EVALUATION OF A 3M PETRIFILM ON-FARM MILK CULTURE SYSTEM FOR USE IN SELECTIVE DRY COW THERAPY

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that the thesis is acceptable in form and content, and that a satisfactory knowledge of the field covered by the thesis was demonstrated by the candidate through an oral examination held on March 28th, 2014.

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ABSTRACT

As an alternative to blanket dry cow therapy (**BDCT**), selective dry cow therapy (**SDCT**) is considered a more judicious approach to antimicrobial use for the purpose of mastitis control during the non-lactating period. The purpose of this research was to evaluate the utility of a 3M Petrifilm-based on-farm milk culture system (**OFCS**) for use in a SDCT program. A randomized clinical trial was conducted using 16 low bulk tank somatic cell count (<250,000 cells/mL) dairy herds from Prince Edward Island (n = 10) and Quebec (n = 6), Canada. When used to detect intramammary infection (**IMI**) in cows at drying off, the OFCS performed well with a sensitivity of 85% and specificity of 73%. By comparing producer-derived Petrifilm results to those obtained using an accurate automated reader, it was determined that producers were able to correctly interpret Petrifilm results with an observed agreement of 91% and kappa value of 0.82.

The study groups comprising the clinical trial were a positive control group consisting of cows receiving blanket application of dry cow therapy (**DCT**) with the addition of an internal teat sealant (**ITS**), and the OFCS group consisting of cows selectively treated at drying off based on OFCS results with ITS alone (Petrifilm negative) or DCT + ITS (Petrifilm positive). No significant differences in postcalving IMI risk and risk of clinical mastitis in the first 120 days of the subsequent lactation were detected between the study groups. Furthermore, no significant differences were observed between study groups regarding test day milk yield and somatic cell count in the first 180 days of the subsequent lactation. Petrifilm-OFCS-based SDCT enabled a reduction in DCT of 21% as compared to BDCT.

The effect of ITS on the interdependence of quarters towards the acquisition of new IMI (**NIMI**) with coagulase negative staphylococci (**CNS**) over the dry period was also investigated. It was demonstrated that in cows that were infused with ITS, the presence of CNS in another quarter at drying off was a risk factor for CNS NIMI, but this association was absent in cows without ITS infusion. This unexpected finding may have resulted from inadvertent introduction of CNS during ITS infusion. In quarters of cows without a CNS IMI at drying off (i.e. without a source of contagious CNS), ITS was protective against CNS NIMI suggesting that CNS infection over the non-lactating period can be partly attributed to CNS species located within the environment.

According to economic analyses, OFCS-based SDCT resulted in a marginally higher total cost per cow than BDCT. However, OFCS-based SDCT provided the combined benefits of lowering DCT treatment risk without increasing the risk of postcalving IMI, and was superior economically to a SDCT program based on somatic cell count and clinical mastitis history. Overall, the Petrifilm OFCS was an accurate diagnostic tool that was effective in reducing DCT use without compromising the health, welfare, and future milk production of the cow.

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DEDICATION

This thesis is dedicated to my amazing husband and partner in life, Jeff,
who inspires me to be the best version of me. Without your love, support, and endless patience, I never would have made it this far.

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LIST OF ABBREVIATIONS

AC	aerobic count
AIC	Akaike's Information Criterion
AMR	antimicrobial resistance
BDCT	blanket dry cow therapy
BTSCC	bulk tank somatic cell count
CBMRN	Canadian Bovine Mastitis Research Network
cfu	colony-forming units
CI	confidence interval
CM	clinical mastitis
CMT	California mastitis test
CNS	coagulase negative staphylococci
DCT	dry cow therapy
DHI	dairy herd improvement
DIM	days in milk
GEE	generalized estimating equations
ICC	intra-class correlation coefficient
IMI	intramammary infection
ITS	internal teat sealant
lnSCC	natural logarithm of the somatic cell count
mL	millilitre

NA	not applicable
NCDF	National Cohort of Dairy Farms
NMC	National Mastitis Council
NIMI	new intramammary infection
NPV	negative predictive value
OFCS	3M Petrifilm on-farm culture system
OR	odds ratio
PPV	positive predictive value
SCC	somatic cell count
SDCT	selective dry cow therapy
SDQT	selective dry quarter therapy
Se	sensitivity
Sp	specificity
subCM	subclinical mastitis

CHAPTER 1. GENERAL INTRODUCTION

1.1 Bovine mastitis

Mastitis is defined as inflammation of the mammary gland. In dairy cows, this inflammation is almost always due to infection with bacteria, but mycotic pathogens, viruses, and trauma can also play causative roles (Radostits et al., 1994). When infection of the mammary gland results in visible changes in the milk, with or without signs of inflammation (heat, pain, redness, and swelling) in the quarter, a diagnosis of clinical mastitis is made. In acute clinical mastitis, systemic signs such as fever, anorexia, tachycardia, tachypnea and dehydration are also exhibited. Subclinical mastitis is characterized only by an increase in inflammatory cells within the milk and is therefore undetectable by the naked eye (Blowey and Edmondson, 2010).

Classification of mastitis is commonly based on the source of infection. Mastitis is considered environmental in origin when the source of the causative pathogen is the environment in which the cow lives. Mastitis pathogens with environmental reservoirs include: non-*Streptococcus agalactiae* streptococci, coliforms, *Trueperella pyogenes*, yeasts, molds, and algae (Blowey and Edmondson, 2010). Contagious mastitis occurs as a result of cow-to-cow transmission of pathogens that reside within the udder. Contagious pathogens include: *Staphylococcus aureus (**Staph. aureus**)*, *Streptococcus agalactiae*, *Mycoplasma* spp., and *Corynebacterium* spp (Blowey and Edmondson, 2010). In addition to the above, coagulase negative staphylococci (**CNS**) are a group of mastitis-causing bacteria comprising both environmental and contagious species (Piessens et al., 2012). Coagulase negative staphylococci and *Corynebacterium* spp. are

considered minor mastitis pathogens because infection with these bacteria generally results in only a minor inflammatory response (Bradley et al., 2012). It is important to note that while the organization of mastitis pathogens into contagious and environmental categories is a classical approach, recent studies have demonstrated that certain species of mastitis-causing bacteria have strain-specific behaviours such that a strain of a contagious pathogen can exhibit environmental properties (i.e. the ability to survive in the environment) or a strain of environmental pathogen can possess contagious properties (i.e. the ability to persist in the mammary gland; Bradley et al., 2012). Mastitis is a very important disease in the dairy industry because it is common, costly, and production-limiting. In Canada, the mean incidence rate for clinical bovine mastitis was reported to be 23 cases per 100 cow-years (Olde Riekerink et al., 2008). As a consequence of treatment, discarded milk, veterinary fees, reduced milk production, and culling, a case of clinical mastitis can incur financial losses averaging CAD$1,050 (Huijps et al., 2008; CBMRN, 2009). Furthermore, estimates of milk yield losses for clinical mastitis average around 300 to 400 kg per case and losses associated with subclinical mastitis can be even greater (Hortet and Seegers, 1998a; Hortet and Seegers, 1998b).

1.2 The dry period

In dairy production, the non-lactating or dry period is a time of rest between successive lactations and is generally 6 to 8 weeks in length. With respect to udder health, the purpose of the dry period is to provide an opportunity for mammary epithelial cells to regress, proliferate, and differentiate with the ultimate goal of maximizing milk production in the subsequent lactation (Capuco et al., 1997). The practice of drying off

cows prior to calving originated in the early 1900s (Arnold and Becker, 1936) and is still widely practiced today, but recently there has been much debate regarding the optimal length of the dry period (van Knegsel et al., 2013). Beyond milk production effects, dry period length has an influence on other important factors in the subsequent lactation such as fertility and the incidence of metabolic disease. While some studies have demonstrated that shortening or abolishing the dry period resulted in increases to production income, improved fertility, and reductions in the frequency of postpartum diseases, others have failed to show any benefit of deviating from the traditional 50 to 60 days (Rastani et al., 2005; Andersen et al., 2005; Kuhn et al., 2006). In a recent meta-analysis of 24 randomized controlled trials, van Knegsel et al. (2013) concluded that shortening of the dry period actually reduced milk production and did not affect fertility nor the incidence of important postpartum diseases such as mastitis, metritis, or displaced abomasums.

1.3 Intramammary infection during the dry period

For almost 65 years, the dry period has been recognized as the stage of the lactation cycle with the highest incidence of new intramammary infection (**NIMI**). In a classic paper by Neave et al. published in 1950, it was reported that the incidence rate for NIMI was six times higher during the first 21 days of the dry period than at any time during the preceding lactation (Neave et al., 1950). Subsequent studies have confirmed an increase in susceptibility to NIMI after drying off and have identified a second high risk period occurring in the late dry period (Oliver and Mitchell, 1983; Smith et al., 1985; Bradley and Green, 2000).

A better understanding of the dynamics of NIMI over the dry period can be had with some general knowledge of mammary gland physiology (for an in-depth review see: Smith and Todhunter, 1982; Bradley and Green, 2004). Three phases of the dry period have been described: active involution, steady-state involution, and colostrogenesis (Smith and Todhunter, 1982). During the first phase of the dry period (involution), there is an increase in susceptibility to NIMI as a result of the cessation of milking and a slow transition to steady-state involution (Sordillo and Nickerson, 1988; Bradley and Green, 2004). In the absence of the milking routine, germicidal teat-dipping stops and bacteria are no longer expelled from the teat canal. Furthermore, the accumulation of milk within the udder and resulting increase in intramammary pressure causes the teat canals to remain open and be at risk for bacterial invasion (Bradley and Green, 2004).

Once fully involuted, protection against NIMI is partially conferred by the presence of a keratin plug which blocks the teat orifice and teat canal (Comalli et al., 1984). Additionally, because of high levels of lactoferrin (an antimicrobial protein), elevated concentrations of leukocytes, and the absence of nutritive milk, the mammary parenchyma is not conducive to bacterial proliferation (Jensen and Eberhart, 1981; Sordillo et al., 1997; Bradley and Green, 2004). Because mastitis pathogens enter the gland via the teat canal, the rate at which the keratin plug is formed is considered to be a key factor in determining the risk of NIMI at the quarter-level. Keratin plug formation can be delayed or absent altogether, thus leaving the teat canal unprotected against pathogen migration (Williamson et al., 1995; Dingwell et al., 2004). In quarters without a functional keratin plug, the risk of NIMI increases by a factor of 1.7, and it is estimated

that 5 to 23% of teats experience a failure or extended delay in plug formation (Williamson et al., 1995; Dingwell et al., 2004).

During the last phase of the dry period, the mammary gland is once again at increased risk for NIMI. With impending parturition, colostrum production is initiated and the accumulation of mammary secretions leads to the dilution of protective factors within the gland (Bradley and Green, 2004). Furthermore, as calving approaches, the keratin plug breaks down leaving the teat canal patent and in danger of bacterial invasion (Oldham et al., 1991).

1.3.1 Impact of dry period intramammary infection on the subsequent lactation

Perhaps of utmost importance when considering the dynamics of intramammary infection (**IMI**) over the dry period are the effects of dry period acquired infections on the subsequent lactation. While clinical mastitis is rare in non-lactating cows, IMI present at calving are a significant cause of clinical mastitis in early lactation (Bradley and Green, 2000; Bradley and Green, 2001; Green et al., 2002). Using DNA fingerprinting, Bradley and Green (2000) estimated that 52% of all cases of clinical coliform mastitis occurring in the first 100 days of lactation can be traced-back to NIMI acquired during the dry period. Regarding the effects on milk production in the subsequent lactation, it is estimated that IMI present at calving, either because of failure to eliminate existing infections or acquisition of new infections, can reduce lactational milk yields by 5% (Hogeveen, 2003; Berry et al., 2004).

1.4 Mastitis control during the dry period

Considering the negative consequences associated with IMI at calving, mastitis control measures during the dry period are particularly important for a healthy and productive subsequent lactation. The aim of mastitis control during the dry period is to minimize the prevalence of IMI at the beginning of the next lactation, and in order to attain that goal, both prevention of NIMI and effective clearing of existing IMI must occur.

1.4.1 Dry cow antibiotic therapy

Dry cow antibiotic therapies, commonly known as dry cow therapy (**DCT**), are long-acting intramammary antimicrobials that are administered to cows at the end of lactation for the purpose of treatment and prevention of IMI. Treatment of IMI during the dry period has benefits over treatment in lactation for reasons such as the following: 1) a higher dose of antimicrobial can be used, 2) in the absence of milking, antimicrobials are maintained within the mammary gland parenchyma for longer periods, 3) DCT are formulated to be slow-release and long-acting, and 4) the risk for contamination of saleable milk is reduced (Blowey and Edmondson, 2010). Consequently, when DCT is used to treat existing IMI present at drying off, high cures rates can be achieved. In a meta-analysis of 22 peer-reviewed studies on the effectiveness of DCT, the cure risk for IMI by any pathogen was estimated at 78%; the spontaneous cure risk (i.e. the risk in untreated quarters) according to the same study was only 46% (Halasa et al., 2009a).

Regarding protection against NIMI, the effectiveness of DCT is uncertain. Despite infusion with DCT at drying off, a large proportion of previously uninfected

quarters will become infected over the dry period (Godden et al., 2003; Arruda et al., 2013). While DCT is considered protective against *Streptococcus* spp., there is a lack of evidence supporting a prophylactic effect against coliforms and questionable efficacy against *Staphylococcus* spp. (Halasa et al., 2009b). With respect to the etiology of dry period NIMI, in the absence of the milking routine and related risk factors for the cow-to-cow transfer of mastitis pathogens, non-lactating cows are at greater risk of infection with environmental pathogens, such as coliforms and non-*Streptococcus agalactiae* streptococci, than they are with contagious pathogens (Oliver and Mitchell, 1983; Smith et al., 1985; Green et al., 2002; Godden et al., 2003; Dingwell et al., 2003). Therefore, an important drawback of DCT is that they are ineffective against coliform bacteria, environmental pathogens which pose an important new infection risk during the dry period. Even when effective against the pathogen of concern, as a result of declining concentration of active compound over time, DCT do not protect against NIMI in the periparturient period when infection rates are known to be high (Oliver et al., 1990).

1.4.2 Internal teat sealants

Internal teat sealants (**ITS**) are a non-antimicrobial alternative to DCT for the prevention of NIMI during the dry period. An initial formulation consisting of bismuth subnitrate in a paraffin base was developed and evaluated in Ireland in the early 1970s but it wasn't until thirty years later that a commercial product became more widely available (Meaney, 1977). The internal teat sealant currently available in North America (Orbeseal, Zoetis Canada Inc., Kirkland, QC) is an inert viscous paste, 65% bismuth subnitrate by weight, that forms a physical barrier within the teat cistern and canal thus

preventing entry of bacteria into the mammary gland (Godden et al., 2003). Internal teat sealants are effective against all bacteria, including Gram-negative coliforms, and have been show to persist within the teat canal for up to 100 days (Woolford et al., 1998). According to the published literature, infusion of ITS in conjunction with DCT significantly reduces the risk of dry period NIMI when compared to DCT alone (Woolford et al., 1998; Huxley et al., 2002; Berry and Hillerton, 2002a; Sanford et al., 2006a; Rabiee and Lean, 2013). Furthermore, in quarters without existent IMI at drying off, ITS are just as effective as DCT at preventing NIMI in non-lactating cows (Woolford et al., 1998; Sanford et al., 2006a; Rabiee and Lean, 2013; Kromker et al., 2013).

1.5 Blanket dry cow therapy

In the late 1960s, with the knowledge that cows are highly susceptible to NIMI during the non-lactating period, mastitis researchers made the recommendation to infuse all quarters of all cows with DCT after the last milking prior to drying off (Neave et al., 1969). This practice, know as blanket or total dry cow therapy (**BDCT**), became a mainstay of mastitis control following its inclusion in the Five Point Mastitis Control Plan described by Neave et al. (1969), and it has persisted to the current day as a control point in the National Mastitis Council's Recommended Mastitis Control Program (National Mastitis Council, 2006). Previous work has shown that when the Five Point Mastitis Control plan is in place, contagious pathogens are better controlled and reductions in bulk tank somatic cell count (**BTSCC**) are observed (Berry et al., 1997; Bradley and Green, 2004). Since the promotion of BDCT in Canada, *Streptococcus*

agalactiae is nearly eradicated and the average provincial geometric-mean BTSCC in 2005 was 225,000 cells/mL (Olde Riekerink et al., 2006; Olde Riekerink et al., 2010).

1.6 Issues surrounding antimicrobial use in dairy production

Concerns regarding antimicrobial drug use in food animal production systems as a contributor to the development of antimicrobial resistance (**AMR**) are increasing across the world (White and McDermott, 2001; Call et al., 2008; Oliver and Murinda, 2012; World Health Organization, 1997). Consequently, a significant challenge facing the dairy industry is the mounting pressure to reduce the amount of antimicrobials used in production. The most common reason for antimicrobial use in dairy cows is for the treatment and prevention of mastitis (Erskine et al., 2003; Pol and Ruegg, 2007b; Thomson et al., 2008; USDA, 2008). According to recent investigations into antimicrobial drug use on Canadian dairy farms, antimicrobials were administered via an intramammary route at an average rate of 5 animal defined-daily doses / 1,000 cow-days which represents over a third of all routes (Saini et al., 2012a). With respect to antimicrobial use in the form of DCT, it is estimated that 72% of herds in the United States and 88% of herds in Canada practice BDCT (USDA, 2008; Dufour et al., 2012). This is in contrast to many Nordic countries where antimicrobial use is highly regulated and BDCT is rarely used (Ekman and Østerås, 2003).

In lactating cows, current scientific evidence has failed to support a clear trend of increased resistance among mastitis pathogens to the antimicrobials commonly used in mastitis therapy (Erskine et al., 2002; Makovec and Ruegg, 2003; Pol and Ruegg, 2007a). With regard to antimicrobial use in non-lactating cows, studies focused on the effect of

DCT on AMR are lacking in the published literature (Rajala-Schultz et al., 2009). In a study of AMR among CNS species isolated before and after treatment with DCT, Rajala-Schultz et al. (2009) identified an association between DCT and resistance, but only in older cows with history of high somatic cell count (**SCC**) at drying off and clinical mastitis in the succeeding lactation. Even though the relationship between antimicrobial drug use in dairy production and AMR remains unclear (Call et al., 2008; Saini et al., 2012b), both human and veterinary antimicrobial drug use is considered a driving force for the development of resistance (Levy and Marshall, 2004; Call et al., 2008), thus motivating research into alternative practices to manage herd health.

An additional consideration when antimicrobials are administered to dairy cows is the risk of inadvertent contamination of milk (Berry and Hillerton, 2002b; Robert et al., 2006a). According to the US National Milk Drug Residue Data Base for the fiscal year 2011, 0.021% (2.1 per 10,000 samples) of milk tanker samples were positive for antimicrobial residues (Food and Drug Administration, 2012). Although the contamination of milk with antimicrobial compounds is uncommon, studies have estimated that 75 to 93% of violations are due to intramammary treatments of which one third are non-lactating preparations (Booth, 1982; Booth and Harding, 1986; McEwen et al., 1991).

1.7 Selective dry cow therapy

In view of today's well-managed farms with low prevalence of contagious mastitis pathogens and capable of maintaining a low BTSCC, reconsideration of the practice of BDCT is warranted. Firstly, not all cows have an IMI at drying off.

Considering that the prevalence of IMI at the end of lactation is estimated at 28 to 41% of cows, BDCT does result in the over-usage of antimicrobials for the purpose of eliminating existing infections (Browning et al., 1994; Sanford et al., 2006b; Torres et al., 2008; Torres et al., 2009). In light of heightened awareness and concern over the development of AMR in both human and veterinary medicine, it will become increasingly difficult to defend practices that support the non-therapeutic use of antimicrobials.

As previously discussed, DCT is not protective in the late dry period as a result of decreasing levels of active compound over time; simultaneously, the late dry period is characterized by a high rate of NIMI. Thus, there is an important lapse of protection that exists when DCT is the single form of treatment at drying off. This gap in protection can be overcome by the addition of an ITS to the end-of-lactation treatment protocol. Furthermore, because studies have demonstrated that in quarters without an IMI at drying off, ITS are just as effective as DCT for the prevention of NIMI, ITS can be used as a sole treatment in the absence of existing infection. Choosing cows for DCT treatment based on known or suspected infection status at the end of lactation is known as selective dry cow therapy (**SDCT**). Selective dry cow therapy has the potential to reduce the amount of antimicrobials used in dairy production and is considered a more targeted approach to the control of IMI during the dry period (Berry and Hillerton, 2002b; Robert et al., 2006b; Rajala-Schultz et al., 2011). Addition of an ITS to a SDCT treatment protocol will ensure that all quarters will have some form of protection against NIMI during the dry period.

1.7.1 Selection procedures in selective dry cow therapy

The success of a SDCT program depends on the accurate identification of a cow's IMI status at drying off so that appropriate treatment decisions can be made, and this is a consistent theme in SDCT research. A common protocol for SDCT is based on monthly SCC and clinical mastitis history such that cows with a low SCC (generally < 200,000 cells/mL) in late lactation and no clinical mastitis are considered to have low probability of IMI at drying off and are not infused with DCT (Torres et al., 2008; McDougall, 2010; Rajala-Schultz et al., 2011). Benefits of this approach include no additional costs for herds already enrolled in a monthly milk testing program, and the convenience of readily accessible data, either in paper or electronic format, from which selective treatment decisions can be made. Important disadvantages do exist however, as monthly SCC and clinical mastitis criteria lack accuracy and may be a poor reflection of a cow's current IMI status considering the potential delay between the last milk test and the end of lactation.

With respect to measures of test accuracy as it relates to the diagnosis of IMI, sensitivity (**Se**) is the proportion of cows with an IMI that are identified as test positive; conversely, test specificity (**Sp**) is the proportion of cows without an IMI that are identified as test negative (Dohoo et al., 2009). While both Se and Sp are important to consider when choosing a test for SDCT, the potential risk associated with not treating an infected cow will be minimized by a test with high Se. In the published literature, reports for the Se and Sp of monthly SCC and clinical mastitis criteria for the detection of IMI at drying off range from 58.4% to 69.4% and 62.7% to 71.5%, respectively (Torres et al., 2008; McDougall, 2010; Rajala-Schultz et al., 2011).

A popular cow-side test, the California Mastitis Test (**CMT**), has also been investigated as a diagnostic tool for SDCT (Sanford et al., 2006b; Bhutto et al., 2012). The California mastitis test is an inexpensive and simple test for the detection of subclinical IMI. California mastitis test scores range from 0 (negative) to 3 (strong positive) based on the viscosity of the reaction between CMT reagent and a milk sample. As the number of somatic cells in a sample increase, the viscosity of the reaction, and thus the test score, also increases (Sanford et al., 2006b). Using a CMT score cut point of > 0 and quarter milk samples collected on the day of drying off, the Se and Sp of the CMT to identify cows with IMI were estimated at 70% and 48%, respectively (Sanford et al., 2006b).

In terms of false negative diagnoses, SDCT programs that rely on the CMT or on monthly SCC and CM history to dictate treatments will fail to detect and subsequently treat up to 30 and 40% of infected cows, respectively. Perhaps of less concern from a health standpoint but resulting in the continued overuse of antimicrobials, these criteria will also result in the unnecessary treatment of numerous uninfected cows.

1.8 On-farm milk culture systems

Rapid on-farm milk culture systems have recently become available for use in dairy production. Investigations into the Minnesota Easy Culture System (University of Minnesota, Saint Paul) and the 3M Petrifilm on-farm culture system (Maritime Quality Milk, University of Prince Edward Island, Charlottetown, PE) have shown that farm-based mastitis diagnostics is useful for the selective antibiotic treatment of mastitis in lactating cows (McCarron et al., 2009b; MacDonald et al., 2011; Lago et al., 2011a; Lago

et al., 2011b). These culture systems enable producers to culture milk directly on-farm and obtain results within 24 hours. In addition to the benefits to producers when treating lactating cows, on-farm culture has the potential to allow dairy producers to make SDCT treatment decisions as well.

The Minnesota Easy Culture System consists of a bi-plate system for the differentiation between Gram positive and Gram negative organisms, or a tri-plate system which contains an additional agar that is selective for streptococci. To the authors' knowledge, investigations into the application of the Minnesota Easy Culture System for use in SDCT has not appeared in the published literature.

Petrifilms are a ready-made culture medium containing standard method nutrients and an indicator dye that allows for the identification and enumeration of individual bacterial colonies. Three types of Petrifilms have been show to be useful in mastitis detection and control: 1) the Aerobic Count Petrifilm (AC Petrifilm), 2) the Coliform Count Petrifilm (CC Petrifilm), and 3) the Staph Express Petrifilm (Leslie et al., 2005; Silva et al., 2005; McCarron et al., 2009a; McCarron et al., 2009b; Wallace et al., 2011). With respect to the distinguishing features of each product, the AC supports growth of all aerobic bacteria, the CC will only grow coliform bacteria, and the Staph express allows for the differentiation of *Staph. aureus* from other *Staphylococcus* spp. For the purpose of SDCT, where the interest is in the identification of overall infection status rather than in the species-specific classification of mastitis, the AC Petrifilm is the most suitable. In a preliminary laboratory study to assess the ability of the on-farm culture system to detect IMI at drying off, the AC Petrifilm correctly identified infected cows with 100% Se at

colony-count cut points of 5, 10 and 20; using the same colony counts, the Sp was 70, 82, and 84%, respectively (McLaughlin et al., 2010).

1.9 Level of dry cow therapy treatment decision

As the diagnosis of IMI can be made at both the quarter and the cow level, so can the decision to treat with dry cow therapy. Treatment decisions made at the quarter level would result in greater reductions in antimicrobial use when compared to selective therapy at the cow level. However, due to evidence that quarters of a cow do not act independently with respect to the risk of NIMI, research supports that DCT treatment decisions be made at the cow level (Berry et al., 2003; Robert et al., 2006a). Considering that ITS create a barrier to infection, it is plausible that quarter interdependence towards dry period NIMI would be reduced in the presence of an ITS. Since the advent of ITS, only one study has reported the potential impact of ITS on quarter interdependence over the dry period. In cows treated with ITS at drying off, the observed distribution of quarters with an IMI at calving was not different than what was expected based on the binomial distribution (Berry et al., 2003). The outcome in that study, prevalence of IMI at calving, was a combination of both cure of existing IMI and prevention of NIMI, therefore no evaluation of the effect of ITS on the interdependence of quarters towards acquisition of NIMI was performed. Results of an analysis to determine the effect of ITS on the within-cow clustering of dry period NIMI could be used to make recommendations regarding the level of treatment in SDCT treatment protocols that also employ ITS.

1.10 Economics of selective dry cow therapy programs

In North America, BDCT is the most popular method of mastitis control during the non-lactating period. Economic outcomes are a major influence on farm management decisions, therefore if SDCT is going to gain favour with dairy producers, it must be, at minimum, a revenue neutral alternative to BDCT. According to a cow-level analysis of the economics of mastitis control over the dry period, treatment with DCT was the only economically viable option when considering cows with existing IMI (Berry et al., 2004). Regarding cows without IMI at the end of lactation, DCT and ITS were financially equivalent choices for the prevention of NIMI (Berry et al., 2004). At the herd level, there is uncertainty regarding which dry period protocol (BDCT or SDCT) is the most cost-effective. Using stochastic modeling of the dynamics of IMI over the dry period, Huijps and Hogeveen (2007) estimated the economic consequences of BDCT, SDCT and no DCT, and determined that SDCT resulted in the lowest annual cost. In contrast, Halasa et al. (2010), who in addition to BDCT and SDCT also combined these protocols with an ITS, identified BDCT without ITS to be the most economical option. In both studies, differences between protocols were small and the importance of herd-specific factors, particularly the incidence of NIMI over the dry period, was recognized. Results were presented with the caveat that conclusions regarding the most economical approach should be made based on farm-specific calculations. While previous research has estimated the economic consequences of SDCT based on SCC criteria (Hujips and Hogeveen, 2007; Halasa et al., 2010), the financial implications of SDCT based on on-farm culture have yet to be reported.

1.11 Summary

The dry period is a very important stage of the lactation cycle with respect to udder health. It is during this time that the mammary gland regenerates secretory tissues in preparation for the next lactation and therefore provides an ideal opportunity to treat existing IMI. Concurrently, non-lactating cows are at increased risk for the development of NIMI as previous research has demonstrated that rates of NIMI are higher in the dry period than at any point during lactation. The most common approach to dry period mastitis control in North America is to infuse all quarters of all cows with DCT after the last milking and this practice is known as BDCT. As a result of concerns regarding the association between antimicrobial drug use in food animal production systems and the development of AMR, dairy producers are facing pressure to reduce antimicrobial use on their farms. While DCT is protective against sensitive pathogens in the early dry period, DCT are not effective in the late dry period, when NIMI rates are known to be elevated. As an alternative to DCT, ITS do not contain antimicrobials but prevent dry period NIMI by creating a physical barrier within the teat cistern and canal. Internal teat sealants are broad-spectrum and have been shown to persist right up until calving. In quarters without IMI at drying off, ITS are just as effective as DCT in prevention of dry period NIMI, suggesting that ITS can be used as a sole treatment at drying off when existent IMI is not a concern. The selective DCT treatment of cows at drying off based on infection status is known as SDCT, and has the potential to reduce the amount of antimicrobials used on dairy farms. In order to be successful, SDCT required a method to determine a cow's end-of-lactation infection status so that appropriate treatment decisions can be made. Previous SDCT protocols have relied on monthly SCC combined with clinical

mastitis history, or the CMT to diagnose IMI at drying off. Studies show that these methods lack accuracy, thus when used to target DCT treatment at drying off, numerous infected cows are left untreated and many cows are treated unnecessarily. Recently, a Petrifilm-based OFCS has been validated for use in the selective antimicrobial treatment of mastitis in lactating cows. These techniques allow producers to culture milk samples directly on the farm and make treatment decisions within 24 hours. The same on-farm culture system, when used to culture milk samples collected prior to drying off, could be used to make targeted DCT treatment decisions as well.

1.12 Objectives and clinical trial description

The overall objective of this thesis was to evaluate a Petrifilm-based on-farm culture system for use in a SDCT program. The study was designed as a randomized clinical trial with two treatment groups: 1) BDCT plus infusion of ITS (the positive control group); 2) SDCT based on results obtained using an Petrifilm OFCS (the study group). For cows in the study group, a composite milk sample was collected on the day prior to drying off and was cultured using the on-farm culture system. On the day of drying off, Petrifilm results were interpreted by the producer and cows with a positive Petrifilm result received DCT and ITS, while cows with a negative Petrifilm result did not receive DCT but were infused solely with an ITS.

Specific objectives were:

- Chapter 2: To determine the test characteristics of the Petrifilm on-farm culture system when used to detect intramammary infection at the end of lactation;
- Chapter 3: To compare the risk of intramammary infection at calving and the risk of a first case of clinical mastitis during the first 120 days of the subsequent lactation between the two treatment groups (quarter-level analysis);
- Chapter 4: To compare the somatic cell count and milk production in the first 180 days of the subsequent lactation between the two treatment groups (cow-level analysis);
- Chapter 5: To evaluate the interdependence of quarters towards new intramammary infection with CNS over the dry period and the effect of treatment with internal teat sealants;
- Chapter 6: To determine the economic outcome of Petrifilm-based SDCT and to make comparisons to BDCT, and to SDCT based on somatic cell count and clinical mastitis history criteria

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CHAPTER 2

EVALUATION OF A 3M PETRIFILM ON-FARM CULTURE SYSTEM FOR THE DETECTION OF INTRAMAMMARY INFECTION AT THE END OF LACTATION

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2.1 ABSTRACT

The purpose of this study was to evaluate a 3M Petrifilm-based on-farm culture system for the detection of intramammary infection (IMI) in low somatic cell count (SCC) cows (<200,000 cells/ml) at drying off. The main objectives were to determine the test characteristics and the predictive values of the Petrifilm on-farm culture system. The ability of dairy producers to correctly classify cows as infected or uninfected based on Petrifilm culture and a set colony count threshold was also assessed. A total of 360 cows originating from 16 low bulk tank SCC (<250,000 cells/mL) dairy herds were enrolled at drying off. Enrolled cows had an expected dry period of 30 to 90 days, a SCC <200,000 cells/mL on the last 3 tests prior to drying off, no clinical mastitis in the same time period, and no antibiotic treatment in the last 14 days. Quarter milk samples were collected on the day prior to drying off, and a composite milk sample was created by combining 5 mL of milk from each quarter sample. Composite milk samples were cultured on-farm using the Petrifilm culture system, which provided results within 24 hours. Quarter milk samples were cultured in a reference laboratory, and the results were aggregated to the cow level. On the day of drying off, the Petrifilm was read by the producer and cows were classified as positive if ≥5 colonies (equivalent to 50 colony forming units/mL) were present. When read by the producer, 47.8% of the cows cultured negative on Petrifilm and were infused with only an internal teat sealant at drying off. The test characteristics of the Petrifilm on-farm culture system were calculated by comparing the producer-derived Petrifilm results to those obtained by standard laboratory

culture. The sensitivity and specificity of the Petrifilm on-farm culture system were 85.2% (78.5 – 90.5) and 73.2% (66.4 – 79.3), respectively. The negative predictive value of the Petrifilm test system was high (86.6%) when estimated using the prevalence of IMI in this data set, and the positive predictive value was moderate (70.9%). An automated 3M Petrifilm reader was used to obtain accurate colony counts. The agreement between Petrifilm results obtained by the producer and those obtained by the automated Petrifilm reader was high, with a kappa value of 0.82 (0.75 – 0.89).

2.2 INTRODUCTION

Mastitis is an important production limiting disease of dairy cattle and is endemic in the dairy industry (Halasa et al., 2007). Efforts to treat and prevent mastitis represent the primary motivation behind antibiotic usage on dairy farms worldwide (Erskine et al., 2003; Thomson et al., 2008; USDA, 2008; Saini et al., 2012). The introduction of the Five Point Mastitis Control Plan in the late 1960s (Neave et al., 1969), and it's further development into a ten point plan (National Mastitis Council, 2006), has resulted in a significant reduction in the prevalence of contagious mastitis pathogens and a consequential reduction in bulk tank somatic cell counts (Berry et al., 1997; Bradley and Green, 2004). In compliance with the ten point plan, most dairy producers in North America treat all quarters of all cows with a dry cow intramammary antibiotic (DCT) at the end of lactation (USDA, 2008; Dufour et al., 2012). This practice is commonly known as blanket dry cow therapy (BDCT) and its purpose is twofold: (1) to clear up

any existing intramammary infections (IMI) and (2) to prevent new infections from being acquired during the dry period.

Owing to increasing public awareness of the practices common to food animal agriculture, consumers have expressed concern with respect to antibiotic use on dairy farms (White and McDermott, 2001). Consequently, a significant challenge facing the dairy industry is the mounting pressure to reduce the amount of antibiotics used in production. In 2010, with the aim of controlling antibiotic resistance, the Ministry of Economic Affairs, Agriculture and Innovation in the Netherlands called for a mandatory 50% reduction in antibiotic use on all farms by 2013, based on 2009 levels (SDA, 2012). In 2012, the Food and Drug Administration (FDA) of the United States presented a guidance document titled: The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals. According to the FDA, the use of antimicrobials for the purpose of prophylaxis requires veterinary oversight, as well as justification of the medical rationale for such use (FDA, 2012). In view of today's well managed farms, capable of maintaining a low bulk tank somatic cell count (BTSCC), reconsideration of the practice of BDCT is warranted.

As an alternative to BDCT, selective dry cow therapy (SDCT) has the potential to reduce the amount of antibiotics used in dairy production. First introduced in 1978, the goal of SDCT is to reserve DCT for cows which would benefit from treatment (Rindsig et al., 1978; Rindsig et al., 1979). Therefore, cows not suspected of having an IMI at drying off would enter the dry period without having received intramammary antibiotics. The addition of an internal teat sealant (ITS) can improve the success of a SDCT

program. An ITS is a non-antibiotic infusion that has been shown to be effective at preventing new IMI throughout the entire dry period (Woolford et al., 1998; Berry and Hillerton, 2002a; Huxley et al., 2002; Sanford et al., 2006a). Selective dry cow therapy plus internal teat sealant (SDCT + ITS) is a dry cow approach that would provide the therapeutic benefits of DCT for cows with an IMI at the end of lactation, in addition to the prophylactic effects of ITS.

In order to be successful, a SDCT+ITS program requires an easy, cost-effective, and rapid method to identify cows with an IMI at the time of drying off (Sanford et al., 2006b). On-farm systems for the bacteriological culture of milk have been validated for the detection of mastitis pathogens in clinical milk samples (McCarron et al., 2009; Lago et al., 2011; MacDonald et al., 2011; Wallace et al., 2011). An important benefit of on-farm culture is the provision of prompt results, allowing producers to make informed and targeted treatment decisions for cases of clinical mastitis. Recent investigations into 3M Petrifilms (3M Canada, London, Ontario) have shown that they are a useful tool for the selective antibiotic treatment of mastitis in lactating cows (McCarron et al., 2009; MacDonald et al., 2011). These techniques allow the producer to culture milk directly on-farm and obtain results within 24 hours. In addition to the benefits to the producer when treating lactating cows, Petrifilm-based on-farm culture has the potential to allow the dairy producer to detect IMI at drying off as well.

The objective of the present study was to determine the test characteristics and predictive values of a Petrifilm on-farm culture system when used in low BTSCC herds to make treatment decisions on low somatic cell count (SCC) cows at drying off, and to

evaluate the ability of dairy producers to correctly classify cows as infected and uninfected based on a set colony count threshold.

2.3 MATERIALS AND METHODS

2.3.1 Herd and animal selection

In order to be considered for inclusion in the trial, herds were required to have an annual average of monthly BTSCC of less than 250,000 cells/mL in the year prior to the study and be enrolled in a dairy herd improvement program. A convenience sample of 16 Canadian dairy herds were selected based on their proximity to the Atlantic Veterinary College, Charlottetown, PE (n = 10) and the Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, QC (n = 6).

Inclusion criteria at the cow level consisted of monthly SCC below 200,000 cells/mL on the last three milk tests prior to drying off, no evidence of clinical mastitis during the same time period, no antibiotic treatment within the last 14 days, ≥ three functional quarters, and an expected dry period of no less than 30 days and no more than 90 days. On the day prior to drying off, eligible cows were subjected to a California Mastitis Test (CMT) by the investigator or a trained technician. Cows with a score of two or greater on any quarter were excluded from the trial. As part of a larger clinical trial evaluating the utility of the Petrifilm on-farm culture system when used in a SDCT program, cows were randomly allocated to either the Petrifilm on-farm culture system group, with selective antibiotic treatment at drying off based on Petrifilm culture results,

or the positive control group which received blanket dry cow therapy. The focus of the present paper is on the data pertaining to the Petrifilm on-farm culture group only (Figure 2.1).

2.3.2 Sampling procedures

Producers scheduled a dry off day every other week to allow for sample collection. On the day prior to drying off (Day -1), quarter level milk samples were collected by the study investigator or a trained technician. Following pre-milking udder preparation as per farm protocol, teat ends were scrubbed with alcohol swabs until clean and initial foremilk streams were discarded into a strip cup. Approximately 30 mL of milk was collected aseptically according to the procedures recommended in the Laboratory Handbook on Bovine Mastitis (National Mastitis Council, 1999). Following quarter level sampling, a composite milk sample was created by combining 5 mL from each quarter sample in a new sample vial. Quarter milk samples were frozen to -20°C before submission to the laboratory for standard bacteriological culture. Composite milk samples were utilized fresh with the Petrifilm on-farm culture system.

2.3.3 Gold standard

Trained laboratory technicians (n = 2) were blinded to the results obtained by the Petrifilm on-farm culture system. Within one month from collection, milk samples were thawed and cultured in a laboratory using standardized methods outlined in the Laboratory Handbook on Bovine Mastitis (National Mastitis Council, 1999). Briefly,

disposable plastic loops were used to streak 0.01 mL of milk on bi-plates containing half Columbia agar + 5% sheep blood and half MacConkey agar. Plates were incubated at 35 ºC and examined for bacterial growth after 24 and 48 hours. Colonies were tentatively identified as staphylococci, streptococci, coliforms, or other pathogens based on colony growth characteristics, morphology, pattern of hemolysis, catalase reaction, and Gram staining. Staphylococcal isolates were tested for coagulase production with the tube coagulase test. API tests (API 20 E and API 20 Strep; bioMérieux (Marcy L'Etoile, France) and the latex agglutination test for streptococcal Lancefield groups were used for the final identification of bacterial organisms as required. For each positive sample, the number of colony forming units (cfu) per 0.01 mL of milk was enumerated up to a maximum of 10 colonies. A quarter was considered infected if ≥ 100 cfu/mL of milk of any pathogenic organism of interest, except coagulase negative staphylococci (CNS), were cultured. For CNS, a definition of ≥ 200 cfu/mL was used. These definitions are in accordance with the recent publication of characterization of IMI based on single sample bacteriological testing (Dohoo et al., 2011). Samples with three or more differing isolates were classified as contaminated. However, when *Staphylococcus aureus* (*Staph. aureus*) was identified in a contaminated sample, it was enumerated and the associated quarter was classified as infected (Reyher et al., 2011).

The quarter level culture results were interpreted in parallel to generate cow level results. If one or more quarter samples were contaminated, a cow was considered infected if at least one of the remaining quarters was infected. If one or more quarter samples were contaminated and no IMI was diagnosed in the remaining samples, the

cow's overall infection status was considered unknown, and the observation was removed from the analyses.

2.3.4 On-farm bacteriological culture

The on-farm culture system was set-up by the investigator or technician on the day prior to drying off. A 3 mL aliquot of composite milk was added to 27 mL of sterile water to make a 1:10 dilution. One millilitre of diluted milk was plated on an AC Petrifilm (3M Canada, London, Ontario) and incubated on-farm at 35°C for 24 hours in a TurboFan Hova-Bator (GQF Manufacturing, Savannah, Georgia, USA). Consistent with a dilution factor of 1:10, a single cfu present on the Petrifilm was equivalent to 10 cfu per mL of milk.

On the scheduled day of drying off (Day 0), the Petrifilm was read by the producer and, in accordance with study protocol, cows were classified as infected if 5 or more colonies were present (equivalent to $\geq$ 50 cfu/mL). In order to determine the performance of the Petrifilm on-farm culture system at different colony count thresholds, producers were asked to record the Petrifilm culture result under the following categories: 0, 1-4, 5-9, and $\geq$ 10 colonies. Cows positive on Petrifilm were treated with DCT + ITS; cows negative on Petrifilm were treated solely with ITS. The Petrifilms were frozen to -20°C at the farm, collected during the subsequent visit, and maintained at -20°C in the laboratory. To evaluate the ability of producers to correctly classify cows into DCT and no DCT groups, the Petrifilms were thawed and subsequently scanned using an

automated 3M Petrifilm reader (3M Canada, London, Ontario) which provided an accurate colony count (Gambrel-Lenarz and Lindberg, 2004).

2.3.5 Statistical analysis

Statistical analysis was performed using Stata/IC 11.0 (StataCorp, College Station, TX). Values of $P \leq 0.05$ were considered statistically significant. The test characteristics of the Petrifilm on-farm culture system were calculated using 2x2 tables (diagt command in Stata). Sensitivity (Se) was defined as the proportion of cows with an IMI, as determined by standard bacteriological culture, that were classified as positive on Petrifilm. Conversely, specificity (Sp) was defined as the proportion of cows without an IMI that were classified as negative on Petrifilm. The predictive values of a test are dependent upon the Se and Sp of the test and the prevalence of disease in the study population (Dohoo et al., 2009). In order to examine the performance of the Petrifilm on-farm culture system in different populations, with varying degrees of subclinical mastitis, the positive (PPV) and negative (NPV) predictive values were calculated for prevalence estimates ranging from 1 to 100%. The PPV was the proportion of Petrifilm positive cows that truly had an IMI, and the NPV was the proportion of Petrifilm negative cows that did not have an IMI. Estimates of Se, Sp and predictive values were calculated at a colony-count threshold of ≥ 5 colonies (the threshold at which treatment decisions were made) and ≥ 10 colonies. The higher cut point was evaluated to determine if increasing the colony count threshold would decrease the number cows treated unnecessarily (i.e. increase Sp) without sacrificing the accuracy of a negative test (i.e. preserve Se).

Generalized estimating equations (GEE) were used to evaluate the effect of clustering of cows within herds on the estimates of Se, Sp, and the confidence intervals (Dohoo et al., 2009). Using the Petrifilm test result as the outcome of interest and herd as a random effect, separate models were examined for gold standard positive and gold standard negative cows. The resulting estimates for Se and Sp, and associated 95% confidence intervals, were compared to those obtained from the unconditional analyses.

The results obtained by the automated 3M Petrifilm reader were used to evaluate the ability of producers to correctly classify cows into DCT and no DCT groups based on on-farm culture results. The level of agreement between the producer-derived results and the automated reader was assessed using McNemar's test for paired data to check for bias, followed by calculation of the kappa statistic.

2.4 RESULTS

2.4.1 Samples

Enrolment of cows from participating herds took place from July 2009 to September 2010. During this time, a total of 1584 cows entered the dry period. Seven hundred and twenty-nine cows (46.0%) ultimately met the inclusion criteria, of which 360 were randomly assigned to the Petrifilm on-farm culture system group and 369 to the positive control group (Figure 2.1). Of the cows in the on-farm culture system group, 16 (4.4%) cows were omitted from the analyses due to contamination of ≥ 1 quarter sample(s) and no IMI diagnosed in the remaining quarters. Eleven of these cows (68.8%)

were diagnosed as infected by the dairy producer based on Petrifilm results and were infused with DCT at drying off. One additional cow was removed due to incomplete records. Overall, data from 343 cows (1368 quarters) from the on-farm culture system group were included in the analyses. The number of cows per herd cultured on-farm using the Petrifilm culture system ranged from 6 to 68 (median = 13).

2.4.2 Gold standard

According to standard bacteriological culture, the cow level prevalence of IMI at drying off in cows meeting the inclusion criteria, i.e. low monthly SCC on the last three milk tests prior to drying off, was 43.4% (n = 149). For the purpose of analysis, the environmental streptococci were grouped together, and included all *Streptococcus* spp. other than *Streptococcus agalactiae*. Gram negative bacteria, other than *Escherichia coli* and *Klebsiella* spp., were also amalgamated into a grouping, and encompassed *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *Pseudomonas* spp., *Serratia* spp., *Salmonella* spp., *Pasteurella multocida*, and other non-differentiated gram negative bacteria. The most common pathogens isolated at the end of lactation were CNS, followed by environmental streptococci, *Corynebacterium* spp., and *Staph. aureus* (Table 2.1). Of the cows with significant growth of any pathogen on standard bacteriological culture, 107 (71.8%) had 1 infected quarter, 31 (20.8%) had 2 infected quarters, and 11 (7.4%) had 3 or 4 infected quarters.

2.4.3 On-farm bacteriological culture

The producer-derived prevalence of IMI at the end of lactation by Petrifilm on-farm culture results was 52.2%. Therefore, at a treatment threshold of ≥ 5 colonies, 47.8% of the cows meeting the inclusion criteria were assigned to the no DCT group and received an internal teat sealant as a sole treatment at drying off. Of the 149 cows with an IMI at drying off according to standard culture, the Petrifilm on-farm culture system, as interpreted by the producer, correctly identified 127 (85.2%) cows as positive and misclassified 22 (14.8%) cows as negative. The breakdown of causative pathogens isolated from the 22 misclassified cows was as follows: 11 CNS, 6 environmental streptococci, 3 *Corynebacterium* spp., and 2 *Staph. aureus*. A false positive Petrifilm result occurred in 52 (15.2%) cows that, according to standard bacteriology, did not have a significant intramammary infection in any quarter at drying off.

2.4.4 Test characteristics and predictive values of the Petrifilm on-farm culture system

The unconditional estimates of Se and Sp of the Petrifilm on-farm culture system at a colony count threshold value of ≥ 5 was 85.2 (95% confidence interval (95% CI): 78.5 – 90.5) and 73.2 (95% CI: 66.4 – 79.3), respectively. At a colony count threshold of ≥ 10 colonies, the estimates of Se and Sp were 71.8 (95% CI: 63.9 – 78.9) and 86.1 (95% CI: 80.4 – 90.6), respectively. The GEE estimates and 95% confidence intervals for Se and Sp were very close to the unconditional estimates (Appendix A), therefore only the unconditional estimates are reported. The test characteristics of the Petrifilm culture

system varied between herds, but the variation was not according to frequency of use. The Se ranged from 0% (n = 1 herd) to 100% (n = 7 herds), with a median of 86.6%; the Sp ranged from 0% (n = 1) to 100% (n = 3), with a median of 75.3%. A Se of 0% was obtained in a herd where a single cow was identified with an IMI according to standard culture, but was misclassified as uninfected on Petrifilm. A Sp of 0% was obtained in a herd where all cows (n = 6) were classified as infected on Petrifilm, but only three cows had an IMI according to standard culture.

The NPV of the Petrifilm on-farm culture system, with a treatment threshold of ≥ 5 colonies, was high (86.6%) when estimated using the prevalence of IMI in this data set. The NPV increased with decreasing prevalence and was lower at a threshold of ≥ 10 colonies (Figure 2.2). High NPV of 80% to 90% were maintained in populations where the proportion of cows with an IMI at drying off ranged from 55% to 35%. The PPV was moderate (70.9%) in the study population when a cut point of ≥ 5 colonies was used and increased slightly when the threshold was increased to ≥ 10 colonies (Figure 2.2).

2.4.5 Agreement between the producers and the automated Petrifilm reader

A total of 265 Petrifilms were available for evaluation by the automated reader. The agreement between the producer and automated reader for the Petrifilm on-farm culture system with a treatment threshold of ≥ 5 colonies is presented in Table 2.2. The level of agreement was high, with an observed agreement of 91.0% and a kappa value of 0.82 (0.75 – 0.89). Because the Se was highest at a threshold of ≥ 5 colonies, the level of agreement calculation was not repeated for the ≥ 10 colonies cut point.

The test characteristics of the Petrifilm on-farm culture system, as compared to standard bacteriology, were calculated for the subset of Petrifilms submitted for evaluation by the automated reader using the producer-derived results and the automated reader results. For the producer-derived results, the Se was 88.0% (80.7 to 93.3) and the Sp was 74.3% (66.5 to 81.1); for the automated reader results, the Se was 89.7% (82.8 to 94.6) and the Sp was 67.6% (59.4 to 75.0).

2.5 DISCUSSION

2.5.1 Test characteristics of the Petrifilm on-farm culture system

The goal of a SDCT+ITS program is to reserve intramammary antibiotics for cows that are suspected of having an IMI at drying off. A significant limitation of SDCT+ITS is that, in most cases, a cow's true infection status is not known. Therefore, essential to the success of a SDCT+ITS program is a method to identify a cow's IMI status at drying off, and the method should have high Se to maximize the treatment of infected cows (Sanford et al., 2006b). When used to detect IMI in low SCC cows at drying off, the Petrifilm on-farm culture system, with a colony count threshold of ≥ 5 colonies, had reasonably high Se (85.2%). A test with high Se will have few false negative results. As a result, dairy producers could use the on-farm culture system to make selective dry cow treatment decisions with the confidence that few infected cows would be missed. The Sp (73.2%) of the Petrifilm on-farm culture system using a treatment threshold of ≥ 5 colonies was moderate. The quantity of milk plated on the

Petrifilm (0.1 mL) is larger than that used in standard culture (0.01 mL), and this discrepancy is partially accounted for by the lower cfu threshold used with the Petrifilm. Increased recovery of pathogens on Petrifilm due to a larger inoculation volume would result in an increase in the number of apparent false positive diagnoses, and would therefore decrease the Sp of the test. The Sp of the Petrifilm on-farm culture system is reported with the caution that false positive Petrifilm results could in fact be true positives that were missed by standard bacteriological culture.

2.5.2 Treatment threshold

The colony count threshold used in this study was derived from McCarron et al. (2009), where a treatment cut point of ≥ 5 colonies maximized the Se of the Petrifilm for the detection of pathogens in mastitic milk samples. In the present study, increasing the colony count threshold to ≥ 10 colonies improved the Sp of the test, but unfavourably decreased the Se to 71.8%. As a result, if the higher threshold was used, fewer cows would be treated unnecessarily, but at the unacceptable expense of increased misdiagnosis of truly infected cows. From a clinical perspective, the cost of a false negative diagnosis (i.e. failing to treat an infected cow) is much greater than the cost of a false positive diagnosis (i.e. treating an uninfected cow), therefore for the purpose of SDCT, a treatment cut point of ≥ 5 colonies is recommended.

2.5.3 Petrifilm on-farm culture versus other diagnostic methods

Historically, SDCT programs have relied on prior information, such as monthly SCC and clinical mastitis (CM) history, to determine a cow's infection status at the end of lactation. In a previous study by Torres et al. (2008), evaluating the performance of cow level monthly SCC and CM history for the identification of infected and uninfected cows at drying off, it was determined that the accuracy of the test method was optimized by using three months SCC records with a threshold of 200,000 cells/mL for cows without CM during lactation, and a threshold of 100,000 cells/mL for cows with a case of CM in the first 90 days of lactation. Using single quarter sample bacteriological culture aggregated to the cow level as the gold standard, the Se and Sp of the criteria described above were 69.4% and 63.3%, respectively (Torres et al., 2008). In a similar study carried out in Atlantic Canada, a maximum Se of 64.9% was obtained using data from three months prior to drying off and a cow level SCC cut-off of 150,000 cells/mL (McCarron, 2012). Given the results of these prior studies, monthly SCC data, with or without CM history, are not sensitive enough and should not be recommended to be used alone as a guide for SDCT. Conversely, in the present study, when Petrifilm on-farm culture was applied to a SDCT program in addition to SCC and CM criteria, the Se of the overall IMI screening method was greatly improved. In effect, the criteria used to select cows for enrolment in this study included low monthly SCC and the absence of CM in the last 120 days prior to drying off. However, despite following these commonly accepted SDCT protocols, the prevalence of IMI in the group of cows that met the inclusion criteria was 43.4% according to standard bacteriological culture. The

diagnostic information currently used by dairy producers is therefore inadequate for the detection IMI at the time of drying off. As a result, the implementation of a SDCT program that relies on these criteria can put the health of a cow at risk, not only for the length of the dry period but into the next lactation as well. With the addition of Petrifilm-based on farm culture to the SDCT+ITS program protocol, 85.2% of the cows with an IMI at drying off, as determined by standard bacteriology, were detected and subsequently received the therapeutic benefit of DCT.

A popular cow-side test, the California Mastitis Test, has also been evaluated for use in a SDCT program (Sanford et al., 2006b). Like the Petrifilm on-farm culture system, the advantages of the CMT are its on-farm application and speed with which results are produced. Using a CMT score cut point of > 0 on quarter milk samples, and generating cow level results by parallel interpretation of quarter level outcomes, the Se and Sp of the CMT to identify cows with IMI on the day of drying off were estimated at 70% and 48%, respectively (Sanford et al., 2006b). Using the CMT alone to select cows for DCT would result in an unacceptably high proportion of infected cows being missed, and an even higher proportion of uninfected cows receiving antibiotics. Furthermore, although the CMT is relatively simple to use, it can be difficult to get accurate and consistent results due to the subjectivity of the scoring. In the present study, cows were excluded from the trial if they had a CMT score of two or greater in any quarter on the day prior to drying off. The addition of the CMT to the enrolment criteria resulted in only 7 additional cows being excluded from the trial, and therefore provided little

information beyond what was obtained using selection criteria based on SCC and CM history.

2.5.4 Predictive values of the Petrifilm on-farm culture system

Knowledge of the test characteristics of the Petrifilm on-farm culture system enabled the calculation of the predictive values of a positive or negative Petrifilm across populations with varying levels of IMI at drying off. As illustrated in Figure 2.2, when the proportion of cows with an IMI at drying off increased, the NPV decreased. For example, at a low prevalence of 30% and a high prevalence of 70%, the NPV of the Petrifilm on-farm culture system was 92.0% and 68.0%, respectively. Translating the NPV to the probability that a Petrifilm negative cow truly has an IMI (positive predictive value of a negative test; 1 – NPV; Dohoo et al., 2009), the proportion of infected cows that would not receive DCT would be very small (8%) for low prevalence herds, but unacceptable (32%) for high prevalence herds. Consequently, when considering implementing a SDCT program, assessment of a herd's current level of udder health (pre-test probability) is an important first consideration.

The positive predictive value of the Petrifilm on-farm culture system was moderate at a treatment threshold of ≥ 5 colonies, and as a result a number of uninfected cows received DCT at drying off. In North America, blanket dry cow therapy is customary, as it is estimated that 72% of herds in the United States and 88% of herds in Canada utilize this form of mastitis control over the dry period (USDA, 2008; Dufour, 2012). While selective dry cow therapy has received interest by the dairy industry as a

means to reduce antibiotic use on dairy farms, in order for it to be attractive to North American dairy farmers, SDCT must be a low risk alternative to the established practice of BDCT. Thus, although increasing the treatment threshold to ≥ 10 colonies would have resulted in a greater decrease in the unnecessary antibiotic treatment of uninfected cows, and may be more favourable in the eyes of the general public, the subsequent increase in the risk of missing infected cows would undoubtedly affect the willingness of dairy producers to adopt the alternative strategy.

2.5.5 Agreement between producer-derived Petrifilm results and automated Petrifilm reader

The level of agreement between the producer-derived Petrifilm results and the automated reader was high indicating that the producers were able to accurately classify the cows into infected and uninfected categories. Correspondingly, the test characteristics of the Petrifilm on-farm culture system based on producer-derived results were very close to the true test characteristics determined using the automated reader results. Interpretation of the AC Petrifilm is made easy by the presence of an indicator dye that stains the colonies a bright pink colour. Furthermore, enumeration of colonies to five or greater made it simple to classify cows into treatment categories. As a result, producers performed well with only minimal training at the outset of the trial. The level of agreement reported in this study is higher than reported in a previous study by MacDonald et al. (2011). In that field trial, when clinical milk samples were cultured on-farm using the culture system, the agreement between the producer-derived results and

the automated reader was 67.9% with a kappa value of 0.33 (MacDonald et al., 2011). It is possible that due to low incidence rates of CM (range = 1 to 24 cases; mean = 6.3 cases), the producers failed to obtain enough practice to become proficient in identification and enumeration of colonies on the Petrifilm (MacDonald et al., 2011). In the present study, all producers read at least six Petrifilms during the course of the study, and the majority (n = 12 herds) read twelve or more. It is very likely that the increased exposure to Petrifilms provided additional practice, enabling producers to obtain more accurate Petrifilm results. Additionally, in the earlier study, clots present in clinical milk samples could be mistaken for bacterial growth on Petrifilms, resulting in lower accuracy. The milk samples used in this study were from clinically normal quarters, therefore no clots should have interfered with Petrifilm interpretation.

2.5.6 Gold standard definition of intramammary infection

It is difficult to compare the performance of the Petrifilm on-farm culture system to previously published detection methods, as studies differ with respect to the definition of IMI used as gold standard. The detection limit of the gold standard test used in this study was quite rigorous, in accordance with the recent publication of new definitions of IMI based on single sample bacteriological testing (Dohoo et al., 2011). It is recognized that standard bacteriological culture is not a perfect test (Dohoo et al., 2011), therefore a strict classification system to define IMI at the quarter level was used with the aim of identifying as many existing infections as possible. Using a threshold of 200 cfu of CNS from a 1.0 mL milk sample resulted in a higher proportion of cows diagnosed with

significant growth than had a definition of ≥ 1000 cfu/mL based on NMC guidelines been used (National Mastitis Council, 1987). For the CNS isolates identified in the quarter milk samples, 47.7% (n = 82) displayed a growth of ≥ 1000 cfu/mL of milk. Correspondingly at the cow level, 54.4% (n = 68) of the cows with an IMI due to CNS had cultures with a growth of ≥ 1000 cfu/mL of milk. Evaluating the performance of the Petrifilm on-farm culture system using the NMC guidelines for significant growth of CNS resulted in a very small improvement in Se (86.3% vs. 85.2%) and a drop in Sp (62.7% vs. 75.2%). As a result of a larger inoculation volume and a lower cfu/mL threshold used with the on-farm culture system, the Petrifilm detected cases of CNS IMI that were missed by standard bacteriological culture using NMC guidelines, resulting in a drop in Sp. Mastitis as a result of IMI with CNS is typically mild and subclinical (Pyörälä and Taponen, 2009). Previous research has focused on CNS infection during lactation, and is conflicting with respect to the effects of CNS on milk production, as well as on the susceptibility of a quarter to infection with a major mastitis pathogen (Reyher et al., 2012). Research into the outcome of CNS infection at drying off is scarce in the literature. In a study by Berry and Hillerton (2002b) investigating SDCT, the risk of a new *Streptococcus uberis* infection at calving, in quarters that did not receive DCT, was 4.2 greater when CNS was isolated at drying off then if no pathogen had been cultured. For the same group of cows, the risk of a new coliform infection was 4.7 times greater (Berry and Hillerton, 2002b). Furthermore, while the diagnosis of CNS in a milk sample usually terminates at the group level, individual species of CNS differ in their effects on udder health, and the determination of the relevance of different CNS species requires an

accurate and practical method to make species-specific diagnoses (Supré et al., 2011). Until identification of different CNS isolates is possible, and until further research into the consequences of CNS infection at drying off is available, the most prudent course of action would be to treat all affected cows with DCT.

2.5.7 Effect of contamination on Petrifilm results

A limitation of the on-farm culture system is the inability of the Petrifilm to distinguish contamination from infection. The AC Petrifilm does not allow for species identification, making it impossible to determine if the presence of colonies represents significant growth of pathogens. A cow incorrectly classified as positive by on-farm culture would receive DCT when no antibiotic treatment was indicated, thus the risk associated with a false positive test result is considered minimal. Following well-documented procedures outlined in the Laboratory Handbook on Bovine Mastitis (National Mastitis Council, 1999), low sample contamination rates were achieved in this study. Attention to strict aseptic technique when collecting samples for use with the Petrifilm on-farm culture system would help to reduce unnecessary antibiotic use. Additionally, periodic submission of milk samples to a diagnostic bacteriology laboratory, particularly if a herd is experiencing a higher than expected proportion of Petrifilm positive cows, should be recommended as a means to assess quarter sampling technique and the quality of the resultant milk samples used with the on-farm culture system.

2.5.8 Effect of sample type and sample storage

In the present study, application of the Petrifilm on-farm culture system was at the level of the cow. The culture system was implemented in this manner to reflect current SDCT practice, where treatment decisions are most frequently made the at the cow level. Gold standard diagnoses at the cow level were originally measured at the quarter level. Petrifilm diagnoses were based on composite samples, which are measurements at the cow level. The Se of composite samples is lower than that of quarter samples due to the dilution effect (Reyher and Dohoo, 2011), therefore the Petrifilm on-farm culture system had an inherent limitation when compared to standard bacteriological culture. Despite this constraint, the Petrifilm on-farm culture system correctly identified 85.2% of infected cows.

In order to transport samples to the bacteriology laboratory, and to comply with weekly sample processing capacity, it was necessary to freeze and maintain all milk samples at -20°C. Freezing of the milk samples may have had an impact on the isolation of pathogens by gold standard bacteriological culture. While Murdough et al. (1996) reported that freezing of milk samples for 6 weeks did not affect the viability of *Staph. aureus*, CNS, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Corynebacterium bovis*, or *E.coli*, Schukken et al. (1989) observed that freezing up to 16 weeks resulted in a decrease in positive cultures for *E. coli* and *Truperella pyogenes*, an increase in the recovery of CNS, and no effect on streptococci and *Staph. aureus*. As a result of freezing the milk samples prior to gold standard bacteriological culture, the estimates of Se and Sp of the Petrifilm on-farm culture system may be somewhat over or under-estimated.

2.5.9 Future directions

Following the procedures outlined in the current study, 46.0% of the cows dried-off during the enrolment period met the inclusion criteria, and 47.8% of those cows cultured negative on-farm and did not receive dry cow antibiotics. Therefore, when the Petrifilm on-farm culture system was applied at the cow level, a total reduction in DCT of 22% was realized. Application of the Petrifilm on-farm culture system at the level of the individual mammary quarter would allow for DCT treatment decisions to be made at the quarter level, thus reducing even further the amount of antibiotics used in dairy production.

In the present study, 82.7% of quarters did not have an IMI at drying off according to standard bacteriology. By comparison, at the cow level, just under half of the cows were identified as uninfected by Petrifilm on-farm culture. Had the Petrifilm on-farm culture system been applied at the quarter level, an even greater reduction in the use of DCT would have been realized, potentially leading to greater cost savings and a more judicious use of antimicrobials. Moreover, quarter level culturing would result in an improvement in the sensitivity when compared to composite culturing (Reyher and Dohoo, 2011), thus reducing the false negative rate of the Petrifilm on-farm culture system.

2.6 CONCLUSION

Petrifilm-based bacteriological culture allows producers to culture milk directly on-farm and obtain results within 24 hours. The Petrifilm on-farm culture system, when used to diagnose IMI in low SCC cows at drying off, had a reasonably high Se and a high NPV, and application of the Petrifilm on-farm culture system in a selective dry cow therapy program enabled a reduction in the use of long-acting intramammary antibiotics at drying off.

The success of a SDCT program can be determined by the prevalence of intramammary infection at freshening and in early lactation, as well as the cure rates achieved over the dry period. The information presented in this paper comprises data from a clinical trial comparing Petrifilm-based SDCT+ITS to BDCT+ITS. Further analysis of the data from the clinical trial will cover aspects of the measured success of SDCT, as well as the economics of Petrifilm-based SDCT.

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Table 2.1 Distribution of pathogens isolated in quarter milk samples collected on the day prior to drying off from cows with a low somatic cell count (< 200 000 cells/mL) on the last three milk tests prior to the end of lactation. Results are presented as the number of quarters and cows (as a percentage of total quarters or cows) infected with a particular organism1.

Pathogen	Number of quarters (% of total quarters)	Number of cows (% of total cows)
No significant growth	1134 (82.7)	194 (56.6)
Coagulase negative staphylococci	172 (12.5)	125 (36.4)
Environmental streptococci[2]	16 (1.2)	14 (4.1)
Corynebacterium spp.	13 (0.9)	11 (3.2)
Staphylococcus aureus	7 (0.5)	7 (2.0)
Escherichia coli	2 (0.1)	2 (0.6)
Trueperella pyogenes	1 (0.1)	1 (0.3)
Other gram negative[3]	1 (0.1)	1 (0.3)
Fungi	1 (0.1)	1 (0.3)
Contaminated	40 (2.9)	-
No sample	4 (0.3)	-

[1] Number of quarters (or cows) may add up to more than the total number of quarters (or cows) included in the analysis as quarters (or cows) could be infected with more than one pathogen.
[2] Environmental streptococci include all streptococci other than *Streptococcus agalactiae.*
[3] Other Gram negatives include: *Serratia* spp., *Citrobacter* spp., *Proteus* spp., *Salmonella* spp., *Pseudomonas* spp., *Pasteurella multocida.*

Table 2.2 Agreement between producers and an automated Petrifilm reader for the Petrifilm on-farm culture system, when used to diagnose intramammary infection in low somatic cell count cows (< 200 000 cells/mL) at the end of lactation, with an infection threshold of ≥ 5 colonies. Cells displaying the disagreement between producers and the automated reader, resulting in the misclassification of a cow into treatment categories, are shaded.

			Producer				
			Negative		Positive		
		Colony count	0	1 to 4	5 to 9	≥10	Total
Automated reader	Negative	0	24	4	0	0	28
		1 to 4	20	58	5	1	84
	Positive	5 to 9	4	8	11	0	23
		≥10	1	5	19	105	130
		Total	49	75	35	106	265

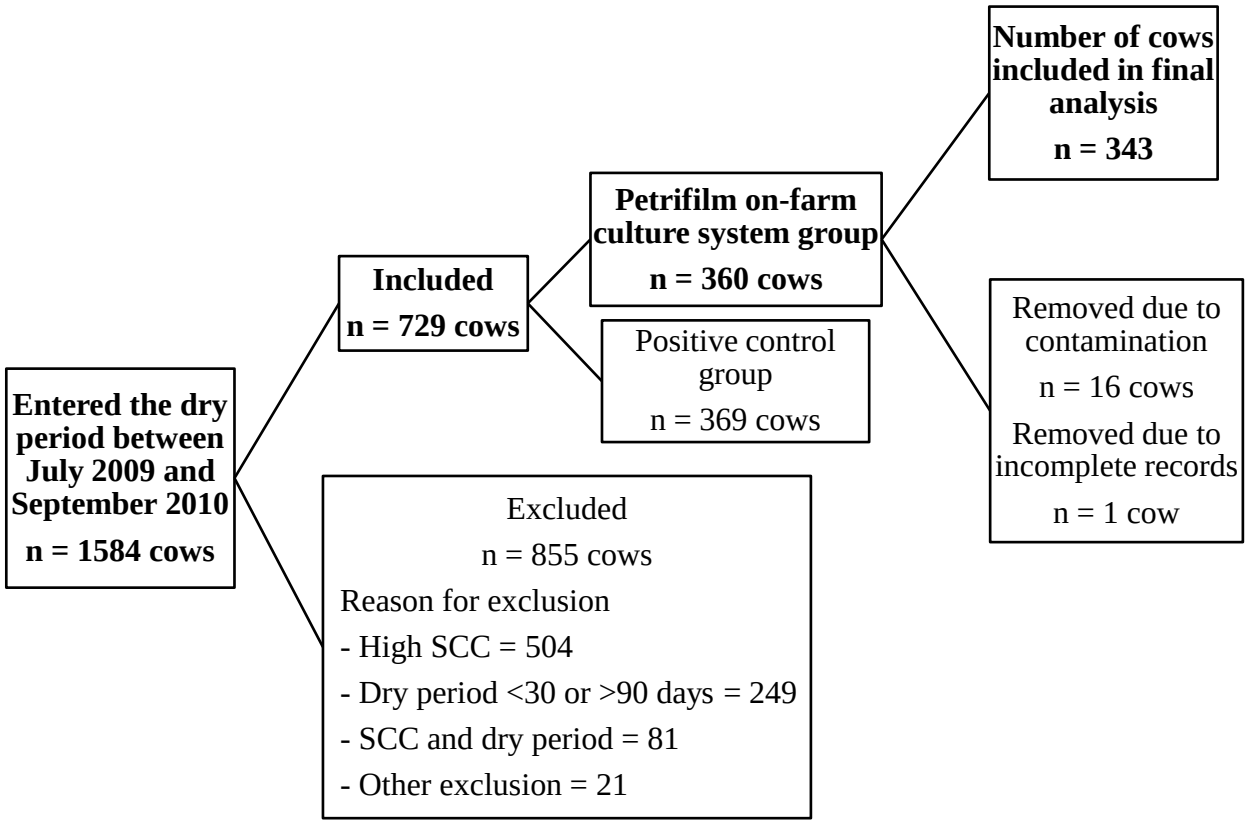

Figure 2.1 Tree diagram illustrating the enrolment of cows in the field trial for the evaluation of a Petrifilm on-farm culture system for the detection of intramammary infection at the end of lactation. (SCC: somatic cell count)

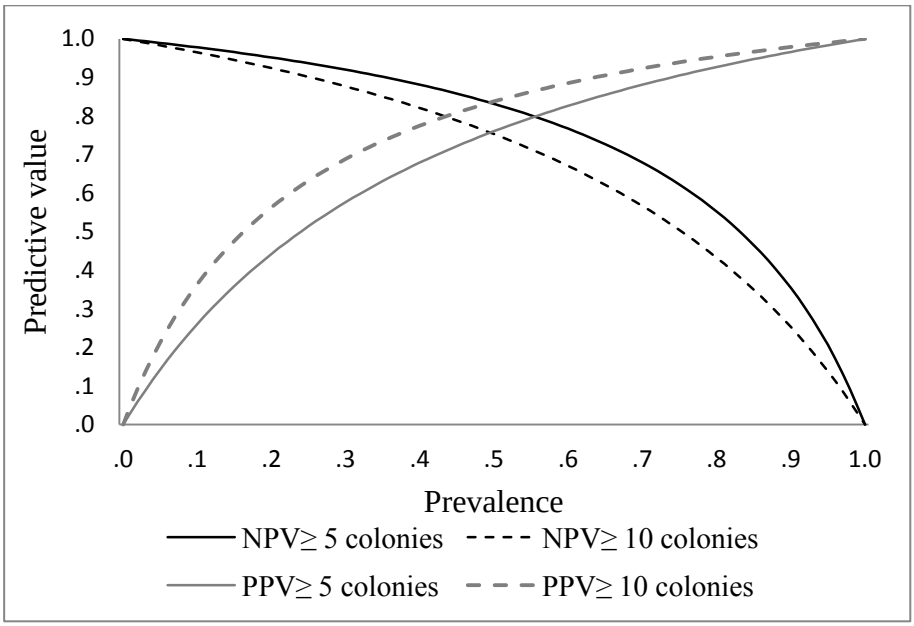

Figure 2.2 Positive predictive values (PPV) and negative predictive values (NPV) for the Petrifilm on-farm culture system, at colony count threshold values of 5 and 10 colonies, when used to diagnose intramammary infection (IMI) in low somatic cell count cows (< 200 000 cells/mL) at drying off, for prevalences of IMI ranging from 0 to 100

CHAPTER 3

EVALUATION OF SELECTIVE DRY COW TREATMENT FOLLOWING ON-FARM CULTURE: RISK OF POST-CALVING INTRAMAMMARY INFECTION AND CLINICAL MASTITIS IN THE SUBSEQUENT LACTATION

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3.1 ABSTRACT

The objective of the study was to evaluate the utility of a Petrifilm-based on-farm culture system when used to make selective antimicrobial treatment decisions on low somatic cell count cows (< 200,000 cells/mL) at drying off. A total of 729 cows from 16 commercial dairy herds with a low bulk tank somatic cell count (< 250,000 cells/mL) were randomly assigned to receive either blanket dry cow therapy or Petrifilm-based selective dry cow therapy. Cows belonging to the blanket dry cow therapy group were infused with a commercial dry cow antimicrobial product (**DCT**) and an internal teat sealant (**ITS**) at drying off. Using composite milk samples collected on the day prior to drying off, cows in the selective dry cow therapy group were treated at drying off based on the results obtained by the Petrifilm on-farm culture system with DCT + ITS (Petrifilm culture positive), or ITS alone (Petrifilm culture negative). Quarters of all cows were sampled for standard laboratory bacteriology on the day prior to drying off, at 3 to 4 days in milk (**DIM**), at 5 to 18 DIM, and from first cases of clinical mastitis occurring within 120 DIM.

Multilevel logistic regression was used to assess the effect of study group (blanket or selective dry cow therapy), and resulting dry cow treatment (DCT + ITS, or ITS alone), on the risk of intramammary infection (**IMI**) at calving and the risk of a first case of clinical mastitis between calving and 120 DIM. According to univariable analysis, there was no difference between study groups with respect to quarter level cure risk and new IMI risk over the dry period. Likewise, the risk of IMI at calving and the risk of clinical mastitis in the first 120 DIM was not different between quarters belonging to

cows in the blanket dry cow therapy group and quarters belonging to cows in the selective dry cow therapy group. The results of this study indicate that selective dry cow therapy based on results obtained by the Petrifilm on-farm culture system achieved the same level of success with respect to treatment and prevention of IMI over the dry period as blanket dry cow therapy, and did not affect the risk of clinical mastitis in the first 120 days of the subsequent lactation.

3.2 INTRODUCTION

3.2.1 Dry Cow Therapy Practices in North America

In North America, the preferred mastitis control strategy during the dry period is the infusion of a long-acting intramammary antimicrobial product (**DCT**) in all quarters of all cows. This practice, known as blanket dry cow therapy (**BDCT**), has been prescribed by the National Mastitis Council (**NMC**) as part of their Recommended Mastitis Control Program (National Mastitis Council, 2006). In the United States and Canada, uptake of BDCT is estimated at 72% and 88% of all dairy herds, respectively (USDA, 2008; Dufour et al., 2012). The function of DCT in mastitis control is twofold: 1) the cure of IMI present at drying off and 2) the prevention of new IMI in the early dry period. Historically, BDCT has played an important role in the overall reduction in the prevalence of contagious mastitis pathogens, and a consequential reduction in bulk tank somatic cell counts (**BTSCC**) (Hillerton et al., 1995; Bradley, 2002; Ruegg, 2012). Since the promotion of BDCT in Canada in the late 1960s, *Streptococcus agalactiae* is nearly eradicated and the average provincial geometric-mean BTSCC in 2005 was 225,000

cells/mL (Olde Riekerink et al., 2006; Olde Riekerink et al., 2010). In light of these changes in the epidemiology of mastitis, it is argued that the practice of BDCT by all herds is no longer required (Robert et al., 2006b; Huijps and Hogeveen, 2007; Rajala-Schultz et al., 2011). In support of this argument, there is growing concern regarding the impact of antimicrobial use in dairy production systems on the emergence of antimicrobial resistant bacteria (Call et al., 2008; Oliver et al., 2011). In dairy production, antimicrobials are most frequently used for the purpose of treatment and prevention of mastitis (Saini et al., 2012; USDA, 2008), but the relationship between antimicrobial drug use and the development of resistance in bacteria is unclear (Call et al., 2008; Saini et al., 2012). Furthermore, studies examining the association between the use of DCT and antimicrobial resistance are sparse (Rajala-Schultz et al., 2009). Nonetheless, antimicrobial drug use is considered a driving force for the development of resistance (Call et al., 2008), thus motivating research into alternative practices which have the potential to limit their application.

3.2.2 Internal Teat Sealants and Selective Dry Cow Therapy

Antimicrobials still play a vital role in dry period mastitis control. Dry cow intramammary antibiotics achieve high cure rates for susceptible pathogens (Gruet et al., 2001; Bradley and Green, 2004; Halasa et al., 2009a), and DCT remains effective in the prevention of new IMI by sensitive pathogens in the early dry period (Williamson et al., 1995; Berry and Hillerton, 2002b; Halasa et al., 2009b). Nevertheless, new IMI still occurs despite DCT when the causative pathogens are non-susceptible, or in the late dry period when antibiotic levels have fallen below the minimum inhibitory concentration

(Oliver et al., 1990; Bradley and Green, 2000). Obstacles to the prevention of new IMI during the dry period can be overcome by the addition of an internal teat sealant (**ITS**) to the dry cow protocol. Internal teat sealants are a non-antimicrobial alternative to DCT for the prevention of IMI during the non-lactating period, and the effectiveness of ITS is well-supported in the literature (Berry and Hillerton, 2002a; Huxley et al., 2002; Sanford et al., 2006a). In studies that have compared ITS to DCT in quarters uninfected at drying off, ITS performed just as well as DCT with respect to the prevention of new IMI (Woolford et al., 1998; Sanford et al., 2006a), suggesting that ITS can be used alone in the absence of IMI at the end of lactation. Antibiotic treatment of cows at the end of lactation based on IMI status is a dry period mastitis control strategy known as selective dry cow therapy (**SDCT**). The addition of an ITS to a SDCT protocol ensures that all quarters have some protection against new IMI, thus reducing the necessity for the prophylactic use of antimicrobials over the non-lactating period.

3.2.3 Selection Procedures for Dry Cow Therapy

While previous research supports the use of ITS alone in uninfected quarters, failing to treat an infected quarter with DCT results in financial losses (Berry et al., 2004). Therefore, an accurate selection method is required to correctly identify quarters free of IMI at drying off (Huxley et al., 2002; Robert et al., 2008; Torres et al., 2008). Selection procedures based on standard bacteriological culture (Robinson et al., 1988; Browning et al., 1990; Browning et al., 1994), somatic cell count (**SCC**) and clinical mastitis history (Rindsig et al., 1978; Torres et al., 2008; Rajala-Schultz et al., 2011), the California Mastitis Test (Rindsig et al., 1978; Sanford et al., 2006b; Bhutto et al., 2012),

and NAGase (Hassan et al., 1999) have been described in the literature with varying degrees of accuracy. The most commonly used selection method is based on monthly SCC which has a reported sensitivity of 70% and specificity of 63% (Torres et al., 2008). The Petrifilm-based on-farm culture system is an easy to interpret, accurate, and quick diagnostic tool that enables dairy producers to culture milk directly on the farm and obtain results within 24 hours (McCarron et al., 2009; Cameron et al., 2013). The Petrifilm-based on-farm culture system performed well when used to diagnose IMI in low SCC cows at drying off, with a sensitivity of 85.2% and specificity of 73.2% (Cameron et al., 2013). The aim of the research outlined in this paper was to evaluate the use of a Petrifilm-based on-farm culture system in a selective dry cow therapy program. The main objectives were to compare IMI risk at calving between quarters of cows receiving blanket dry cow therapy with the addition of an ITS and quarters of cows selectively treated based on the results of the Petrifilm on-farm culture system with DCT + ITS, or ITS alone. A comparison between the two study groups was also made regarding the risk of a first case of clinical mastitis between calving and 120 days in milk (**DIM**) of the subsequent lactation.

3.3 MATERIALS AND METHODS

3.3.1 A Priori Sample Size Calculation

Sample size calculations were based on a published prevalence of IMI at calving of approximately 20% when both DCT and ITS were used at drying off (Godden et al., 2003; Sanford et al., 2006a). With a significance level of 95% (two-sided) and a power

of 80%, it was determined that approximately 2,280 quarters (570 cows with 285 being in each group) would be required to detect a minimum change in risk of IMI at calving from 20% to 25% (margin of non-inferiority of 5%). This calculation assumes that quarters within a cow are independent, although research has shown interdependence of quarters with respect to IMI (Barkema et al., 1997; Berry and Meaney, 2006). To account for clustering of quarters within cows, an adjustment to the sample size was made using a published intraclass correlation coefficient (ρ) of 0.20 (Barkema et al., 1997; Berry and Meaney, 2006) resulting in a sample size of 3,632 quarters (908 cows, with 454 per group).

3.3.2 Herd Selection

In order to be considered for inclusion, herds were required to have an annual average of monthly BTSCC below 250,000 cells/mL over the last 12 months and be enrolled in a dairy herd improvement program. A convenience sample of 16 Canadian dairy herds from Prince Edward Island (n = 10) and Quebec (n = 6) were enrolled in the randomized clinical trial. Eligible herds were selected based on willingness to adhere to the study protocol and proximity to the veterinary college in each province.

3.3.3 Cow Selection

Cow level inclusion criteria consisted of monthly SCC below 200,000 cells/mL on the last three milk tests prior to drying off, no clinical mastitis in the same time period, an expected dry period of 30 to 90 days, no antibiotic treatment in the last 14 days, and at least 3 functional quarters. On the day prior to drying off, eligible cows were subjected

to a California Mastitis Test by the trained study personnel, and cows with a score of two or greater in any quarter were excluded from the trial.

Based on an analysis of the Canadian Bovine Mastitis Research Network cohort study, it was expected that 30% of cows would fail to meet the inclusion criteria for SCC less than 200,000 cells/mL (unpublished data). Additional exclusions based on other criteria were estimated at 10% of cows. Therefore, a pre-exclusion sample of approximately 1,500 cows would be required to achieve the required sample size of 908 cows.

3.3.4 Pre-Dry Sampling and Random Allocation to Treatment Groups

Producers scheduled a dry off day every other week to allow for cow enrolment and sample collection. On the day prior to drying off (Day -1), single quarter level milk samples were collected by the study personnel. Following pre-milking udder preparation as per farm protocol, teat ends were scrubbed clean with alcohol swabs and initial foremilk streams were discarded into a strip cup. Approximately 30 mL of milk was collected aseptically according to the procedures recommended in the Laboratory Handbook on Bovine Mastitis (National Mastitis Council, 1999). Following sample collection (**DO** = dry off sample), cows were randomly assigned to either the blanket dry cow therapy (BDCT) or the Petrifilm-based selective dry cow therapy (SDCT) study group according to a randomization table unique to each herd, with a block size of six thus randomizing six cows at a time, three to BDCT and three to SDCT.

3.3.5 On-Farm Bacteriological Culture and Treatment at Drying Off

The study personnel set-up the Petrifilm-based on-farm culture system for cows assigned to SDCT. A composite sample was created by combining 5 mL of milk from each quarter sample into a new sample vial. A 3 mL aliquot of composite milk was added to 27 mL of sterile water to make a 1:10 dilution (McCarron et al., 2009). One milliliter of diluted milk was plated on an Aerobic Count **(AC)** Petrifilm (3M Canada, London, Ontario) and incubated on-farm at 35°C for 24 hours in a TurboFan Hova-Bator (GQF Manufacturing, Savannah, Georgia, USA). The Aerobic Count Petrifilm is a ready-made culture medium used for the detection of all aerobic bacteria and contains an indicator dye which facilitates colony enumeration. On the scheduled day of drying off (Day 0), the Petrifilm was read by the producer and, in accordance with study protocol, cows were classified as positive if 5 or more colonies were present (equivalent to ≥ 50 cfu/mL of milk). Cows negative on Petrifilm were treated solely with an ITS composed of 65% bismuth subnitrate (Orbeseal, Pfizer Animal Health, Kirkland, Quebec, Canada) at drying off. Cows positive on Petrifilm were treated with a long-acting intramammary formulation of 500 mg ceftiofur hydrochloride (Spectramast DC, Pfizer Animal Health, Kirkland, Quebec, Canada) followed by an ITS. Producers recorded the number of colonies present on the Petrifilm, as well as the treatment applied, to verify compliance with the study protocol. Cows in the BDCT group were infused with ceftiofur followed by an ITS in all four quarters, and again producers recorded the treatments given. All cows were dried-off abruptly and dry cow treatments were administered by the producer or farm personnel immediately after the last milking. Teats were prepared aseptically prior to treatment, and were disinfected post treatment with a commercial teat dip.

3.3.6 Post-Calving Sampling and Record Keeping

Post-calving quarter milk samples were aseptically collected by the producer between 3 and 4 DIM (**PC1** = post-calving sample 1) and by the study personnel between day 5 and 18 DIM (**PC2** = post-calving sample 2) using the methods outlined for pre-dry off sampling. At the time of sample collection, the producer and the study personnel recorded the occurrence of any post-partum disease (such as milk fever, mastitis, or retained placenta) and any given antibiotic treatment. The antibiotic treatment of a cow prior to the collection of the first post-calving sample resulted in the removal of the entire observation from the analyses. All post-enrolment exclusions were enumerated and reported separately from pre-enrolment exclusions to allow for comparison between groups.

3.3.7 Clinical Mastitis Sampling

Clinical mastitis cases occurring in participating cows in the first 120 days of the next lactation were recorded and sampled by the producer prior to treatment. Only the first clinical mastitis episode per quarter was considered in the analysis. Clinical mastitis was defined as visible changes in milk, with or without heat, swelling or redness in the quarter, and with or without signs of systemic disease. Milk samples were collected aseptically and frozen on farm until collection by the study personnel during their visit every other week.

3.3.8 Laboratory Bacteriological Culture

Subsequent to collection, all milk samples were frozen at -20°C before shipment to the Maritime Quality Milk research laboratory at the University of Prince Edward Island. Laboratory technicians were blinded to the results obtained by the Petrifilm on-farm culture system. Laboratory bacteriological culture was performed according to procedures outlined in the NMC's Laboratory Handbook on Bovine Mastitis (National Mastitis Council, 1999). A quarter was considered infected if ≥ 100 cfu/mL of milk of any pathogenic organism of interest, except for coagulase negative staphylococci (**CNS**), were cultured. For CNS, a definition of ≥ 200 cfu/mL was used. These definitions are in accordance with the recent publication of characterization of IMI based on single sample bacteriological testing (Dohoo et al., 2011b). Samples with three or more differing isolates were classified as contaminated, and isolation of *Bacillus* spp. was considered non-significant growth. However, when *Staphylococcus aureus* (***Staph. aureus***) was identified in a contaminated sample, it was enumerated and the associated quarter was classified as infected (Reyher et al., 2011).

For the purpose of analysis, Gram-negative bacteria, other than *Escherichia coli* (***E. coli***) and *Klebsiella* spp., were amalgamated into a grouping and encompassed *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *Pseudomonas* spp., *Serratia* spp., *Salmonella* spp., *Pasteurella multocida*, and other non-differentiated Gram-negative bacteria. Coagulase negative staphylococci were not differentiated beyond the group level, but if two phenotypically different species of CNS displayed growth on culture, each isolate was enumerated and reported.

3.3.9 Definitions

Post-Calving Intramammary Infection. Two definitions for post-calving IMI were applied, considering either PC1 alone, or both PC1 and PC2 interpreted in parallel. For the first definition, a quarter was considered to have an IMI if one or two pathogens were isolated in PC1. If PC1 was missing or contaminated, the observation was dropped from the analysis. For the second definition, a quarter was considered infected if either PC1 or PC2 displayed growth of one or two pathogens. In cases where one of the post-calving samples was missing or was classified as contaminated, the quarter infection status was determined solely by the results of the remaining sample. If both post-calving samples were contaminated or missing, the associated quarter was removed from the analysis.

Dry Period Cure. Dry period cure was determined at both the pathogen and quarter levels. A pathogen isolated in DO was considered cured over the dry period if it was absent in both PC1 and PC2. A quarter was considered cured when a pathogen isolated in DO was not recovered in either PC1 or PC2. If a quarter was infected with two pathogens at drying off, the absence of both pathogens in PC1 and PC2 was required for that quarter to be labeled cured. In cases where one, or both, of the post-calving samples were missing or contaminated, the associated quarter was dropped from the analysis.

New Intramammary Infection. Two definitions of new intramammary infection were investigated. According to both definitions, a quarter was defined as having a new IMI if a pathogen was cultured at calving that was not present in DO. Therefore, both uninfected and infected quarters were considered at risk for new IMI during the dry period. For the first definition, only PC1 was considered. The second definition was based on parallel interpretation of PC1 and PC2, thus if a pathogen was present in either post-calving

samples, but was not present in DO, that quarter was considered newly infected over the dry period.

3.3.10 Statistical Analysis

Statistical analysis was performed using Stata/IC 11.0 (StataCorp, College Station, TX). The experimental unit for statistical analysis was the individual mammary quarter. Univariable analysis of the effect of study group was carried out using Fisher's exact test for the following outcomes: risk of IMI at drying off, risk of IMI at calving, risk for cure over the dry period, and risk of new IMI during the dry period.

Multilevel logistic regression models were applied to evaluate the effect of study group, and treatment within the study group, on 1) the risk of IMI at calving, and 2) the risk of a first case of clinical mastitis between calving and 120 DIM. Random effects for cows and herds were incorporated in the models to account for clustering of quarters within cows, and cows within herds. The main predictor of interest 'treatment group' was specified at the cow level, and was created by combining the variables study group (BDCT or SDCT) and subsequent treatment at drying off (DCT + ITS, or ITS alone), thus comprising three levels: BDCT, Petrifilm positive, and Petrifilm negative. Additional explanatory variables considered for inclusion in the models included region, herd size, housing type (tie-stall or free-stall), season at calving, parity at calving (2, 3, or 4+), last recorded 24 hour milk yield (in Kg) prior to drying off, length of the dry period, quarter infection status at drying off as determined by standard bacteriologic culture, and quarter position (front or hind). The variable for season at calving was created using three month intervals, such that winter was designated as December to February, spring

as March to May, summer as June to August, and fall as September to November. All variables were explored using descriptive statistics and graphical analyses where appropriate. Continuous predictors were evaluated for linearity with the logit of the outcomes using a locally weighted scatter plot smoothed (lowess) curve.

The primary predictor of interest 'treatment group' was forced into all models regardless of statistical significance. Intramammary infection status at drying off according to gold standard bacteriological culture was also retained in all models in order to control for the confounding effect of this variable on the association of treatment group with both the risk of IMI at calving and the risk of clinical mastitis in the subsequent lactation. Unconditional associations between the other explanatory variables and the outcomes were examined using simple univariable logistic regression, and variables with P values < 0.30 were offered for inclusion in the multivariable models. A backwards stepwise procedure was used to determine the final models, and all two-way interactions involving the main predictor 'treatment group' were evaluated. Of particular interest, an interaction between 'treatment group' and quarter infection status at drying off as determined by standard bacteriologic culture was created to examine the effects of a false negative and a false positive Petrifilm result. Significance was declared at a P value < 0.05, but variables were retained in the models with a P value < 0.10, or if they were involved in a significant two-way interaction. Once the final models were reached, the fit was evaluated by examination of residual plots (Dohoo et al., 2009).

3.4 RESULTS

3.4.1 Cow Enrolment and Descriptive Statistics

During the cow enrolment period of July 2009 to September 2010, a total of 1,584 cows from the 16 participating herds entered the dry period. The number of cows dried-off per farm ranged from 42 to 263, with a median of 81 cows. Details of cow enrolment and a flow chart of study units can be found in Figure 3.1. The most common reason for exclusion from the trial was high SCC (504/855; 58.9% of exclusions), followed by an anticipated long or short dry period (249/855; 29.1% of exclusions). The addition of the CMT to the enrolment criteria resulted in only 7 additional cows being excluded from the trial and therefore provided little information beyond what was obtained using selection criteria based on SCC and clinical mastitis history. The proportion of cows per farm eligible for inclusion ranged from 28.3% (15/53) to 69.4% (145/209), and ultimately a total of 729 (46.0%) cows were enrolled in the trial. Of these cows, 369 (1,476 quarters) were assigned to the BDCT group and 360 (1,440 quarters) were assigned to the SDCT group (Figure 3.1). Post-enrolment exclusions and attrition resulted in the loss of 126 cows for the following reasons: no post-calving sample was collected (n = 9), death (n = 10), sold prior to calving (n = 11), abortion (n = 2), not pregnant (n = 2), an actual dry period greater than 90 days or shorter than 30 days (n = 51), other antibiotic treatment prior to post-calving sample collection (n = 40), and contamination of all post-calving milk samples (n = 1). Sixteen cows that received antibiotic treatment prior to sample collection were treated for mastitis (BDCT = 7 cows; SDCT = 9 cows). An additional 114 quarters were dropped due to contamination, and 11 quarters were non-lactating.

There were no significant differences between study groups with respect to post-enrolment exclusion or attrition. Thus, 1,157 BDCT group quarters (n = 305 cows) and 1,130 SDCT quarters (n = 298 cows) were available for analysis (Figure 3.1). The cow level prevalence of IMI at drying off in the SDCT group according to Petrifilm on-farm culture was 54.4% (162/298). Therefore, at a Petrifilm treatment threshold of ≥ 50 cfu/mL, 45.6% (136/298) of the cows were classified as uninfected and did not receive a long-acting intramammary antibiotic at drying off. Descriptive statistics for the cows included in the final analysis can be found in Table 3.1.

3.4.2 Risk of Intramammary Infection at Drying Off and Post-Calving

The standard bacteriological culture results from quarter level milk samples collected at the end of lactation and at calving are shown in Tables 3.2 and 3.3. Despite random allocation into study groups, the proportion of quarters with an IMI at drying off was higher in the SDCT group than in the BDCT group, but this difference was only borderline significant (BDCT: 12.4% (10.5, 14.3) vs. SDCT: 15.1% (13.0, 17.2); P = 0.054). In the SDCT group, a false negative Petrifilm result occurred in 20 cows and as a result, 23 quarters were misclassified as negative and did not receive DCT at drying off. Out of the group of cows (n = 162) that cultured positive on Petrifilm, 67.3% had at least one quarter with an IMI at drying off according to standard culture.

Following calving, the prevalence of IMI was not different between study groups, whether post-calving IMI status was determined by the first post-calving sample (BDCT: 11.1% (9.0, 13.2) vs. SDCT: 10.6% (8.6, 12.7); P = 0.77) or by parallel interpretation of both post-calving samples (BDCT: 15.3% (13.2, 17.4) vs. SDCT: 15.8% (13.7, 18.0); P =

0.72). As the second definition resulted in higher statistical power and represented a more liberal definition of post-calving IMI, it was examined as the outcome in the multivariable model. Coagulase negative staphylococci were the most commonly isolated pathogens at all sampling periods. The next most common pathogens isolated at drying off were *Corynebacterium* spp. and environmental streptococci, followed by *Staph. aureus.* After calving, environmental streptococci and *Staph. aureus* were the next most prevalent pathogens.

3.4.3 Cure Risk Over the Dry Period

Quarter level cure risk over the dry period was high and was not different between study groups (BDCT: 84.5% (76.0, 90.9); SDCT: 89.0% (81.9, 94.0); $P = 0.33$). There was no significant difference in species-specific cure risks between quarters that received BDCT and quarters that received SDCT. The cure risk for *Staph. aureus* in the BDCT group was 100% (5/5), but in the SDCT group the cure risk was 75% (3/4). In the SDCT group, the quarter infected with *Staph. aureus* that failed to cure belonged to a cow that was misdiagnosed by the Petrifilm on-farm culture system and therefore that quarter did not receive DCT at drying off. The same was true for a SDCT group quarter that remained infected with *Streptococcus dysgalactiae* and one quarter that remained infected with CNS from drying off to calving. However, the overall apparent self-cure risk for quarters misdiagnosed by the Petrifilm on-farm culture system was 87.0% (20/23). Pathogens isolated from the quarters with spontaneous cure were CNS (n = 12), nondifferentiated streptococci (n = 5), and *Corynebacterium* spp. (n = 3).

3.4.4 New Intramammary Infection Risk Over the Dry Period

When new intramammary infection risk was examined using only the first post-calving sample, the results were similar to those obtained using both post-calving samples (Appendix B). Because analyses using both post-calving samples resulted in greater power, and a more liberal definition of new IMI, only those results are presented. The pathogen-specific and quarter-level new IMI risks are presented in Table 3.4. Overall, there was no significant difference between quarters that received BDCT and quarters under SDCT based on Petrifilm results. There were more quarters with new IMI caused by fungi and yeast in the BDCT group than in the SDCT group (0.9% vs. 0.2%; $P = 0.04$). The majority of new infections were caused by CNS, followed by environmental streptococci and *Staph. aureus*.

3.4.5 Unconditional Analyses and Final Multilevel Model for the Risk of Intramammary Infection at Calving

Unconditional associations between independent variables and the probability of IMI at calving are presented in Table 3.5. Significantly associated with the risk of IMI post-calving were IMI status at drying off according to standard culture, season at drying off, parity at calving, and quarter position. Twenty-four hour milk yield on the last test prior to drying off was borderline significant (Odds ratio (OR) 1.02 (1.00, 1.04)).

The results of the final multivariable logistic model are presented in Table 3.6. In the final model, treatment group was not significantly associated with the probability of IMI at calving ($P = 0.95$). Similarly, the following independent variables were not associated with the risk of IMI at calving and were dropped from the final model: region,

herd size, housing type, 24 hour milk yield on the last test, parity at calving, and length of the dry period. Neither intramammary infection status at drying off according to standard bacteriological culture ($P = 0.29$), nor the interaction term between infection status and treatment group ($P = 0.36$) were significant. Quarter position was a significant predictor in the model, with hind quarters having a higher odds of IMI (OR 1.36 (1.05, 1.75)). Season at calving was also significant, with quarters belonging to cows calving in the summer and the fall having a higher likelihood of IMI (OR 1.95 (1.21, 3.15); OR 1.75 (1.10, 2.78), respectively) than quarters of cows calving in the winter (referent category). Evaluation of residual plots did not indicate a problem with the fit of the final model.

3.4.6 Risk of Clinical Mastitis in the First 120 Days of Lactation

Clinical mastitis data were available for 11 herds, for a total of 620 enrolled cows (2,480 quarters). Prior to analysis, a number of cows were dropped for the following reasons: death (n = 9), sold (n = 8), abortion (n = 1), not pregnant (n = 2), dry period greater than 90 days or shorter than 30 days (n = 37), and other antibiotic treatment (n = 37). An additional 9 quarters were non-lactating, and 70 quarters were removed due to contamination of the sample collected at drying off. Thus, 525 cows (2,025 quarters) were used in the analysis, 264 (1,019 quarters) belonging to the BDCT group and 261 (1,006 quarters) belonging to the SDCT group.

In total, there were forty-five reported cases of clinical mastitis, with 24 cases occurring in the BDCT group and 21 cases occurring in the SDCT group. The overall quarter level incidence of clinical mastitis in the first 120 days of lactation was 2.2% (45/2,025) and at the cow level the incidence was 7.4% (39/525). The most commonly

isolated pathogens were *E. coli* (9/45), CNS (8/45), and *Staph. aureus* (7/45). A diagnosis of no microbial growth was made in 22.2% (10/45) of the samples. In the final multivariable logistic model, neither treatment group ($P = 0.58$) nor infection status at drying off based on standard culture ($P = 0.22$) were statistically significant. No association between the other tested variables and the risk of clinical mastitis was found. Evaluation of residual plots did not indicate a problem with the fit of the final model.

3.5 DISCUSSION

This is the first study evaluating the utility of a Petrifilm-based on-farm culture system in a selective dry cow therapy program. Considering that blanket dry cow therapy remains the mainstay of dry period mastitis control in North America, the acceptance of SDCT by dairy producers necessitates a cautious approach, particularly in the introductory phase. As a result, a tiered approach to the selection protocol was used, creating a starting point of low BTSCC herds and low SCC cows and this must be taken into account when interpreting the results of the study. Following the procedures outlined in the current study, 46.0% of the cows dried-off during the enrolment period met the inclusion criteria and, after accounting for post-enrolment exclusions, 45.6% of cows assigned to SDCT cultured negative on-farm and did not receive dry cow antibiotics. Therefore, when the Petrifilm on-farm culture system was applied at the cow level, a total reduction in DCT of 21% was realized.

3.5.1 Risk of Intramammary Infection at Calving

According to the present study, the selective antibiotic treatment of low SCC cows at the end of lactation based on Petrifilm on-farm culture results was just as effective in the treatment and prevention of IMI during the dry period as BDCT in herds with a low BTSCC (< 250,000 cells/mL). The success of a selective dry cow therapy program depends greatly on the ability of the selection protocol to accurately identify cows with an IMI at the time of drying off so that DCT can be applied judiciously, while minimizing the risk of failing to treat an infected cow. The Petrifilm on-farm culture system enabled the detection of cows with an IMI at the end of lactation and as a result, SDCT based on Petrifilm culture results achieved the same level of success as BDCT with regards to infection status at calving.

Exploration of an interaction between treatment group and IMI status at drying off according to standard bacteriological culture demonstrated that misclassification of cows by the Petrifilm on-farm culture system did not have a negative effect on the outcome at calving. With respect to misdiagnosis, of greatest concern would be a false negative Petrifilm result at the cow level resulting in an infected quarter not receiving the therapeutic benefits of DCT. In the current study, only three infected quarters belonging to cows with a false negative Petrifilm result remained infected with the same pathogen at calving, including one quarter chronically infected with *Staph. aureus*. Despite misdiagnosis at drying off, 87% (20/23) of infected and untreated quarters experienced an apparent self-cure over the dry period. Most importantly, the occurrence of a quarter level false negative diagnosis was infrequent (13.5%; 23/171 quarters), and therefore did not result in an increase in the risk of IMI post-calving when compared to BDCT.

In contrast to a false negative diagnosis, the consequence of a false positive diagnosis would be the unnecessary antimicrobial treatment of quarters without an IMI at drying off and is less of a concern from a clinical standpoint. A limitation of the on-farm culture system is the inability of the Petrifilm to distinguish non-significant growth and contamination from intramammary infection. The AC Petrifilm does not allow for species identification making it impossible to determine if colonies represent a single or multiple species, or if growth is that of a recognized mastitis pathogen. Strict aseptic technique must be applied when collecting samples for use with the on-farm culture system in order to minimize the false positive rate and the unnecessary antimicrobial treatment of mammary quarters. The Petrifilm on-farm culture system was applied at the cow level because, for the majority of selective dry cow therapy programs, this is the level at which treatment decisions are made. As a result, many uninfected quarters were treated because they belonged to a cow that had at least one infected quarter. Application of the on-farm culture system at the quarter level would result in a greater reduction in unnecessary antibiotic use, but would require individual quarter culture, which in turn would demand more materials and labor. The literature has shown interdependence of quarters for the acquisition of new IMI during the dry period, leading to the recommendation that DCT be applied to all quarters of a cow, as opposed to only the affected quarter(s) (Browning et al., 1990; Berry et al., 2003; Robert et al., 2006a). However, quarter interdependence toward new IMI may be reduced when effective prevention strategies are in place or when the risk of cross-quarter contamination is low (Barkema et al., 1997; Berry et al., 2003; Robert et al., 2006a).

3.5.2 Prevalence of Intramammary Infection at Drying Off

The prevalence of intramammary infection at drying off was low, reflecting the nature of the herds and the cows meeting the inclusion criteria, i.e. low BTSCC at the herd level, and low monthly SCC prior to drying off at the cow level. According to results obtained from the Canadian National Cohort of Dairy Farms (**NCDF**) from milk samples collected between 4 and 2 weeks before drying off, and again between 2 weeks and drying off (n = 1,681 cows originating from 90 herds), the prevalence of IMI at the quarter level was 66.3%, and the majority of these infections were caused by minor pathogens (60.2%) [D. Haines (Université de Montréal, St-Hyacinthe, QC, Canada), personal communication (Jan. 15, 2013)]. The farms recruited in the NCDF were chosen to represent a uniform distribution of BTSCC, from ≤ 150,000 cells/mL to > 300,000 cells/mL, and therefore included herds with higher BTSCC than found in the current study. In the NCDF, *Staph. aureus* was the most commonly isolated major pathogen, and the third most commonly isolated pathogen overall, found in 4.2% of quarters prior to drying off [D. Haines (Université de Montréal, St-Hyacinthe, QC, Canada), personal communication (Jan. 15, 2013)]. In the present study, *Staph. aureus* was the fourth most commonly isolated pathogen at drying off, but the prevalence at the quarter level was only 0.4% (9 quarters positive with *Staph. aureus* out of 2,287 quarters cultured). *Staphylococcus aureus* remains an important mastitis pathogen in the Canadian dairy industry (Reyher et al., 2011). Lactational cures rates for *Staph. aureus* are generally < 50% at the quarter-level (Roy and Keefe, 2012). As a result, it is common to treat cows infected with *Staph. aureus* at drying off, where cure rates of up to 77% have been reported (Halasa et al., 2009). For the present study, inclusion criteria at the herd level

included low BTSCC because the likelihood of high prevalence of *Staph. aureus* and other major contagious pathogens would be low. In herds where major contagious pathogens dominate and are prevalent, the practice of BDCT remains an important control measure.

In the present study, CNS were the most frequently isolated pathogens in both study groups at all sampling points. Coagulase negative staphylococci are considered to be an emerging mastitis pathogen as they are the most common cause of IMI in many countries, however the importance of CNS as a group of mastitis pathogens remains unclear (Pyörälä and Taponen, 2009; Schukken et al., 2009; Reyher et al., 2012). Some studies have shown that CNS infection can be protective against major pathogen infection during lactation and over the dry period (Rainard and Poutrel, 1988; Østerås and Sandvik, 1996; Green et al., 2005), while other studies have declared an increase in susceptibility to major pathogens when a CNS infection is established (Hogan et al., 1988; Berry and Hillerton, 2002a; Berry and Hillerton, 2002b). Until further research establishes the true nature of CNS infection, the most prudent recommendation would be to treat all cases CNS IMI present at the end of lactation. However, according to the results of this study, leaving a quarter infected with CNS untreated at drying off may not necessarily result in a negative outcome at calving, as an apparent self-cure rate of 92.3% (12/13) for CNS was achieved in untreated quarters that were misdiagnosed at drying off by the Petrifilm on-farm culture system.

3.5.3 Cure Risk Over the Dry Period

Cure risks over the dry period were high in both study groups, and were similar to those reported in other studies in quarters receiving DCT + ITS (Godden et al., 2003; Newton et al., 2008; Bradley et al., 2011). In the final multivariable model, IMI status at drying off according to standard bacteriological culture was not associated with the risk of IMI post-calving. Dry cow antibiotic therapy was therefore effective in eliminating IMI in both study groups, and more specifically for the SDCT group, DCT was applied in a manner which successfully eliminated IMI to the same degree as BDCT. A high cure risk was maintained in infected quarters that did not receive DCT as a result of a false negative Petrifilm result. High apparent self-cure rates in cows with low SCC at drying off have been reported in other studies (Harmon et al., 1986; Huxley et al., 2002; Bradley et al., 2010). In the current study, the low SCC nature of the cows might indicate that some of the pathogens isolated at drying off may have represented teat canal infections that were more easily eliminated.

3.5.4 New Intramammary Infection Risk Over the Dry Period

The risk of new IMI over the dry period was low, and was similar between BDCT and SDCT groups. A notable aspect of this study was the application of an ITS in all quarters of all cows, as opposed to reserving ITS for quarters of cows selected not to receive DCT. The use of an ITS in all quarters of all cows as a tool for the prevention of IMI during the dry period is well-supported by the literature (Sanford et al., 2006a; Newton et al., 2008; Bradley et al., 2010). The use of an ITS alone to prevent IMI in quarters uninfected at the end of lactation is also established by prior studies that have

shown that ITS is just as effective as DCT in the prevention of IMI in quarters that are bacteriologically negative at drying off (Woolford et al., 1998; Sanford et al., 2006a). However, when ITS are used alone, strict asepsis and partial insertion technique is recommended. Internal teat sealants contain no antibiotic, therefore it is essential to minimize the risk of accidental introduction of bacteria during infusion. In the present study, all non-lactating cow treatments were administered by the producers. The producers were able to maintain asepsis during infusion of ITS, and as a result, the risk of new IMI was not higher in quarters that received ITS as a sole treatment at drying off. Interestingly, the risk of new IMI caused by fungi and yeast, typically associated with poor aseptic technique (National Mastitis Council, 1999) was numerically higher in the quarters receiving BDCT, however the numbers were too small to draw definitive conclusions.

In comparison to the results of the current study, other studies examining selective dry cow therapy have reported a higher risk of new IMI in selectively treated cows than in cows receiving BDCT (Rindsig et al., 1978; Berry and Hillerton, 2002b). In the earlier studies, cows not receiving DCT were left unprotected during the dry period. It has been shown that up to 23.4% of teats have not formed a protective keratin plug within the streak canal by 6 weeks dry (Dingwell et al., 2004). Mammary quarters lacking a functional keratin plug remain susceptible to infection, and as a result, the odds of new IMI in an open quarter is 1.7 times higher compared to a closed quarter (Dingwell et al., 2004). In the current study, the use of an ITS in all cows, and specifically in cows not infused with DCT, resulted in the protection of all quarters against new IMI over the dry period.

3.5.5 Misclassification Bias

In the current study, post-calving IMI status was modeled using an outcome based on parallel interpretation of milk samples collected between 3 and 4 DIM and between 5 and 18 DIM. Parallel interpretation of milk culture results has the benefit of increasing sensitivity to detect IMI over series interpretation or single sample culturing (Dohoo et al., 2011a). Additionally, parallel interpretation minimized the loss of study units due to missing or contaminated samples. However, because IMI status was determined using samples collected post-calving, it is possible that infections detected in those samples represented infections that were acquired in the post-calving period. Consequently, the risk of IMI post-calving included not only the risk of IMI over dry period, but also the risk up to 18 days into the next lactation. In fact, the prevalence of IMI was higher when calculated using both post-calving samples than when only the first sample was considered. When considering only the first post-calving sample, which was collected between 3 to 4 days in lactation and would be more representative of IMI status at calving, the risk of IMI between study groups was not different. Lastly, because the same sampling protocol was used in both study groups, any misclassification bias resulting from the timing of the post-calving samples would be nondifferential, that is the risk of misclassification error would be the same in both groups. Nondifferential misclassification errors bias the estimate of the odds ratio towards the null, therefore it is possible that a type II error (i.e. the false conclusion that study group did not affect the risk of IMI post-calving) occurred as a result of the sampling protocol used (Dohoo et al., 2009).

3.5.6 Clinical Mastitis in the First 120 Days in Milk

Infections acquired during the dry period can be a cause of clinical mastitis in early lactation (Green et al., 2002;2007). Therefore, prevention of IMI during the dry period can have the additional benefit of reducing clinical mastitis in early lactation and the associated negative effects on milk production and reproduction. In this study, the risk of clinical mastitis in the first 120 days of lactation was the same between treatment groups. Furthermore, quarters negative on Petrifilm, that did not receive DCT but were infused with only a ITS at drying off, were not at increased risk for an episode of clinical mastitis in early lactation when compared to quarters infused with DCT + ITS. The use of an ITS alone, and in combination with DCT, has been shown to be protective against clinical mastitis in early lactation by others (Godden et al., 2003; Sanford et al., 2006a; Newton et al., 2008). Overall, the risk of clinical mastitis was low, however compliance of study participants with this portion of the study may have been incomplete, therefore these results should be interpreted with caution due to the reduced sample size. Despite incomplete compliance regarding clinical mastitis reporting and sampling, there is no reason to believe that under-reporting was greater in one study group.

3.5.7 Association of Outcome with Other Predictors

While the main focus of this study was to compare Petrifilm-based SDCT to BDCT, the collection of additional data provided an opportunity to evaluate the impact of other factors on the risk of IMI at calving. According to the data, hind quarters were more likely to have an IMI post-calving than quarters located in the front. Prior studies

examining quarter interdependence for IMI and clinical mastitis have reported a higher prevalence in hind quarters when compared to front quarters (Adkinson et al., 1993; Barkema et al., 1997). Conversely, the majority of studies evaluating the use of ITS have failed to report a quarter effect (Berry and Hillerton, 2002a; Huxley et al., 2002; Godden et al., 2003).

Another association revealed in the final multivariable model was the effect of season on IMI risk at calving. According to the data, quarters belonging to cows that calved in the summer and fall were more likely to have an IMI post-calving than quarters belonging to cows that calved in the winter. Others have hypothesized that higher temperatures and increased humidity in the summer and early fall result in a greater pathogen load in the environment (Smith et al., 1985; Todhunter et al., 1995), as well as increased cow susceptibility due to immunosupression from heat stress (Smith et al., 1985; Carroll and Forsberg, 2007). Similarly, different housing and nutrition in the warmer months may explain some of the seasonality effect (Godden et al., 2003).

3.5.8 Limitations of the Study

According to the initial study design, exclusion rates based on SCC data from the NCDF were expected to be approximately 30% of cows. It was also estimated that an additional 10% of cows would be excluded based on other criteria. Ultimately, 37% of cows were ineligible due to high SCC, and an additional 17% were excluded for other reasons. As a result of higher than anticipated exclusion rates, in addition to post-enrolment losses, the final sample size used in the analysis was smaller than what was declared required to maintain a power of 0.80. The concern with small sample sizes is a

lack of power to detect differences where differences exist, especially when the difference is small. While at no time were there trends in treatment group factor effects approaching significance, it should be noted that maintaining a power of 0.80 using the effective sample size attained in this study, the smallest detectable difference between BDCT and SDCT group would be 6.5%.

3.6 CONCLUSIONS

Following the selection protocol outlined in the current study, which included criteria based on historical SCC with the addition of Petrifilm on-farm culture, a total reduction in DCT of 21% was realized. The use of a Petrifilm-based on-farm culture system to make targeted DCT treatment decisions on cows with a low SCC at the end of lactation did not affect the risk of IMI at calving nor the risk of clinical mastitis in the first 120 DIM, as compared to BDCT. While application of the Petrifilm on-farm culture system at the cow level (i.e. composite milk samples and cow level treatment) lead to a modest decrease in the use of DCT in the study herds, use of the culture system at the quarter level (i.e. quarter samples and quarter level treatment) has the potential to effect an even greater reduction in the use of intramammary antimicrobials at the end of lactation and is the focus of a forthcoming research project. Analysis of the economics of Petrifilm-based SDCT is currently being conducted.

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Table 3.1. Descriptive statistics for cows enrolled in a randomized clinical trial to evaluate the use of a Petrifilm-based on-farm culture system in a selective dry cow therapy program

	BDCT[1] (n = 305)				SDCT[2] (n = 298)			
	Number	Mean	SD	Range	Number	Mean	SD	Range
Milk yield at last test (Kg)		22.3	5.7	5.5-41.6		22.2	5.6	6.1-42.4
Dry period length		59	11	30-90		59	11	32-89
Parity at calving								
2	145				144			
3	77				64			
4 or above	83				90			
Season at calving								
Winter	77				69			
Spring	56				54			
Summer	79				78			
Fall	93				97			

[1]BDCT: Cows receiving blanket dry cow therapy plus internal teat sealant at drying off

[2]SDCT: Cows selectively treated based on Petrifilm on-farm culture results with dry cow antibiotic and internal teat sealant, or internal teat sealant alone, at drying off

Table 3.2. Prevalence of intramammary infection (IMI) and bacterial species isolated on the day prior to drying off in quarters receiving blanket dry cow therapy plus internal teat sealant (BDCT) and quarters selectively treated based on Petrifilm on-farm culture results with dry cow antibiotic (DCT) and internal teat sealant (ITS), or ITS alone (SDCT). Definition of IMI: All pathogens except coagulase negative staphylococci (CNS) ≥ 100 cfu per 1.0 mL of milk; CNS ≥ 200 cfu per 1.0 mL of milk (Dohoo et al., 2011b)

	BDCT	**SDCT**		
	n = 1157	Overall n = 1130	ITS only n = 530	DCT + ITS n = 600
Total quarter IMI (%)	143 (12.4)†	171 (15.1)†	23 (4.4)	148 (24.7)
Bacterial species cultured (%)				
Staphylococcus aureus	5 (0.4)	4 (0.4)	1 (0.2)	3(0.5)
Streptococcus uberis	1 (0.1)	1 (0.1)	0 (0)	1 (0.2)
Streptococcus dysgalactiae	0 (0)	1 (0.1)	1 (0.2)	0 (0)
Nondifferentiated streptococci[1]	8 (0.7)	11 (1.0)	5 (0.9)	6 (1.0)
Total environmental streptococci	9 (0.8)	13 (1.2)	6 (1.1)	7 (1.2)
Escherichia coli	2 (0.2)	2 (0.2)	0 (0)	2 (0.3)
Nondifferentiated Gram-negative bacteria	2 (0.2)	1 (0.1)	0 (0)	1 (0.2)
Total Gram-negative bacteria	4 (0.4)	3 (0.3)	0 (0)	3 (0.5)
Fungi & Yeast	1 (0.1)	1 (0.1)	0 (0)	1 (0.2)
Coagulase negative staphylococci	117 (10.1)*	148 (13.1)*	13 (2.5)	135 (22.5)
Corynebacterium spp.	16 (1.4)	9 (0.8)	3 (0.6)	6 (1.0)

† *P* value < 0.10; * *P* value < 0.05; *P* values from univariable analysis

[1] Including *Enterococcus* spp.; does not include *Streptococcus agalactiae*

Table 3.3. Prevalence of intramammary infection (IMI) and bacterial species isolated within 18 days after calving in quarters receiving blanket dry cow therapy plus internal teat sealant (BDCT) and quarters selectively treated based on Petrifilm on-farm culture results with dry cow antibiotic (DCT) and internal teat sealant (ITS), or ITS alone (SDCT). Definition of IMI: All pathogens except coagulase negative staphylococci (CNS) ≥ 100 cfu per 1.0 mL of milk; CNS ≥ 200 cfu per 1.0 mL of milk (Dohoo et al., 2011b)

	BDCT	**SDCT**		
		Overall	ITS only	DCT + ITS
	n = 1157	n = 1130	n = 530	n = 600
Total quarter IMI (%)	177 (15.3)	179 (15.8)	75 (14.2)	104 (17.3)
Bacterial species cultured (%)				
Staphylococcus aureus	16 (1.4)	18 (1.6)	12 (2.3)	6 (1.0)
Streptococcus uberis	1 (0.1)	1 (0.1)	0 (0)	1 (0.2)
Streptococcus dysgalactiae	3 (0.3)	2 (0.2)	1 (0.2)	1 (0.2)
Nondifferentiated streptococci[1]	24 (2.1)	24 (2.1)	11 (2.1)	13 (2.2)
Total environmental streptococci	28 (2.4)	27 (2.4)	12 (2.3)	15 (2.5)
Escherichia coli	2 (0.2)	4 (0.4)	1 (0.2)	3 (0.5)
Nondifferentiated Gram-negative bacteria	5 (0.4)	2 (0.2)	0 (0)	2 (0.3)
Total Gram-negative bacteria	7 (0.6)	6 (0.5)	1 (0.2)	5 (0.8)
Fungi & Yeast	10 (0.9)*	2 (0.2)*	1 (0.2)	1 (0.2)
Coagulase negative staphylococci	117 (10.1)	127 (11.2)	52 (9.8)	75 (12.5)
Corynebacterium spp.	11 (1.0)	10 (0.9)	2 (0.4)	8 (1.3)

* *P* value < 0.05; *P* values from univariable analysis

[1] Including *Enterococcus* spp.; does not include *Streptococcus agalactiae*

Table 3.4. Species-specific and overall apparent new intramammary infection risk over the dry period for quarters receiving blanket dry cow therapy plus internal teat sealant (BDCT) and quarters selectively treated based on Petrifilm on-farm culture results with dry cow antibiotic and internal teat sealant, or internal teat sealant alone (SDCT)

	BDCT	**SDCT**		
Pathogen	n = 1157	Overall n = 1130	ITS only n = 530	DCT + ITS n = 600
Staphylococcus aureus	16 (1.4)	17 (1.5)	11 (2.1)	6 (1.0)
Streptococcus uberis	1 (0.1)	1 (0.1)	0 (0)	1 (0.2)
Streptococcus dysgalactiae	3 (0.3)	1 (0.1)	0 (0)	1 (0.2)
Nondifferentiated streptococci[1]	24 (2.1)	24 (2.2)	11 (2.1)	13 (2.2)
Total environmental streptococci	28 (2.4)	26 (2.3)	11 (2.1)	15 (2.5)
Escherichia coli	2 (0.2)	4 (0.4)	1 (0.2)	3 (0.5)
Nondifferentiated Gram-negative bacteria	5 (0.4)	2 (0.2)	0 (0)	2 (0.3)
Total Gram-negative pathogens	7 (0.6)	6 (0.5)	1 (0.2)	5 (0.8)
Fungi & Yeast	10 (0.9)*	2 (0.2)*	1 (0.2)	1 (0.2)
Coagulase negative staphylococci	100 (9.6)	114 (11.6)	51 (9.9)	63 (13.5)
Corynebacterium spp.	11 (1.0)	10 (0.9)	2 (0.4)	8 (1.3)
Total pathogen count	172	175	77	98
Quarter-level[2]	160 (13.8)	164 (14.5)	72 (13.6)	92 (15.3)

* *P* value < 0.05; *P* values from univariable analysis

[1] Including *Enterococcus* spp.; does not include *Streptococcus agalactiae*

[2] A quarter may have been infected with up to two different pathogens

Table 3.5. Unconditional associations between independent variables and the risk of intramammary infection at calving for quarters receiving blanket dry cow therapy plus internal teat sealant (BDCT) and quarters selectively treated based on Petrifilm on-farm culture results with dry cow antibiotic and internal teat sealant, or internal teat sealant alone (SDCT)

Variable	N (qtrs)	Proportion with IMI (%)	*P* value
Treatment group			0.318
BDCT	1,157	15.3	
Petrifilm neg.	530	14.2	
Petrifilm pos.	600	17.3	
IMI at drying off			0.018
Yes	314	20.1	
No	1,973	14.9	
Region			0.304
PEI	1,409	16.2	
Quebec	878	14.6	
Herd size	OR = 1.00[1]		0.217
Housing type			0.157
Free-stall	1,866	16.1	
Tie-stall	421	13.3	
Season at calving			0.003
Winter	571	11.7	
Spring	427	13.3	
Summer	598	18.6	
Fall	691	17.5	
Parity at calving			0.048
2	1,107	14.8	
3	528	18.9	
4+	652	14.1	
24 hour milk yield	OR = 1.02[1]		0.065
Length of dry period	OR = 1.01[1]		0.286
Quarter position			0.030
Front	1,149	13.9	
Hind	1,138	17.2	

[1]Odds ratio from simple logistic regression

Table 3.6. Final multilevel model for the risk of intramammary infection post-calving in quarters receiving blanket dry cow therapy plus internal teat sealant (BDCT) and quarters selectively treated based on Petrifilm on-farm culture results with dry cow antibiotic and internal teat sealant, or internal teat sealant alone (SDCT)

Variable	Coefficient	SE	Odds ratio	95% CI	*P* value	Overall *P* value
Constant	-2.758	0.280	NA	NA	NA	
Treatment group						
BDCT	Reference	NA	NA	NA	NA	0.945
Petrifilm negative	-0.031	0.217	0.97	0.63, 1.48	0.888	
Petrifilm positive	0.051	0.201	1.05	0.71, 1.56	0.801	
IMI status at drying off						
Uninfected	Reference	NA	NA	NA	NA	
Infected	0.204	0.194	1.23	0.84, 1.79	0.292	
Quarter position						
Front	Reference	NA	NA	NA	NA	
Hind	0.305	0.129	1.36	1.05, 1.75	0.018	
Season at calving						
Winter	Reference	NA	NA	NA	NA	0.021
Spring	0.173	0.272	1.19	0.70, 2.03	0.525	
Summer	0.668	0.244	1.95	1.21, 3.15	0.006	
Fall	0.557	0.237	1.75	1.10, 2.78	0.019	

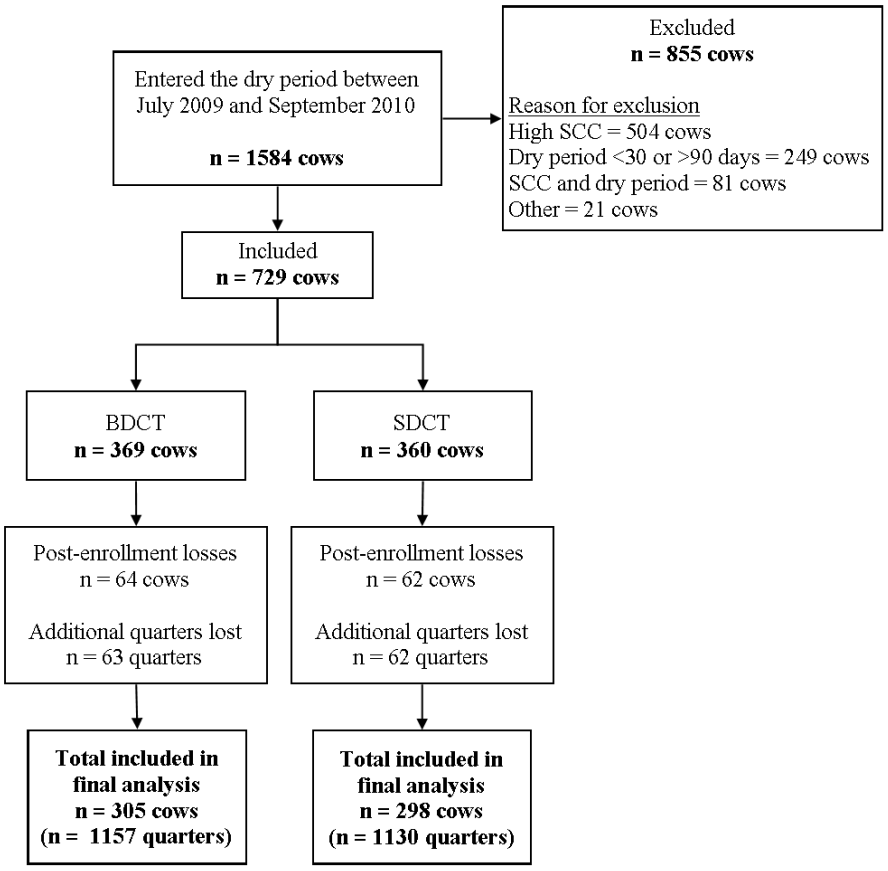

Figure 3.1. Enrolment of cows in the randomized clinical trial to evaluate the use of a Petrifilm-based on-farm culture system in a selective dry cow therapy program (BDCT: cows receiving blanket dry cow therapy plus internal teat sealant at drying off; SDCT: cows selectively treated based on Petrifilm on-farm culture results with dry cow antibiotic and internal teat sealant, or internal teat sealant alone, at drying off).

CHAPTER 4

EVALUATION OF SELECTIVE DRY COW TREATMENT FOLLOWING ON-FARM CULTURE: MILK YIELD AND SOMATIC CELL COUNT IN THE SUBSEQUENT LACTATION

4.1 ABSTRACT

Compared to total dry cow therapy, the selective antimicrobial treatment of cows based upon on-farm culture-results has the potential to reduce the amount of antibiotics used in dairy production. The objective of the study was to determine the effect of a Petrifilm on-farm culture-based selective dry cow therapy program on milk yield and somatic cell count in the following lactation. A total of 729 low somatic cell count (< 200,000 cells/mL) cows from 16 commercial dairy herds with a low bulk tank somatic cell count (< 250,000 cells/mL) were randomly assigned to receive either blanket dry cow therapy or Petrifilm-based selective dry cow therapy. Cows belonging to the blanket dry cow therapy group were infused with a commercial dry cow antimicrobial product (**DCT**) and an internal teat sealant (**ITS**) at drying off. Using composite milk samples collected on the day prior to drying off, cows in the selective dry cow therapy group were treated at drying off based on the results obtained by the Petrifilm on-farm culture system with DCT + ITS (Petrifilm culture positive), or ITS alone (Petrifilm culture negative). Milk test day records for the following lactation were obtained from Dairy Herd Improvement for all cows enrolled in the trial.

Repeated measures linear mixed models were used to assess the effect of study group (blanket or selective dry cow therapy) on test day milk production and somatic cell count over the first 180 days of the lactation of the subsequent lactation. According to the final multivariable models, when low somatic cell count cows were selectively treated with DCT at drying off based on results obtained using the Petrifilm on-farm culture

system, there was no effect on milk production or somatic cell count in the subsequent lactation when compared to cows receiving blanket dry cow therapy. The results of this study indicate that selective dry cow therapy based on results obtained by the Petrifilm on-farm culture system enabled a reduction in the use of DCT without negatively impacting milk production and milk quality.

Key words: selective dry cow therapy, Petrifilm, somatic cell count, milk yield

4.2 INTRODUCTION

4.2.1 The economic importance of mastitis on dairy farms

Mastitis is a production limiting and economically important disease of dairy cows (Seegers et al., 2003; Hand et al., 2012). Beyond the direct costs associated with the treatment of mastitis, there are financial ramifications due to decreased milk production and the potential to incur penalties as a result of poor milk quality. According to an analysis of Dairy Herd Improvement (**DHI**) test day production records from 2,835 Ontario dairy herds, in comparison to a referent SCC value of ≤ 100,000 cells/mL, estimates for 24 hour milk production losses at a cow-level somatic cell count (**SCC**) of 200,000 cells/mL ranged from 0.35 kg to 1.09 kg depending on parity and production levels; at an SCC of 2,000,000 cells/mL, production losses ranged from 1.49 to 4.7 kg (Hand et al., 2012). The somatic cell count at the level of the bulk tank is an important indicator of the quality of milk produced at the farm. Consequently, bulk tank somatic

cell counts (**BTSCC**) that exceed regulatory limits can result in financial penalties and the suspension of the right to market milk. Thus, management of both clinical and subclinical mastitis on a dairy farm is very important for the profitability of the farming enterprise.

4.2.2 Mastitis control over the dry period

With regard to mastitis control, the dry period is considered a critical component of the milk production cycle on the basis of two main factors: 1) for existing intramammary infections **(IMI)**, high cure rates can be achieved, and 2) the rate of new intramammary infection **(NIMI)** is greater in the periparturient period than at any point during lactation (Smith et al., 1985; Dingwell et al., 2003; Halasa et al., 2009). In North America, blanket dry cow therapy **(BDCT)** is the most common approach to mastitis control during the dry period (USDA, 2008; Dufour et al., 2012). In accordance with BDCT, all quarters of all cows are infused with a long-acting intramammary antimicrobial **(DCT)** at the end of lactation. As concerns regarding the emergence of antimicrobial resistant pathogens are on the rise, dairy producers are facing increasing pressure to reduce antibiotic usage on their farms. For dairy herds with a low prevalence of contagious mastitis and a consistently low BTSCC, an alternative approach to mastitis control over the dry period would be to target antimicrobial treatment at cows with an IMI at drying off, a protocol known as selective dry cow therapy **(SDCT)**. With the advent of internal teat sealants **(ITS)**, producers have at their disposal a non-antimicrobial product that has proven to be just as efficacious as DCT in the prevention of IMI during

the non-lactating period (Woolford et al., 1998; Sanford et al., 2006). The addition of an ITS to a SDCT program ensures that all quarters have some form of protection against dry period NIMI.

4.2.3 Petrifilm-based selective dry cow therapy plus internal teat sealant

In North America, estimates for compliance with a BDCT protocol range from 72% of herds in the United States to 88% of herds in Canada (USDA, 2008; Dufour et al., 2012). In order for dairy producers to consider SDCT, the outcomes of the subsequent lactation regarding udder health, milk production, and milk quality must be equivalent to those achieved by BDCT. The majority of studies evaluating SDCT and ITS have focused on the risk of IMI and clinical mastitis in the subsequent lactation, whereas published reports examining the effects on milk yield and SCC are sparse. A consistent theme in SDCT research is that the success of such a program depends on the ability to determine a cow's IMI status at the end of lactation so that the appropriate treatment can be applied (Huxley et al., 2002; Robert et al., 2008; Torres et al., 2008). A Petrifilm-based on-farm culture system has recently been validated for use in a SDCT program. When used in low BTSCC herds (<250,000 cells/mL) to diagnose IMI in cows with a low SCC (<200,000 cells/mL) prior to drying off, the culture system performed well with a sensitivity of 85% and specificity of 73% (Cameron et al., 2013). Furthermore, Petrifilm-based SDCT did not affect the risk of IMI at calving, nor the risk of a first case of clinical mastitis in the first 120 days of lactation, when compared to BDCT (Cameron et al., 2014). The aim of the research outlined in this paper was to evaluate the milk

quality and production outcomes in the subsequent lactation following Petrifilm-based SDCT. The main objectives were to compare milk production and SCC in the first 180 days of lactation between cows receiving BDCT with the addition of an ITS and cows selectively treated based on the results of the Petrifilm on-farm culture system with DCT and an ITS, or with an ITS alone.

4.3 MATERIALS AND METHODS

4.3.1 Trial design

Cows in a convenience sample of dairy herds from Quebec (n = 6) and Prince Edward Island (n = 10) that entered the dry period between July 2009 and September 2010 were considered for inclusion in the trial. Herd inclusion criteria included an average BTSCC below 250,000 cells/mL over the last 12 months and participation in a DHI program with regular milk testing. Herd size ranged from 44 to 251 cows with a median of 75 cows, and the total number of cows entering the dry period during the study period was 1,584. Enrolled cows had monthly SCC <200,000 cells/mL on the last three tests prior to drying off, no clinical mastitis in the same time period, an expected dry period of 30 to 90 days, no antibiotic treatment in the last 14 days, at least 3 functional quarters, and all quarters scoring <2 on the California mastitis test on the day prior to drying off.

Single quarter milk samples were collected on the day prior to drying off, at 3 to 4 days post-calving, and again at 5 to 18 days post-calving. The first post-calving sample

was collected by the producer or farm personnel; all other samples were collected by the study personnel. Milk samples were frozen at -20°C before shipment to the Maritime Quality Milk research laboratory at the University of Prince Edward Island. Following pre-drying off sample collection, cows were randomly assigned to either the blanket dry cow therapy (**BDCT+ITS)** study group or the Petrifilm-based selective dry cow therapy (**SDCT+ITS**) study group according to a randomization table unique to each herd, with a block size of six thus randomizing six cows at a time, three to BDCT and three to SDCT. On the day of drying off, cows in the BDCT+ITS group were infused with a long-acting intramammary formulation of 500 mg ceftiofur hydrochloride (Spectramast DC, Zoetis Canada, Kirkland, Quebec, Canada) followed by an ITS composed of 65% bismuth subnitrate (Orbeseal, Zoetis Canada, Kirkland, Quebec, Canada) in all four quarters. For cows assigned to the SDCT+ITS group, a composite milk sample was cultured on-farm using the Petrifilm on-farm culture system. The Petrifilm on-farm culture was set-up by the study personnel on the day prior to drying off as follows: a composite milk sample was created by combining 5 mL from each quarter sample into a new sample vial. A 3 mL aliquot of composite milk was added to 27 mL of sterile water to make a 1:10 dilution. One millilitre of diluted milk was plated on a total aerobic count Petrifilm (3M Canada, London, Ontario) and incubated on-farm at 35°C for 24 hours in a TurboFan Hova-Bator (GQF Manufacturing, Savannah, Georgia, USA). Consistent with a dilution factor of 1:10, a single colony forming unit (**cfu**) present on the Petrifilm was equivalent to 10 cfu per mL of milk. On the scheduled day of drying off (Day 0), the Petrifilm was read by the producer and, in accordance with study protocol, cows were classified as

positive if 5 or more colonies were present (equivalent to ≥50 cfu/mL of milk). Cows negative on Petrifilm were treated solely with an ITS at drying off; cows positive on Petrifilm were treated with 500 mg ceftiofur hydrochloride followed by an ITS. All treatments were applied by the producer or farm personnel immediately following the last milking, and treatments were recorded to verify compliance with the study protocol.

4.3.2 Bacteriology

Within one month from collection, milk samples were thawed and cultured in a laboratory using standardized methods outlined in the Laboratory Handbook on Bovine Mastitis (National Mastitis Council, 1999). Trained laboratory technicians (n = 2) were blinded to the results obtained by the Petrifilm on-farm culture system. Briefly, disposable plastic loops were used to streak 0.01 mL of milk on bi-plates containing half Columbia agar + 5% sheep blood and half MacConkey agar. Plates were incubated at 35 ^{o}C and examined for bacterial growth after 24 and 48 hours. Colonies were tentatively identified as staphylococci, streptococci, coliforms, or other pathogens based on colony growth characteristics, morphology, pattern of hemolysis, catalase reaction, and Gram staining. Staphylococcal isolates were tested for coagulase production with the tube coagulase test. API tests (API 20 E and API 20 Strep; bioMérieux (Marcy L'Etoile, France) and the latex agglutination test for streptococcal Lancefield groups were used for the final identification of bacterial organisms as required. For each positive sample, the number of cfu per 0.01 mL of milk was enumerated up to a maximum of 10 colonies. A quarter was considered infected if ≥ 100 cfu/mL of milk of any pathogenic organism of

interest, except coagulase negative staphylococci (**CNS**), were cultured. For CNS, a definition of ≥ 200 cfu/mL was used. These definitions are in accordance with the recent publication of characterization of IMI based on single sample bacteriological testing (Dohoo et al., 2011). Samples with three or more differing isolates were classified as contaminated. However, when *Staphylococcus aureus* (***Staph. aureus***) was identified in a contaminated sample, it was enumerated and the associated quarter was classified as infected (Reyher et al., 2011). Organisms classified as "other Gram positive", which were mainly *Bacillus* spp., were assumed to be associated with environmental contamination and considered non-significant growth. No samples collected during the trial displayed growth of *Streptococcus agalactiae*.

The quarter level culture results were interpreted in parallel to determine the cow level infection status. If one or more quarter samples were contaminated, a cow was considered infected if at least one of the remaining quarters was infected. If one or more quarter samples were contaminated and no IMI was diagnosed in the remaining samples, the cow's overall infection status was considered unknown, and the observation was removed from the analyses. Throughout this manuscript, infection status or IMI refers to the classification of a cow based on parallel interpretation of quarter level standard bacteriological culture results, whereas Petrifilm on-farm culture results are referred to simply as Petrifilm positive or Petrifilm negative.

4.3.3 Data collection

Data on milk yields and SCC were recorded by DHI technicians on a minimum of ten separate occasions spaced over the course of twelve months. Data files were provided by DHI and the following information was extracted for each participating cow: test day 24 hour milk yield (in kg) and SCC during the first 180 days of lactation (consisting of 5 to 6 milk tests per cow), days in milk at the time of the milk test, parity, and the previous lactation 305 day milk production (in kg). Additional data collected at the time of collection of post-calving milk samples included the occurrence of post-partum disease (such as metritis, displaced abomasum, or mastitis) and antimicrobial treatment prior to sample collection.

4.3.4 Statistical analysis

Statistical analysis was performed using Stata/IC 11.0 (StataCorp, College Station, TX). Two outcomes of interest were analyzed: test day 24 hour milk production and test day SCC during the first 180 days of lactation, and all analyses were at the level of the cow test-day. Somatic cell counts (in '000/mL) were modelled using the natural logarithm of SCC (**lnSCC**). The data consisted of repeated measurements (up to 6 milk tests) within a study subject, therefore milk yield and lnSCC were modelled using a repeated measures linear mixed model. Different correlation structures were assessed (first order autoregressive, first order moving average, unstructured, Toeplitz, and exponential), and the most appropriate structure was determined by likelihood ratio tests and Akaike's Information Criteria (**AIC**). For both outcomes, an unstructured correlation

structure provided the best fit for the repeated measures data and was used in the final models. A random effect for herd was included in all models to account for the clustering of cows at the herd level. As the main predictor, study group (BDCT+ITS or SDCT+ITS) was forced into the models. Other variables considered for inclusion were previous lactation 305 day milk production, infection status at drying off according to gold standard bacteriological culture, length of the dry period, parity at calving (2, 3, or 4+), calving season, occurrence of post-partum disease (other than mastitis), days in milk on the day of the milk test, herd size, housing type (free-stall [n = 11] vs tie-stall [n = 5]), and region. Dry period was scaled by subtracting 30 days from each observed value, which was the minimum possible value as dictated by the selection criteria. Previous lactation 305 day milk production was centered at the median value of 9,916 kg and scaled to reflect a change of 1,000 kg. The variable for calving season was created using three month intervals, such that winter was designated as December to February, spring as March to May, summer as June to August, and fall as September to November. Within the milk yield model, the effect of days in milk **(DIM)** was estimated with the addition of Wilmink's function **($DIM^{-0.05}$)**, as illustrated by Schaeffer et al. (2000). To assess nonlinear trends for DIM in the lnSCC model, fractional polynomials were used and the best fitting model was determined by the deviance difference. Unconditional associations between the various independent variables and the dependent variable of interest were assessed using simple linear regression, and variables with a *P* value of 0.25 or less were offered as potential predictors in the multivariable models. With respect to model building, a backwards stepwise procedure was used to select the best model, and

significance was declared at a *P* value <0.05. Once the variables to be included in the final model were determined, all first-order interactions with study group were examined and retained if significant. Additionally, an interaction between DIM and parity was also explored within each model. Dropped variables were re-introduced into the model and were retained if they changed the other model coefficients substantially. A random coefficient term for study group was evaluated at the herd level, but did not result in an improvement of the analysis of either milk yield or lnSCC based on a likelihood ratio test. The herd-level contextual effect of Petrifilm on-farm culture results was assessed by the inclusion of an additional variable, representing the proportion of Petrifilm positive cows for a particular herd, but was non-significant in both the milk yield and lnSCC models. The assumptions of the final models were assessed through examination of the residuals at the various levels.

4.4 RESULTS

4.4.1 Descriptive statistics

In total, 729 cows were enrolled of which 369 were assigned to BDCT+ITS and 360 were assigned to SDCT+ITS. The proportion of cows per herd eligible for inclusion ranged from 28.3% (15/53) to 69.4% (145/209), and the overall inclusion probability was 46% (729/1584). Post-enrolment exclusions and attrition resulted in the loss of 129 cows for the following reasons: death (n = 15), sold (n = 11), or culled (n = 18) prior to the first milk test, abortion (n = 2), not pregnant (n = 2), dry period greater

than 90 days or shorter than 30 days (n = 51), and contamination of all pre-drying off milk samples (n = 30). There was no significant difference between study groups with respect to post-enrolment exclusion or attrition. Thus, 307 BDCT+ITS group cows and 293 SDCT+ITS group cows were available for analysis. The cow level prevalence of IMI at drying off in the SDCT+ITS group according to Petrifilm on-farm culture was 53.2% (156/293). Therefore, at a Petrifilm treatment threshold of ≥50 cfu/mL, 46.8% (137/293) of the cows were classified as uninfected and did not receive a long-acting intramammary antibiotic at drying off. Descriptive statistics for the cows included in the final analysis can be found in Table 4.1. Despite random allocation into treatment groups, the prevalence of IMI at drying off as determined by standard bacteriological culture was higher in cows assigned to SDCT+ITS than that observed in cows assigned to BDCT+ITS ($P = 0.005$). The prevalence of IMI post-calving was not different between the study groups ($P = 0.72$).

4.4.2 Test day milk production

The results of the final repeated measures linear mixed model for milk production are presented in Table 4.2. When controlling for the other independent variables included in the model, test day milk production was not different between cows receiving BDCT+ITS and cows selectively treated based on Petrifilm results (Least square means: BDCT+ITS = 39.3kg (95% CI: 37.9, 40.8) vs SDCT+ITS = 39.0 kg (95% CI: 37.6, 40.5); $P = 0.43$). For every 1,000 kg increase in the previous lactation 305 day milk yield average daily milk production increased by 2.04 kg ($P < 0.01$). According to simple

linear regression, higher milk production was observed for cows in their third or greater lactation than for cows in their second lactation. However, in the multivariable model, which adjusted for previous lactation production, cows in their third lactation ($P < 0.01$) and fourth or greater lactation ($P < 0.01$) produced less than cows in their second lactation. For example, a cow achieving a production of 10,000 kg in her first lactation produced more milk in her second lactation than a cow who achieved that level in her 4^{th} lactation produced in her 5^{th} lactation. Calving season was also a significant predictor of milk production ($P < 0.01$), with cows calving in the summer having lower production than cows calving during any other season. Milk production increased with increasing duration of the dry period beyond 30 days (to a maximum of 90 days as dictated by the inclusion criteria; $P < 0.01$), and the occurrence of post-partum disease other than mastitis reduced milk yield by an average of 2.64 kg per day. Stage of lactation, modelled by the variables DIM and $DIM^{-0.05}$, followed what is considered a standard lactation curve with an initial rise to a peak around 50 days, followed by a gradual decline over time (Macciotta et al., 2005). Illustrated in Figure 4.1 is a graph of test day 24 hour milk yield by DIM.

4.4.3 Test day somatic cell count

The results of the final repeated measures linear mixed model for lnSCC are presented in Table 4.3. After adjusting for other independent variables included in the final model, there was no significant difference in test day lnSCC between cows receiving BDCT+ITS and cows selectively treated at drying off based on Petrifilm results

($P = 0.82$). Parity was significantly associated with lnSCC, with cows in their third lactation ($P = 0.03$) and fourth or greater lactation ($P < 0.01$) having a higher average lnSCC than cows in their second lactation. Cows with an IMI at drying off according to standard bacteriological culture had a higher test day lnSCC during the first 180 days of the next lactation than cows uninfected at drying off ($P = 0.02$). Fractional polynomials suggested that DIM was best modelled using the power terms 0.5 and -0.5. Illustrated in Figure 4.2 is a graph of test day SCC by DIM. A similar pattern was apparent between groups with a rapid decrease in SCC from calving to approximately 50 DIM, followed by a gradual increase over time.

4.5 DISCUSSION

4.5.1 Test day milk production

It is estimated that intramammary infections present at calving, either as a result of failure to eliminate existing infections or acquisition of new infections, can reduce lactational milk yields by 5% (Hogeveen, 2003; Berry et al., 2004). Thus, minimizing the prevalence of IMI post-partum is important for achieving optimum milk production. The successful outcome of a SDCT program depends on the accurate diagnosis of infection status at drying off so that DCT is applied appropriately and judiciously for the purpose of eliminating existing IMI. Furthermore, as prior studies have demonstrated that untreated quarters are at higher risk for the development of NIMI over the dry period,

it is also important that a method to protect quarters against NIMI be in place, especially for cows not infused with DCT (Rindsig et al., 1978; Berry and Hillerton, 2002). According to the present study, when low SCC cows were selectively treated with DCT at drying off based on results obtained using the Petrifilm on-farm culture system, there was no effect on milk production in the subsequent lactation when compared to cows receiving BDCT. The Petrifilm on-farm culture system provided sensitive results thus enabling the accurate diagnosis of cows with an IMI at the end of lactation (Cameron et al., 2013). Moreover, internal teat sealants were used in all cows thus ensuring some measure of prophylaxis against IMI for all quarters. While internal teat sealants have been evaluated for use in SDCT in terms of infection status at calving and clinical mastitis in the subsequent lactation, no other studies to date have published data pertaining to the effect of SDCT + ITS on milk production. In a decision tree analysis evaluating dry cow protocols by Berry et al. (2004), comparison were made between cows receiving DCT and negative controls, as well as cows receiving ITS and negative controls, and it was determined that DCT had a positive effect on milk yield but there was no effect of ITS. The data originated from two separate clinical trials involving cows that were either uninfected or infected with CNS or *Corynebacterium* spp. at drying off, and assignment to treatment groups was based on randomization. In previous works evaluating the effect of DCT on milk production, when random assignment to treatment groups was used, DCT displayed a significant positive effect on production when compared to a negative control (Østerås and Sandvik, 1996; Berry et al., 1997; Berry et al., 2004). However, when selection procedures were put in place, DCT lost its

production-related effect. According to Rajala-Schultz et al. (2011), there was no effect of DCT on milk production when BDCT was compared to no treatment in cows with a low SCC and no clinical mastitis prior to drying off. This is in agreement with the current study, further demonstrating the importance of the selection protocol for SDCT.

With regards to the effect of parity on milk production, the data indicated that cows entering their second lactation produced more milk than cows beginning their third or greater lactation. However, because the final model also controlled for previous lactation milk production, this finding actually reflected the greater capacity of second parity cows to increase their milk production over the previous lactation than higher parity cows. This, coupled with the significant positive association between previous lactation production and current production, is in agreement with Rajala-Schultz et al. (2011). Season of calving as a predictor of milk production has also been explored elsewhere, and the results of this trial are in accord with previous research (Wilmink, 1987; Rajala-Schultz et al., 2011). Finally, the occurrence of post-partum disease has been shown to be a risk factor for decreased milk production (Wilson et al., 2004), therefore it was not surprising that cows experiencing one of the recorded disease events sometime between calving and 18 DIM had lower milk production than that observed in cows that remained healthy. While mastitis events were recorded, mastitis was not included in the list of post-partum diseases. Mastitis in the post-partum period was considered an intervening variable between study group and milk yield, and inclusion of mastitis would have resulted in a biased measure of the effect of study group on milk production (Dohoo et al., 2009).

4.5.2 Test day somatic cell count

In the current study, the selective antimicrobial treatment of cows at the end of lactation based on Petrifilm culture results did not affect the SCC in the following lactation when compared to cows that received total antimicrobial therapy at drying off. In earlier studies comparing BDCT to a negative control group, cows receiving DCT had a lower SCC in the subsequent lactation than cows left untreated over the dry period (McNab and Meek, 1991; Østerås and Sandvik, 1996; McDougall, 2010; Rajala-Schultz et al., 2011). This illustrates the importance of providing some form of protection, such as ITS, for quarters of cows that do not receive DCT. Comparisons of the effect of ITS alone vs. DCT on SCC are infrequent in the literature. In a split-udder trial involving cows without an IMI at drying off, when the SCC at 1 to 8 DIM was compared between quarter receiving ITS and quarters infused with DCT, the proportion of quarters with an SCC >200,000 was 37% in the ITS group and 36% in the DCT group (Sanford et al., 2006). In an observational study by Green et al. (2008) examining dry period risk factors associated with high SCC in early lactation, it was concluded that DCT and ITS had the same effect on SCC in early lactation when used in cows with a low SCC prior to drying off.

Regarding the other significant associations revealed in the final regression model, the findings are in agreement with previous research. Parity as a significant predictor of SCC has been reported elsewhere and is commonly attributed to increased risk of NIMI and clinical mastitis (Laevens et al., 1997; Walsh et al., 2007; Pantoja et al.,

2009). Cows with an intramammary infection at drying off according to gold standard laboratory culture had a higher SCC in the subsequent lactation than cows uninfected at drying off. Predisposition to intramammary infection as a result of poor udder hygiene and teat morphology has been described in the literature (Schreiner and Ruegg, 2003; O'Reilly et al., 2006), and previous IMI has been shown to be a risk factor for the development of NIMI (Zadoks et al., 2001; Green et al., 2002).

4.6 CONCLUSION

In conclusion, while much research has examined the outcome of SDCT on intramammary infection at calving and clinical mastitis in early lactation, investigations into milk production and milk quality outcomes are lacking. The use of a Petrifilm-based on-farm culture system to make targeted DCT treatment decisions on cows with a low SCC at the end of lactation did not affect milk production and somatic cell counts in the subsequent lactation, as compared to BDCT. Overall, Petrifilm-based SDCT with the addition of an ITS enabled a reduction in the use of antimicrobials on low BTSCC dairy farms without affecting the health, welfare, and future milk production of the cow.

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Table 4.1. Descriptive statistics for cows enrolled in a randomized clinical trial to evaluate the use of a Petrifilm-based on-farm culture system in a selective dry cow therapy program.

	BDCT+ITS[1] (n = 307)	SDCT+ITS[2] (n = 293)
Somatic cell count x 10^{-3}/mL[3]	36 (12, 228)	39 (14, 268)
24 hour milk production (kg)[4]	41.4 (8.2)	40.8 (8.2)
Previous 305 day milk production (kg)[5]	9,995 (1,865)	9,903 (1,766)
Length of the dry period (days)[5]	59 (11)	60 (12)
Parity at calving		
2	149	142
3	78	64
4+	80	87
Calving season		
Spring (Mar – May)	58	57
Summer (Jun – Aug)	79	76
Fall (Sep – Nov)	94	93
Winter (Dec – Feb)	76	67
Prevalence of post-partum disease[6]	5.9	5.2
% of cows with IMI[7] at drying off	33.2*	44.4*
% of cows with IMI[7] after calving	38.7	40.1

*$P = 0.005$

[1] BDCT+ITS: blanket dry cow therapy (DCT) plus internal teat sealant (ITS)

[2] SDCT+ITS: selective dry cow therapy based on Petrifilm on-farm culture results with ITS alone (Petrifilm negative), or DCT and ITS (Petrifilm positive)

[3]Median (10^{th} and 90^{th} percentile) somatic cell count in the first 180 days of the subsequent lactation

[4]Mean (standard deviation) 24 hour milk production in the first 180 days of the subsequent lactation

[5]Mean (standard deviation)

[6]Excluding clinical mastitis. Occurring within the first 18 days of lactation

[7] IMI: intramammary infection. Definition of IMI: All pathogens except coagulase negative staphylococci (CNS) $\geq$ 100 cfu per 1.0 mL of milk; CNS $\geq$ 200 cfu per 1.0 mL of milk (Dohoo et al., 2011)

Table 4.2. Final multilevel model evaluating the effect of Petrifilm-based selective dry cow therapy on twenty-four hour milk production (kg) in the first 180 days of the subsequent lactation.

Variable	Coefficient	SE	*P* value	Overall *P* value
Study group[1]				
BDCT+ITS	reference			
SDCT+ITS	-0.29	0.37		0.43
Length of the dry period[2]	0.05	0.02		<0.01
Calving season				
Summer (Jun – Aug)	reference			< 0.01
Fall (Sep – Nov)	1.12	0.50	0.02	
Winter (Dec – Feb)	2.61	0.53	<0.01	
Spring (Mar – May)	2.05	0.57	<0.01	
Days in milk (DIM)	-0.09	0.003		< 0.01
$DIM^{(-0.05)3}$	-21.49	0.89		< 0.01
Parity				
2^{nd} lactation	reference			<0.01
3^{rd} lactation	-1.93	0.53	<0.01	
$\geq 4^{th}$ lactation	-3.58	0.55	<0.01	
Previous 305 milk[4]	2.04	0.13		< 0.01
Post-partum disease[5]	-2.64	0.82		< 0.01
Intercept	49.66	0.99		
Variance estimate				
Herd-level variance	4.87	2.18		

[1]BDCT+ITS: blanket dry cow therapy (BDCT) plus internal teat sealant (ITS); SDCT+ITS: selective dry cow therapy (SDCT) based on Petrifilm on-farm culture results with ITS alone (Petrifilm negative), or DCT and ITS (Petrifilm positive)
[2]Scaled at 30 days
[3]Wilmink's function $= e^{DIM\ (-0.05)}$
[4]Centered at 9,916 kg and scaled to reflect a 1,000 kg change
[5]Other than mastitis; occurring within the first 18 days of the subsequent lactation

Table 4.3. Final multilevel model evaluating the effect of Petrifilm-based selective dry cow therapy on natural log somatic cell count (lnSCC) in the first 180 days of the subsequent lactation.

Variable	Coefficient	Standard Error	*P* value	Overall *P* value
Study group[1]				
BDCT+ITS	reference			
SDCT+ITS	0.02	0.08		0.82
Parity				
2nd lactation	reference			< 0.01
3rd lactation	0.21	0.09	0.03	
≥ 4th lactation	0.32	0.09	< 0.01	
Days in milk$^{(-0.5)}$	6.94	0.60		< 0.01
Days in milk$^{(0.5)}$	0.14	0.01		< 0.01
IMI status at drying off[2]	0.19	0.08		0.02
Intercept	1.55	0.22		
Variance estimate				
Herd-level variance	0.04	0.03		

[1]BDCT+ITS: blanket dry cow therapy (BDCT) plus internal teat sealant (ITS); SDCT+ITS: selective dry cow therapy (SDCT) based on Petrifilm on-farm culture results with ITS alone (Petrifilm negative), or DCT and ITS (Petrifilm positive)
[2]IMI: intramammary infection. Definition of IMI: All pathogens except coagulase negative staphylococci (CNS) ≥ 100 cfu per 1.0 mL of milk; CNS ≥ 200 cfu per 1.0 mL of milk (Dohoo et al., 2011)

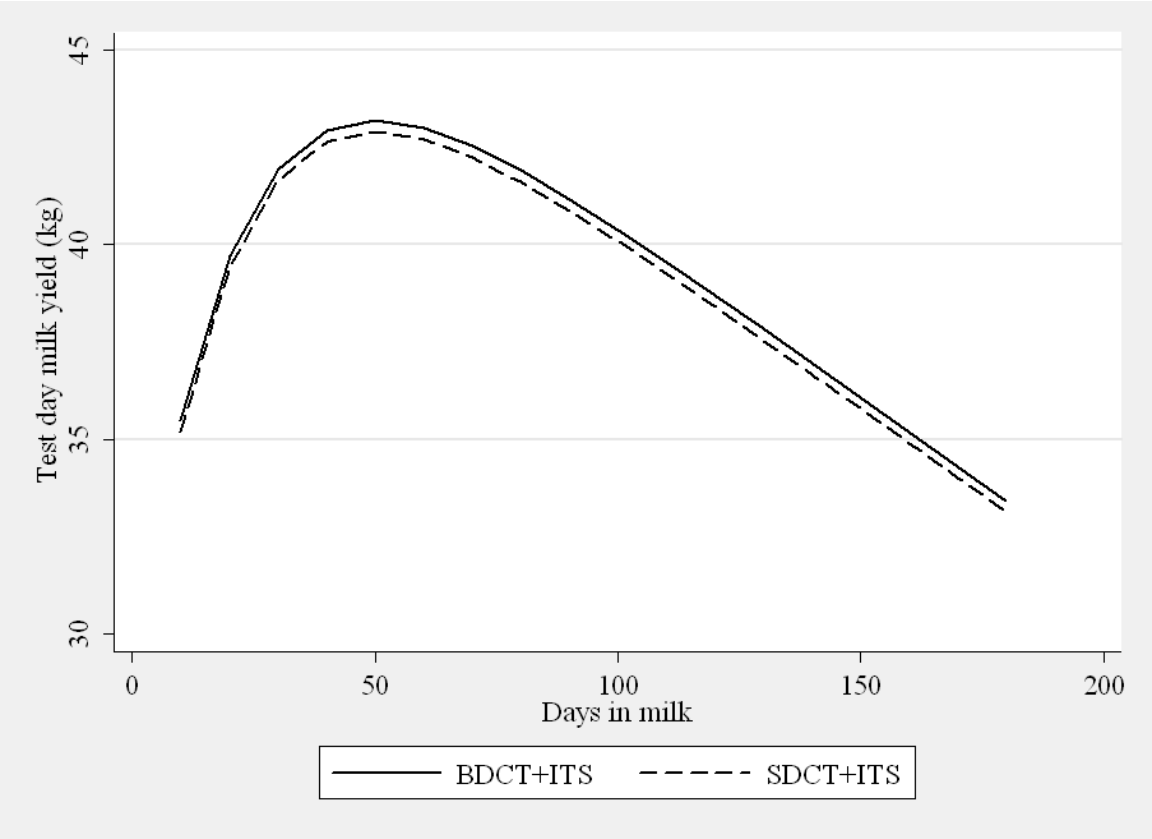

Figure 4.1. Graph of test day milk production (least-squares means) by days in milk for cows receiving blanket dry cow antimicrobial therapy plus internal teat sealant (BDCT+ITS), and cows selectively treated at drying off based on Petrifilm on-farm culture results (SDCT+ITS).

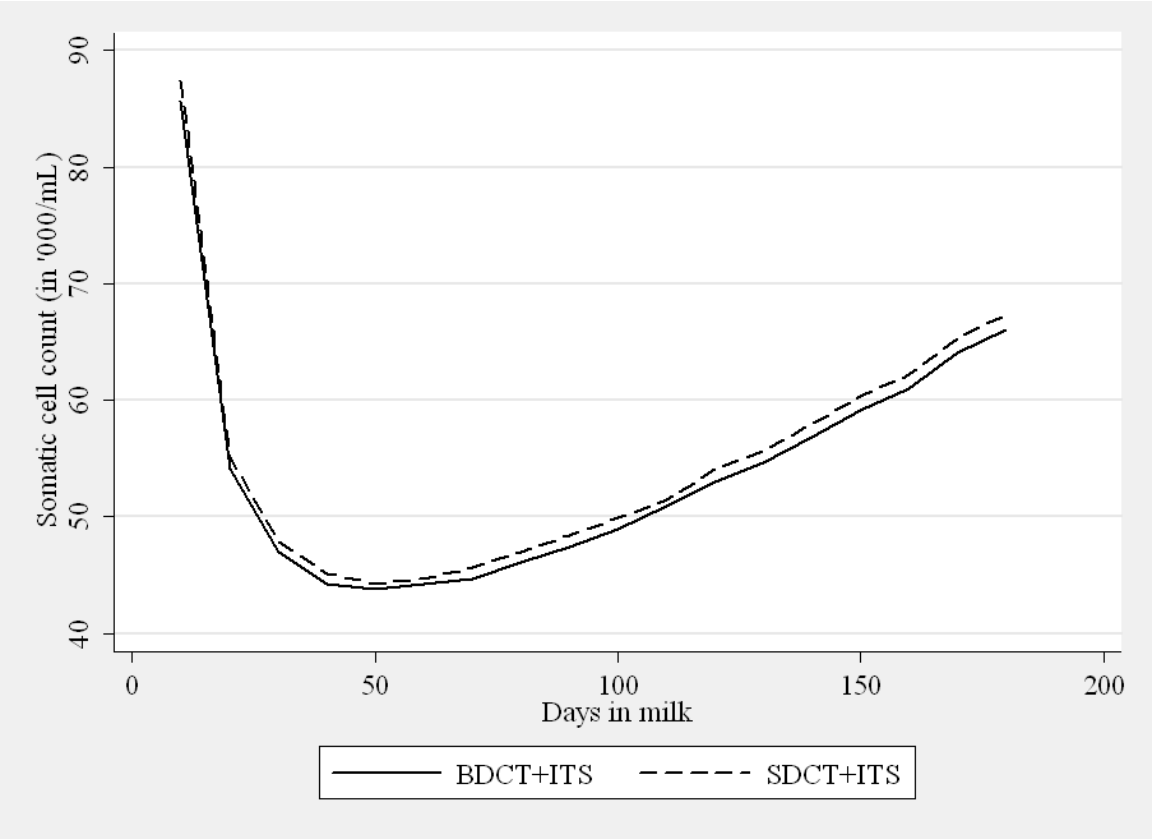

Figure 4.2. Graph of somatic cell count (least-squares means) by days in milk for cows receiving blanket dry cow antimicrobial therapy plus internal teat sealant (BDCT+ITS), and cows selectively treated at drying off based on Petrifilm on-farm culture results (SDCT+ITS).

CHAPTER 5

INTERDEPENDENCE OF MAMMARY QUARTERS TOWARDS NEW INTRAMAMMARY INFECTION WITH COAGULASE NEGATIVE STAPHYLOCOCCI DURING THE DRY PERIOD AND THE EFFECT OF INTERNAL TEAT SEALANTS

5.1 ABSTRACT

For herds with a low prevalence of contagious mastitis pathogens, selective dry cow therapy (**SDCT**) is an approach to mastitis control over the dry period with the potential to reduce antimicrobial usage on dairy farms. While selective treatment decisions are usually made at the cow level, knowledge of quarter level infection status would enable selective treatment of individual mammary quarters. Selective dry quarter therapy (**SDQT**) would result in even greater reductions in antimicrobial use when compared to SDCT, but an important barrier to SDQT is that prior research has demonstrated that quarters are not independent in their risk for new intramammary infection (**NIMI**) in the dry period. It is suggested that interdependence of quarters towards NIMI in non-lactating cows may occur as a result of cross-contamination between teats. Considering that the method of action of internal teat sealants (**ITS**) is to create a barrier to bacterial invasion of the mammary gland, it is possible that ITS may reduce the within-cow transmission of pathogens and thus diminish or eliminate interdependence. The main objective of the study was to determine the effect of ITS on the interdependence of mammary quarters towards the acquisition of coagulase negative staphylococci (**CNS**) NIMI over the dry period, and the data used in the analysis originated from a SDCT trial and a cohort study with longitudinal milk sampling. A generalized estimating equation logistic model was used to evaluate an interaction between infusion with ITS and the presence of CNS at drying off on the acquisition of CNS NIMI during the dry period. Other factors considered included: parity at drying off,

quarter location, infusion with dry cow therapy, infection with any other pathogen at drying off (as a measure of susceptibility), and prevalence of CNS in the herd. Of the main effects investigated in the analysis, only herd prevalence of CNS was significantly associated with dry period CNS NIMI such that increasing herd prevalence increased the risk of CNS NIMI. There was no evidence to support that infusion with ITS resulted in a reduction in the risk for the within cow transfer of CNS from an infected quarter to an uninfected quarter. Surprisingly, in quarters that were infused with ITS, the presence of CNS in another quarter at drying off significantly increased the risk of CNS NIMI over the dry period. This may have occurred as a result of inadvertent introduction of teat end CNS into the mammary gland during infusion of ITS. Regarding quarters of cows without a CNS intramammary infection at drying off, the odds of CNS NIMI was lower for quarters treated with ITS, suggesting that dry period CNS NIMI can be partly attributed to CNS species located within the environment. Steps to lower levels of CNS within the herd (e.g. through better management of dry cow housing, changes in bedding type, etc.) will play an important role in reducing incidence of CNS NIMI over the dry period. While independence was not demonstrated, there is still a need for quarter level clinical trials to truly assess if SDQT in combination with ITS is a viable approach to dry period mastitis control that will promote udder health and concurrently lower DCT use.

5.2 INTRODUCTION

5.2.1 Mastitis control during the non-lactating period

Conceived in the late 1960s for the purpose of controlling contagious mastitis, the practice of blanket application of dry cow antimicrobial therapy (dry cow therapy; **DCT**) is still widely practiced today (Neave et al., 1969; Berry and Hillerton, 2002; USDA, 2008; Dufour et al., 2012b). Due in part to concerns regarding a possible link between antimicrobial use in food-producing animals and the development of resistance in bacteria, the requirement for blanket application of DCT (**BDCT**) is being called into question (Østerås et al., 1991; Berry and Hillerton, 2002; Torres et al., 2008). For herds with a low prevalence of contagious pathogens and capable of maintaining a low bulk tank somatic cell count (**BTSCC**), an alternative to BDCT would be to reserve DCT for cows suspected or known to have an intramammary infection (**IMI**) at drying off (Rindsig et al., 1978). This practice is known as selective dry cow therapy (**SDCT**) and it is considered a more judicious approach to the control of IMI over the non-lactating period (Browning et al., 1994; Torres et al., 2008).

5.2.2 Level of treatment (cow vs. quarter) for selective application of DCT

While selective treatment decisions are usually made at the cow level, with knowledge of quarter level infection status it would be possible to make selective DCT decisions for individual mammary quarters. Selective dry quarter therapy (**SDQT**) would result in even greater reductions in antimicrobial use when compared to SDCT (Østerås

and Sandvik, 1996; Berry et al., 2003; Robert et al., 2006a). With the advent of on-farm culture systems, producers have at their disposal a rapid test for the detection of IMI that could enable them to determine quarter infection status and subsequently treat only infected quarters (Cameron et al., 2013). However, an important barrier to SDQT is that prior research has demonstrated that quarters are not independent in their risk for the acquisition of new intramammary infection (**NIMI**) in the dry period. According to a study of NIMI over the dry period in cows under SDCT, it was reported that uninfected cows and cows infected in two or more quarters occurred more frequently than expected based on a random distribution, thus supporting interdependence of quarters during the dry period. Consequent to these results, the authors' concluded that selective treatment decisions should be made at the cow level (Robert et al., 2006a).

5.2.3 Internal teat sealants

Also available for the purpose of managing IMI during the dry period are intramammary infusions known as internal teat sealants (**ITS**). Internal teat sealants do not contain antimicrobials but protect against NIMI by creating a physical barrier in the teat thus preventing bacterial invasion and colonization (Godden et al., 2003). It has been suggested that interdependence of quarters towards NIMI in non-lactating cows partly occurs as a result of cross-contamination between teats (e.g. while in a prone position; Robert et al., 2006a). Considering that the method of action of ITS is to create a barrier to bacterial invasion of the mammary gland, it is possible that ITS may reduce the within-cow transmission of pathogens and thus diminish or eliminate interdependence. Since

the emergence of ITS, only one study has investigated the effect of ITS on the within-cow dynamics of dry period NIMI. In that study, it was reported that ITS may have resulted in quarter independence, but the authors' failed to achieve conclusive results because of low statistical power (Berry et al., 2003). Moreover, the outcome of that investigation was prevalence of IMI at calving, and therefore the analyses considered both the acquisition of NIMI and the cure of existing IMI over the dry period.

5.2.4 Coagulase negative staphylococci in non-lactating cows

Regarding the importance of coagulase negative staphylococci (**CNS**) during the dry period, it has been reported that CNS are the most common cause of dry period NIMI and that prevalence of CNS is highest around calving (Dingwell et al., 2002; Taponen et al., 2006; Robert et al., 2006a; Bradley et al., 2010; Arruda et al., 2013). According to deterministic modeling of the transmission dynamics of CNS IMI, transmission during lactation alone is not able to maintain the presence of CNS within a herd (Reksen et al., 2012). Rather, persistence of CNS in a herd requires the acquisition of CNS NIMI at drying off, during the dry period, and around calving, thus illustrating the importance of preventative measures against CNS NIMI around the non-lactating period (Reksen et al., 2012). While intramammary infection with CNS typically results in moderate increases in somatic cell count (**SCC**) and rarely develops into clinical mastitis, research has shown that the role of CNS is of greater importance in herds with a low BTSCC (Schukken et al., 2009; Pyörälä and Taponen, 2009; Sampimon et al., 2010). Schukken et al. (2009) observed that in herds with an average BTSCC < 200,000 cells/mL, CNS-infected

quarters contributed more somatic cells to the bulk tank than quarters infected with a major pathogen. Considering that adoption of SDCT is only recommended for herds capable of maintaining a low BTSCC and with good control of major contagious pathogens, it is likely that CNS are an important causal agent of subclinical mastitis in herds already practising or thinking about converting to SDCT.

5.2.5 Study objectives

The main objective of the current study was to determine the effect of ITS on the interdependence of mammary quarters towards the acquisition of CNS NIMI over the dry period. Results of this study could then be used to guide recommendations regarding the level of treatment (quarter versus cow) in selective DCT treatment protocols.

5.3 MATERIALS AND METHODS

5.3.1 Sources of data

The data used in the analysis of the effect of ITS on the interdependence of mammary quarters towards NIMI during the dry period originated from two separate sources: a SDCT trial and a longitudinal cohort study. Treatments in both sources were applied at the cow level, but infection status was determined at the quarter level.

Selective dry cow therapy trial. A clinical trial was conducted to evaluate the utility of an on-farm milk culture system when used in a SDCT program and involved sixteen dairy herds with a low BTSCC (<250,000 cells/mL) (Cameron et al., 2013; Cameron et

al., 2014). Within each herd, cows with a low SCC (<200,000 cells/mL) on the last three milk tests prior to drying off and a California mastitis test score <2 in all quarters on the day before drying off were randomly allocated to receive BDCT, consisting of an infusion of DCT followed by an ITS, or SDCT. The selection of cows for infusion with DCT in the latter group was based on results obtained by a Petrifilm-based on-farm culture system. Using a composite milk sample collected on the day prior to drying off, cows with a positive Petrifilm result were infused with DCT and ITS, and cows with a negative Petrifilm result were infused with ITS alone. Each herd contributed cows that were treated with DCT + ITS and cows that were treated with ITS alone to the final dataset.

Sample collection for submission to standard bacteriological culture consisted of 1) a single quarter level sample collected by trained technicians on the day prior to drying off (dry off sample; **DO**), 2) a single quarter level sample collected by the dairy producer at 3 to 4 days postcalving (fresh cow sample 1; **FC1**), and 3) a single quarter level sample collected by a technician at 5 to 18 days in milk (fresh cow sample 2; **FC2**). The results of standard culture, and not on-farm culture, were used in the analysis of the current research.

National Cohort of Dairy Farms, Canadian Bovine Mastitis Research Network. Established by the Canadian Bovine Mastitis Research Network (**CBMRN**), the National Cohort of Dairy Farms (**NCDF**) is a national data collection platform for the purpose of epidemiological mastitis research (Reyher et al., 2011). In 2006 and 2007, 91 commercial dairy farms originating from six provinces across Canada were recruited.

Over the course of two years (2007 and 2008), repeated quarter milk samples were collected from both clinical mastitis cases and randomly selected clinically normal cows at various points during lactation, at drying off, and following calving. While participating farms in the NCDF were selected based on BTSCC to achieve a uniform distribution of BTSCC from ≤ 150,000 cells/mL to > 300,000 cells/mL, for the research in question, only samples collected from herds with BTSCC of 250,000 cells/mL or less were used. Furthermore, because individual cow data pertaining to treatments at drying off were not available, only herds practising BDCT (ascertained by a questionnaire on the subject of dry period management) were included. Not all herds used ITS, and of the herds that used ITS, not all practiced blanket application of ITS. For the analyses, regarding the herds that used ITS, only herds that infused ITS in all cows at drying off were included. Therefore the final dataset included only herds that practiced BDCT (n = 30), and a proportion of those herds also practiced blanket application of ITS (n = 11).

In the NCDF, dry period samples were collected largely by farm personnel and consisted of 1) single quarter level samples collected between 4 and 2 weeks before drying off, 2) single quarter level samples collected between 2 weeks before drying off and drying off, 3) single quarter level samples collected within 24 hours postcalving (considered the FC1 sample), and 4) single quarter level samples collected between 1 and 2 weeks postcalving (considered the FC2 sample). In order to be consistent with the SDCT trial with respect to samples collected at drying off, only those collected within two weeks prior to drying off were used in the analysis (considered the DO sample).

There were no restrictions for inclusion at the cow-level, therefore all cows with pre-drying off and postcalving milk culture data were eligible for inclusion in the analysis.

5.3.2 Milk sample collection, storage, and culture

Techniques used for sample collection, bacteriological culture, and species identification in the SDCT trial were based on those used by the NCDF, which in turn used guidelines set forth by the National Mastitis Council (National Mastitis Council, 1999). All samples were collected aseptically and were maintained at -20°C until cultured in the laboratory. In the NCDF, samples were processed at one of four laboratories using standardized methods, while samples of the SDCT trial were analyzed at a single laboratory common to the NCDF. A description of the techniques used can be found in Cameron et al., (2014). Samples that displayed growth of 3 or more pathogens were considered contaminated. If *Staphylococcus aureus* was among the pathogens isolated in a contaminated sample, it was considered significant growth and was enumerated, and isolation of *Bacillus* spp. was classified as non-significant growth associated with environmental contamination.

5.3.3 Definitions

Intramammary infection. A quarter was considered infected if ≥ 100 colony-forming units (**cfu**)/mL of milk of any pathogenic organism of interest, except for CNS, were cultured. For CNS, a definition of ≥ 200 cfu/mL was used. These definitions are in accordance with the recent publication of characterization of IMI based on single sample

bacteriological testing, and is in agreement with the National Mastitis Council (Dohoo et al., 2011; Lopez-Benavides et al., 2012).

Dry period new intramammary infection. A quarter was considered to have acquired a CNS NIMI over the dry period if it was uninfected at drying off but classified as infected with CNS postcalving. Postcalving infection status was determined by parallel interpretation of FC1 and FC2. According to parallel interpretation, a quarter was considered infected if either FC1 or FC2 displayed significant growth. In cases where one of the postcalving samples was missing or was classified as contaminated, the quarter infection status was determined solely by the results of the remaining sample.

Complete sample set. Only cows with complete sets of samples were included in the analysis. A complete set consisted of interpretable results from all four quarters for DO and at least FC1 or FC2. Therefore, cows with fewer than 4 functional quarters were excluded.

5.3.4 Statistical modeling procedure

The outcome investigated was dry period acquired NIMI caused by CNS. Analyses using multilevel logistic models with random effects for herds and cows revealed very little variance at the herd level, thus a general estimating equation (**GEE**) with a logit link, a binomial error distribution, and an exchangeable correlation structure for quarters within cows was used (xtgee in Stata/IC 11.0). General estimating equations have the benefit of producing population averaged estimates and make no assumption regarding the distribution of the random effects, but can only incorporate a single level of

clustering. The main predictors in the model were a cow level predictor for the presence of an IMI with CNS at drying off and infusion with ITS at drying off. The presence of CNS at drying off was modeled as a binary predictor with a value of 1 if CNS was isolated in at least one quarter, other than the quarter at risk of NIMI, based on the samples taken prior to the end of lactation. To evaluate the effect of ITS on the interdependence of mammary quarters towards CNS NIMI, an interaction between the ITS treatment variable and the variable for the presence of IMI due to CNS at drying off was assessed. Additional predictors considered potential confounders were parity at drying off (dichotomized into parity one and parity two or greater), quarter location, and infusion with DCT. Susceptibility to intramammary infection was modeled by a dichotomous cow-level predictor for the presence of an IMI with any pathogen other than CNS at drying off, and a herd level variable for the prevalence of CNS was created using the culture results from each herd (Reyher et al., 2013). To assess for nonlinear trends of herd prevalence, the fractional polynomial method was used and the best fitting model was determined by the deviance difference. All biologically relevant interactions between the primary predictors and other variables (e.g. interaction between CNS at drying off and infusion with DCT) were examined and included in the final model when significant. Statistical analysis was performed using Stata/IC 11.0 (StataCorp, College Station, TX). Because of the relatively small numbers of NIMIs, and hence relatively low power of the study, statistical significance was defined as $P \leq 0.10$.

5.4 RESULTS

5.4.1 Data and descriptive statistics

The final dataset contained information from 9,644 quarter samples obtained from 910 cows (3,640 unique quarters). The SDCT trial provided data on 579 cows originating from 16 herds; the NCDF provided information on 331 cows originating from 30 herds. For the herds of the NCDF, 36.7% (11/30) used ITS in all cows at drying off. In total, 189 cows were missed during the FC1 sampling (756 quarter samples), and 130 cows were missed during the FC2 sampling (520 quarter samples). Of the quarters sampled at drying off, 2,847 (78.2%) did not have an IMI and were therefore considered at risk of acquiring a CNS NIMI over the dry period. Table 5.1 contains a summary of the data included in the analysis stratified by data source. According to standard bacteriological results, the apparent overall and CNS-specific intramammary infection pressures were greater in the herds comprising the NCDF than found in the SDCT (Table 5.1). Cows of the NCDF had more CNS and non-CNS IMI at drying off than cows enrolled in the SDCT trial, and herd prevalence of CNS was significantly higher in the NCDF population (Table 5.1). Over the dry period, 449 previously uninfected quarters became infected with CNS for an overall quarter incidence of 15.8% (449/2,847).

5.4.2 Generalized estimating equation results

The results of the GEE model are given in Table 5.2. With respect to odds ratios (**OR**), values > 1 indicate that the predictor is associated with an increased risk for CNS

NIMI, values < 1 indicate that the predictor is associated with protection against CNS NIMI, and values = 1 indicate no association with CNS NIMI. Of the main effects investigated in the analysis, only herd prevalence of CNS was significantly associated with dry period CNS NIMI. Herd prevalence was best represented by a linear term and according to the GEE model, as herd prevalence increased, the odds of CNS NIMI also increased.

Quarter interdependence towards CNS NIMI during the dry period was investigated by an interaction term between CNS infection in another quarter at drying off and infusion with ITS, and this term was significant with a *P*-value of 0.058 (Table 5.2). When CNS was present at drying off (i.e. isolation of CNS in at least one quarter other than the one of interest), the odds of acquiring a dry period CNS NIMI was not significantly different for quarters infused with ITS when compared to quarters not infused with ITS (OR = 1.04, 95% confidence interval (**CI**) = 0.69, 1.56). Regarding quarters of cows without a CNS IMI at drying off, the odds of CNS NIMI was lower for quarters treated with ITS (OR = 0.59, 95% CI = 0.35, 0.99). Furthermore, when holding ITS infusion status constant (i.e. all quarters infused with ITS or all quarters without ITS), in quarters infused with ITS, the presence of CNS in another quarter at drying off increased the odds of incident dry period CNS infection (OR = 1.49, 95% CI = 1.08, 2.06), but no significant effect of CNS at drying off was observed for quarters without ITS treatment (OR = 0.85, 95% CI = 0.52, 1.39).

The correlation between quarters of cows was modeled using an exchangeable structure with a reported within cow correlation coefficient of 0.09.

5.5 DISCUSSION

The main objective of these analyses was to evaluate the effect of ITS on the interdependence of mammary quarters towards the acquisition of CNS NIMI during the dry period and the potential implications for selective dry cow therapy. While it would have been interesting to repeat the analyses for other mastitis-causing organisms, low NIMI incidence rates in the data precluded the investigation of all pathogens except for CNS.

5.5.1 Interdependence towards new intramammary infection over the dry period

In accordance with the results of the current study, ITS did not reduce the interdependence of quarters towards CNS NIMI during the dry period. There was no evidence to support that infusion with ITS resulted in a reduction in the risk for the within cow transfer of CNS from an infected quarter to an uninfected quarter. Surprisingly, in quarters that were infused with ITS, the presence of CNS in another quarter at drying off significantly increased the risk of CNS NIMI over the dry period, but this effect was not found in quarters without ITS treatment. Internal teat sealants contain no antimicrobial, therefore strict adherence to aseptic technique during infusion is necessary to prevent inadvertent introduction of pathogens (Crispie et al., 2004). It is possible that the presence of CNS in one quarter increased the likelihood of teat end colonization in a second quarter, and infusion with ITS facilitated the transfer of CNS from the teat end

into the mammary gland. Alternatively, misclassification of quarters with respect to CNS infection status at drying off may have contributed to the observed results. The sensitivity of bacteriological culture using a definition of CNS infection of ≥ 200 cfu/mL is estimated at 56% (Dohoo et al., 2011), so potentially several negative quarters were in fact positive for CNS. It stands to reason that, because cows with one CNS positive quarter would be more likely to have a second positive quarter, the misclassification occurred more frequently in quarters of cows identified with a CNS infection at drying off. Infusion of a misclassified quarter (i.e. truly infected quarter classified as uninfected) with ITS may have caused the progression of the infection from a mild form with low bacterial counts to a more severe form with a greater pathogen load and thus detectable by bacteriological culture in samples collected postcalving. Regarding DCT treatment in conjunction with ITS, 98% (400/410) of cows with a CNS IMI identified at drying off were infused with DCT. Therefore, in order for either hypotheses presented above to be true, DCT would have to be poorly effective against CNS, either for protection against early dry period NIMI or cure of existing NIMI. A discussion of DCT can be found below.

In quarters belonging to cows without a CNS IMI at drying off, ITS did reduce the incidence of dry period CNS NIMI. These results suggest that CNS infection over the non-lactating period can be partly attributed to CNS species located within the environment. More than ten different CNS species have been isolated from mastitic milk samples, however CNS are normally not identified to species level but treated as a uniform group (Pyörälä and Taponen, 2009). While commonly considered opportunistic

pathogens found on the skin of cows, research has established that CNS originate from multiple sources, including both the cow (such as infected glands and bovine skin) as well as the farm environment, and that reservoir type and mode of transmission are species-specific (Piessens et al., 2011; Piessens et al., 2012). In the current research, speciation of CNS was not performed thus preventing analyses at the species-level. Knowledge of species distribution within CNS positive samples may have provided insight into the route by which cows acquired CNS NIMI over the dry period, potentially lending support to the hypothesis that CNS infections were environmental in origin. Prior studies on risk factors related to CNS IMI have reported that the environment is an important source of infection for both pre-calving heifers and cows (Sampimon et al., 2009; Piepers et al., 2011). Furthermore, in Sampimon et al. (2010), it was surmised environmental CNS would predominate in herds with a low BTSCC because maintenance of low BTSCC requires adherence to the Five Point Mastitis Control Plan, a mastitis control strategy which aims to reduce the prevalence of contagious pathogens and thus contagious species of CNS.

5.5.2 Dry cow antimicrobial therapy

According to analyses in the present study, DCT did not prevent infection with CNS over the dry period. This is in agreement with the results a meta-analysis summarizing the effect of DCT on dry period NIMI, where it was concluded that DCT was not effective against CNS (Robert et al., 2006b). Coagulase negative staphylococci are repeatedly identified as the primary cause of dry period NIMI despite treatment with

DCT causing speculation regarding the prophylactic effect of DCT against CNS (Robert et al., 2006a; Bradley et al., 2010; Arruda et al., 2013). A peak in infection rate in the late dry period is well-supported by the literature (Oliver and Mitchell, 1983; Smith et al., 1985; Bradley and Green, 2000). It has also been demonstrated that, as a result of declining levels of active compound over time, DCT are not effective against NIMI in periparturient cows (Oliver et al., 1990). Thus, for teats exposed to CNS in the late dry period, infusion with DCT offers little to no protection against the establishment of infection. Moreover, antimicrobial resistance among CNS has been observed and may explain a lack of prophylactic and therapeutic effects (Makovec and Ruegg, 2003; Pitkälä et al., 2004; Østerås et al., 2006; Rajala-Schultz et al., 2009). However, others consider CNS to respond well to antimicrobial treatment with reported DCT cure rates of 80 to 90% (Pyörälä and Taponen, 2009). Disagreement among researchers may be the result of misclassification bias. The reported incidence rate of CNS NIMI is high, and concurrently the duration of infection is short (Dufour et al., 2012a). In the absence of strain typing, it is possible that an IMI found at drying off was not the same CNS that was identified at calving, and thus it would appear that DCT was ineffective at clearing the prevalent CNS IMI when in fact a cure was achieved.

5.5.3 Quarter location

In the current study, the risk of acquiring a CNS NIMI over the dry period was not different for different quarters within the udder. This is in contrast with Robert et al. (2006a) who, in a study that evaluated the interdependence of quarters towards NIMI

over the dry period, observed that front quarters had more CNS NIMI than did hindquarters. However, in analyses of all mastitis pathogens in aggregate, Berry et al. (2003) also failed to detect an effect of quarter location on the prevalence of postcalving IMI. During lactation, cross-contamination between quarters due to proximity has been suggested as an explanation for interdependence and this hypothesis has been extended to the non-lactating period as well (Adkinson et al., 1993; Robert et al., 2006a; Reyher et al., 2013). Adkinson et al. (1993) reported that teat ends of the hindquarters were located closest together and indeed, in studies based on lactational data only, CNS infections have been more frequently isolated in hindquarters than front quarters (Barkema et al., 1997; Berry and Meaney, 2006). The physical proximity hypothesis for interdependence over the dry period has not been upheld by prior research (Berry et al., 2003; Robert et al., 2006a), and it is not supported by the results of this study.

5.5.4 Susceptibility parameter

In order to account for differences in the predilection of cows towards NIMI, a susceptibility parameter consisting of the presence of IMI by a pathogen other than CNS at drying off was included in the model. Some of the specific factors affecting the susceptibility of a cow to NIMI that were not already controlled for in the analysis included stage of lactation, conformation, and immune system competency. In a similar analysis using the complete NCDF dataset (i.e. including both lactational samples, pre-drying-off, and postcalving samples for a total of 18,433 complete sample sets), Reyher et al. (2013) identified an increased risk for CNS NIMI in cows with an IMI by another

pathogen in one or more quarters at the previous sampling. This effect was not observed in the current study and may be the result of low power. A discussion on power in the present study can be found below. It is possible that susceptibility to NIMI was greater for cows in the NCDF dataset than for cows in the SDCT dataset but when the two datasets were combined, the effect of cow susceptibility was lost. Based on the samples taken, the apparent overall and CNS-specific intramammary infection pressures were greater in the herds comprising the NCDF. These observed differences in infection pressures are likely the result of the study protocol within each data source. For the NCDF, cows were chosen at random with no restrictions, thus all cows entering the dry period were eligible for inclusion. For the SDCT trial, cows had to meet an SCC criterion of less than 200,000 cells/mL on the last three milk tests prior to drying off, and consequently only 46% of cows dried-off during the study period were enrolled. Thus, cows comprising the NCDF (33% of the total sample size) were potentially a mix of both low and high SCC cows with associated low and high probabilities of infection at drying off. In contrast, cows comprising the SDCT trial (67% of the total sample size) were all low SCC and therefore less likely to be infected at drying off. When combined, because the SDCT trial made up most of the data and had low power to detect the effect of IMI at drying off, the susceptibility parameter was non-significant.

5.5.5 Clustering of new intramammary infections

According to preliminary analyses using multilevel logistic regression with random effects of cows and herds, very little variance existed at the herd level. With

inclusion of the predictor for herd prevalence of CNS, a large proportion of farm factors associated the occurrence of CNS NIMI were accounted for and thus the remaining unexplained variation within farms was minimal. In the analysis using the complete NCDF dataset, Reyher et al. (2013) also identified only a small amount of herd-level variance when herd prevalence was included in the model. While a within herd intra-class correlation coefficient (**ICC**) was not calculated, multilevel logistic regression identified considerably less variance within herds than within cows, thus the ICC within herds would be smaller than the ICC within cows and this is in agreement with others (Barkema et al., 1997; Piepers et al., 2011; Reyher et al., 2013). Clustering of NIMI within a cow is expected for contagious pathogens, particularly during lactation given that the milking routine presents many opportunities for the between quarter within cow transfer of microorganisms. During the dry period however, the risk factors associated with milking are absent thus cross-contamination between teats would be less common. In the current study, the level of clustering within a cow was low (estimated ICC of 0.09) and thus consistent with expectations. Prior studies have also identified cow-level clustering of NIMI within non-lactating cows, therefore some within-cow transmission must occur (Berry et al., 2003; Robert et al., 2006a). Others have attributed this apparent interdependence to direct contact between teat ends when a cow lies down, or to common risk factors shared by the quarters of a cow such as immune competency and conformation (Berry et al., 2003; Robert et al., 2006a).

5.5.6 Herd prevalence of CNS

The association between herd prevalence of CNS and the risk of CNS NIMI was expected. Considering species of CNS that exhibit contagious properties, as the number of cows harbouring CNS increases, the risk of infection due to exposure to an infected quarter will also increase. As previously discussed, the likelihood of cow-to-cow or within cow transfer of pathogens is expected to be lower during the dry period than during lactation. However, non-lactating cows remain at risk of infection by pathogens within the environment. For species of CNS that are environmental in nature, as levels within the environment increase, the risk of exposure would increase, and thus a higher prevalence and incidence of CNS IMI would be expected. With respect to herd-level risk factors associated the incidence of CNS NIMI, bedding type has been identified as important in the epidemiology of CNS. According to analyses by Dufour et al. (2012a), sand and wood-based products were associated with decreased risk of CNS NIMI when compared to straw bedding.

The dataset used in the present study consisted of a combination of data from two separate sources – an SDCT trial comprising cows with a low SCC prior to drying off and a longitudinal cohort study without SCC restrictions. As previously noted, these sources differed with respect to degree of infection, with cows comprising the NCDF having more prevalent and incident IMI than cows of the SDCT trial. Inclusion of a herd level variable for the prevalence of CNS provided a surrogate measure of data source, thus enabling some control of the confounding effect of the existing inherent differences between the NCDF and SDCT trial.

5.5.7 Power and Type II error

As a result of low numbers of CNS NIMI within the sample of quarters included in the analysis, it is possible that the current study lacked power thus resulting in Type II error. Type II error occurs when a study fails to identify an effect when in fact the effect exists. The power of a study is dependent upon both the sample size and the size of the effect being investigated, among other factors. Of particular interest in the current study where the main association of interest consisted of an interaction term, analysis of interactions necessitates a larger sample size than analysis of main effects only. Thus, the significance level was relaxed to $P < 0.10$ to reduce the risk of making a Type II error.

Regarding the impact of CNS IMI misclassification in this study, the resulting bias would almost certainly be nondifferential and therefore towards the null hypothesis. Statistical methods to adjust for CNS IMI misclassification have been described in the literature and could have been used in the current study (Dufour et al., 2012). However, it has also been demonstrated that the potential gains in power that result from adjusting for the imperfect sensitivity and specificity of bacteriological culture would be offset by the resulting increase in effect size and thus considerable changes in statistical significance were not expected, therefore no adjustments were made.

5.6 CONCLUSION

The purpose of the analyses presented in this research was to determine if ITS induced independence of quarters for the acquisition of CNS NIMI during the dry period, and no evidence that ITS reduced interdependence was found. As a result of low NIMI risk over the dry period, a very large dataset would be required in order to repeat the analyses for pathogens other than CNS. Herd prevalence was an important predictor in the current study, therefore steps to lower levels of CNS within the herd (e.g. through better management of dry cow housing, changes in bedding type, etc.) will play an important role in reducing incidence of CNS NIMI over the dry period. Coagulase negative staphylococci are group of mastitis pathogens comprising multiple species, with different reservoirs and modes of transmission, and various degrees of AMR. Speciation and antimicrobial susceptibility testing of CNS was not done in the current study, therefore the authors were forced to speculate on the importance of AMR and the source of infection. While independence was not demonstrated, there is still a need for quarter level clinical trials to truly assess if SDQT, enabled by knowledge of quarter infection status (e.g. obtained by an on-farm culture system) and using ITS, is a viable approach to dry period mastitis control that will promote udder health, concurrently lower DCT use, and potentially incur an economic benefit for dairy producers.

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Table 5.1. Summary of data included in the analysis of quarter interdependence toward new intramammary infection with coagulase negative staphylococci (CNS) over the dry period and the effect of internal teat sealants. Sources of data for the analysis were a selective dry cow therapy trial and the National Cohort of Dairy Farms, a longitudinal cohort study for the purpose of mastitis research.

	Selective dry cow therapy trial	National Cohort of Dairy Farms
Number of cows	579	331
Parity at drying off >1 (% of cows)	289 (49.9)	162 (48.9)
Infection with CNS at drying off (% of cows)***	174 (30.1)	236 (71.3)
Infection with other pathogen at drying off (% of cows)***	44 (7.6)	101 (30.5)
Dry cow therapy treatment risk (% of cows)***	436 (75.3)	331 (100)
Internal teat sealant treatment risk (% of cows)***	579 (100)	123 (37.2)
Dry period acquired CNS infection (% of quarters)***	170 (8.4)	279 (34.4)
Herd prevalence of CNS (% of samples)[1, ***] (25^{th} percentile, median, 75^{th} percentile)	8.4 (5.3, 8.3, 10.9)	35.2 (26.5, 34.3, 42.4)

[1]Prevalence of CNS based on samples taken within each herd

† $P \leq 0.10$, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$; P-values from univariable analysis

Table 5.2. Results of a generalized estimating equation used to model the incidence of new intramammary infection (NIMI) with coagulase negative staphylococci (CNS) over the dry period. Sources of data for the analysis were a selective dry cow therapy trial and a longitudinal cohort study.

Variable	Coefficient	SE	*P*-value	Odds ratio	95% CI
Constant	-2.44	0.358	N/A	N/A	N/A
Quarter position					
Left front (reference level)	—	—	—	—	—
Left hind	-0.001	0.151	0.995	1.00	0.74, 1.34
Right front	0.031	0.149	0.838	1.03	0.77, 1.38
Right hind	-0.066	0.154	0.669	0.94	0.69, 1.27
Parity at drying off >1	-0.027	0.125	0.831	0.97	0.76, 1.24
Dry cow therapy	0.055	0.204	0.787	1.06	0.71, 1.58
Other infection(s) at drying off	-0.167	0.194	0.390	0.85	0.58, 1.24
Herd prevalence of CNS	0.061	0.007	<0.000	1.06	1.05, 1.08
CNS IMI at drying off ($CNS_{d/o}$)[1]	-0.159	0.250	0.524	0.85	0.52, 1.39
Internal teat sealant (ITS)	-0.522	0.266	0.050	0.59	0.35, 1.00
$CNS_{d/o}$ x ITS	0.559	0.294	0.058	1.75	0.98, 3.11

Contrasts

$CNS_{d/o}$	ITS	vs.	$CNS_{d/o}$	ITS	OR (95% confidence interval)
Yes	No	vs.	Yes	Yes	1.04 (0.69, 1.56)
No	No	vs.	No	Yes	0.59 (0.35, 0.99)
No	Yes	vs.	Yes	Yes	1.49 (1.08, 2.06)
No	No	vs.	Yes	No	0.85 (0.52, 1.39)

[1]Presence of CNS in at least quarter other than the one at risk of CNS NIMI

CHAPTER 6

ECONOMIC ASSESSMENT OF A 3M PETRIFILM ON-FARM MILK CULTURE SYSTEM USED IN A SELECTIVE DRY COW THERAPY PROGRAM

6.1 ABSTRACT

In many countries, blanket dry cow therapy (**BDCT**) is the standard protocol for mastitis control during the dry period. In response to concerns regarding antimicrobial resistance, selective dry cow therapy (**SDCT**) has been suggested as an alternative with the potential to reduce antimicrobial use. Recently, a Petrifilm-based on-farm culture system (**OFCS**) has been developed and validated for SDCT. Previous research has estimated the economic consequences of SDCT based on somatic cell count (**SCC**) and clinical mastitis criteria, but the financial implications of SDCT based on OFCS have yet to be reported. Using stochastic Monte Carlo models, the objective of this study was to determine the economic effect of SDCT according to results of the OFCS (**OFCS-SDCT**) and to make comparisons to BDCT, BDCT with the addition of an internal teat sealant (**BDCT+ITS**), and SDCT based on SCC and clinical mastitis criteria (**SCC-SDCT**). Additionally, the impact of the accuracy of the OFCS was examined by comparing observed results with those obtained from a theoretical test with perfect sensitivity (**Se**) and specificity (**Sp**). The input parameters were based on data from a clinical trial evaluating the use of an OFCS in SDCT, information obtained from the published literature, and expert opinion. Regarding reductions in antimicrobial usage, a drop in DCT use of 23.8% was observed under OFCS-SDCT, compared to a mean reduction of 47.5% achieved by SCC-SDCT. While the average cost per eligible cow was similar for BDCT (mean: $100.29; range: $81.50, $119.09), BDCT+ITS (mean: $110.87; range: $94.55, $127.19) and OFCS-SDCT (mean: $114.16; range: $95.19, $133.66), they were

considerably higher for SCC-SDCT, and SCC-SDCT had the greatest variation (mean: $147.60; range: $116.86, $178.33). For the SCC-SDCT protocol, the model assumed the diagnostic accuracy of the SCC and CM criteria based on data from the clinical trial, but greater accuracy has been reported in the literature. Using test characteristics of SCC and CM criteria based the published literature lowered the total cost (mean: $132.82; range: $105.67, $159.98), but SCC-SDCT still remained the most expensive and most variable option. Considering a theoretical diagnostic test with perfect Se and Sp, the average total cost per cow was $93.55 (range: $78.10, $109.10). The results showed that while OFCS-SDCT resulted in a higher total cost per cow than either BDCT scenarios, it provided the combined benefits of lowering DCT treatment risk without increasing the risk of IMI at calving. Future research direction should be the development of diagnostic methods with even greater accuracy, for better tests will result in larger reductions in DCT use, lower risk of IMI at calving, and potentially greater cost-savings for dairy producers.

6.2 INTRODUCTION

6.2.1 Dry period mastitis control

The importance of the dry period with respect to mastitis control on a dairy farm is well-recognized. Research into the dynamics of intramammary infection (**IMI**) over the dry period in the 1960s lead to the recommendation that all cows be infused with a long-acting intramammary antimicrobial at the end of lactation and it is estimated that 88% of Canadian dairy herds and 72% of American dairy herds follow this protocol

(Neave et al., 1969; National Mastitis Council, 2006; USDA, 2008; Dufour et al., 2012). The role of dry cow antibiotic therapy (**DCT**) is to cure IMI that are present at the end of lactation and to prevent the acquisition of new intramammary infections (**NIMI**). According to meta-analysis of dry cow interventions, quarters infused with DCT have higher cure risk and lower NIMI risk over the dry period than untreated quarters (Halasa et al., 2009a; Halasa et al., 2009b). However, as a result of decreasing levels of active antibiotic over time, DCT is not protective in the late dry and periparturient periods when rates of NIMI are elevated (Smith et al., 1985; Oliver et al., 1990; Dingwell et al., 2003). With the development of internal teat sealants (**ITS**), the prevention of dry period NIMI can now be accomplished without the use of antimicrobials (Woolford et al., 1998; Sanford et al., 2006). Furthermore, internal teat sealants have the benefit of being protective against NIMI throughout the entire length of the dry period, and quarters infused with an ITS, either alone or in combination with DCT, have a reduced risk of NIMI when compared to quarters treated with only DCT (Woolford et al., 1998; Sanford et al., 2006; Halasa et al., 2009b). In cows that don't require the therapeutic effects of DCT, i.e. without an IMI at drying off, prophylaxis against NIMI can be provided by ITS alone, therefore the requirement for blanket DCT treatment (**BDCT**) of all cows at the end of lactation is debatable.

6.2.2 Selective dry cow therapy

Choosing cows for DCT treatment based on suspected or known infection status at the end of lactation is known as selective dry cow therapy (**SDCT**). Selective dry cow

therapy can result in a reduction in the use of antimicrobials in dairy production and is considered a more judicious approach to antimicrobial use for mastitis control during the dry period. However, since BDCT is the mainstay for dry period mastitis control in many countries, for producers to be motivated to switch to a SDCT protocol, the udder health and financial outcomes of SDCT must be at least equivalent to that of BDCT.

For selective dry cow therapy, treatment decisions are often based on somatic cell count (**SCC**) and clinical mastitis (**CM**) history such that cows with a monthly SCC of < 200,000 cell/mL on all of the last three milk tests prior to drying off and no CM are considered low risk for IMI and are therefore not infused with DCT after the last milking (Torres et al., 2008; Rajala-Schultz et al., 2011). According to the literature, approximately 70% of cows with an IMI at drying off will be detected, and subsequently treated with DCT, using this method (Torres et al., 2008). Recently, an on-farm culture system (**OFCS**) has been developed and validated for SDCT (Cameron et al., 2013; Cameron et al., 2014). The system utilizes 3M Aerobic Count Petrifilms to culture composite milk samples directly on the farm and provides results within 24 hours, enabling producers to make selective treatment decisions based on the current IMI status of a cow. While former selective dry cow therapy protocols have relied on historical SCC and CM records to make treatment decisions, the accuracy of this approach was greatly improved when Petrifilm-based on-farm culture was added to the protocol with a sensitivity (**Se**) of 85.2% and specificity (**Sp**) of 73.2% (Cameron et al., 2013). Furthermore, when OFCS-based SDCT was used in low bulk tank SCC herds (< 250,000 cells/mL) on low SCC cows (< 200,000 cells/mL), the risk of IMI at calving and CM in

the first 120 days of the next lactation was equivalent to that observed in cows under BDCT (Cameron et al., 2014).

6.2.3 Study objectives

Previous research has estimated the economic consequences of SDCT based on SCC criteria (Berry et al., 2004; Huijps and Hogeveen, 2007; Halasa et al., 2010), but the financial implications of SDCT based on OFCS have yet to be reported. The objective of this analysis was to determine the economic effect of selectively treating cows with DCT at drying off according to results of a Petrifilm-based OFCS and to make comparisons to both a BDCT protocol as well as an SCC and CM based selective treatment approach. Additionally, the impact of the accuracy of the OFCS was examined by comparing observed results with those obtained from a theoretical test with perfect Se and Sp.

6.3 MATERIALS AND METHODS

6.3.1 Clinical trial evaluation of OFCS-based SDCT

Details of the clinical trial and on-farm culturing technique have been described in detail elsewhere (Cameron et al., 2014). Briefly, inclusion criteria at the herd level consisted of an annual average of monthly bulk tank SCC of less than 250,000 cells/mL in the year prior to the study and enrolment in a dairy herd improvement (**DHI**) program. A convenience sample of 16 predominately Holstein-Friesian dairy herds were selected based on their proximity to the Atlantic Veterinary College, Charlottetown, PE (n = 10)

and the Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, QC (n = 6). Cows were selected for inclusion in the trial if they had a low SCC (< 200,000 cells/mL) on the last 3 milk tests prior to drying off, no CM in the same time period, no antibiotic treatment in the last 14 days, a California mastitis test score <2 in all quarters on the day before drying off, and an expected dry period of 30 to 90 days. On the day prior to drying-off, eligible cows were randomly assigned to BDCT or OFCS-based SDCT. Cows allocated to BDCT were infused in all four quarters with a long-acting intramammary formulation of 500 mg ceftiofur hydrochloride (Spectramast DC, Zoetis, Kirkland, Quebec, Canada) followed by an ITS composed of 65% bismuth subnitrate (Orbeseal, Zoetis, Kirkland, Quebec, Canada) at drying off. Cows assigned to OFCS-based SDCT had composite milk samples cultured on-farm using the Petrifilm OFCS on the day prior to drying off. Cows were considered positive for IMI if ≥ 5 colonies were present on the Aerobic Count (**AC**) Petrifilm (3M Canada, London, Ontario) after 24 hours incubation (equivalent to 50 colony forming units (**cfu**)/mL of milk). At drying off, all quarters of cows with a positive Petrifilm result were infused with ceftiofur followed by an ITS, and all quarters of cows negative on Petrifilm were infused solely with an ITS.

Single quarter level milk samples were collected from all participating cows on the day prior to drying off, at 3 to 4 days in milk (**DIM**) and at 5 to 18 DIM. Samples were maintained at -20°C and submitted to the Maritime Quality Milk research laboratory at the University of Prince Edward Island for laboratory culture. Intramammary infection based on standard bacteriology was defined as the presence of ≥

100 cfu/mL of milk of any pathogenic organism of interest, except for coagulase negative staphylococci (**CNS**). For CNS, a definition of ≥ 200 cfu/mL was used (Dohoo et al., 2011). Samples with three or more differing isolates were classified as contaminated and isolation of *Bacillus* spp. was assumed to be associated with environmental contamination and considered non-significant growth (Reyher et al., 2013). Postcalving infection status was determined by parallel interpretation of the two milk samples obtained after calving. According to parallel interpretation, a quarter was considered infected if either sample displayed significant growth. In cases where one of the post-calving samples was missing or was classified as contaminated, the quarter infection status was determined solely by the results of the remaining sample.

6.3.2 Dry cow therapy strategies

In this investigation, the economic effects of four different dry cow therapy protocols were evaluated: blanket dry cow therapy (**BDCT**), BDCT with the addition of an internal teat sealer (**BDCT+ITS**), SCC and CM guided selective dry cow therapy (**SCC-SDCT**), and Petrifilm OFCS-based selective dry cow therapy (**OFCS-SDCT**). Within each protocol, cows meeting the inclusion criteria outlined in the clinical trial comprised the subset of the herd for which calculations were carried out, therefore comparisons between interventions were based solely on cows with low SCC and no CM in the last 3 months prior to the end of lactation. This approach was used to uphold the recommended practice of total dry cow antibiotic therapy for all quarters of cows with subclinical mastitis or a recent history of clinical mastitis. Figure 6.1 illustrates

treatments and DCT selection procedures where applicable for each protocol. All quarters of all cows in both BDCT and BDCT+ITS protocols received an infusion of DCT at drying off, and ITS were administered to all cows considered in the BDCT+ITS, SCC-SDCT, and OFCS-SDCT strategies. In the SCC-SDCT intervention, the selection protocol for DCT consisted of the inclusion criteria of the clinical trial and all eligible cows were considered uninfected and did not receive any DCT at drying off. For the OFCS-SDCT protocol, DCT treatment decisions were based on on-farm culture results and only cows positive on the AC Petrifilm were treated with DCT.

6.3.3 Model description

A stochastic Monte Carlo simulation model was created using @Risk 6.2.0 (Palisade Corporation, Ithaca, New York, United States) Microsoft Excel add-in software to estimate the cost of the four dry period interventions.

6.3.3.1 Input parameters. The input parameters used to simulate the dynamics of IMI over the dry period are listed in Table 6.1. Estimates for dry period cure risk and NIMI risk for DCT alone, ITS alone, and combined DCT+ITS were modeled by a summary estimate based on existing peer-reviewed literature. Using PubMed (U.S. National Library of Medicine, Bethesda, MD), a search of the literature for clinical trials evaluating DCT and ITS published between the years 2000 and 2013 was conducted. Keywords used in the search included: teat sealant, teat seal, Orbeseal, dry cow, dry period, dry off, treatment, therapy, intramammary infection, and mastitis. Publications had to be peer-reviewed, report quarter-level outcome data for each of the intervention

groups, and report the number of quarters in each group. An overall estimate of the effect (either cure risk or NIMI risk) was computed using meta-analysis methodology which appropriately weights the estimates of each study using the standard errors of the estimates (metan command; Stata/IC 11.2, College Station, TX, USA).

The economic input parameters used to estimate the resulting costs associated with each intervention were based on data from the clinical trial, information obtained from the published literature, or expert opinion (Table 6.2). Input values for the amount of labour time required for on-farm culture and application of dry cow treatments were based on expert opinion. Expert opinion consisted of estimates obtained from clinical faculty members (n = 5) of the Atlantic Veterinary College at the University of Prince Edward Island, Canada. The experts had knowledge and experience in mastitis treatment and control, and were familiar with regional dairy practices. Input variables consisting of expert opinion were modeled using a PERT distribution in the form of most likely, minimum, and maximum (Van Hauwermeiren et al., 2009).

6.3.3.2 Dynamics of intramammary infection over the dry period. The dynamics of IMI over the dry period was modeled via a series of consecutive steps as outlined in Figure 6.2. Each iteration (10,000 per dry period scenario) of the simulation modeled an individual cow and began with the cow's IMI status at drying off as determined by the prevalence of IMI at the end of lactation for the cows enrolled in the source clinical trial (Table 6.1). Depending on the dry period protocol in question, each cow had a probability of receiving DCT at drying off. The probability of treatment with DCT was 1 for both BDCT protocols; for SCC-SDCT, the treatment risk was 0 since all cows

considered in the model had low SCC and no CM prior to drying off as per the selection criteria. According to OFCS-SDCT, the DCT treatment risk was dependent on the Se and Sp of the OFCS previously established by the clinical trial described above (Cameron et al., 2013). In the model, Se and Sp were represented using a Beta distribution as described in Greiner and Gardner (2000). The shape parameters of the Beta distribution were modelled using $\alpha = k + 1$ and $\beta = n - k + 1$, where k denotes the number of correctly classified test results and n denotes the number of truly diseased (for Se) or truly non-diseased (for Sp) animals (Vose et al., 1996). The probability of infusion with DCT for cows with an IMI at drying off was equal to the prevalence of IMI at drying off multiplied by the Se of the OFCS. Conversely, the probability of infusion with DCT for cows uninfected at drying off was equal to 1 – the prevalence of IMI at drying off multiplied by 1 - the Sp of the OFCS.

For cows infected at drying off, the risk for a cure was dependent on the treatment applied (Table 6.1). Cows that failed to cure were considered chronic and were not at risk for NIMI. Cows uninfected at drying off and cows achieving a dry period cure were at risk for NIMI, and risk was again dependent upon the treatment(s) administered at drying off (Table 6.1). Cows infected during the dry period or chronically infected due to failure to cure were considered infected at calving and were at risk for clinical mastitis in the next lactation. The probability of clinical mastitis as a result of IMI acquired during lactation was assumed to be the same for all cows and was not factored into the model. Values for dry period cure risk and NIMI risk taken from the literature were estimates at the quarter level, therefore the end values for the probability of IMI at calving

represented quarter level risk. In order to aggregate the quarter level values to the cow level, a multiplier based on the average number of quarters infected per cow with an IMI at calving in the clinical trial (equal to 1.3; Cameron et al., unpublished data) was applied. Finally, to estimate the proportion of IMI at calving that would become clinical during the next lactation, a weighted average risk of CM was calculated based on the top three most commonly isolated pathogens at calving in the clinical trial and CM risk values for these pathogens obtained from the published literature (Table 6.1). It was assumed that infections that did not become clinical would result in subclinical mastitis.

6.3.3.3 Economic outcomes. The economic outcomes were estimated by first simulating the total number of cows eligible for inclusion using the values for herd size, the proportion of cows entering the dry period, and the risk for inclusion based on the selection criteria (Table 6.2). Next, the expenses associated with each of the dry cow therapy protocols were estimated. The expenses considered were the cost of DCT and ITS, labour costs incurred during intramammary treatment and related to on-farm culture, the cost of the OFCS, and the cost of clinical and subclinical mastitis (Table 6.2). It was assumed that herds were already enrolled in DHI, therefore no additional cost for monthly SCC determination was considered. The cost of a case of clinical mastitis was estimated using a spreadsheet tool produced by the Canadian Bovine Mastitis Research Network (**CBMRN**; Huijps and Hogeveen, 2011) and based on a published work by Huijps et al (2008). The probability of culling as a result of clinical mastitis was set at 15% (Huijps et al., 2008) and the average culling cost was estimated at $2000 (Huijps and Hogeveen, 2011). The cost of clinical mastitis was modeled as a weighted average

value for both non-culled and culled cases (representing 85% and 15% of cases respectively), and costs were calculated using both the mean Canadian milk price for 2012 (Canadian Dairy Information Centre, 2012) as well as the mean world milk price for 2012 as reported by International Farm Comparison Network (International Farm Comparison Network, 2014). With respect to the cost of subclinical mastitis, the cost was assumed to be the result of reduced milk production only (i.e. no treatment or veterinary fees), and once again the CBMRN tool and both milk prices were used. The total cost associated with each dry cow therapy protocol was reported as cost incurred per eligible cow.

To determine the indifference point at which the cost of OFCS-SDCT became equivalent to less costly protocols, the Se and Sp of the OFCS were changed independently, with all other variables remaining unchanged, until the mean total cost per cow for OFCS-SDCT approached that of the less expensive options.

6.3.3.4 Alternative scenarios. To examine model behaviour using a perfect diagnostic test, a model was run using a theoretical test with perfect Se and Sp. The results from that model were used to determine the maximum cost such a test could incur that would result in an overall cost equal to that of BDCT+ITS, while simultaneously reducing the use of DCT.

For the SCC-SDCT scenario, the model assumed the diagnostic accuracy of the SCC and CM criteria based on data from the clinical trial. According to a study by Torres et al. (2008), a Se of 69.8% and Sp of 50.6% was achieved using three months of data, an SCC threshold of < 200,000 cells/mL, and no CM during the entire lactation to

identify uninfected cows (Table 6.1). The gold standard definition of IMI used by Torres et al. differed slightly from the source clinical trial for pathogens other than *Staphylococcus aureus* or *Streptococcus agalactiae* as the requirement was set at ≥ 500 cfu/mL. For the purpose of comparison to the SCC-SDCT model, a second SCC and CM-based SDCT model was run with test characteristics obtained from Torres et al (2008). Using the reported values for Se and Sp and the prevalence of IMI at drying off (p(D +)) from the clinical trial, the predictive value of low SCC on the last three milk tests and no CM in the same time frame (the predictive value of a negative test; PV-) was calculated using the following formula (Dohoo et al., 2009):

$$PV- = \frac{p(D-) * Sp}{p(D-) * Sp + p(D+) * (1 - Se)}$$

The predictive value was then used to estimate the proportion of truly negative and falsely negative cows observed under this scenario.

6.4 RESULTS

6.4.1 Standard bacteriological culture

The pathogen data used in modelling treatment scenario outcomes was based on the clinical trial (described above). According to the results of standard bacteriological culture of quarter-level milk samples collected at drying off and within the first 18 days of lactation aggregated to the cow level, CNS (32.1%), environmental streptococci (3.7%), and *Corynebacterium* spp. (3.2%) were the most frequently isolated pathogens at

drying off; after calving, CNS (29.8%), environmental streptococci (7.8%), and *Staphylococcus aureus* (4.7%) were most commonly identified (Cameron et al., 2014).

6.4.2 Summary estimates for treatment dependent cure risk and new intramammary infection risk

The summary estimates for treatment dependent dry period cure risk and NIMI risk based on peer-reviewed literature are presented in Table 6.1. Intramammary infection cure risk was comparable for DCT alone and combined DCT and ITS, whereas infected quarters infused with ITS alone (considered a spontaneous cure risk) had cure risks approximately half that of DCT treated quarters. With respect to new intramammary infections, quarters treated with ITS, either alone or in conjunction with DCT, had lower risk than quarters infused with DCT alone.

6.4.3 Results for BDCT, BDCT+ITS, SCC-SDCT, and OFCS-SDCT protocols

The results of the stochastic models are shown in Table 6.3. For the following, the average value is reported with the 5^{th} and 95^{th} percentiles in parentheses. In all protocols, 38 (28, 50) cows were deemed eligible for inclusion. Under OFCS-SDCT, 49.8% (46.2%, 53.4%) of eligible cows were selected to receive DCT. Considering the average values for inclusion risk and DCT treatment risk, a drop in DCT use of 23.8% was observed under OFCS-SDCT, compared to a mean reduction of 47.5% achieved by SCC-SDCT.

While SCC-SDCT resulted in the largest decrease in DCT usage, the risk of IMI at calving, and similarly the risk for CM in the next lactation, was highest for this scenario. For the remaining scenarios, intramammary infection risk at calving was lowest for BDCT+ITS, followed by OFCS –SDCT and BDCT, but the differences between these protocols was small.

The cost of dry cow treatments including labour was highest for BDCT+ITS, followed by OFCS-SDCT; SCC-SDCT had the lowest treatment related costs. For the OFCS-SDCT scenario, the cost of the OFCS was $8.43 ($7.42, $9.58) per eligible cow.

The overall cost of the dry period intervention per eligible cow was lowest in the BDCT scenario and highest for SCC-SDCT. While average total costs were similar for both BDCT scenarios and for OFCS-SDCT, they were considerably higher for SCC-SDCT, and SCC-SDCT displayed the most variation. A graphical representation of total costs per dry cow therapy protocol is illustrated in Figure 6.3. The indifference point at which the total cost per cow for OFCS-SDCT became equivalent to BDCT+ITS was reached at a Se of 92% (mean total cost per eligible cow $110.86) or at a Sp of 93% (mean total cost per eligible cow $110.81).

6.4.4 Results for alternative scenarios

The results of the alternative models are listed in Tables 6.3 and 6.4. Considering a theoretical diagnostic test with perfect Se and Sp, total costs for materials and labour incurred by the test could amount to a maximum of $17.32 ($16.45, $18.09) and be equivalent to costs associated with BDCT+ITS (Table 6.3).

When compared to the clinical trial-based SCC-SDCT protocol, estimations using Torres et al., (2008) test characteristics resulted in a lower false negative risk (32.5% vs. 39.4%) and a reduction in the proportion of cows with an IMI at calving (33.0% vs. 37.4%; Table 6.4). As a result, the mean estimated cost for a SDCT scenario based on SCC and CM history was reduced to $132.82 (vs. $147.60 for SCC-SDCT; Table 6.4).

6.5 DISCUSSION

6.5.1 Total cost per eligible cow

Using stochastic modeling, the economic outcome of an OFCS guided SDCT program was compared to BDCT with and without the addition of an ITS, and SCC-based SDCT. The most cost-effective dry period treatment intervention was BDCT without ITS and this is in agreement with Halasa et al. (2010). Addition of an ITS to the BDCT protocol resulted in a higher net cost due to the additional labour and material expenses, however the overall probability of IMI at calving was lowest for this option. While the current study, which focused on herds with low bulk tank SCC and presumably low infection pressures, failed to demonstrate the economic advantage of ITS, it is possible that considering herds with a greater infection risk may have produced different results. Prior research has demonstrated that the cost-effectiveness of ITS is most sensitive to the rate of NIMI during the dry period and that in herds with high rates of IMI during the dry period, BDCT with that addition of ITS becomes economically advantageous over BDCT (Halasa et al., 2010).

In the majority of cases, economic outcomes guide farm management decision making and producers could use analyses such as the one presented here to help make farm specific decisions. Producers wanting to minimize the costs of dry period interventions will choose BDCT. While cost differences between treatment protocols did exist, the choice of treatment strategy will also depend on non-economic factors. For producers motivated to adopt a more targeted approach when administering antimicrobial products, the scenario resulting in the greatest reduction in DCT use would be the most appealing. In that case, a producer could choose OFCS-SDCT with the knowledge that a reduction in DCT can be achieved without increased risk of IMI at calving and only a moderate increase in cost per cow.

Presently in North America there are no policies preventing conventional dairy producers from using BDCT and therefore producers may not be highly motivated to switch to SDCT. Conversely, in certain European countries the pressure to reduce antimicrobial use in food animal production is high, resulting in stricter regulations regarding their application. For instance, in Norway where the use of antimicrobial drugs is highly regulated, SDCT is the recommended practice and BDCT is never used (Østerås and Sølverød, 2009). In the Netherlands, the Ministry of Economic Affairs, Agriculture and Innovation called for a mandatory 50% reduction in antimicrobial use on all farms by 2013 based on 2009 levels (SDA, 2012). Historically, Dutch dairy producers have relied on BDCT for dry period mastitis control (Lam et al., 2013), however with the recent introduction of financial penalties for failing to reduce antibiotic usage, transitioning to an effective SDCT program is one method by which producers can meet the restrictions

outlined by the current Dutch strategy. When more attention is focused on the use of antimicrobials in food animal production systems, alternatives that promote a more judicious approach to antimicrobial treatment of dairy cows will become more important. In 2012, the Food and Drug Administration of the United States (**FDA**) presented a guidance document titled: The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals. The purpose of this document was to outline a set of general practices that promote the judicious use of antimicrobial drugs in food animal agriculture (FDA, 2012). While the outlined recommendations were nonbinding, the creation of this guidance document reflects the increasing importance of the issue of antimicrobial use in food producing animals in the United States.

6.5.2 Accuracy of the selection protocol in SDCT

The success of a SDCT program is dependent upon the accuracy of the treatment selection protocol used. For SDCT to be economically beneficial, both test Se (defined as the proportion of cows with an IMI identified as infected by the test) and test Sp (defined as the proportion of cows without an IMI identified as uninfected by the test) are important. As sensitivity increases, the risk of failing to treat cows with an IMI at drying off decreases; as Sp increases, the proportion of cows receiving unnecessary DCT treatment decreases. The OFCS-SDCT economic model became a cost indifferent option to BDCT+ITS when the OFCS achieved a high Se of 92% or a high Sp of 93%.

While no test for mastitis has perfect Se and Sp, even a theoretical perfect test will have to be priced such that the cost of implementation is equivalent to, or less than, the

cost of current DCT practices. According to the estimates of this study and using BDCT+ITS as the base scenario, the combined cost of materials and labour required to obtain perfect information regarding a cow's IMI status at drying off should not exceed $17 per eligible cow. Implementation of SDCT based on a perfect diagnostic test would result in a 28.8% reduction in DCT use. By comparison, OFCS-SDCT resulted in a 23.8% DCT reduction, marginally lower than a perfect test but at an average cost of $3.29 more than BDCT+ITS per eligible cow. Within the current study, selective treatment decisions were made at the cow-level and achieved modest decreases in DCT use, but greater reductions in DCT use could be effected when treatment decisions are made at the quarter level. Application of the OFCS at the quarter level would enable selective dry quarter treatment, but would result in higher material and labour costs associated with on-farm culture. Quarter-level culture has the benefit of increased Se over cow-level composite sample culture (Reyher and Dohoo, 2011), therefore it is possible that reductions in false negative test results and missed treatments, leading to decreases in IMI at calving and lactational mastitis, might offset the increased costs of quarter-level culture.

6.5.3 Selective dry cow therapy based on SCC and CM history

The main objective of this research was to determine the economic viability of Petrifilm on-farm culture-based SDCT. As part of that objective, a comparison was made with SDCT based on SCC and CM history. Not only did SCC-SDCT bear the highest average total cost, but the variation in total cost was greatest for this protocol, and thus

would result in the most risk. For herds enrolled in DHI, this option does not incur additional costs but as the results show, the economic success of such an approach depends greatly on the ability of this method to accurately determine the infection status of a cow. Following SCC-SDCT and using data from the clinical trial, a large percentage of infected cows would not have received treatment. Consequently, the risks of IMI at calving and mastitis during lactation were high for this scenario. One of the assumptions of the model was that all infections present at calving, either as a result of failing to cure or newly acquired infections, would result in either CM or subclinical mastitis in the subsequent lactation. In reality, self-cure of IMI does occur, therefore the estimates for the effect of IMI at calving are overestimated.

Using Torres et al. (2008) test characteristics, the outcome of SCC and CM-based SDCT was more favourable. As a result of a different gold standard definition of IMI used in Torres et al., a proportion of the infections detected in the clinical trial would have been classified as non-significant according to Torres et al., thus explaining a lower apparent false negative rate. For the clinical trial described in this paper, the definition of IMI was chosen with the aim of identifying as many existing infections as possible, as described by Dohoo et al. (2011) and is in agreement with the National Mastitis Council (Lopez-Benavides et al., 2012). Furthermore, the criteria used by Torres et al. differed slightly from those outlined in the selection criteria of the clinical trial such that the requirement for no clinical mastitis extended to the entire lactation as opposed to the last three months of lactation. A more rigorous inclusion criterion would result in higher Se (and lower false negative risk), but at the expense of lower Sp (and a higher false positive

risk). Finally, when considering using monthly SCC data to made SDCT decisions, an important factor to consider is the potential effect of the test date interval. Because milk test-day intervals are typically one month or more in length, the information may not reflect the actual state of the cow at the end of lactation. Conversely, Petrifilm on-farm culture, when performed on the day prior to drying off, provides producers with an up-to-date assessment of a cow's IMI status.

6.5.4 Cure risk and NIMI risk

According to the models used in this analysis, no consideration was given to the variation in cure risk and NIMI risk that exists for different pathogens. The most prevalent pathogen at drying off in the trial dataset was CNS. As a group, CNS is considered to respond well to antimicrobial treatment with reported DCT cure rates of 80 to 90% (Pyörälä and Taponen, 2009) and the DCT cure rates used in the modeling of the dynamics of IMI fall within this range. Coagulase negative staphylococci were also the most commonly isolated pathogen post-calving. This was not an unexpected finding as in many countries including Canada, CNS are the most common cause of IMI and are considered to be an emerging mastitis pathogen (Pyörälä and Taponen, 2009; Schukken et al., 2009; Reyher et al., 2012).

6.5.5 Risk of IMI at calving

With respect to the effect of dry period treatments on the dynamics of IMI over the dry period, the probability of IMI at calving was marginally higher for OFCS-SDCT

as compared to BDCT+ITS. Previous work has demonstrated that the Petrifilm OFCS is an accurate tool for the identification of IMI at drying off, providing timely results with good Se and Sp. Moreover, the selective treatment of cows at the end of lactation based on Petrifilm culture results did not increase the risk of IMI at calving nor the risk of CM in the first 120 days of the subsequent lactation. Selective dry cow therapy based on SCC resulted in nearly twice the risk of IMI at calving, suggesting that, while initial antibiotic use was minimized, a treatment selection protocol based on monthly SCC alone would risk higher mastitis incidence versus the other approaches evaluated. Surprisingly, the risk of IMI at calving was higher for BDCT+ITS than for SDCT based on a perfect diagnostic test. According to the summary estimate of NIMI risk based on published literature, quarters treated with ITS alone had a lower risk of NIMI than quarters treated with both ITS and DCT, and thus produced the observed outcome.

6.6 CONCLUSIONS

Stochastic models were built to determine the costs associated with Petrifilm OFCS-based SDCT, and to make comparisons with BDCT, BDCT+ITS, and SCC-SDCT. Although blanket dry cow therapy without the addition of ITS was economically the best option and SCC-SDCT was the most costly, the estimated average cost per cow for BDCT, BDCT+ITS, and OFCS-SDCT were similar. Regardless of the test characteristics used (clinical trial data or Torres et al., 2008), an SDCT protocol based on SCC and CM history always resulted in the highest and most variable costs. The results

showed that while OFCS-SDCT resulted in a numerically higher total cost per cow than either BDCT scenarios, it provided the combined benefits of lowering DCT treatment risk without increasing the risk of IMI at calving. When industry goals are to target antimicrobial use for the purpose of therapy rather than prophylaxis, there will be an opportunity for implementation of OFCS-based SDCT. Future directions for SDCT research include: 1) the development of diagnostic methods with even greater accuracy, for better tests will result in larger reductions in DCT use, lower risk of IMI at calving, and potentially greater cost-savings for dairy producers, and 2) evaluation of the economic effects of selective treatment at the quarter level, for quarter level treatments could also reduce the necessity for DCT in dairy herds.

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6.7 REFERENCES

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Table 6.1 The values (%) used to model the dynamics of intramammary infection (IMI) across the dry period for the following dry period interventions: blanket dry cow therapy (BDCT), BDCT plus internal teat sealant (ITS), selective dry cow therapy (SDCT) based on results of an on-farm culture system, and SDCT based on clinical mastitis and somatic cell count history.

Input parameter	Value
3M Petrifilm on-farm culture system sensitivity[1]	85.2
3M Petrifilm on-farm culture system specificity[1]	73.2
Prevalence of IMI at drying off[2]	39.4
Probability of cure with dry cow therapy (DCT) alone[3]	85.3
Probability of cure with DCT + internal teat sealant (ITS)[4]	88.0
Probability of cure with ITS alone[5]	46.4
Probability of new IMI with DCT alone[6]	12.5
Probability of new IMI with DCT+ITS[7]	11.0
Probability of new IMI with ITS alone[8]	9.7
Probability of clinical mastitis given IMI at calving[9]	20.0

[1]Cameron et al., 2013
[2]Data from clinical trial; Cameron et al. (unpublished data)
[3]Huxley et al., 2002; Berry and Hillerton, 2002b; Godden et al., 2003; Cook et al., 2005; Newton et al., 2008; Bradley et al., 2010; Bradley et al., 2011; Arruda et al., 2013
[4]Godden et al., 2003; Cook et al., 2005; Newton et al., 2008; Bradley et al., 2010; Bradley et al., 2011; Cameron et al., 2014
[5]Huxley et al., 2002; Berry and Hillerton, 2002a; Bradley et al., 2010; Cameron et al., 2014
[6]Huxley et al., 2002; Berry and Hillerton, 2002b; Godden et al., 2003; Cook et al., 2005; Sanford et al., 2006; Berry and Hillerton, 2007; Newton et al., 2008; Bradley et al., 2010; Bradley et al., 2011; Arruda et al., 2013
[7]Godden et al., 2003; Cook et al., 2005; Sanford et al., 2006; Berry and Hillerton, 2007; Newton et al., 2008; Bradley et al., 2010; Bradley et al., 2011; Cameron et al., 2014
[8]Huxley et al., 2002; Berry and Hillerton, 2002a; Sanford et al., 2006; Bradley et al., 2010; Cameron et al., 2014
[9]Todhunter et al., 1995; Swinkels et al., 2005; Taponen et al., 2006; Cameron et al., 2014

Table 6.2 The input parameters used to estimate the total cost per eligible cow for the following dry period interventions: blanket dry cow therapy (BDCT), BDCT plus internal teat sealant (ITS), selective dry cow therapy (SDCT) based on results of an on-farm culture system (OFCS), and SDCT based on clinical mastitis and somatic cell count history.

Input parameter	Distribution	Value	Source
Herd size	fixed	100	Basis for analysis
Proportion of cows dried off, %	fixed	80	Data from clinical trial; Cameron et al. (unpublished data)
Inclusion risk, %	triangular	46.0 (28.3, 69.4)[1]	Cameron et al., 2014
OFCS, CAD$/cow	triangular	6.00 (5.00, 8.00)[1]	Maritime Quality Milk[2]; 3M Canada[3]
DCT, CAD$/cow	fixed	22.40	Commercial products[4]
ITS, CAD$/cow	fixed	20.48	Commercial products[4]
Labour, CAD$/hour	triangular	16.00 (10.00, 16.00)[1]	MacDonald et al., 2011; Government of Canada Labour Program minimum wage database[5]
Labour time, minutes			
OFCS	PERT	9 (5, 15)[1]	Experts[6]
DCT or ITS alone	PERT	3 (1, 5)[1]	Experts[6]
DCT + ITS	PERT	5 (2, 7)[1]	Experts[6]
Clinical mastitis, CAD$/case	discrete	600, 800[7]	Canadian Bovine Mastitis Research Network[8]; Huijps et al., 2008
Subclinical mastitis, CAD$/lactation	discrete	170, 325[7]	Canadian Bovine Mastitis Research Network[8]; Huijps et al., 2008

[1]Most likely (minimum, maximum)
[2]www.milkquality.ca (accessed January 20th, 2014)
[3]www.3m.com/intl/ca/ (accessed January 20th, 2014)
[4]Values obtained from the pharmacy of the Atlantic Veterinary College at the University of Prince Edward Island, Charlottetown, PE
[5]Average minimum wage used as minimum value; http://srv116.services.gc.ca/dimt-wid/sm-mw/rpt1.aspx?lang=eng (accessed January 20th, 2014)
[6]See acknowledgements for details
[7]Low value based on World milk price; high value based on Canadian milk price; each value modeled with 50% probability
[8]http://www.medvet.umontreal.ca/rcrmb/en/page.php?p=13&tm=s (accessed January 20th, 2014)

Table 6.3 Results of stochastic models to determine the total cost (CAD$) per eligible cow for the following dry period interventions: blanket dry cow therapy (BDCT), BDCT plus internal teat sealant (BDCT+ITS), selective dry cow therapy based on results of an on-farm culture system (OFCS-SDCT), SDCT based on clinical mastitis and somatic cell count history (SCC-SDCT), and SDCT based on a theoretical perfect test. Results are presented as the average value with 5th and 95th percentiles in parentheses where applicable.

Item	OFCS-SDCT	SCC-SDCT	BDCT+ITS	BDCT	Perfect Test SDCT
Eligible cows, n	38 (28, 50)	38 (28, 50)	38 (28, 50)	38 (28, 50)	38 (28, 50)
DCT[1], %	49.8 (46.2, 53.4)	0	100	100	39.4
$IMI^{2}_{calving}$, %	21.6 (20.8, 22.6)	37.4	19.8	22.8	18.7
CM[3] risk, %	4.3 (4.2, 4.5)	7.5	4.0	4.6	3.7
OFCS cost	8.43 (7.42, 9.58)	N/A	N/A	N/A	N/A
Dry cow treatments (materials and labour)	32.56 (31.73, 33.42)	21.18 (21.05, 21.27)	44.05 (43.82, 44.20)	23.10 (22.97, 23.19)	30.19 (30.02, 30.31)
CM[3] cost	30.30 (25.18, 35.74)	52.36 (44.88, 59.84)	27.68 (23.72, 31.63)	31.97 (27.41, 36.54)	26.24 (22.50, 29.99)
SubCM[4] cost	42.86 (28.53, 58.13)	74.05 (50.87, 97.24)	39.15 (26.89, 51.40)	45.22 (31.06, 59.38)	37.12 (25.49, 48.74)
Total costs per eligible cow	114.16 (95.19, 133.66)	147.60 (116.86, 178.33)	110.87 (94.55, 127.19)	100.29 (81.50, 119.09)	93.55 (78.10, 109.01)

[1]DCT: dry cow therapy
[2]IMI: intramammary infection
[3]CM: clinical mastitis
[4]subCM: subclinical mastitis

Table 6.4 Results of stochastic models to determine the total cost (CAD$) per eligible cow for selective dry cow therapy (SDCT) based on clinical mastitis (CM) and somatic cell count history (SCC) such that cows with SCC <200,000 cells/mL on the last three milk tests prior to drying off and no CM would not receive dry cow therapy (DCT) at drying off. The SCC-SDCT model used data collected as part of a clinical trial evaluating an on-farm culture system for use in a SDCT program (Cameron et al., 2013 and 2014); the Torres-SDCT model used test characteristics for SCC and CM selection criteria as reported by Torres et al. (2008; sensitivity: 69.8%, specificity: 50.6%). Results are presented as the average value with 5th and 95th percentiles in parentheses where applicable.

Item	SCC-SDCT	Torres-SDCT
Eligible cows, n	38 (28, 50)	38 (28, 50)
DCT, %	0	0
IMI[1]$_{calving}$, %	37.4	33.0
CM risk, %	7.5	6.6
Dry cow treatments (materials and labour)	21.18 (21.05, 21.27)	21.18 (21.05, 21.27)
CM cost	52.36 (44.88, 59.84)	46.24 (39.64, 52.85)
SubCM[2] cost	74.05 (50.87, 97.24)	65.40 (44.92, 85.88)
Total costs per eligible cow	147.60 (116.86, 178.33)	132.82 (105.67, 159.98)

[1]IMI: intramammary infection
[2]subCM: subclinical mastitis

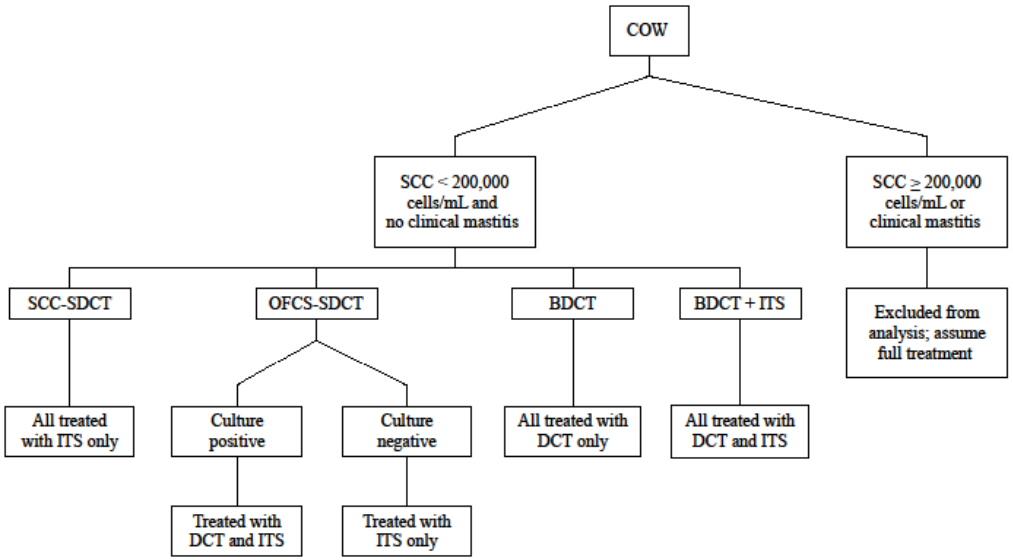

Figure 6.1 Flowchart describing dry cow therapy protocols modeled using stochastic methods to estimate the cost of dry period mastitis control. Blanket dry cow therapy (BDCT), BDCT plus internal teat sealant (BDCT+ITS), selective dry cow therapy based on results of an on-farm culture system (OFCS-SDCT), and SDCT based on clinical mastitis and somatic cell count history (SCC-SDCT). SCC: somatic cell count; DCT: dry cow antibiotic therapy; ITS: internal teat sealant.

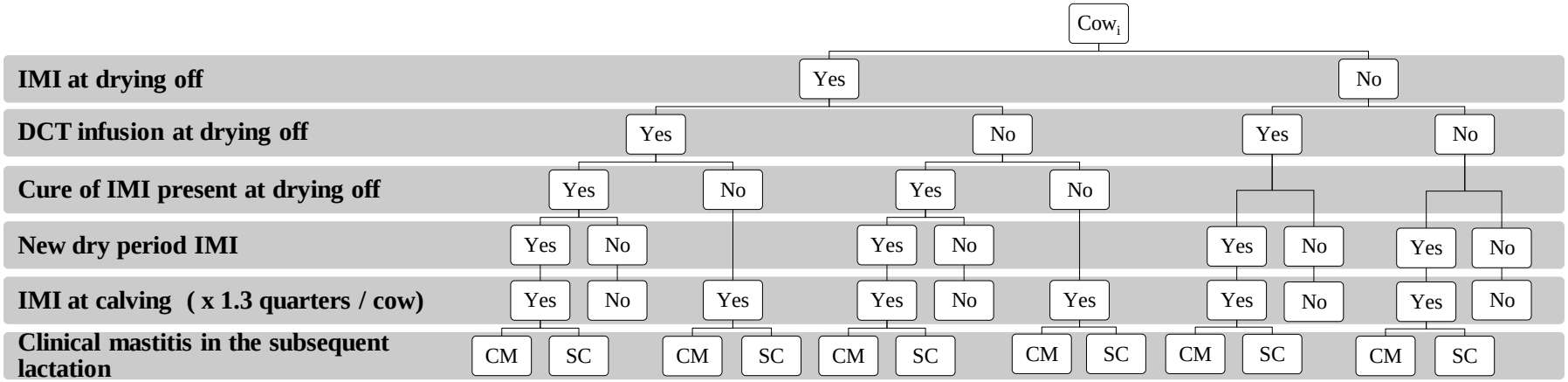

Figure 6.2 Event tree for the dynamics of intramammary infection (IMI) over the dry period for cows under either blanket or selective dry cow therapy protocols. DCT: dry cow therapy; CM: clinical mastitis; SC: subclinical mastitis.

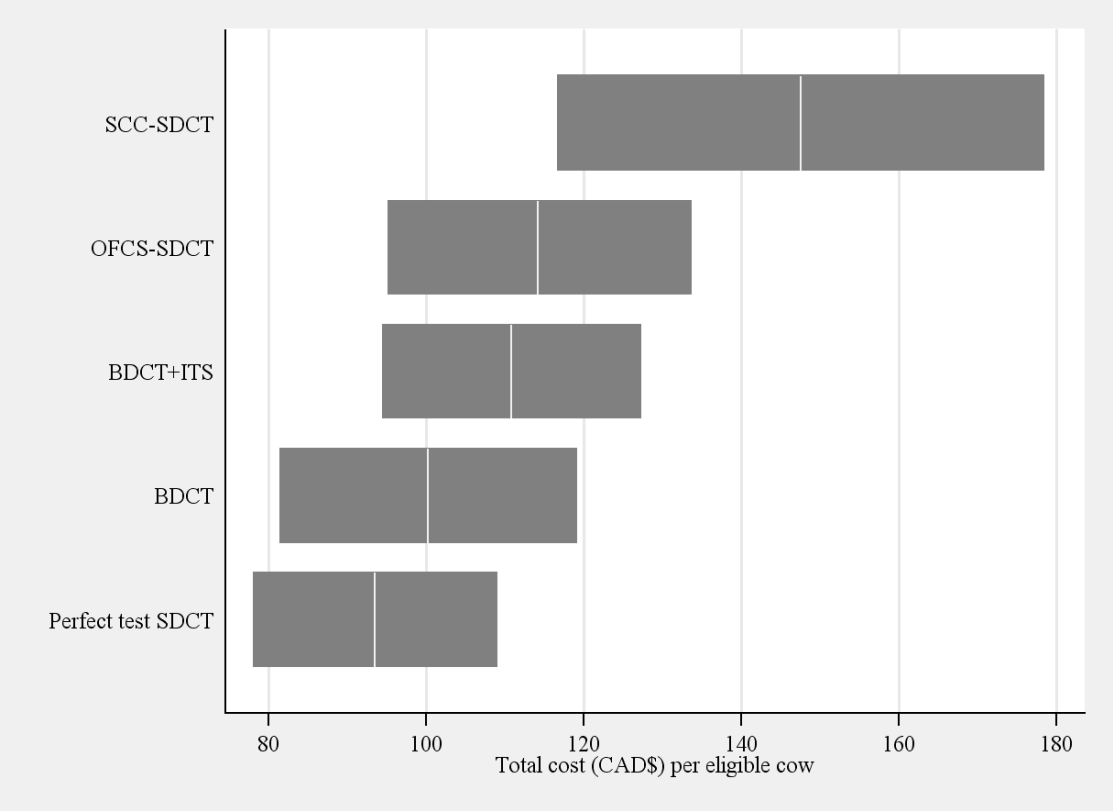

Figure 6.3 Graphical representation of the cost of various dry period mastitis control programs as estimated by stochastic models. Bars extend from 5th to 95th percentiles with average value located at the white line. Blanket dry cow therapy (BDCT), BDCT plus internal teat sealant (BDCT+ITS), selective dry cow therapy based on results of an on-farm culture system (OFCS-SDCT), SDCT based on clinical mastitis and somatic cell count history (SCC-SDCT), and SDCT based on a theoretical test with perfect accuracy.

CHAPTER 7. SUMMARY AND GENERAL DISCUSSION

7.1. GENERAL DISCUSSION

7.1.1 Rationale for study

Mastitis is the most common disease in dairy cattle worldwide, and as a result it is the number one indication for antimicrobial use in the dairy industry (Seegers et al., 2003; Halasa et al., 2007; Pol and Ruegg, 2007; Saini et al., 2012). In many countries, including Canada and the United States, mastitis control during the dry period includes treatment of all cows with dry cow antimicrobial therapy (**DCT**) at the end of lactation (Barkema et al., 1998; Berry and Hillerton, 2002; Robert et al., 2006b; USDA, 2008; Dufour et al., 2012). The purpose of DCT is to cure existing intramammary infections (**IMI**) and prevent new intramammary infections (**NIMI**). In response to growing concerns regarding the use of antimicrobials in food-producing animals, selective dry cow therapy (**SDCT**) is being promoted as an alternative to a blanket application approach (blanket dry cow therapy: **BDCT**), with the potential to reduce the amount of antimicrobials used in dairy production (Rindsig et al., 1978; Østerås et al., 1991). According to a SDCT protocol, DCT is reserved for cows with an IMI at drying off, therefore the success of a SDCT program requires the accurate identification of a cow's end-of-lactation infection status.

Previous studies have investigated DCT selection strategies based on California mastitis test (**CMT**) scores, as well as monthly somatic cell counts (**SCC**) combined with clinical mastitis history. In general, reliance on these methods to dictate the infusion of

DCT will result in the failure to detect, and subsequently treat, approximately 30% of infected cows. While less concerning from a mastitis control standpoint, these criteria also lack specificity and consequently the risk for DCT treatment of uninfected cows remains high (Sanford et al., 2006b; Torres et al., 2008; McDougall, 2010; Rajala-Schultz et al., 2011). With the advent of on-farm systems for the bacteriological culture of milk, producers have at their disposal a diagnostic tool for the detection of IMI with a quick turnaround time. The 3M Petrifilm on-farm culture system (**OFCS**) was developed by McCarron et al. (2009) for the purpose of selective treatment of clinical mastitis in lactating cows. In laboratory trials to assess the ability of the OFCS to detect IMI at drying off, the 3M Aerobic Count Petrifilm correctly identified infected cows with 100% sensitivity at colony cut-off values of 5, 10 and 20. Using the same cut-off values, the specificity was 70, 82 and 84%, respectively (McLaughlin et al., 2010). To fully evaluate the effectiveness of the OFCS for use in a SDCT program, application of the technology at the farm level was necessary to assess the ability of dairy producers to diagnose infection status at drying off, and to follow-up with outcomes of the subsequent lactation and the economic effects of OFCS-based SDCT.

7.1.2 Study Design

A clinical trial was conducted using 16 predominantly Holstein dairy herds from Prince Edward Island (n = 10) and Quebec (n = 6) with a 12 month average bulk tank SCC <250,000 cells/mL prior to the commencement of the study. Only low bulk tank SCC herds were enrolled because of the recommendation that SDCT be reserved for

herds with good control of contagious mastitis pathogens and a low prevalence of IMI at drying off. The Petrifilm on-farm culture system was set-up on each of the participating farms and producers were provided with DCT and internal teat sealants (**ITS**) for the treatment of cows at the end of lactation. Within each herd, cows with a monthly SCC <200,000 cells/mL on the last three tests prior to the end of lactation, no clinical mastitis in the same time-frame, and a CMT score of <2 in all quarters on the day prior to drying off were randomly assigned to either the study group or the positive control group. Eligibility criteria at the cow-level were chosen to reflect the criteria commonly used by current SDCT protocols (Torres et al., 2008). Treatments in the positive control group (BDCT) consisted of infusion with DCT followed by ITS in all quarters. Treatments in the study group (**OFCS-based SDCT**) were dependent upon the results of the OFCS when used to process composite milk samples collected on the day prior to drying off. Cows with a positive Petrifilm result received DCT plus ITS, while cows with a negative Petrifilm result received only ITS. Blanket application of ITS was used in order to provide some form of prophylaxis against NIMI, which was particularly important in cows not receiving DCT at drying off (Sanford et al., 2006a). Considering that the clinical trial involved only commercial herds, it was expected that participating dairy producers would be averse to leaving cows completely unprotected against NIMI for the duration of the dry period.

While treatment decisions in the current trial were made at the cow-level, a theme throughout the thesis was that treatment decisions at the quarter-level could lead to even greater reductions in DCT use on dairy farms. Previous work has demonstrated quarter

interdependence towards dry period NIMI, thus recommendations are to make selective DCT treatment decisions at the cow-level (Berry et al., 2003; Robert et al., 2006a). In order to determine the effect of ITS on the interdependence of quarters towards dry period NIMI, a second dataset was combined with the data from the clinical trial to provide information from cows not infused with ITS. The additional dataset consisted of a longitudinal cohort study that was conducted from 2007 to 2008 and involved 91 Canadian herds (Reyher et al., 2011). For the current research, herds within the cohort study were chosen based on bulk tank SCC (12 month average <250,000 cells/mL) in order to be comparable to herds included in the clinical trial.

7.2. GENERAL THESIS OVERVIEW

This thesis evaluated a 3M Petrifilm-based OFCS for use in a SDCT program in Canada. The hypothesis was that OFCS-based SDCT would enable a reduction in the use of DCT without adverse effects on cow health, welfare, and milk production in the subsequent lactation. An initial step consisted of determination of the test characteristics and predictive values of the OFCS when used to detect IMI in cows at the end of lactation. Comparisons were then made between cows under OFCS-based SDCT and cows receiving BDCT for: 1) the risk of IMI at calving, 2) the risk of a first case of clinical mastitis in the initial 120 days of the subsequent lactation, and 3) test day milk yield and SCC over the first 180 days in milk. The economical outcomes of OFCS-based SDCT were estimated and comparisons were made with BDCT and with SDCT based on SCC and clinical mastitis criteria. Finally, the effect of ITS on the interdependence of

quarters towards dry period NIMI caused by CNS was assessed so that recommendations with respect to level of treatment (quarter versus cow) could be made for SDCT protocols that also employ infusion of ITS.

7.3. SUMMARY OF RESULTS

7.3.1 Accuracy of the on-farm culture system when used to detect IMI at drying-off

In total, 343 cows originating from 16 herds were used to assess the performance of the OFCS when used to detect IMI at drying off. According to standard bacteriological culture, the prevalence of IMI at drying off was 43.3%, while the producer-derived prevalence of IMI by Petrifilm on-farm culture results was 52.2%. Using a Petrifilm colony count threshold of ≥5 colonies (equivalent to 50 colony-forming units (**cfu**)/mL), the sensitivity and specificity of the OFCS were 85.2% and 73.2%, respectively. Increasing the threshold to ≥10 colonies resulted in an improvement in specificity (86.1%), but lowered the sensitivity (71.8%). The on-farm culture system demonstrated reasonably high sensitivity for detecting IMI at drying off, therefore when used in a SDCT program few infected cows would be missed. While increasing the threshold to ≥10 colonies would result in a reduction in the unnecessary treatment of uninfected cows, the downside would be an increase in the misdiagnosis of infected cows, thus a treatment threshold of ≥5 colonies is recommended. Compared to selective treatment criteria based on SCC and clinical mastitis history, or the CMT, the OFCS was a more accurate method to determine a cow's infection status at the end of lactation. In fact, enrolment criteria used in the clinical trial included requirements for low SCC with

no clinical mastitis in the last three months of lactation and a CMT score <2 in all quarters prior to inclusion, yet 43.3% of cows were identified as infected at drying off according to standard bacteriology. As a result, the implementation of a SDCT program that relies only on these criteria will result in failure to treat many infected cows.

Using the prevalence of IMI at drying off in the current dataset, the negative predictive value (**NPV**) of the Petrifilm OFCS was 86.6%. The negative predictive value increased with decreasing prevalence, and a NPV of 80 to 90% was maintained in populations where the proportion of cows with an IMI at drying off ranged from 55% to 35%. Selective dry cow therapy should only be recommended to herds with good control of contagious pathogens and low bulk tank SCC. Therefore, an essential first step when contemplating implementation of SDCT on a farm is to determine the existing udder health status of the herd.

A total of 265 Petrifilms were available for evaluation by an automated reader to obtain accurate colony counts. The level of agreement between producer-derived results, using a threshold of ≥5 colonies, and those of the automated reader was high with a kappa of 0.82 and observed agreement of 91%. Aerobic Count Petrifilms contain an indicator dye which stains colonies bright pink and this makes it easy to identify and enumerate individual bacterial colonies. Furthermore, classification of cows into treatment categories using a threshold of 5 colonies was straightforward, therefore producers became quickly proficient at reading Petrifilms despite only minimal training at the onset of the trial.

7.3.2 Risk of IMI at calving and risk of clinical mastitis in the subsequent lactation

The objective of dry period management regarding udder health is to minimize the proportion of infected quarters at the beginning of the subsequent lactation (Dingwell et al., 2003). Thus, an important measure of success for OFCS-based SDCT is undoubtedly the prevalence of IMI at calving. In total, 1,157 BDCT group quarters (n = 305 cows) and 1,130 OFCS-based SDCT quarters (n = 298 cows) were available for an analysis of risk of IMI at calving. The inclusion criteria of the clinical trial were met by 46% of the cows. Using a Petrifilm treatment threshold of ≥5 colonies (50 cfu/mL), 45.6% (136/298) of the cows in the OFCS-based SDCT group were classified as uninfected and did not receive DCT at drying off. Therefore, when the OFCS was used to dictate the infusion of intramammary antimicrobials, a total reduction in DCT of 21% was realized.

According to univariable analysis, quarter level cure risk over the dry period was high and was not different between study groups (BDCT: 84.5% (95% CI: 76.0, 90.9); OFCS-based SDCT: 89.0% (95% CI: 81.9, 94.0); $P = 0.33$). Likewise, there was no significant difference between quarters that received BDCT and quarters under OFCS-based SDCT with respect to the risk of dry period NIMI (BDCT: 13.8% (95% CI: 11.8, 15.8); OFCS-based SDCT: 14.5% (95% CI : 12.5, 16.6); $P = 0.64$). A multilevel logistic regression model was applied to evaluate the effect of study group, and treatment within the study group, on the probability of postcalving IMI. In the final model, the risk of postcalving IMI at the quarter-level was not different between study groups, regardless of

OFCS result and subsequent treatment at drying off (BDCT: 15.3%; OFCS-based SDCT: 15.8%; $P = 0.95$).

Intramammary infections present at calving are a significant cause of clinical mastitis in early lactation, therefore an investigation was also undertaken to compare clinical mastitis risk in the first 120 days of the subsequent lactation (Bradley and Green, 2000; Green et al., 2002). Clinical mastitis data were available for 11 herds, 264 cows (1,019 quarters) belonging to the BDCT group and 261 cows (1,006 quarters) belonging to the OFCS-based SDCT group. In total, there were 45 reported cases of clinical mastitis, with 24 cases occurring in the BDCT group and 21 cases occurring in the OFCS-based SDCT group. In the final multivariable logistic model, neither study group ($P = 0.58$) nor infection status at drying off based on standard culture ($P = 0.22$) were statistically significant.

According to the present study, the selective antibiotic treatment of low SCC cows at the end of lactation based on OFCS results was just as effective in the treatment and prevention of IMI during the dry period as BDCT in herds with a low BTSCC (< 250,000 cells/mL). The Petrifilm on-farm culture system enabled the detection of cows with an IMI at the end of lactation and as a result, OFCS-based SDCT achieved the same level of success as BDCT with regards to infection status at calving and risk of clinical mastitis in early lactation.

7.3.3 Somatic cell count and milk yield in the subsequent lactation

The presence of IMI at calving, either as a result of failure to eliminate existing infections or acquisition of new infections, can reduce lactational milk yields by 5% (Hogeveen, 2003; Berry et al., 2004). Thus, management of IMI over the dry period is critical for the optimization of milk production and for profitability. The aim of the research outlined in Chapter 4 was to evaluate the milk quality and production outcomes in the subsequent lactation following OFCS-based SDCT.

A total of 307 BDCT group cows and 293 OFCS-based SDCT group cows were included in the analysis. Using repeated measures linear mixed models, two outcomes of interest were investigated: test day 24 hour milk production and test day natural logarithm SCC (**lnSCC**) during the first 180 days of lactation. After controlling for the other independent variables included in the model (such as stage of lactation, parity, and previous milk production), test day milk production was not different between cows receiving BDCT and cows selectively treated based on OFCS results (Least square means: BDCT = 39.3 kg (95% CI: 37.9, 40.8) vs OFCS-based SDCT = 39.0 kg (95% CI: 37.6, 40.5); $P = 0.43$). Similarly, after adjustment for parity, stage of lactation, and infection status at drying off, there was no significant difference in test day lnSCC between cows receiving BDCT and cows selectively treated at drying off based on Petrifilm results ($P = 0.82$). With respect to SCC over the first 180 days in milk, a similar pattern was observed in both study groups with a rapid decrease in SCC from calving to approximately 50 DIM, followed by a gradual increase over time.

While much research has examined the outcome of SDCT on IMI at calving and clinical mastitis in early lactation, investigations into milk production and milk quality outcomes are lacking. In comparison to BDCT, OFCS-based SDCT enabled a reduction in the use of antimicrobials on low bulk tank SCC farms without affecting milk production and SCC in the subsequent lactation.

7.3.4 Quarter interdependence toward dry period NIMI and effect of ITS

While selective dry cow antimicrobial treatment decisions are usually made at the cow-level, with knowledge of quarter infection status at drying off, it would be possible to make selective DCT decisions for individual mammary quarters. Selective dry quarter therapy (**SDQT**) would result in even greater reductions in antimicrobial use when compared to SDCT (Østerås and Sandvik, 1996; Berry et al., 2003; Robert et al., 2006a), and the OFCS could be used to determine quarter-level infection status. While prior research has demonstrated that quarters are not independent in their risk for the acquisition of NIMI in the dry period (Berry et al., 2003; Robert et al., 2006a), the hypothesis investigated in Chapter 5 was that infusion of ITS would reduce the within-cow transmission of pathogens and thus eliminate interdependence. The data used in the analysis included both data from the clinical trial, and data from a longitudinal cohort study known as the National Cohort of Dairy Farms (**NCDF**; Reyher et al., 2011). Data from the NCDF was used to provide information on cows not infused with ITS at drying off. The SDCT trial provided data on 579 cows originating from 16 herds; the NCDF provided information on 331 cows originating from 30 herds. Using a generalized

estimating equation logistic model, the outcome investigated was dry period acquired NIMI caused by coagulase negative staphylococci (**CNS**). Low incidence of NIMI caused by other pathogens precluded the evaluation of any additional organisms.

According to the final model, in quarters that were infused with ITS, the presence of CNS in another quarter at drying off significantly increased the risk of CNS NIMI over the dry period, but this effect was not found in quarters without ITS treatment. This unexpected association may have occurred as a result of introduction of teat end infection into the mammary gland during infusion of ITS. Internal teat sealants do not contain antimicrobials, so strict asepsis must be maintained during infusion. In quarters belonging to cows without a CNS IMI at drying off, ITS did reduce the incidence of dry period CNS NIMI, suggesting that CNS infection over the non-lactating period can be partly attributed to CNS species located within the environment. Not surprisingly, increasing herd prevalence was also associated with increasing risk of CNS NIMI. Efforts to decrease the prevalence of CNS at the herd level is thus important for reducing the risk for dry period CNS NIMI at the quarter-level.

In summary, there was no evidence to support that infusion with ITS resulted in a reduction in risk for the within cow transfer of CNS from an infected quarter to an uninfected quarter. Rather, it appeared that in cows infused with ITS, the presence of CNS in another quarter at drying off increased the risk of CNS NIMI, but this was not true for cows without ITS. This unexpected result warrants further investigations into the dynamics of CNS IMI over the dry period, including determination of the prophylactic and therapeutic effect of DCT, as well as the potential for transfer of CNS during ITS

infusion. Future research would greatly benefit from speciation of CNS as prior studies have demonstrated that reservoir, transmission mode, and virulence of CNS are species-specific (Supre et al., 2011; Piessens et al., 2012). Molecular fingerprinting techniques would also enhance further research and would enable researchers to determine if prevalent CNS IMI is a source of incident CNS NIMI for the same cow. In the current study, it was an initial objective to investigate different mastitis pathogens however, in order to repeat the analysis for other pathogens, a large dataset would be required due to low risk of dry period NIMI by non-CNS pathogens in herds with a low average bulk tank SCC.

7.3.5 Economic impact

For on-farm culture system-based SDCT to be attractive to dairy producers in countries where BDCT is the preferred approach, the financial ramifications of implementation must not be a deterrent. Prior studies have estimated the economic consequences of SDCT based on SCC criteria (Berry et al., 2004; Huijps and Hogeveen, 2007; Halasa et al., 2010), but the financial implications of OFCS-based SDCT have yet to be reported. In Chapter 6, the economic outcomes of OFCS-based SDCT were evaluated and comparisons were made to BDCT, and to SDCT based-on SCC and clinical mastitis criteria. Stochastic Monte Carlo models were used to model the dynamics of IMI over the dry period and estimate the cost of dry period intervention for cows with a low SCC and no clinical mastitis prior to drying off (i.e. cows eligible for SDCT as per the inclusion criteria of the clinical trial). According to the final models,

BDCT was the most economical approach, but there was considerable overlap in range of total cost with OFCS-based SDCT, and with a BDCT approach that also included ITS. Included among the protocols was a SDCT approach based on SCC and clinical mastitis history. The initial model was run using the performance of these criteria as determined by the clinical trial. Better accuracy of SCC and clinical mastitis criteria have been described by others, and when the SCC-SDCT model was run an additional time using test characteristics from Torres et al.(2008), the economics were improved but total average cost was still higher than for OFCS-SDCT. Insufficient sensitivity of SCC and clinical mastitis criteria meant that many infected cows were left untreated, thus the increased cost of SCC-based SDCT resulted from a greater proportion of IMI at calving than that in any of the other protocols. Finally, a theoretical perfect test with 100% sensitivity and specificity was used to estimate how much such a test could cost and be equivalent to BDCT+ITS. Including both materials and labour, the cost of a test providing perfect information could amount to $17 per cow, and implementation of a SDCT program using this test would result in an estimated 28.8% reduction in DCT use. By comparison, OFCS-SDCT resulted in a 23.8% DCT reduction, marginally lower than a perfect test but at an average cost of $3.29 more than BDCT+ITS per eligible cow.

Without a clearly financially optimal option, the choice of dry period mastitis control program would depend on the motivation of the individual dairy producer. Producers looking to minimize cost would be most attracted to BDCT. Producers interested in a more targeted approach to antibiotic use would be motivated to implement OFCS-based SDCT, and could do so with the knowledge that that a reduction in DCT can

be achieved without increased risk to IMI at calving and only moderate increase in cost per cow. In conclusion, BDCT was economically the best option, but the cost of OFCS-based SDCT was only marginally more. When industry goals are to target antimicrobial use for the purpose of therapy rather than prophylaxis, there will be an opportunity for implementation of OFCS-based SDCT.

7.4. CONCLUDING REMARKS AND FUTURE RESEARCH DIRECTIONS

This research determined that the Petrifilm-based on-farm culture system was a quick, simple, and accurate tool for the detection of IMI present at the end of lactation. Using the Petrifilm on-farm milk culture system, producers could implement SDCT on their farms with the knowledge that few infected cows would be missed. Internal teat sealants were used in all cows to provide some form of protection against NIMI, which was of particular concern for quarters of cows in the OFCS-based SDCT that were Petrifilm negative. As a result of the accurate identification and subsequent treatment of IMI present at drying off, OFCS-based SDCT achieved the same level of success with respect to udder health as BDCT. Likewise, when compared to BDCT, OFCS-based SDCT did not affect milk production or milk quality in the subsequent lactation.

By using the OFCS to target DCT treatment of cows at the end of lactation, a reduction in DCT use of approximately 21% was realized. According to results obtained by the OFCS using composite samples, 47.8% of cows in the SDCT group did not have an IMI at drying off. By comparison at the quarter level, 82.7% of quarters were

uninfected at the end of lactation as per standard bacteriology. While application of the OFCS at the cow level (i.e. composite milk samples and cow level treatment) lead to a modest decrease in the use of DCT in the study herds, use of the culture system at the quarter level (i.e. quarter samples and quarter level treatment) has the potential to effect an even greater reduction in the use of DCT. Moreover, quarter level culturing would result in an improvement in the sensitivity of the OFCS when compared to composite culturing (Reyher and Dohoo, 2011), thus reducing the false negative rate of the Petrifilm on-farm culture system.

Quarter interdependence towards NIMI over the dry period has been demonstrated by others, thus the leading recommendation for selective DCT programs is to treat at the cow-level (Berry et al., 2003; Robert et al., 2006a). Internal teat sealants provide a barrier to NIMI and could hypothetically reduce the within cow transfer of IMI, but studies of this potential effect are lacking in the literature. In the present thesis, the application of an ITS did not reduce the risk of within-cow transfer of CNS from an infected quarter to an uninfected quarter. Instead, in quarters infused with ITS, the presence of CNS in another quarter at drying off significantly increased the risk of CNS NIMI over the dry period. This association did not occur in cows without ITS. Internal teat sealants contain no antimicrobial, therefore infusion with ITS may introduce bacteria into the mammary gland and result in NIMI if asceptic protocols are not adhered to. Incorporation of an antimicrobial compound into ITS with local activity in the teat canal and cistern has been proposed to decrease the risk of introducing NIMI during their administration (Crispie et al., 2004; Crispie et al., 2005; Petrovski et al., 2011). If such

products were to become commercially available, it would be of interest to investigate how an ITS with antimicrobial properties might impact within cow transfer of IMI in the dry period.

According to economic analyses, OFCS-based SDCT was a revenue neutral option to BDCT+ITS. Currently in North America, there are no regulations preventing the application of DCT in all quarters of all cows at drying off, thus enthusiasm for SDCT is likely more dependent on economic outcomes as opposed to motivation to reduce antimicrobial use. Future directions for research should include the development and evaluation of diagnostic methods with even greater accuracy, for better tests will result in larger reductions in DCT use, a lower risk of IMI at calving, and potentially greater cost-savings for dairy producers. Revisiting selective therapy at the quarter level, application of the OFCS at the quarter-level and subsequent SDQT would result in greater reductions in DCT use, but would result in higher labour and OFCS costs. Considering that quarter level culturing would improve the sensitivity of the OFCS, the increases in cost could potentially be offset by the greater accuracy when targeting DCT at drying off. If future research were to include application of the OFCS at the quarter level and SDQT, an important consideration would be an assessment of economical outcomes.

It is understood that the attributes of an ideal test for use in SDCT are quick and accurate results, cow-side application, low-cost, and easy-to-use. As mastitis research focuses on the importance of CNS as an emerging mastitis pathogen, perhaps of interest for future development would be a on-farm diagnostic tool for the speciation of CNS. If

producers had knowledge of which species of CNS would likely respond to DCT , which species are likely to self-cure, and which species would likely persist in spite of treatment, such a tool would enable them to select cows for treatment, no treatment, and culling based on the species of CNS isolated. Targeting end-of-lactation treatment of CNS infected cows on a case-by-case manner would certainly assist in reducing the spread and prevalence of CNS within a herd.

In conclusion, selective dry cow therapy based on Petrifilm on-farm culture system results was successful when used in low SCC cows originating from low bulk tank SCC herds and resulted in a modest decrease in DCT use. As awareness of the importance of judicious antibiotic use in food animal production increases, interest in SDCT will also increase and the availability of an accurate, cost-effective, and quick method to identify the end-of-lactation infection status of cows will be required. The findings of this thesis are supportive of on-farm culture-based SDCT, but more research is warranted.

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APPENDIX A (Chapter 2)

Generalized estimating equation logistic model for population-averaged estimate of sensitivity of the Petrifilm on-farm culture system

```
.  xi: xtgee  res_petri5 if res_gs2_dc == 1 & b_random_num==1, family(binomial 1) link(logit)
>  corr(exchangeable) vce(robust)

Iteration 1: tolerance = .00579122
Iteration 2: tolerance = .00091136
Iteration 3: tolerance = .00014216
Iteration 4: tolerance = .00002213
Iteration 5: tolerance = 3.445e-06
Iteration 6: tolerance = 5.363e-07

GEE population-averaged model                   Number of obs      =       149
Group variable:                farm_id_num      Number of groups   =        16
Link:                                logit      Obs per group: min =         1
Family:                           binomial                     avg =       9.3
Correlation:                  exchangeable                     max =        35
                                                Wald chi2(0)       =         .
Scale parameter:                         1      Prob > chi2        =         .

                           (Std. Err. adjusted for clustering on farm_id_num)
------------------------------------------------------------------------------
             |               Semirobust
  res_petri5 |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       _cons |    1.734246   .2266138     7.65   0.000     1.290091    2.178401
------------------------------------------------------------------------------

.
end of do-file

. display exp(1.734246)
5.664655

. display 5.664655/ (1+5.664655)
.84995472

. display exp(1.290091)
3.6331172

. display 3.6331172 / (1+3.6331172)
.78416259

. display exp(2.178401)
8.8321723

. display 8.8321723 / (1+8.8321723)
.89829308
```

Generalized estimating equation logistic model for population-averaged estimate of specificity of the Petrifilm on-farm culture system

```
.  xtgee  res_petri5_Sp if res_gs2_dc == 0, family(binomial 1) link(logit) corr(exchangeable)
>  vce(robust)

Iteration 1: tolerance = .01212454
Iteration 2: tolerance = .00056668
Iteration 3: tolerance = .00003186
Iteration 4: tolerance = 1.804e-06
Iteration 5: tolerance = 1.022e-07

GEE population-averaged model                   Number of obs      =       194
Group variable:                 farm_id_num     Number of groups   =        16
Link:                                 logit     Obs per group: min =         2
Family:                            binomial                    avg =      12.1
Correlation:                   exchangeable                    max =        38
                                                Wald chi2(0)       =         .
Scale parameter:                           1    Prob > chi2        =         .

                            (Std. Err. adjusted for clustering on farm_id_num)
------------------------------------------------------------------------------
             |               Semirobust
res_petri5~p |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       _cons |   .9790897   .2038736     4.80   0.000     .5795047    1.378675
------------------------------------------------------------------------------

.
end of do-file

. display exp(.9790897)
2.6620319

. display 2.6620319/(1+2.6620319)
.72692756

. display exp(.5795047 )
1.785154

. display 1.785154/(1+1.785154)
.64095343

. display exp(1.378675)
3.9696384

. display 3.9696384 /(1+3.9696384)
.79877812
```

APPENDIX B (Chapter 3)

Species-specific and overall apparent new intramammary infection risk over the dry period for quarters receiving blanket dry cow therapy plus internal teat sealant (BDCT) and quarters selectively treated based on Petrifilm on-farm culture results with dry cow antibiotic and internal teat sealant, or internal teat sealant alone (SDCT). New intramammary infections were determined using single quarter milk samples collected between 3 and 4 days in milk.

	BDCT (n = 1,017)	**SDCT** (n = 1,023)
Pathogen		
Staphylococcus aureus	8 (0.8)	12 (1.2)
Streptococcus uberis	1 (0.1)	0 (0)
Streptococcus dysgalactiae	2 (0.2)	0 (0)
Nondifferentiated streptococci[1]	11 (1.1)	12 (1.2)
Total environmental streptococci	14 (1.4)	12 (1.2)
Escherichia coli	2 (0.2)	2 (0.2)
Nondifferentiated Gram-negative bacteria	3 (0.3)	1 (0.1)
Total Gram-negative pathogens	5 (0.5)	3 (0.3)
Fungi & Yeast	5 (0.5)	2 (0.2)
Coagulase negative staphylococci	58 (6.4)	62 (7.0)
Corynebacterium spp.	4 (0.4)	4 (0.4)
Total pathogen count	94	95
Quarter-level[2]	92 (9.1)	95 (9.3)

[1] Including *Enterococcus* spp.; does not include *Streptococcus agalactiae*
[2] A quarter may have been infected with up to two different pathogens