d intervals. Only milk yields until 320 DIM were considered for analysis.

6.3.7. Statistical analysis

6.3.7.1. Complete data set

The main interest of the analysis was to compare milk yield during the following lactation after treatment allocation between low and high-SCC cows at cow level. Milk yields were modeled using repeated measures analysis (PROC MIXED) in SAS® 9.1 (SAS Institute Inc., Cary, NC, USA), accounting for the correlated data according to the following linear model [18, 19]:

\[ y = X\beta + Zu + e \]  

Where:

- \( y \) is the vector of observed milk yields,
- \( X \) is the known matrix of covariates,
- \( \beta \) is the unknown fixed effects parameter vector,
- \( Z \) is the known design matrix,
- \( u \) is the vector of unknown random herd effect parameters assumed to be independent and identically distributed (i.e., i.i.d) \( N(0, \sigma_h^2) \), and
- \( e \) is the unobserved vector of Gaussian random errors associated with the \( j \)th cow assumed to be i.i.d. \( N(0, \sigma^2) \). To account for non-independence of observations obtained on the same cow over time, four correlation structures were tested (compound symmetry, variance components, first order autoregressive, and unstructured).

Independent variables considered during analysis were classification status of the cows based on SCC of the previous lactation and CM history (i.e., low and high-SCC cows), DCT, presence of IMI at dry-off, SCS, lactation number, CM during the current lactation, previous days dry (i.e., length of dry period, PRVDD), AMP305PL, calving season, and stage of lactation.
Presence of IMI at dry-off was a dichotomous variable based on microbiological culture results from single quarter milk samples collected at dry-off (Infected=1; Uninfected=0). Lactation was categorized in two groups (second and third or higher). An indicator variable for CM with 5 levels was created: 1= CM occurring 1-15 days, 2= CM occurring 16-30 days, 3= CM occurring 31-60 days, 4= CM occurring 61-90 days, and 5= No CM until the end of follow-up (reference group). All continuous variables were centered at the median of the data: SCS at 2.8, PRVDD at 57 days, and AMP305PL at 10,556 kg (scaled to reflect a 1,000-kg change). Herd was included in the model as a random effect.

To model the effect of calving season on milk yield, the day of calving was transformed into two variables, sine(calving day) and cosine(calving day), following the method used by Schukken et al. (1992) and Østerås et al. (2006):

\[
\text{Sine (calving day)} = \sin [2 \times \pi \times (\text{calving day}/365)]
\]

\[
\text{Cosine (calving day)} = \cos [2 \times \pi \times (\text{calving day}/365)]
\]

6.3.7.2. Low-SCC data set

The same analyses were performed using data from low-SCC cows only and the main interest was to compare the effect of treatment (i.e., DCT) on milk yield during the following lactation. Continuous variables were centered at the median of the low-SCC data: SCS at 2.2, PRVDD at 57 days, and AMP305PL was centered at 10,709 kg (scaled to reflect a 1,000-kg change).

6.3.7.3 Modeling procedure

Initially, the correlation structure that best fit the data was chosen based on the Akaike’s information criterion (AIC) and Schwarz’ Bayesian criterion (BIC). A
saturated model with all independent variables was run with each of the covariance structures. The model with the correlation structure with the lowest AIC and BIC was selected. Next, univariable models were built for each independent variable against the outcome. Variables with p-value $\leq 0.25$ from the Wald $\chi^2$ test or likelihood ratio test (LRT) were included in the initial multivariable model. The risk factor of interest (i.e., low and high-SCC classification for the complete data set and DCT for the low-SCC data set) was forced in the models. Backward selection procedure was used to run a sequence of models adjusted for the independent variables previously selected, dropping each variable individually. Variables with significant LRT ($p<0.05$) were kept in the model.

To evaluate whether a variable was a confounder, the change in the coefficient of the risk factor of interest was calculated after the variable had been dropped from the model. Any variable that induced a change $\geq 10\%$ was kept in the model as a potential confounder. After a model where all independent variables were significant was found, biologically plausible two-way interactions were tested. These were: 1) classification status x presence of IMI at dry-off; 2) classification status x parity; 3) Presence of IMI at dry-off x parity; 4) DCT x presence of IMI at dry-off; and 5) DCT x parity. Interaction terms were kept in the model if they were significant (LRT: $P<0.05$). The AIC of the model with the interaction term were also evaluated to assess the fit of the model.

6.4. Results

6.4.1. Complete Data-Set

Data from a total of 394 low and 368 high-SCC cows were used in the analysis. Four high-SCC cows (0.5\%) were dropped from the analysis because both
sets of samples collected at dry-off were contaminated. Additionally, 16 low and 30 high-SCC cows were dropped from the analysis because of missing test-day data. A total of 6,121 observations from 378 low and 334 high-SCC cows were available for analysis. On average, cows had information from five test-days (ranging from 1 to 11).

Second lactation cows represented 56.8% of the data, with 43.2% of cows with three or more lactations (up to 11). The distribution of calvings was uniform around the year with 20, 24, 34, and 22% of cows calving during December-February, March-May, June-August, and September-November, respectively. The prevalence of IMI at dry-off for low and high-SCC cows was 22.5% (95% CI: 18.6-26.7) and 48.7% (95% CI: 43.9-53.5), respectively (Fisher’s exact P=0.000). Prevalence of infection at calving was 54.6% (95% C.I.: 49.5-59.6) and 54.8% (95% C.I.: 49.4-60.0) for low and high-SCC cows, respectively (Fisher’s exact P=1.00).

The percentage of cows that had a case of CM during the first 90 DIM was 26.8% (n=220), with 12.1% of cases in low and 14.7% in high-SCC cows. The majority of the cases occurred during the first month after calving; total distribution of cases during the first 90 DIM is shown in Table 6.1.

6.4.1.2. Milk yields in low and high-SCC cows

Averages and standard deviations for daily milk yields and SCC of current lactation, PRVDD and AMP305PL are shown in Table 6.2. On average cows produced 37.6 kg of milk/test-day (±10.1), with a minimum of 1.3 kg and a maximum of 79.4 kg per test-day. The overall median of SCC was 81,000 cells/ml, with 57,000 and 123,000 cells/ml for low and high-SCC cows, respectively. Overall SCS average was 3.01 (± 2.4) with a median of 2.8. The median of days dry was 57, with a
minimum of 15 and maximum of 180. Low-SCC cows had significantly higher AMP305PL than high-SCC cows (P=0.0026).

The first order autoregressive correlation structure fit the data best. Results from the mixed model are presented in Table 6.3.

The adjusted estimated average of daily milk yield was 27.6 kg. The difference in the milk yield between low and high-SCC cows was not statistically significant (P=0.9742). However, high-SCC cows had higher milk production during the first 10 DIM than low-SCC cows, but with an estimated loss of 0.7 kg of milk/day over the entire lactation (Figure 6.1). The effects of DCT, IMI at dry-off, and PRVDD were not statistically significant, but were important confounders of the effect of previous lactation SCC on milk yield.

There was a highly significant reduction of milk yield of 0.6 kg/day for every one unit increase of SCS above 2.8 (P<0.0001). Parity was also significantly associated with daily milk yield, cows in their third or higher lactation produced less milk than second lactation cows (P<0.0001).

Cows that experienced a case of CM produced less milk than cows without CM during early lactation. The highest losses were for cows with CM between 16-30 DIM, with 3.5 kg less milk than healthy cows (P=0.0469). Thus, assuming a lactation of 305 d, the estimated total lost of milk, if a CM case occurred between 16 and 30 DIM, would be between 962 and 1,011 kg. Stratification of the data by parity revealed that older cows lost significantly more milk, than cows with two lactations, during the first month of lactation, for cases that occurred during 1 to15 DIM and 16 to 30 DIM (data not shown, P<0.05).
Actual 305-d milk production from the previous lactation was significantly associated with daily milk yield in the subsequent lactation: for every 1,000 kg produced above 10,556 kg in the previous lactation, the average daily milk yield over the next lactation increased by 1.8 kg of milk/test day (P= <0.0001).

6.4.2. Low-SCC cows data set

Among the low-SCC cows, 180 were untreated (47.6%) and 198 received DCT at dry-off (52.4%). There was no difference in the prevalence of IMI at dry-off between experimental groups (Fisher’s exact P=0.206). Untreated low-SCC cows had IMI prevalence of 19.8% at dry-off (95% CI: 14.7-25.8) and treated low-SCC cows had 25.0% (95% CI: 19.4-31.3). At dry-off, most of the quarter samples were negative (81.1%), indicating that the classification based on SCC and CM history accurately identified uninfected cows. Prevalence of infection at calving was 57.1% (95% CI: 49.9-64.1) and 52.1% (95% CI: 44.8-59.3) for untreated and treated cows, respectively (P=0.36).

Total number of cases for untreated and treated low-SCC cows is shown in Table 6.1. Of the untreated and treated cows, 12.5 and 11.3 % had a case of CM, respectively (P=0.789). The majority of the cases occurred during the first two weeks of lactation (61.6%), and more than 90% of the cases occurred in cows that calved during the months of March throughout August (i.e., spring and summer).

6.4.2.1. Milk yields in untreated and treated low-SCC cows

The effect of treatment was the main interest of the analysis when modeling daily milk yield. Somatic cell scores, parity, PRVDD, AMP305PL, and season of calving were significantly associated with milk yield. The presence of an IMI at dry-off was neither statistically significant nor a confounder of the effect of DCT on milk yield.
yield, thus it was not included in the final model. The best correlation structure was first order autoregressive. Results from the mixed model are presented in Table 6.4.

Comparison of baseline data for untreated and treated low-SCC cows did not reveal differences in AMP305PL (P=0.4207). The adjusted estimated average daily milk yield for low-SCC cows was 29.2 kg. There were no statistical differences in milk yield between untreated and treated low-SCC cows (P=0.1506). Both low-SCC groups had similar lactation curves, as can be observed in Figure 6.2. The estimated average effect of DCT suggested that treated cows produced 0.8 kg more milk daily than untreated low-SCC cows. However, when the fitted model was stratified by herd no differences in milk production were observed between treated and untreated cows in two of the herds, in one herd treated low-SCC produced significantly more milk than untreated herd mates, and in one of the herds treated low-SCC produced less milk than untreated low-SCC cows (results not shown).

Milk yield significantly decreased by 0.6 kg for every one unit increase of SCS above 2.2 (P<0.0001). Parity was significantly associated with milk yield, cows in their third or higher lactation produced less milk than second lactation cows (P<0.0001).

Occurrence of CM was not significantly associated with milk yield, however cows that experienced a case of CM during early lactation produced less milk than cows without CM during lactation. The highest losses were for cows with CM between 16 and 30 DIM, with 1.3 kg less milk than healthy cows (P=0.5622). Thus, assuming a lactation of 305-d, the estimated total loss of milk, if a CM case occurred between 16 and 30 DIM, would be between 346.8 and 357.5 kg/lactation. Stratification of data by parity revealed that cows with three or more lactations lost
more milk per day than second lactation cows during the first 15 DIM (data not shown, P=0.05).

For every one-day increase in number of days dry above 57, there was a decrease of 0.04 kg of milk per day (P=0.0328) and for every 1,000 kg produced above 10,794.6 kg in the previous lactation there was an increase of 1.9 kg of milk/test day.

6.5. Discussion

Incidence of clinical mastitis in dairy cows is generally higher during early lactation, with the majority of cases occurring during the first month of lactation [8, 11, 31] and even during the first week post-partum [8, 26, 36]. The percentage of CM that occurs during the first month of lactation has been reported to vary from 21 to 67% [8, 10, 11, 21, 25, 35]. Our results, in both data sets, are in agreement with these studies with most of the clinical cases (60%) occurring during the first month of lactation and with the vast majority taking place during the first 15 DIM (>55%).

Cows with high milk yield have a higher risk of contracting CM and despite the disease, cows with mastitis usually produce more milk than healthy cows [26, 27, 30, 36]. This makes the selection of the reference level for estimating milk losses due to mastitis challenging and requires careful consideration in interpretation of the results. The reference group in the current analyses was cows without clinical mastitis during the complete follow-up, thus our results most likely underestimate the losses in milk yield due to CM. Adjustment of the effect of other diseases also occurring during early lactation (e.g., ketosis, displacement of abomasums, hypocalcaemia) is important to obtain a better estimate of the effect of CM on milk yield. In the present analyses, the effect of other diseases on milk yield can not be ruled out.
The negative effect of CM on milk yield was severe in the complete data set, with significant losses of 1.5 and 3.5 kg/day for cases occurring during the first 2 week and between 16 to 30 days in milk, respectively. It appears that cows that suffered subclinical mastitis at the end of the previous lactation (i.e., high-SCC cows) are more likely to contract CM in early lactation than those cows that were healthy (i.e., low-SCC cows), thus experiencing the higher losses due to the disease.

Milk losses of 75 to 95 kg of milk during the 60-d period after occurrence of CM have been reported for first lactation and older cows, respectively [1]. Rajala-Schultz et al (1999) found that cows with CM before the peak, lost from 1.1 to 2.5 kg of milk during the first 15 days after the onset of the case, depending on the lactation number. Moreover, losses from 15 to 28 days ranged from 0.7 to 2.3 kg/day [26]. Even though our comparison was not to the pre-mastitis milk yield potential of the cows with CM, in the complete data set, cows that experienced a case of CM during the first 15 DIM or between 15 and 30 DIM had losses similar to those reported by Bartlett et al (1991) and Rajala-Schultz (1999). When only low-SCC cows were analyzed, losses during the first 15 DIM and 16-30 DIM were relatively lower than those reported by Rajala-Schultz et al (1999) for cows with three or more lactations.

Increases of SCC during lactation have been reported to be associated with reduction of milk production [9, 13]. A one point increase in SCS has been associated with a loss of 0.7 kg/day of milk yield [28]. In this study, in both data sets, an increase of one unit in SCS above 2.8 (complete data set) or 2.2 (low-SCC data set), significantly reduced daily milk yield approximately 0.6 kg/day (P<0.0001), regardless of DCT in agreement with losses reported by Reneau (1986) and Hortet and Seegers (1998). However, in another study, loss of milk yield was associated with
increased SCC only in cows randomly assigned to be left without DCT at dry-off [20].

Initial milk yield in cows with high SCC in the previous lactation have been reported to be higher than milk yield of cows with low SCC during the first two months of lactation of the following lactation [24]. However, the authors reported that cows with high SCC were unable to maintain the same level of production for the rest of the lactation. It appears from figure 6.1 that in the present study, cows that had high-SCC at dry-off had initially higher milk yield than low-SCC cows, but only for a short period of time. This likely indicates the effect of subclinical mastitis on milk yield as suggested by Østerås and Sandvik (1996). Low levels of SCC at the end of the previous lactation is indicative of an efficient level of primary barrier of defense against mastitis pathogens (e.g., integrity of teat sphincter, teat canal, and teat skin) that enables these cows to maintain a higher level of production, than cows with high-SCC.

Increased losses due to CM in multiparous cows, as compared to primiparous cows, were reported by Bartlett et al (1991) and Rajala-Schultz (1999). The same effect was observed in the present study, where cows with three lactations or more lost more milk per day than cows with two lactations, in both data sets. Overall, cows with three or more lactations had lower milk yields than second lactation cows. A possible explanation is the presence of subclinical mastitis in older cows that induced higher losses due to increased SCC as compared with younger cows [15].

Length of the dry period to maximize milk production was recently investigated by Kuhn et al (2006). The authors reported that the minimum length of the optimal dry period depended on parity, with a minimum of 40-45 days for second
lactation cows and 55-65 days for cows with more lactations to avoid milk losses. Moreover, dry periods longer than 60 days was associated with milk losses [17]. In the present study, PRVDD was an important confounder in both data sets. Furthermore, PRVDD was significantly associated with milk yield in the low-SCC data set, cows with PRVDD longer than 57 days had reduced milk production in the following lactation. Association between milk production from previous lactation with milk yield in the subsequent lactation has been reported to be highly significant [24]. This association was found to be highly significantly in the present study also, with an increase of approximately 2 kg of milk/day for every 1,000 kg increase above the median AMP305PL.

Most of the studies on SDCT have evaluated the effect of treatment on prevalence of mastitis pathogens and occurrence of clinical mastitis [5-7, 23, 32], but few have assessed the effect of SDCT on milk yield [24]. Our results suggest that the presence of IMI at dry-off did not reduce milk yield in the following lactation for cows that have low-SCC at dry-off. Efforts to determine the effect of DCT on milk yield and milk quality are needed to improve the management of cows at dry-off. Some of the studies that had evaluated milk yield have used a randomized design to allocate cows to either receive DCT or not to receive DCT at the end of lactation. Results from those studies suggested an increase in milk production in cows treated at dry-off as compared with cows without DCT for the complete lactation [14, 20] or for the first 120 DIM [3]. Strengths of the present study were firstly, treatment of all cows suspected to be infected (high-SCC cows) and secondly, random allocation of uninfected cows to DCT groups. Thus, the effect of DCT on healthy udders was assessed. Even though the mixed models suggested an increase in daily milk yield in
cows that received DCT, as compared with untreated cows of approximately 0.8 kg/day in both data sets, analysis of the low-SCC data stratified per herd revealed that the effect of DCT varied with farm. There was one farm where treated low-SCC cows produced almost 3 kg/day more than untreated cows, but the opposite was also observed. In another farm low-SCC cows that received DCT lost almost 2 kg/day as compared with untreated cows. Thus, the effect of DCT on milk yield appears to be farm dependent and one general recommendation is not warranted. Most importantly, economic consequences of SDCT under different farm conditions need to be evaluated. A recently published simulation study indicated that, for some farms, SDCT can be the best option economically, while for others, TDCT is definitely the best approach [16].

6.6. Conclusions

Even though milk yield in the following lactation between cows that had elevated SCC at the end of previous lactation and those with low SCC, did not differ significantly, high-SCC cows produced 0.7 kg/day less than low-SCC cows. Occurrence of CM during early lactation and elevated SCS significantly reduced daily milk production.

There were no statistical differences in milk yield of untreated and treated low-SCC, but the effect of DCT on milk yield varied from farm to farm. Increased milk yield of approximately 3 kg/day in dry treated low-SCC cows can be obtained in some herds with losses of almost 2 kg/day in others. Global recommendations, either treating all cows on a herd or selectively treating only infected cows are not warranted. Treating only those cows that have a high likelihood to respond positively to treatment after consideration of farm characteristics is advised.
6.7. References


### Table 6.1: Total number of clinical mastitis cases during early lactation in low and high-SCC cows

<table>
<thead>
<tr>
<th></th>
<th>1-15 DIM(^1)</th>
<th>16-30 DIM</th>
<th>31-60 DIM</th>
<th>61-90 DIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-SCC(^2)</td>
<td>61</td>
<td>7</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Untreated</td>
<td>32</td>
<td>3</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Treated</td>
<td>29</td>
<td>4</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>High-SCC(^3)</td>
<td>58</td>
<td>16</td>
<td>20</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>23</td>
<td>37</td>
<td>41</td>
</tr>
</tbody>
</table>

\(^1\) Days in milk  
\(^2\) Cows without CM and <200,000 cells/ml during the last three mo. of previous lactation, or with CM during the first 90 DIM and <100,000 during the rest of lactation  
\(^3\) Cows with ≥200,000 cells/ml during any of the last three mo. of previous lactation or with CM after 90 DIM

Table 6.1: Total number of clinical mastitis cases during early lactation in low and high-SCC cows
### Table 6.2: Average (SD) of daily milk yield (kg) and somatic cell counts (SCC) from current lactation, previous days dry and actual 305-d milk production of the previous lactation for low\(^1\) and high\(^2\)-SCC cows

<table>
<thead>
<tr>
<th></th>
<th>Milk yield</th>
<th>SCC (x10(^3)/ml)</th>
<th>PRVDD(^3)</th>
<th>AMP305PL(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cows</td>
<td>37.6 (10.1)</td>
<td>377 (960)</td>
<td>58 (17)</td>
<td>10,666 (2010)</td>
</tr>
<tr>
<td>Low-SCC</td>
<td>38.3 (10.1)</td>
<td>263 (720)</td>
<td>57 (14)</td>
<td>10,854 (1999)</td>
</tr>
<tr>
<td>High-SCC</td>
<td>36.8 (10.1)</td>
<td>503 (1,157)</td>
<td>59 (19)</td>
<td>10,461 (2002)</td>
</tr>
</tbody>
</table>

\(^1\)Cows without clinical mastitis (CM) and <200,000 cells/ml during the last three mo. of previous lactation, or with CM during the first 90 days in milk and <100,000 cells/ml during the rest of lactation

\(^2\)Cows with ≥200,000 cells/ml during any of the last three mo. of previous lactation or with CM after 90 days in milk

\(^3\)Previous days dry

\(^4\)Actual 305-d milk production from the previous lactation (kg)
<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>27.56</td>
<td>0.69</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>High-SCC(^1)</td>
<td>-0.65</td>
<td>0.52</td>
<td>0.2116</td>
</tr>
<tr>
<td>DCT(^2)</td>
<td>0.73</td>
<td>0.57</td>
<td>0.2069</td>
</tr>
<tr>
<td>IMI at dry-off</td>
<td>0.28</td>
<td>0.47</td>
<td>0.5399</td>
</tr>
<tr>
<td>SCS(^3)</td>
<td>-0.64</td>
<td>0.05</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Parity (Reference: Second)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥Third</td>
<td>-2.87</td>
<td>0.48</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Mastitis (Reference: Cows without CM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-15 DIM(^4)</td>
<td>-1.47</td>
<td>0.58</td>
<td>0.0117</td>
</tr>
<tr>
<td>16-30 DIM</td>
<td>-3.49</td>
<td>1.27</td>
<td>0.0059</td>
</tr>
<tr>
<td>31-60 DIM</td>
<td>0.09</td>
<td>0.96</td>
<td>0.9237</td>
</tr>
<tr>
<td>61-90 DIM</td>
<td>-0.12</td>
<td>0.92</td>
<td>0.8973</td>
</tr>
<tr>
<td>PRVDD(^5)</td>
<td>-0.02</td>
<td>0.01</td>
<td>0.086</td>
</tr>
<tr>
<td>AMP305PL(^6)</td>
<td>1.75</td>
<td>0.12</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

\(^1\)Cows with ≥200,000 cells/ml during any of the last three mo. of previous lactation or with CM after 90 days in milk. The reference group was cows with <200,000 cells/ml (low-SCC cows). The model was adjusted for stage of lactation.

\(^2\)The reference group was untreated cows.

\(^3\)Somatic cell scores centered at 2.8.

\(^4\)Days in milk.

\(^5\)Previous days dry were centered at 57 days.

\(^6\)Actual 305-d milk production from the previous lactation was centered at 10,556 kg and scaled to reflect a 1,000-kg change.

Table 6.3: Estimated daily milk yield adjusted for the effect of dry cow therapy (DCT), intramammary infections (IMI) at dry-off, somatic cell scores (SCS), parity, occurrence of clinical mastitis (CM), previous days dry (PRVDD), and actual 305-d milk production of the previous lactation for low and high-SCC cows.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>29.15</td>
<td>0.89</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>DCT$^2$</td>
<td>0.80</td>
<td>0.56</td>
<td>0.1506</td>
</tr>
<tr>
<td>SCS$^3$</td>
<td>-0.57</td>
<td>0.08</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Parity (Reference: Second)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥Third</td>
<td>-3.47</td>
<td>0.64</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Mastitis (Reference: Cows without CM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-15 DIM$^4$</td>
<td>-1.09</td>
<td>0.79</td>
<td>0.1661</td>
</tr>
<tr>
<td>16-30 DIM</td>
<td>-1.20</td>
<td>2.07</td>
<td>0.5622</td>
</tr>
<tr>
<td>31-60 DIM</td>
<td>-0.93</td>
<td>1.34</td>
<td>0.4897</td>
</tr>
<tr>
<td>61-90 DIM</td>
<td>-0.46</td>
<td>1.46</td>
<td>0.7526</td>
</tr>
<tr>
<td>PRVDD$^5$</td>
<td>-0.04</td>
<td>0.02</td>
<td>0.0328</td>
</tr>
<tr>
<td>AMP305PL$^6$</td>
<td>1.86</td>
<td>0.17</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Sin(Calving day)</td>
<td>-0.21</td>
<td>0.43</td>
<td>0.6254</td>
</tr>
<tr>
<td>Cos(Calving day)</td>
<td>1.16</td>
<td>0.42</td>
<td>0.007</td>
</tr>
</tbody>
</table>

1Cows without CM and <200,000 cells/ml during the last three mo. of previous lactation or with CM during the first 90 days in milk and <100,000 cells/ml during the rest of lactation. The model was adjusted for stage of lactation
2The reference group were untreated cows
3Somatic cell scores centered at 2.2
4Days in milk
5Previous days dry were centered at 57 days
6Actual 305-d milk production from the previous lactation was centered at 10,709 kg and scaled to reflect a 1,000-kg change

Table 6.4: Estimated daily milk yield for low-SCC$^1$ cows randomly allocated either to receive or not to receive dry-cow therapy (DCT) at dry-off, adjusted for the effect of intramammary infections (IMI) at dry-off, somatic cell scores, parity, occurrence of clinical mastitis (CM), previous days dry, actual 305-d milk production of the previous lactation, and season effect of calving day.
Figure 6.1: Adjusted estimated milk yield for low and high-SCC cows in subsequent lactation after treatment allocation
Figure 6.2: Adjusted estimated milk yield for low-SCC cows randomly allocated either to receive or not to receive dry-cow therapy (DCT) at dry-off in the subsequent lactation.
CONCLUSIONS

Quality of milk begins at the farm with maximization of milk production throughout efficient management. Mastitis control is essential to achieve production of high quality milk, to maximize milk yield, economic return, and welfare of dairy cows. Antimicrobials are an essential tool in the control and treatment of animal diseases but development of antimicrobial resistance might jeopardize the future use of these compounds on animal production medicine. The effect of SDCT on udder health was assessed throughout evaluation of occurrence of clinical mastitis during early lactation and milk production in the following lactation after treatment. The effect of treatment at dry-off varied according with the level of SCC a cow had at the end of previous lactation and herd.

Based on the results form this study, the diagnosis of IMI can be based on a single aseptically collected quarter milk sample when source of infection and type of mastitis pathogen is considered during culture results interpretation. Requiring 100 or more cfu/ml of major contagious pathogens improved identification of cows infected with these pathogens, while requiring at least 500 cfu/ml of other pathogens maximized identification of true infections instead of contaminations.

Cows that had high SCC during the last trimester of the previous lactation were more likely to contract CM during early lactation than cows with low SCC,
regardless of antimicrobial treatment at dry-off. Both, occurrence of clinical mastitis during the first month of lactation and increased SCS significantly reduced milk yield.

The effect of DCT on cows that had low SCC during the last trimester of the previous lactation depended on parity and farm. Uninfected second lactation cows have a higher hazard of contracting clinical mastitis when treated at dry-off than untreated counterparts. Treated cows with more than two lactations had a reduced hazard of contracting clinical mastitis during early lactation as compared to untreated cows in the same parity group. Increased milk yield of approximately 3 kg/day in dry treated cows can be obtained in some herds with losses of almost 2 kg/day in others.

Careful consideration of farm characteristics and SCC of cows at the end of lactation are needed to maximize the benefits of dry cow therapy in dairy herds. Global recommendations, either treating all cows on a herd or selectively treating only infected cows are not warranted. Treating only those cows that have a high likelihood to respond positively to treatment after consideration of farm characteristics is advised. Evaluation of the effect of dry cow therapy on milk quality (i.e., SCC), culling of cows, and its economic impact on a farm basis is needed for optimizing the use of antimicrobials and their impact on udder health and productivity of dairy herds.
LITERATURE CITED

Chapter 1


Chapter 2


90. Smith, K.L. and D.A. Todhunter, \textit{The physiology of mammary glands during the dry period and the relationship to infection}. 1982. Louisville, KY.


Chapter 3


Chapter 4


Chapter 5


### Chapter 6


