Innovative Dry Cow Therapy

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Carolyn Jane Sanford

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Abstract

The ability of an internal teat sealer (ITS) administered at dry-off for the prevention of new intramammary infections (IMI) and for the cure of existing IMI during the dry period was examined. In addition, incidence of clinical mastitis (CM), pattern of somatic cell count (SCC), particulate matter recovery, and milk production during the subsequent lactation were measured. The California mastitis test (CMT) was evaluated for its ability to identify cows and quarters with IMI at dry-off.

The quarter-level CMT results, interpreted in parallel to form a cow-level test result, were an effective way to identify infected cows for treatment of DCT. Using an ITS in conjunction with DCT provided enhanced protection from the development of new IMI during the dry period when compared with DCT alone, although it had no additional benefit on the cure of existing IMI. An ITS alone provided enhanced protection against the development of new environmental IMI during the dry period when compared with DCT alone. There was no reduction in the incidence of CM during the subsequent lactation when an ITS was utilized either in combination with DCT or alone. Quarters treated with ITS have significantly more particulate matter recovered during the first day postpartum when compared with quarters treated with DCT. Internal teat sealers had a negative impact on milk production during the first six months of the subsequent lactation.

In summary, ITS are an efficacious addition to dry cow programs to aid in the prevention of new IMI. The effects of ITS on milk production need to be studied further to help clarify the effect found in the current study. Lastly, the CMT is an inexpensive, effective and efficient way to identify infected cows at dry-off. This finding will be useful to assist farmers in adopting selective dry cow programs.

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List of Abbreviations

CM	Clinical Mastitis
CMT	California Mastitis Test
CFU	Colony Forming Units
CNS	Coagulase-negative Staphylococci
DIM	Days in Milk
DCT	Dry Cow Antibiotic Therapy
DHI	Dairy Herd Improvement
HPLC	High Pressure Liquid Chromatography
IMI	Intramammary Infection
ITS	Internal Teat Sealer
LS	Linear Score
MIC	Minimum Inhibitory Concentration
NPV	Negative Predictive Value
PD-Inf	Pre-Dry Infected Group
PD-Uninf	Pre-Dry Uninfected Group
PPV	Positive Predictive Value
SCC	Somatic Cell Count

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CHAPTER 1 GENERAL INTRODUCTION

1.1. Introduction

The bulk of this thesis focused on development of intramammary infections (IMI) during the dry period. Therefore, a brief overview of dry cow management, causative organisms, risk factors, diagnostic tests, and prevention is provided. A literature review of previous internal teat sealer (ITS) studies is conducted. In addition, this thesis examines the effect of using an ITS administered at dry-off for the prevention of new IMI and its effect on cure rates of IMI during the dry period. In addition, the incidence of clinical mastitis (CM), particulate matter recovery, milk production, and somatic cell count (SCC) during the subsequent lactation were examined. Diagnostic methods to determine whether cows and quarters were infected at dry-off were investigated. Finally, the validation of split-udder clinical trials when dry cow antibiotic therapy (DCT) was administered was examined.

1.2. Dry Cow Management

Dry cow management has been a research focus for the past 50 years. The non-lactating transition period is well recognized as a critical time point in the life of a dairy cow and may result in economic losses to the producer, if not well managed. The dry period has long been documented as a risk period for the development of numerous peri-parturient diseases including ketosis, metritis, subclinical mastitis, and CM (Radostits et al., 1994). Mastitis is the most costly disease of dairy cattle,

therefore elimination and prevention of IMI during the dry period is of great economic importance (Dingwell et al., 2003a). Clinical mastitis occurring in early lactation is extremely frustrating and costly to the dairy producer. Many studies have shown that CM in early lactation is often the result of IMI that are established during the dry period (Bradley & Green, 2000; Green et al., 2002). A recent study found that almost 25% of quarters had an IMI with a major pathogen (defined below) isolated in the peri-parturient period, despite the use of blanket DCT (Green et al., 2005).

In terms of mastitis management during the dry period, there are essentially two separate issues to consider: elimination of existing IMI and the prevention of new IMI (Eberhart, 1986; Browning et al., 1994). Elimination of existing IMI will be reviewed in the dry cow therapy section (1.6.1). Preventing new IMI during the dry period can be very difficult as the udder undergoes major anatomical and physiological changes both at the beginning and end of the non-lactating period (Fig. 1-1). Involution occurs during the first two weeks of the dry period during which time the production of milk has to decrease rapidly, SCC and lactoferrin rise, and concentrations of fat and casein decrease (Hurley, 1989). A keratin plug forms in the base of the teat canal (Comalli et al., 1984). During this time of change, the teat is susceptible to penetration by mastitis-causing organisms. After the udder involutes and teat ends close, the mammary gland becomes resistant to IMI (Cousins et al., 1980). As parturition approaches colostrogenesis begins and the udder parenchyma redevelops. During this time, teat ends may begin to open and leak milk. This is also a high risk period for the penetration of bacteria into the udder (Oliver & Sordillo,

1988). Therefore, the management of the dry period in regards to the control of mastitis must address both critical risk periods.

1.3. Causative Organisms of Mastitis During the Dry Period

1.3.1. Major Pathogens

Major mastitis-causing pathogens of both contagious and environmental etiology need to be considered as problematic during the dry period. Contagious pathogens are present in the udder of some cows at dry-off and should be eliminated during the dry period with an approved DCT to allow the cow to begin the next lactation free from IMI. The most common major contagious organisms include *Staphylococcus aureus, Streptococcus agalactiae*, and *Mycoplasma bovis*, although much success has been seen in the elimination of *S. agalactiae* mastitis due to its susceptibility to antibiotics (Dingwell et al., 2003a). A current study monitoring the prevalence of contagious mastitis pathogens in Canadian dairy farms found *S. agalactiae* in 2.5% of bulk-tank samples (n = 291) which is much lower than older estimates (Olde Riekerink et al., 2005).

A lot of work investigating the impact of environmental pathogens during the dry period has been conducted (Smith et al., 1985; Todhunter et al., 1991; Bradley and Green, 2000; Green et al., 2005). Environmental organisms such as *Escherichia coli, Klebsiella* species, and non-agalactiae Streptococci are the most common cause

of new IMI during the dry period (Smith et al., 1985; Green et al., 2005). One study calculated the average incidence risks of new coliform and non-agalactiae Streptococcal IMI were 16%, 7%, 6%, and 15%, over quarters of the dry period (1st to 4th, respectively) and found that the risk of developing a new IMI was decreased upon DCT administration (Smith et al., 1985). Continual exposure to these pathogens during the non-lactating period provides opportunity for invasion and colonization of the mammary gland. In one study, the prevalence of major IMI approximately doubled over the dry period, because of the increase in both *E. coli* and *Streptococcus uberis* IMI (Green et al., 2005). Another study found significant increases in the prevalence of all *Enterobacteriaceae* between dry-off to the periparturient period (Bradley and Green, 2000). Prevention of IMI by these agents is extremely important as the vast majority of DCT available in North America are targeted against gram-positive bacteria (Smith et al., 1985). Other opportunistic invaders such as yeast and *Prototheca* are also commonly isolated from non-lactating glands (Dingwell et al., 2003a).

1.3.2. Minor Pathogens

Minor pathogens are commonly isolated from the mammary gland during both the lactating and non-lactating periods. A very high prevalence of coagulase-negative staphylococci (CNS) infections have been observed in many studies, as have IMI caused by *Corynebacterium bovis*. The literature provides inconsistent reports as to

the importance of these minor pathogens (Dingwell et al., 2003a). Some studies have shown that IMI with a minor pathogen protects the quarter from the development of a major IMI (Rainard and Poutrel, 1988; Green et al., 2005), while other studies of similar design have found the opposite effect (Hogan et al., 1988; Berry and Hillerton, 2002). Therefore, the importance of these two very heterogeneous groups of pathogens remains unclear at this time.

1.4. Risk Factors for Intramammary Infections

Risk factors that increase the susceptibility to IMI during the dry period have been examined extensively. They can be classified at the herd-, cow-, and quarter-level.

An important herd-level risk factor is dry cow environment. This plays a large role in the prevention of new IMI and includes both housing and climate considerations. Constant exposure to high concentrations of environmental pathogens will increase opportunity for bacterial penetration into the udder causing IMI. Dry cows should be housed in a clean, dry area to decrease environmental contamination (Dingwell et al., 2004). In a UK study, incidence of enterobacterial IMI was significantly higher during the summer than in spring and winter (Bradley and Green, 2000). Similarly, another study showed the highest risk of new environmental IMI occurred in spring and summer (Smith et al., 1985). Other herd-level risks include

on-farm udder-health programs (which may include vaccinations), dry cow nutrition and the overall prevalence of IMI (Dingwell et al., 2003a).

Cow-level risk factors have also been reviewed and they include parity, milk production, immune status, dry period length, genetic selection, and method of drying-off cows (Dingwell et al., 2003a). Older cows are more susceptible to developing new IMI during the dry period than their younger cohorts (Funk et al., 1982; Smith et al., 1985; Bradley and Green, 2000; Østerås and Edge, 2000; Dingwell et al., 2002; Green et al., 2005). There are no consistent findings regarding the relationship between milk production at dry-off and new IMI during the dry period, although most results suggest that cows at a higher level of production at the time of dry-off are more likely to develop a new IMI (Dingwell et al., 2002). However, an earlier study found that cows producing more milk had a lower prevalence of IMI at dry-off, but no relationship between production and new IMI was found (Funk et al., 1982). Proper nutrition should ensure adequate immune function of the dairy cow and aid in preventing mastitis. This factor could be considered both a herd- and cow-level risk factor. Relationships between vitamin E and selenium levels and IMI development have been documented (Smith et al., 1997). One study found that dry period diets containing high supplement of Vitamin E significantly reduced the number of new IMI at calving and CM cases in early lactation when compared with low supplement rations (Weiss et al., 1997). It has been proposed that Vitamin E and selenium mediate resistance mechanisms which provide increased protection to the mammary gland (Radostits et al., 1994). In addition to Vitamin E and selenium, dry

period rations should contain adequate levels of protein, energy, vitamins and minerals to ensure optimal nutrition and immunity (Godden et al., 2004). Negative energy balance has also been associated with the incidence of IMI. Proper nutrition which reduces incidence of periparturient disease will decrease incidence of IMI, as many of these metabolic diseases have been associated with increased risk of mastitis (Godden et al., 2004).

A good review of the effect of dry period length on milk production, composition, animal health and reproductive performance has been conducted (Grummer and Rastani, 2004). The study concluded that beneficial effects of shortened dry periods may be mediated through a decrease in metabolic peripaturient disease as animals with short dry periods could be maintained on lactating rations (Grummer and Rastani, 2004). Effects of shortened dry periods on the occurrence of new IMI are inconsistent. It was concluded that more studies using large numbers of animals in commercial herds should be conducted to better identify associations between dry period length and IMI development (Grummer and Rastani, 2004). One older study found a lower overall incidence of new IMI during dry periods in cows with dry periods less than 30 days (Natzke et al., 1975). However, a more recent study found little evidence of a dry period length effect on CM when cows with four, seven and ten week dry periods were compared (Enevoldsen and Sørensen, 1992). It was concluded that there were much more important predisposing factors to the development of CM than dry period length. Genetic selection for high milk production has been positively correlated with an increased incidence of mastitis

(Rushen, 2001; Oltenacu and Algers, 2005). Milk production has doubled over the past 50 years and with it, a decline in fertility, longevity, and increases in multiple animal health diseases have occurred (Oltenacu and Algers, 2005). While the economics of dairy farming are a top priority, sacrificing animal welfare for gains in production must be addressed. Income earned as a result of higher milk yields may be lost as a result of higher disease occurrence (namely mastitis and lameness) and replacement costs. The method of drying-off cows must also be considered. One study has shown that cows dried off with intermittent milking (milked once a day for seven days prior to dry-off), were less likely to develop new IMI, than cows that were dried off in an abrupt manner (Oliver et al., 1990). However, an older study found no difference in the incidence of new IMI between cows that were blanket dry cow treated (Natzke et al., 1975).

Quarter-level risk factors affecting new IMI development in the non-lactating cow include keratin plug formation, teat end lesions, bacterial populations at teat ends, and current IMI status (Dingwell et al., 2003a). The timely formation of the keratin plug is required for protection of the quarter, and has been studied. It was found that time to closure varies amongst teats, and between 5-23% of teats remained open at the end of the study period (Willamson et al., 1995, Dingwell et al., 2004). Open teats were much more likely to develop new IMI or experience clinical mastitis during the dry period (Willamson et al., 1995; Dingwell et al., 2004).

Teat-end lesions are another quarter-level risk factor that must be considered. One study found that quarters with cracked teat ends were significantly more likely to

develop new IMI than quarters with non-cracked teats (Dingwell et al., 2004). The relationship between teat-end lesions and CM has also been explored. Quarters with CM had more damaged teat ends than healthy quarters (Neijenhuis et al., 2001). Therefore it appears that teat-end pathology can have a substantial impact on the development of IMI.

Populations of bacteria increase at the teat end following dry-off (Eberhart, 1986). Many opportunistic bacteria found on skin or in the environment may be able to establish IMI if the challenge is strong enough. The cessation of both milking and routine teat dipping are thought to predispose the quarter to high numbers of bacteria (Cousins et al., 1980).

An IMI involving either a major or minor pathogen, may make that quarter more susceptible to new IMI during the dry period although reports are not consistent. Quarter infection with a major IMI increased the risk of developing a new IMI caused by a different pathogen during the dry period (Godden et al., 2003). Similarly, in another study, when quarters were sampled at four different time points during the dry period, those infected with *S. uberis* were more likely to have an *E. coli* IMI at another time point, and the reverse synergistic relationship was also demonstrated (Green et al., 2005). An explanation for these findings could be that some quarters are simply more susceptible to developing IMI.

One study found that quarters infected with a minor IMI at dry-off were no more likely to develop a new IMI during the dry period (Godden et al., 2003). However, another dry period clinical trial demonstrated that quarters infected with

either CNS or *Corynebacterium* species were at a higher risk of developing a new IMI (Berry and Hillerton, 2002). In addition, a study that sampled quarters at four different time points during the dry period found that infections with minor pathogens decreased the odds that a quarter would isolate a major IMI at a different time point (Green et al., 2005). These differing results demonstrate the complexity of infection dynamics in the dry period and need for further research in this area. One study which may help explain the great variability in the minor/major IMI relationship demonstrated that 20% of CNS isolates (specifically *Staphylococcus chromogenes*) inhibited the growth of *S. aureus*, *S. dysgalactiae*, and *S. uberis* in vitro (DeVliegher et al., 2004). This finding demonstrates that the effect of minor pathogens against the development of major pathogen IMI is both species- and isolate-specific, which could explain why some studies conclude that minor pathogens are protective, while other studies conclude that they are a risk factor.

1.5. Diagnosis of Intramammary Infections

By definition, there is currently no gold standard for the diagnosis of an IMI. At present, bacteriological culture remains the most trusted diagnostic test. It is accepted as the gold standard for diagnosis of IMI although its sensitivity is not perfect (Erskine and Eberhart,1988). Culture is also expensive and requires at least 24-48 hours from collection of sample to reporting of results. Many clinical cases are treated prior to bacteriological culture results being available. When collecting milk

samples for bacteriology, an aseptic technique must be practiced to prevent the sample from being contaminated. Other tests have been evaluated as surrogates for bacteriological culture and the most popular of these include SCC and the California Mastitis Test (CMT).

Somatic cell counts increase in the udder for various physiologic and pathological reasons. An increase in SCC is often a response to inflammation. The most common cause of this in the bovine mammary gland is IMI. Elevated SCCs are then taken as a predictor of IMI. The choice of a threshold value of SCC for the prediction of IMI will affect the test characteristics, including sensitivity, specificity, positive and negative predictive values (McDermott et al., 1982; Dohoo and Leslie, 1991). One study found that when a cut-off of 200,000 cells/ml was used, SCC was 73% sensitive and 86% specific for diagnosing IMI when compared with milk bacteriology (Dohoo and Leslie, 1991). Another study found an 89% sensitivity and a 75% specificity at the 200,000 cells/mL threshold (McDermott et al., 1982). Advantages of this test include its price, turnaround time, and ease of sampling. The majority of Canadian dairy herds routinely have monthly SCC tests performed on all lactating cows in the herd. However, decisions regarding the use of SCC for treatment decisions should be made at an individual herd-level because the predictive values of the test performance rely on the prevalence of infection (McDermott et al., 1982).

Another inexpensive test that has been studied is the CMT. The CMT is a cow-side test that is an indirect measure of SCC in the milk (Barnum et al., 1961). A

CMT reagent is added to a sample of milk and it reacts with the nuclear material in cells, forming a gel. The level of SCC in the milk sample will affect the amount of gel formation. A CMT is scored numerically from 0-3 based on the degree of gel formation detected. Many studies have compared the CMT with bacteriological culture and while they found good specificity of the test, poor sensitivities are its biggest drawback. This has prevented a more widespread adoption of this test (Barnum and Newbould, 1961; Brookbanks, 1966; Poutrel and Rainard, 1981; Sargeant et al., 2001; Dingwell et al., 2003b; Wallace et al., 2004). Another disadvantage to this test is the subjectiveness of the scoring.

1.6. Prevention of IMI During the Dry Period

1.6.1. Dry Cow Therapy

Recommendations for the antibiotic treatment of all quarters of all cows at dry-off have been in place for over 50 years, from the time when the "5 point plan" was developed (Neave et al., 1950). Blanket DCT has aided the cure of existing IMI and also in the prevention of new infections (Eberhart, 1986). Antibiotic formulations have targeted gram-positive, contagious pathogens, which were historically the most important cause of IMI (Bradley & Green, 2000). More recently, environmental pathogens such as *S. uberis*, *S. dysgalactiae*, and *E. coli* have become more common. Most DCT products provide little to no activity against

coliforms (Smith et al., 1985) and variable spectrum of activity is provided against environmental streptococci. Therapeutic levels of DCT remain in the mammary gland for a maximum of three weeks. Long-acting formulations may maintain therapeutic levels for greater periods. In general, protection is provided during the first high risk period, but the concentration of the antibiotic is below the minimum inhibitory concentration in the two weeks prior to calving when the second period of high risk occurs (Smith et al., 1985).

A lot of work has been done to examine the efficacy of selective dry cow programs as the prophylactic use of antibiotics in food animals is under scrutiny (Eberhart, 1986; Browning et al., 1990; Schukken et al., 1993; Browning et al., 1994; Østerås et al., 1999). Selective DCT decisions should be made at the cow-level and not the quarter-level due to the lack of independence between quarters (Rindsig et al., 1978; Barkema et al., 1997; Berry et al., 2003). Selective DCT programs offer additional challenges of identifying cows with IMI for treatment. Quarters which are not treated with DCT are more susceptible to IMI during the dry period and need additional protection (Schukken et al., 1993; Woolford et al., 1998; Berry and Hillerton, 2002; Dingwell et al., 2002). Some alternatives that have been explored include teat-dipping, external teat sealers and internal teat sealers (Dingwell et al., 2003a).

1.6.2. External Teat Sealers

Alternatives for the prevention of IMI during the dry period such as external teat sealers (ETS) have been studied. Application of a water proof substance after milking at dry-off, to protect the teat from environmental agents, has been investigated with mixed results (Dingwell et al., 2003a). The major drawback to ETS is the lack of adherence to the teat end, thereby requiring frequent applications immediately after the cessation of milking and again just prior to calving as the mammary gland parenchyma begins to redevelop (Timms, 2000).

1.6.3. Internal Teat Sealers

An alternative that has been studied and utilized in Ireland since the late 1970s, and that has been recently introduced and marketed worldwide, is the use of an ITS. This non-antibiotic product formulated from bismuth subnitrate, is infused into the teat cistern and forms a viscous physical barrier preventing environmental organisms from penetrating the mammary gland. Bismuth is a naturally occurring element. The bismuth subnitrate formulation is infused in a mineral oil vehicle. The ITS has excellent persistency throughout the dry period. One study was able to identify it radiographically in all of 19 rear teats, 100 days after intramammary infusion (Woolford et al., 1998).

The licensing and marketing of ITS on a global scale has propelled an upsurge of research in this area of dry cow management. Internal teat sealer clinical trials examining the incidence of new IMI, clinical mastitis and cure rates have been

performed in various countries worldwide (Meaney, 1977; Woolford et al., 1998; Berry and Hillerton, 2002; Huxley et al., 2002; Godden et al., 2003; Cook et al., 2005). The trials are very different in terms of study design, sample size, product composition, geographic location, cow housing, herd representativeness, dry cow management, inclusion and exclusion criteria, definitions of IMI, CM and cure, DCT used and statistical analyses. These differences make direct comparisons of trial results difficult. Some differences in clinical trial design can be attributed to the official registration of ITS within countries. In North America ITS are a veterinary device which are recommended for use with an approved dry cow antibiotic. In Europe and New Zealand, ITS are registered as products. Utilizing DCT in conjunction with ITS in these regions is extra-label drug use. The clinical trials within each region were designed to compliment the product registration. Differences between the cows utilized within these studies, specifically in terms of milk production, SCC, IMI status at dry-off, prevalence of various pathogens, nutrition, teat-end condition and immune status further complicate their comparisons. Nevertheless, some consistency of results has been observed and is described in detail below.

1.6.3.1. Development of New IMI

Consistently throughout the literature, quarters treated with an ITS, either alone or with DCT, have developed significantly fewer new IMI over the dry period

than negative control quarters (Meaney, 1977; Woolford et al., 1998; Berry and Hillerton, 2002) and these incidences are summarized in Table 1-1. When comparisons have been made between ITS and DCT alone, one study found a significant reduction in the number of new IMI (Huxley et al., 2002), whereas, another study found no difference between treatments (Woolford et al., 1998). Studies comparing the efficacy of an ITS used in conjunction with DCT have also been performed. Addition of ITS to DCT significantly enhanced protection against the development of new IMI (Meaney, 1977; Godden et al., 2003; Hillerton and Berry, 2004; Cook et al., 2005), with the exception of a New Zealand study which found no additional benefit to the use of ITS with DCT (Woolford et al., 1998). However, this study compared ITS used in conjunction with a short-acting DCT, against a long-acting DCT product (used alone) that is not available in North America.

Major reductions in the incidence of new IMI caused by *S. uberis* during the dry period were found in New Zealand and in one UK study when using an ITS (Woolford et al., 1998; Berry and Hillerton, 2002). This was also documented as the most predominant new IMI in all treatment groups in both studies. Both US studies found significant reductions in the incidence of environmental streptococcal IMI, but these were not specifically attributed to *S. uberis* (Godden et al., 2003; Cook et al., 2005). Another UK study found the greatest treatment effect when gram-negative pathogens were considered but this was not seen in other studies (Huxley et al., 2002). A low prevalence of IMI due to these gram-negative organisms, resulting in

low power to find species specific effects may explain this inconsistency in some studies (Woolford et al., 1998; Berry and Hillerton, 2002).

The Minnesota study examined the effect of ITS and DCT on SCC post-calving and found a significant reduction at one to three days in milk (DIM) and six to eight DIM, when ITS and DCT were used in combination compared with DCT alone (Godden et al., 2003). However, the Wisconsin study did not find a significant reduction in SCC measured postpartum (Cook et al., 2005).

Preliminary results are available from other ITS studies, but details of the studies are not sufficient to allow for a review (Hassfurther et al., 2003; Falkenberg et al., 2005).

As consistent findings in the reduction of the incidence of new IMI during the dry period have been reported when ITS were used, it appears that ITS work at least as well as DCT in protecting the udder during the dry period. The effects of ITS on the development of new IMI needs to be examined in a large population of cows, which are representative of current industry conditions, including both small and large herds, of different housing types, nutrition, and management styles. Furthermore, clarification as to the performance of ITS used alone in non-infected cows, and ITS used in combination with DCT in infected cows, at dry-off in comparison with DCT is needed.

1.6.3.2. Clinical Mastitis

Dry period IMI are unlikely to become clinical during the non-lactating phase. However, there is evidence that these new IMI have increased probability of becoming clinical in early lactation (Green et al., 2002). Because the primary goal of an ITS is the prevention of new IMI during the dry period, it should also have an effect on the development of CM in the subsequent lactation. Studies comparing the development of CM, from 30 to 150 DIM of lactation have found varying results, making a general statement concerning ITS efficacy against CM difficult (Table 1-2). When ITS, used alone or in conjunction with DCT were compared with a negative control, significant reductions in the incidence of CM have been observed (Woolford et al., 1998). A UK study compared CM rates between ITS and negative control quarters infected at calving and found a significant reduction in the ITS group; however, when CM rates were compared for all study cows in each treatment group, no significant difference was found (Berry and Hillerton, 2002). When ITS alone was compared with DCT, no difference in the incidence of CM was detected (Woolford et al., 1998; Huxley et al., 2002). Finally, in the two studies that compared the efficacy of ITS and DCT with DCT, one study found a significant reduction in the development of CM with the combination treatment (Godden et al., 2003), whereas, the other found no differences (Cook et al., 2005). However, when survival analyses were performed on the CM data, the hazard ratio for the ITS and DCT treated cows was significantly lower than cows treated with DCT alone (Cook et al., 2005).

There are many differences between the ITS studies that may explain the variability of the results. Firstly, differences in the detection and identification of

CM were also shown through-out the studies. The New Zealand study relied on herd managers to record CM for five months post-calving, however no milk samples were taken (Woolford et al., 1998). Therefore study monitors had to rely on the memory and recording accuracy of these herdsmen with training from researchers. Both English studies and the Wisconsin study relied on farm staff to detect and collect milk samples for CM analysis (Berry and Hillerton, 2002; Huxley et al., 2002; Cook et al., 2005). All these studies had low incidence of CM. The herds utilized in the Minnesota study had previously established CM detection protocols and on-farm culture in place prior to commencement of the ITS study (Godden et al., 2003). Therefore these herds had an interest in monitoring and identifying CM. Previous training may have made herd personnel more sensitive and conscientious at detecting and sampling milk that was suspect. This may explain why this study detected significant differences whilst most others did not.

Finally, the predominant causal organisms causing CM varied greatly among the ITS studies and may explain the different findings. In one UK study, the most predominant pathogens causing CM in ITS treated quarters were coliforms, whereas, the negative control quarters were infected with *S. aureus* and *S. uberis* (Berry and Hillerton, 2002). In another UK study, both *E. coli* and *S. uberis* were commonly isolated pathogens from both treatment groups (Huxley et al., 2002). In the Minnesota study, the most predominant CM pathogen isolated from both treatment groups was *E. coli.* Environmental streptococci were very common causes of CM in the DCT treated quarters, whereas a significant reduction in these pathogens, and all

major pathogens combined was observed in the combination treatment group (Godden et al., 2003). Clinical mastitis cases were not cultured at all during the New Zealand trial (Woolford et al., 1998) and culture samples were missed in over half of the CM cases in the Wisconsin study preventing any analysis by pathogen type (Cook et al., 2005). The differences in etiology of CM could have some bearing on the efficacy of DCT, but should not impact the efficacy of ITS in the reduction of CM postpartum.

Two of the ITS studies have also recorded CM cases that developed in the dry period. A significant reduction in dry period CM was found when ITS treated quarters were compared with negative control quarters (Woolford et al., 1998; Berry and Hillerton, 2002). In both of the aforementioned studies, the predominant cause of CM was *S. uberis*.

Real inconsistencies exist in the literature as to the effect of ITS, whether used alone or in conjunction with DCT, on the incidence of CM in early lactation. A further study examining the effect of ITS on CM across a large number cows is warranted in an attempt to clarify these differences.

1.6.3.3. Cure of Existing IMI at Dry-off

Some studies have also examined whether ITS used in addition to DCT in a quarter would have an effect on pathogen cure during the dry period (Table 1-3). No significant differences in the cure of pathogens were observed when treatment of ITS

and DCT were compared with DCT alone (Woolford et al., 1998; Godden et al., 2003; Cooke et al., 2005). A UK study examined the spontaneous elimination rate of pathogens during the dry period in quarters treated with an ITS only. The study found that 63 and 59% of major and minor pathogens were eliminated, respectively (Huxley et al., 2002).

Because of the non-antibiotic nature of ITS, the product should not have a primary beneficial effect on the cure of pathogens isolated at dry-off. However, through some secondary action, such as prevention of DCT leaking from quarters or due to induction of a local immune response to the physical infusion of a foreign substance, it could be possible that ITS increases rate of cure. All studies that examined the effect of ITS on cure rate were performed in a small number of herds (Woolford et al., 1998; Godden et al., 2003; Cook et al., 2005). In addition, quarter cures were estimated using single milk samples. Diagnostic errors, particularly in regards to cure rates are commonplace when single milk samples are utilized without a confirmatory test (Morant et al., 1988). Furthermore, the distribution of pathogens varied considerably between the studies and the prevalence of IMI was considerable. Therefore, a further study to examine any potential ITS effect on cure rates should be performed.

1.6.3.4. Particles in Milk

Because an ITS has a paste-like consistency, it should be removed from the teat canal post-calving before the cow is milked. Industry safety trials have found no dangerous side effects when ITS was fed to newborn calves at four times the standard dose (McHardy and Meaney, 1999). The material can be removed easily by hand-stripping. Due to the ability of the material to move within the mammary gland, on post-mortem examination, particles of ITS material have been seen throughout the alveolar tissue (Meaney, 1977). Several studies have mentioned the presence of ITS particles in the milk for as long as 21 days post-calving but it was not recorded specifically (Meaney, 1977; Berry and Hillerton, 2002).

No studies examining the presence of particulate matter from ITS in the milk post-calving are available. The concern over potential milk particles is of paramount importance to dairy producers who strive to sell quality milk. Furthermore, milking equipment is of great expense, therefore, studies to ensure that the working condition of these units will not be compromised, are essential for the adoption of ITS.

1.6.3.5. Bacteriocin

Some work has also been done to incorporate a bacteriocin into an intramammary teat sealer (Ryan et al., 1998). A bacteriocin is an antibiotic produced by some strains of *S. aureus*. Split udder design challenge trials involving the introduction of *S. aureus* into the mammary gland of uninfected lactating cows was used to test the efficacy of this ITS-bacteriocin combination (Twomey et al., 2000).

After milking, two teats were disinfected and infused with the bacteriocin and ITS combination. The remaining quarters were left as negative controls. Two hours later, the teats were inoculated with *S. aureus*. Eighteen hours later the ITS was removed from the teats, and milk samples were taken prior to milking. At high concentrations of bacteriocin used with ITS, *S. aureus* was significantly less likely to survive in treated quarters than untreated controls, but this finding was not replicated when lower concentrations of bacteriocin were examined (Twomey et al., 2000).

1.6.3.6. ITS Administration

Aseptic techniques are critical for the successful use of ITS. The risks of introducing a mastitis pathogen exists while infusing any product into the mammary gland. Since an ITS has no antibacterial properties, failure to practice clean intramammary infusion techniques has the potential for disaster. If the cleanliness of the teat end is in question, even after it has been scrubbed with alcohol, it should continue to be cleaned until it is free of possible contamination. Similarly, if the sterility of the ITS has been compromised, the tube should be discarded.

Internal teat sealer should be stored in a clean, dry container at room temperature. Cold weather can make infusion of the product difficult, but it should never be placed in warm water. When the product was introduced in the market in New Zealand, farmers were putting the ITS tubes into buckets of warm water to make infusion easier during the colder months. Multiple cases of *Pseudomonas* mastitis

occurred in several herds. The *Pseudomonas* was traced back to the buckets of water (Allison, personal communication). The occurrence of this problem emphasized the importance of keeping infusion tubes as clean as possible. Placing the infusion tubes in coverall pockets once in the barn, provides a safe way to warm syringes without damaging sterility.

1.7. Objectives of Current Research

1.7.1. Overall Goals

The overall objective of this research was to determine if the administration of an ITS administered at dry-off would be able to reduce the incidence of new IMI and CM when used alone in non-infected cows and in conjunction with DCT in infected cows. The incidence of new IMI and CM in quarters treated with ITS, DCT, and a combination of ITS and DCT, were compared.

1.7.2. Methodology

The data collected for this thesis were structured in a hierarchical (clustered) fashion. Outcomes were at the quarter-level, with quarters being clustered within cows, cows clustered within herds, and herds clustered within region. In some instances, there were also repeated measures taken at either the cow- or quarter-level.

If clustering is present in a dataset, it must be accounted for in the analysis in order to obtain valid estimates of the parameter effects and their standard errors (Dohoo et al., 2003). In general, failure to account for clustering will provide parameter estimates with standard errors that are too small and hence overestimate the statistical significance of the results (Dohoo et al., 2003).

To account for this model structure in the analysis, specialized statistical techniques were used. These included generalized linear mixed models, generalized estimating equations, and linear mixed models. When repeated measures were present, correlation structures to allow for dependence among the repeated observations were assessed and added to the model structure.

Latent class models, fit using Bayesian techniques, were used to evaluate test performance characteristics when no gold standard test was available. Bayesian inference involves the incorporation of prior information, expressed through probability distributions, with current data to elicit a posterior estimation of the test parameters and population prevalence of disease (Branscum et al., 2005).

1.7.3. Specific Objectives of the Six Research Chapters

Chapter 2. Objectives: To compare the incidence of new IMI in quarters of uninfected and infected cows, treated with ITS, a combination of ITS and DCT, and DCT during the dry period. Also, to compare the incidence of CM between treatment groups, during the first 60 days post-calving.

Chapter 3. Objectives: To compare the cure rate of infected quarters during the dry period, caused by all, major, and minor pathogens, treated at dry-off with either a combination of ITS and DCT, or DCT alone.

Chapter 4. Objectives: To compare SCC and particulate matter recovered from quarters of uninfected and infected cows, treated with ITS, ITS and DCT and DCT, during the first seven days post-calving.

Chapter 5. Objectives: To compare milk production and SCC in cows treated with ITS, a combination of ITS and DCT, and DCT at dry-off, over the first six months of lactation. To compare the incidence of CM, between treatment groups, during the first 30 days post-calving. To evaluate the ability of producers and veterinarians to follow treatment protocols in a minimal intervention clinical trial.

Chapter 6. Objectives: To assess if cloxacillin infused in two quarters of the udder at dry-off could move into untreated quarters during the first three days of the dry period. To quantify the concentration of cloxacillin detected in untreated quarters. To compare the cloxacillin movement between ipsilateral and contralateral treated quarters.

Chapter 7. Objectives: To characterize the test characteristics of the CMT and SCC performed at dry-off for detecting IMI when no gold standard is available. To utilize latent class models and Bayesian techniques to evaluate the CMT and SCC with bacteriological milk culture. To assess the predictive values of the CMT and SCC to identify cows in a herd for selective DCT.

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Table 1-1 Summary of incidence of new intramammary infections developed during the dry period from previous Internal Teat Sealer trials.

Study	Study Size (n)	Country	Negative Control	Dry Cow Antibiotic	Internal Teat Sealer	Internal Teat Sealer + Antibiotic
Meaney, 1977	14	Ireland	32.0%[a]		3.5%[b]	
	17		32.4%[a]			5.8%[b]
	14		14.0%			7.0%
Woolford et al., 1998	528	New Zealand	12.7%[a]	2.3%[b]	2.4%[b]	1.6%[b]
Berry and Hillerton, 2002	401	England	11.6%[a]		3.4%[b]	
Huxley et al., 2002	505	England		15.4%[a]	11.0%[b]	
Godden et al., 2003	437	United States		25.4%[a]		20.2%[b]
				21.7%[a]		18.8%[a]
Hillerton and Berry, 2004	189	England		6.3%[a]		1.5%[b]
Cook et al., 2005	528	United States		16.7%[a]		8.0%[b]

[a-b]Within a row, values without a common superscript were different ($P < 0.05$)

Table 1-2 Summary of the incidence of clinical mastitis in the subsequent lactation from previous Internal Teat Sealer trials.

Study	Risk DIM	Negative Control	Antibiotic	Internal Teat Sealer	Internal Teat Sealer + Antibiotic
Woolford et al., 1998	60	4.2%[a]	1.9%[b]	1.1%[b]	2.1%[b]
	150	6.3%[a]	3.0%[b]	2.1%[b]	2.8%[b]
Berry and Hillerton, 2002	100	6.5%[a]		1.9%[a]	
Huxley et al., 2002	100		3.6%[a]	3.2%[a]	
Godden et al., 2003	60		8.0%[a]		5.9%[b]
Cook et al., 2005	100		29.1%[a]		23.7%[a]

[a-b]Within a row, values without a common superscript were different ($P < 0.05$)

Table 1-3 Summary of cure rates of intramammary infections during the dry period from previous Internal Teat Sealer trials.

Study	Antibiotic	Internal Teat Sealer	Internal Teat Sealer + Antibiotic
Woolford et al., 1998	87.0%[a]		83.0%[a]
Huxley et al., 2002[b]	69.7%[a]	63.0%[a]	
Huxley et al., 2002[c]	83.3%[a]	59.0%[a]	
Godden et al., 2003	88.2%[a]		91.3%[a]
Cook et al., 2005	80.6%[a]		90.1%[a]

[a] Within a row, values without a common superscript were different ($P < 0.05$)
[b] Major pathogen cure rates
[c] Minor pathogen cure rates

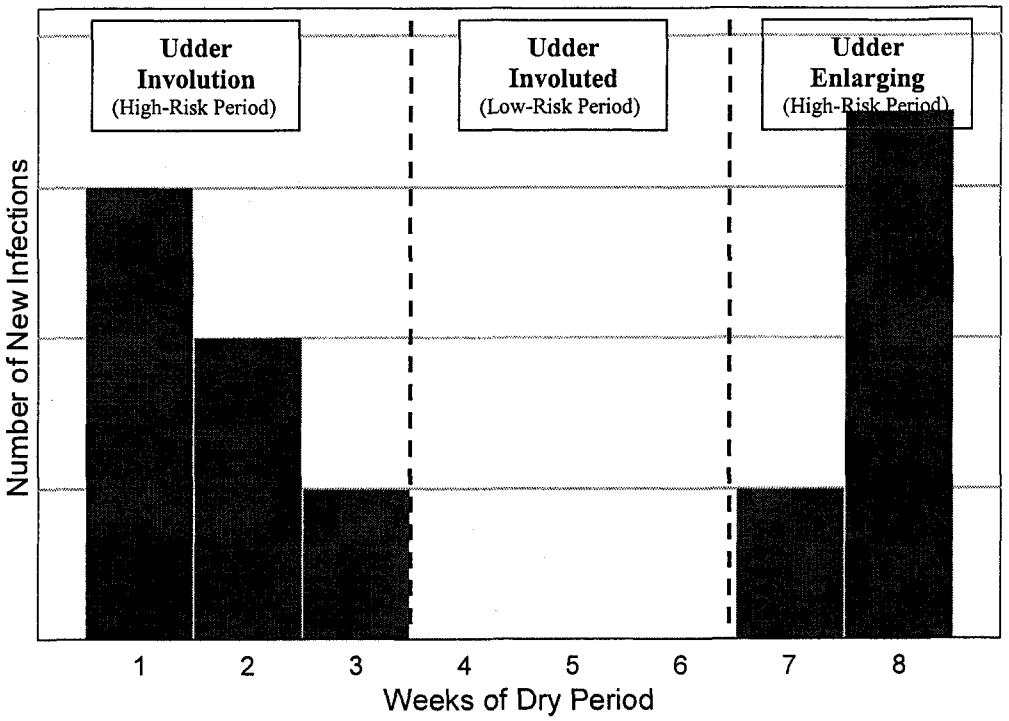

Figure 1-1 Important risk periods during the dry period, for the development of new IMI (adapted from Radostits et al., 1994).

CHAPTER 2 EFFICACY OF AN INTERNAL TEAT SEALER FOR THE PREVENTION OF NEW INTRAMAMMARY INFECTIONS DURING THE DRY PERIOD

2.1. Abstract

The objectives of this study were to determine the efficacy of an internal teat sealer (ITS) administered at dry-off for the prevention of new intramammary infections (IMI) during the dry period, and of clinical mastitis during the first 60 days in milk (DIM). In total 939 dairy cows from 16 herds were enrolled. Milk samples were collected aseptically at two weeks prior to the planned dry-off date, on the dry-off date and again at 1-8 DIM. Cows were allocated into two groups based on late lactation bacteriology results: Pre-Dry Uninfected if no quarter had an IMI, and Pre-Dry Infected if at least one quarter had an IMI. Quarters within cows were randomized to treatment (dry cow antibiotic (DCT), ITS, or ITS and DCT) based on group assignment, in a split udder design. Quarters of Pre-Dry Uninfected cows were treated with DCT or ITS only, while quarters of Pre-Dry Infected cows were treated with DCT or ITS and DCT. Quarters which were treated with both ITS and DCT were significantly less likely to acquire a new IMI during the dry period caused by all pathogens (OR = 0.51), major pathogens (OR = 0.49), environmental pathogens (OR = 0.47) and non-agalactiae streptococci (OR = 0.41), when compared with quarters treated with DCT alone. No significant differences on the incidence of CM were found between treatments in either the Pre-Dry Uninfected or Pre-Dry Infected cows. Using ITS in conjunction with DCT at dry-off significantly decreases the development of new IMI at calving.

2.2. Introduction

Dry cow management continues to be an evolving area of dairy research. The dry period is a critical time for reducing the risk of postpartum diseases, including mastitis. During the dry period, there are two critical risk times when the bovine udder is particularly susceptible to the development of new IMI (Neave et al., 1950; Cousins et al., 1980; Smith et al., 1985; Eberhart, 1986). The first period of increased risk starts when milking stops and continues for the first 21 days when the udder is undergoing involution. Once the udder is fully involuted and a natural teat canal keratin plug is formed, the udder is highly resistant to new IMI (Oliver and Sordillo, 1988). The second period of increased risk begins just prior to calving and continues throughout the peripartum period, while the udder is undergoing colostrogenesis and the cow is experiencing immunological, metabolic and management changes. Currently, the accepted practice for the prevention of new IMI during the dry period is the treatment of all cows and quarters with an approved intramammary dry cow antibiotic (Eberhart, 1986). This approach, termed blanket dry cow therapy (DCT), is recommended by the NMC (National Mastitis Council, 1999). This method has been successful in decreasing the incidence of new IMI during the dry period, and also eliminates many existing IMI present at the end of lactation (Smith et al., 1985; Schukken et al., 1993). While DCT is effective, it is not without limitations. Therapeutic levels of antibiotics are present in the mammary gland for a limited period of time (Oliver et al., 1990). Also commercially available dry cow antibiotics

are targeted specifically against gram-positive bacteria (Bradley and Green, 2001). The mammary gland is left largely unprotected against gram-negative pathogens throughout the dry period, and is also left unprotected against gram-positive organisms in the period just prior to calving. Risk factors for new IMI during the dry period have been reviewed in detail (Dingwell et al., 2004). During the dry period, a keratin plug forms in the streak canal and base of the teat cistern to provide a natural physical barrier for the mammary gland. Several studies have shown that not all teats close during the dry period and between 5 and 23% of teats have not formed a keratin plug by day 50 of the dry period (Williamson et al., 1995; Dingwell et al., 2004). These open quarters were 1.8 times more likely to develop a new IMI (Dingwell et al., 2004).

The introduction of OrbeSeal* (Pfizer Animal Health, Montreal, Quebec), an internal teat sealer (ITS) into the global dairy marketplace has stimulated further research in this area. The ITS composed of 65% bismuth subnitrate, functions as an inert physical barrier in the teat cistern and its goal is to prevent the penetration of bacteria from the environment into the udder. As a non-antibiotic device to block the teat canal, ITS offer a unique opportunity to dairy producers. They can be used alone in non-infected cows, as a means to prevent new IMI and decrease use of antibiotics as part of a selective dry cow program. Alternatively, ITS can be used in conjunction with DCT to provide protection beyond the scope achieved with DCT alone. The incorporation of an ITS in the dry-off management practices of dairy farms should have an impact on the number of open teats during the dry period. Bismuth subnitrate

inserted at dry-off remained present at the base of the teat cistern for 100 days after infusion (Woolford et al., 1998). Persistency for this length of time would also extend protection of teats after therapeutic activity of DCT has expired, into the second period of increased susceptibility just prior to calving, when teats are opening up.

An ITS made of 25% bismuth subnitrate was studied in Ireland in several small trials to test the efficacy of the product for the prevention of new IMI during the dry period (Meaney, 1977). This paste-like product has since been modified to its current formulation, OrbeSeal*, and has been the subject of several clinical trials worldwide over the last seven years (Woolford et al., 1998; Berry and Hillerton, 2002; Huxley et al., 2002; Godden et al., 2003; Cook et al., 2005). In previous studies, incorporation of an ITS at dry-off, whether used alone or in conjunction with DCT, lowered the incidence of new IMI post-calving when compared with a negative control (Woolford et al., 1998; Berry and Hillerton, 2002). In both these studies, the protective effect was largely against *Streptococcus uberis* as this reflected the majority of new IMI identified. Similarly, new IMI at calving were decreased when an ITS used in conjunction with DCT was compared with DCT alone (Godden et al., 2003; Cook et al., 2005). The use of an ITS alone was protective against new IMI by *Escherichia coli*, *Enterobacteriaceae* and all major pathogens combined when compared with DCT (Huxley et al., 2002).

Effectiveness of an ITS in reducing the number of cases of clinical mastitis (CM) has been investigated in studies that examined the first 60 to 150 days of lactation (Woolford et al., 1998; Huxley et al., 2002; Berry and Hillerton, 2002;

Godden et al., 2003; Cook et al., 2005). Some studies found that ITS decreased the incidence of CM (Woolford et al., 1998; Godden et al., 2003). Huxley et al. (2002) found no significant difference in the incidence of CM between treatment groups.

The main objective of the current study was to compare three different treatments, DCT, ITS alone, and ITS used in conjunction with DCT, for the prevention of new IMI during the dry period, as well as to assess its impact on the incidence of CM occurring during the first 60 days of lactation. The study was designed to answer the following: 1) in cows with no quarter infected two weeks before dry-off, is an ITS (used alone) more efficacious in preventing new IMI in the dry period when compared to DCT alone, 2) in cows with at least one quarter infected two weeks before dry-off, does the addition of an ITS to DCT provide a more efficacious therapy for preventing new IMI, when compared to DCT alone. The current study design reflected the potential use of ITS in Canadian dairy herds in a selective dry cow program (treatment based on herd, and cow factors) or as an adjunct to DCT. A large number of cows from multiple herds, with varying housing conditions, were used to increase the external validity of the research.

2.3. Material and Methods

2.3.1. Herd and Animal Selection

All lactating cows in a convenience sample of 939 cows from 16 herds from three provinces (Ontario (4 herds), Prince Edward Island (6 herds) and Québec (5 herds)) in Canada and from Kansas (1 herd) USA, were selected to participate in this study based on their proximity to participating academic institutions. A conscious effort was made to select herds that were representative of each region, with the exception of two university research herds (1 in Ontario and 1 in Kansas). Eight of the study herds were housed in free-stall operations. The remaining eight herds were housed in tie-stalls. All milking cows in each herd were eligible to participate if they had at least three functional quarters, were confirmed pregnant, and had an expected dry period length between 30 and 90 days.

2.3.2. Sample Collection Schedule and Treatment Allocation

All herds were visited once a week on the same day by trained study personnel. This approach required that herds adopted this weekly visit day as their dry-off day. Two weeks prior to the scheduled dry-off day (Day -14), single quarter milk samples were collected aseptically from all eligible cows according to NMC methods (National Mastitis Council, 1999). Samples were frozen at -20°C prior to shipping and submission to the Mastitis Research Laboratory at the University of Guelph. Bacteriology results from these milk samples were taken at this point in time in order to split the cows into two groups for allocation of treatment (Pre-Dry

Uninfected and Pre-Dry Infected), based on a single culture. Cows with all four quarters free of IMI (based on the Day -14 culture results) were assigned to the Pre-Dry Uninfected Group (PD-Uninf), and cows with one or more quarters found to have an IMI or a contaminated sample were assigned to the Pre-Dry Infected Group (PD-Inf). Within each group, quarters within cows were sequentially assigned to one of two treatments in a split udder design (Fig.2-1). In PD-Uninf cows, ipsilateral quarters were randomly assigned to receive either an intramammary infusion of ITS (OrbeSeal*, Pfizer Animal Health, Quebec, Canada) or an intramammary infusion of 500mg benzathine cloxacillin DCT (DryClox®, Ayerst Veterinary Laboratories, Guelph, Canada) which has a milk withdrawal time of 30 days. In PD-Inf cows, ipsilateral quarters were randomly assigned to receive intramammary infusions of both ITS and DCT, or an intramammary infusion of DCT only. In quarters that received both ITS and DCT, the DCT was administered first.

On the day of dry-off, single quarter milk samples were collected aseptically and split for submission for both bacteriology and SCC analysis. Following sampling and milking, the quarters were infused with the assigned treatment protocol. Prior to treatment infusion, teats were prepared aseptically, and a partial insertion technique was used. Quarters were massaged after infusion of DCT. For quarters treated with both ITS and DCT, the DCT was infused first and the udder massaged. The ITS was then infused with no subsequent massaging. Teats were disinfected using a commercial teat dip and cows were moved to each farm's dry cow area. After calving between 1-8 DIM, single post-calving milk samples were collected

aseptically and split for submission for both bacteriology and SCC analysis. All milk samples were collected after the cows were prepared for milking as per the farm's usual practice.

In seven of the study herds, during the first 60 days of lactation, milk samples were collected by the producer, from any quarter(s) which they identified as CM cases. Clinical mastitis was defined as abnormal milk with or without signs of heat, redness or swelling in the quarter, and with or without clinical disease in the cow. Samples were frozen on the farm and collected weekly for submission for bacteriology.

2.3.3. Bacteriological Culture Procedures

All laboratory procedures were performed by the same individual, and in accordance with NMC recommendations (National Mastitis Council, 1999). The laboratory staff was blinded to treatments. An inoculum of 0.01 ml of milk was plated onto a Columbia base agar containing 5% sheep blood. Plates were incubated at 37°C and examined for bacterial growth at 24 and 48 h. Colonies were tentatively identified as staphylococci, streptococci, coliform, or other pathogens based on colony growth, morphology and appearance, pattern of hemolysis, catalase reaction, and Gram staining. Staphylococcal isolates were tested for coagulase production with the tube coagulase test. Streptococcal isolates were further subcultured with agar containing esculin. Gram-negative bacteria were plated on MacConkey agar to facilitate identification. Gross appearance and reaction to citrate were used to differentiate *E. coli* and *Klebsiella* sp. For each positive quarter, the number of colony forming units (CFU) per 0.01 ml milk was reported in one of four categories: 1 to 5, 6 to 10, 11 to 50, or > 50 CFU.

2.3.4. Definitions

Bacteriological culture result and SCC were used in series to define infection. Therefore, all definitions of IMI below include a SCC of >200,000 cells/ml.

Intramammary Infection : Isolation of any major mastitis causing organism (i.e. *Staphylococcus aureus, Streptococcus agalactiae,* non-agalactiae streptococci, *E. coli, Klebsiella* spp., *Enterobacter* spp., *Aeromonas* spp., *Citrobacter* spp., *Proteus* spp., *Pseudomonas* spp., *Serratia* spp., *Salmonella* spp., *Tatumella* spp., *Prototheca* spp., *Arcanobacterium pyogenes*, yeast, and mold) or >10 CFU per 0.01ml of coagulase-negative staphylococci (CNS) resulted in a quarter being defined as having an IMI.

Major IMI: Isolation of any mastitis-causing organism as listed above, except CNS, was classified as major IMI. Major IMI were subdivided into 2 classes: contagious IMI (data not shown) and environmental IMI (see below). Environmental IMI were further sub-classified as gram-negative IMI, and non-agalactiae streptococci IMI.

Environmental IMI: Isolation of any non-agalactiae streptococci, gram-negative environmental spp. (listed below), *Prototheca* spp., *A. pyogenes*, yeast, or mold were classified as environmental IMI.

Gram-Negative IMI: Isolation of any gram-negative mastitis causing organism (i.e. true coliforms, such as: *E. coli, Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., and other gram-negatives, such as: *Aeromonas* spp., *Proteus* spp., *Pseudomonas* spp., *Serratia* spp., *Salmonella* spp., *Tatumella* spp.) were classified as gram-negative IMI.

Non-agalactiae streptococci IMI: Isolation of any non-agalactiae streptococci which included *Streptococcus dysgalactiae*, *S. uberis*, *Enterococcus* spp., *Aerococcus* spp., and other non-differentiated *Streptococcus* spp. were classified as non-agalactiae streptococci IMI.

Contaminated Sample: A milk sample with three or more isolates was considered contaminated.

New Intramammary Infection: Quarters that were free from IMI on the dry-off sample but which had an IMI at the post-calving sample were defined as new IMI. Quarters that were infected with a different bacteriological species at post-calving than were present at dry-off were also considered new IMI.

2.3.5. Statistical Analysis

Data of a hierarchical nature were used. The primary experimental units were quarters which were nested within cows, cows were nested within herds, and herds were nested within region. Consequently, a generalized linear mixed model (Rabe-Hesketh and Skrondal, 2005) with a binomial family and a logit link which accounted for the clustering at multiple levels was used to analyze risk factors for the probability of a quarter developing a new infection by including random effects for herds and cows. Specialized statistical techniques to account for the interdependency of quarters

within a cow and cows within a herd were necessary to ensure that we received valid estimates with correct standard errors (Berry et al., 2003).

The Cows were blocked into two groups (PD-Uninf and PD-Inf) based on quarter bacteriology results prior to dry-off (day -14). A split udder design was used to randomize treatment of quarters within cows, within each infection group. The main predictor of interest was treatment and it had 3 levels: ITS, DCT, and ITS used in conjunction with DCT. Models with the following outcomes were tested: new IMI caused by any pathogen, new IMI caused by major pathogens, new IMI caused by environmental pathogens, new IMI caused by gram-negative pathogens, and new IMI caused by non-agalactiae streptococci. The group variable was forced into each model to account for DCT being a treatment option in both the PD-Uninf and PD-Inf groups. Variables considered in all models included herd-level factors such as herd size and housing type, cow-level factors such as parity at freshening, dry period length and season, and quarter-level factors such as quarter, infection status of quarter at dry-off, *Corynebacterium bovis* infection status of quarter at dry-off. Because there were less than five regions, which was the highest level of the hierarchy, this variable was used as a fixed effect (Dohoo et al., 2003). However, because region contributed nothing to the models, it was removed.

Unconditional associations between all predictors and the dichotomous outcome (new IMI) were evaluated using a simple logistic regression model to select potential predictors for the full models. All predictors with a $P < 0.30$ for the unconditional association with the outcome were retained for evaluation in the

multivariable model. A backwards step-wise elimination process was utilized for model reduction, using a significance level of $P < 0.10$. P-values < 0.05 were considered significant, and P-values between 0.06 and 0.10 were retained in the models and their importance discussed. All predictors were assessed for significant interactions and confounding before they were removed from the multivariable models.

2.4. Results

2.4.1. Descriptive Statistics

There were 939 Holstein-Friesian dairy cows (3731 quarters) enrolled in this trial. A total of 519 and 420 cows were allocated into the PD-uninf and PD-inf groups, respectively. Exclusion criteria for the study cows and quarters are described in Table 2-1. Fifty-eight cows did not finish the study. A further 146 cows were excluded from the analysis because of dry period length, antibiotic administration outside the study protocol, contamination of all milk samples or having post-calving milk samples taken after 8 DIM. Finally, 165 quarters were excluded from the analysis because of contamination or incorrect treatment at dry-off. This left data from 734 cows (2771 quarters) for analysis. No significant differences in exclusions (cows or quarters) were found (overall or reason specific) between PD-Inf and PD-Uninf groups.

The average herd size was 123 (median 100, range 40-380). Mean parity at calving for the study cows was 3.3 (median 3, range 2-10). Average dry period length was 58 days (SD 18). Seasonal breakdown of dry periods are as follows: winter 27, spring 20, summer 27, fall 26%. Proportions of quarters with SCC > 200,000 cells/mL were not significantly different between treatments within groups. In the PD-Uninf, 29% of the quarters treated with an ITS and 28% of the quarters treated with cloxacillin had SCC > 200,000 cells/mL at the end of lactation. In the PD-Inf, 35% of the quarters that received either treatment had SCC > 200,000 cells/mL at the end of lactation. At the time or the first post-calving sample (1 to 8 DIM), 37% of quarters treated with ITS and 36% of quarters treated with cloxacillin had SCC > 200,000 cells/mL in the PD-Uninf. Similarly in the PD-Inf, 42% of quarters treated with an ITS in conjunction with DCT and 40% of quarters treated with cloxacillin alone had SCC > 200,000 cells/mL at the first post-calving sample.

2.4.2. Incidence of New IMI

Bacteriological results of milk cultures taken at dry-off and post-calving, broken down by group and treatment are shown in Tables 2-2 and 2-3. In quarters of cows in the PD-Uninf group, the prevalence of IMI was higher post-calving, than at dry-off (Table 2-2). In contrast, in the PD-Inf group, the prevalence of IMI post-calving was lower than at dry-off (Table 2-3). An increase in environmental streptococci and gram-negative pathogen IMI was evident primarily in cows in the PD-Uninf group, where quarter infection increased up to three fold depending on the treatment administered at dry-off. This increase was less evident in cows in the PD-Inf group.

Table 2-4 lists the prevalence of IMI by bacterial species in all quarters of all cows by DIM at the time of the post-calving sample ($\leq$3 DIM, $\leq$5 DIM, $\leq$8 DIM). Prevalence is marginally higher in the immediate post-calving samples but not substantially different from the period up to 8 DIM. This pattern was consistent across treatment groups (data not shown). Using samples taken up to 8 DIM provides much greater statistical power and therefore the full dataset was used for model building.

Results of generalized linear mixed models for the effects of treatment on development of new IMI are summarized in Tables 2-5 (PD-Inf) and 2-6 (PD-Uninf). Although results are presented separately for each group of cows, the estimates were

derived from a single model, which included data from both groups and included a group term.

When all pathogens were considered, quarters treated with ITS and DCT were significantly less likely to develop a new IMI at calving than quarters treated with DCT (OR = 0.51; 95% CI = 0.30-0.85) in the PD-Inf. No difference was found between ITS or DCT treated quarters in the PD-Uninf. Right front quarters were significantly more likely to develop a new IMI, compared with left front quarters (OR = 1.60; 95% CI = 1.03-2.49).

When major pathogens were considered, quarters treated with both ITS and DCT were significantly less likely to develop a new IMI at calving than quarters treated with DCT (OR = 0.49; 95% CI = 0.28-0.89) in the PD-Inf. No difference in the development of new major pathogen IMI was found between ITS or DCT treated quarters in the PD-Uninf.

When environmental pathogens were considered, quarters treated with both ITS and DCT were significantly less likely to develop a new IMI at calving than quarters treated with DCT (OR = 0.47; 95% CI = 0.23-0.93) in the PD-Inf. In the PD-Uninf, quarters treated with ITS tended to be less likely to develop a new environmental IMI at calving than quarters treated with DCT (OR = 0.54; 0.29-1.02). Cows in their 4^{th} or greater lactation (at calving) were significantly more likely to develop new IMI caused by an environmental pathogen, compared with 2^{nd} lactation cows (OR = 2.00; 95% CI = 1.05-3.82).

When gram-negative pathogens were considered, there were no treatment differences among any groups. Quarters infected at dry-off with *C. bovis* were significantly more likely to develop a new IMI caused by gram-negatives compared to culture-negative quarters (OR = 2.60; 95% CI = 1.11-6.11).

When non-agalactiae streptococci pathogens were considered, quarters treated with ITS and DCT were significantly less likely to develop a new IMI at calving than quarters treated with DCT (OR = 0.41; 95% CI = 0.18-0.91) in the PD-Inf. No difference was found between ITS or DCT treated quarters in the PD-Uninf for new non-agalactiae streptococci IMI.

Within each different model the majority of the unexplained variation was between cows within the same herd. There was little evidence of any unexplained variation between herds (Appendix A).

2.4.3. Incidence of CM During the First 60 DIM

In the CM component of the research trial, data was available from 328 cows (1304 quarters) from seven herds (6 from PEI and 1 from Ontario). Of these cows, 215 were from the PD-Uninf and 113 were from the PD-Inf. A total of 303 cows (1195 quarters) were utilized in the analysis. Exclusions from the analysis were due to dry period length, antibiotic therapy (outside of study protocol), abortions, sale, death or incorrect dry-off treatment. These exclusions included two of the CM cases.

In total, CM was reported in 31 quarters in 27 cows during the 60 days post-calving, resulting in a 60-day quarter incidence and cow incidence risks of 2.4% and

9.6%, respectively. Table 2-7 shows the multivariable regression analysis models for clinical cases due to all pathogens and environmental pathogens, respectively.

No significant differences between treatments were found in either the PD-Uninf or PD-Inf for either CM caused by all pathogens or environmental pathogens. The only significant predictor of CM was parity. Cows that were in their 3rd or 4th lactation (at calving) were at a higher risk of developing a case of CM than cows in their 2nd lactation caused by all pathogens (OR = 4.2; 95% CI = 1.22-14.5) or by environmental pathogens (OR = 6.96; 95% CI = 1.63-29.4). Cows in their 5th lactation and greater also appeared to be at higher risk, but the difference between these cows and 2nd lactation cows was only borderline significant for all pathogens (OR = 2.98; 95% CI = 0.88-10.2) and environmental pathogens (OR = 3.60; 95% CI = 0.83-15.6), respectively.

2.5. Discussion

This is the first study in Canada to evaluate the efficacy of ITS. Herds with geographic representativeness of the Canadian dairy industry were selected (>70% of milk sales and farms in Canada from the three provinces where herds were enrolled) (Dairy Farmers of Canada, 2002). The current study was unique in that it compared three different treatments: ITS alone, ITS used in conjunction with DCT, and DCT alone, in two subpopulations of cows (PD-Uninf and PD-Inf). This reflected the practical on-farm application of ITS, whereby producers may choose to use an ITS

alone in cows they identify as not infected when entering the dry period, or they may use it in combination with DCT.

The current study provides evidence that the use of ITS in conjunction with DCT enhances dry period protection against the development of new IMI caused by all pathogens, major pathogens, environmental pathogens and non-agalactiae streptococci when used in cows with an IMI in late lactation. This finding was similar to a Minnesota study comparing the efficacy of an ITS and DCT with DCT alone, on the development of new IMI (Godden et al., 2003). Woolford et al. (1998) reported that the use of both an ITS and DCT did not enhance dry period protection beyond the level of that achieved by either an ITS alone or long-acting DCT alone. The significant treatment effect from the New Zealand study was seen in comparison with negative control quarters, and was particularly seen against *S. uberis* (Woolford et al., 1998). The marked difference between the Canadian and New Zealand dairy industries with respect to housing, calving and dry cow management makes further comparisons of the results of these two studies difficult. A treatment effect against *S. uberis*, similar to that noted in the New Zealand study, was found by UK researchers who compared the use of ITS with a negative control (Berry and Hillerton, 2002). In Canada, a very high proportion of herds use blanket DCT (Gill et al., 1990). Comparison of product performance against a negative control, while of scientific interest, is not consistent with industry practice in Canada.

There were no significant treatment effects of ITS when used alone and compared to DCT, in late-lactation uninfected cows. However, a strong trend existed

for the reduction of new IMI caused by environmental pathogens in quarters treated with an ITS. When the environmental pathogens were split into gram-negative pathogens and environmental streptococci and similar analyses were performed, the trend was not evident. Huxley et al. (2002) found a significant treatment effect when comparing an ITS alone against DCT with regards to all major pathogens combined, *E. coli,* and all *Enterobacteriaceae.* In the current study, there was insufficient power to evaluate the effects of ITS on individual pathogens. Management differences between the UK and Canada may have accounted for the differences between the results of Huxley et al. (2002) and the current study. The cows in the UK study were from a large number of herds in one particular region in England and were housed in very different conditions (predominantly on pasture with winter housing indoors). Herds in the current study were in either full-confinement or partial-confinement (seasonal) housing. It is possible that the confinement aspect of management overwhelms the quarter with gram-negative exposure to the point that an ITS used alone is not able to prevent a significantly higher number of IMI compared with DCT.

Achieving the objectives of this study relied upon the determination of quarter-infection status at dry-off and at calving. New quarter IMI were defined using milk bacteriological culture to isolate pathogens from quarters that were "free" from IMI at dry-off, or the isolation of different pathogens post-calving than were present at dry-off. The current study used single quarter milk samples in series with quarter SCC to determine IMI status. Therefore, quarters had to have pathogens isolated on culture, as well as a SCC greater than 200,000 cells/ml to be considered

infected. This series infection criterion of culture and SCC was used to increase the specificity of the definition of IMI. The most serious concern with the classification of IMI used in the current study is low sensitivity. While increasing the number of samples taken pre-partum or postpartum will increase the possibility that IMI will be detected, the specificity of the classification would decrease. Decrease in specificity could be due to laboratory contamination or streak canal IMI. Regardless of the number of samples taken, errors will occur. Using one postpartum milk sample for bacteriological culture introduces misclassification bias, that is, a rearrangement of study quarters/cows into incorrect categories because of failure to detect IMI that were present. This bias would not be expected to be different between the treatment groups, and therefore would be non-differential misclassification. When the outcome is dichotomous, non-differential misclassifications always biases the measure of association towards the null (Rothman and Greenland, 1998; Dohoo et al., 2003). Therefore, the estimates of treatment effects from the current study data would be conservative. Furthermore, there was potentially a real reduction in new IMI caused by environmental pathogens in the ITS group, but only a strong trend was statistically evident due to the conservative estimates.

In order to detect new IMI that occurred during the dry period, the ideal sampling time frame should be at dry-off and again post-calving before the cow is milked. This strategy was employed in the majority of other ITS studies (Woolford et al., 1998; Berry et al., 2002; Huxley et al., 2002) in an attempt to ensure that they were not detecting IMI that occurred post-calving. Godden et al. (2003) collected

post-calving samples at 1-3 DIM and again at 6-8 DIM acknowledging that they could be detecting new IMI which developed postpartum. The sampling strategy from the current study has limitations as new IMI that developed post-calving, and not just during the dry period, may have been detected. However, the proportion of cows infected remained stable for the first 8 DIM and in fact, decreased marginally. Again, even if some new infections or spontaneous cures occurred in the early postpartum period, this would lead to a non-differential misclassification bias, because quarters receiving different treatments would not be more likely than others to develop or cure IMI post-calving. The DCT used in this trial has a milk withdrawal of 30 days and would have no residual effect postpartum. The ITS is removed prior to the first milking and would also have no residual effect.

Intramammary infections that originate in the dry period may be undetected until clinical signs appear, typically early in lactation (Bradley and Green, 2000). Evidence from two other ITS clinical trials (Godden et al., 2003; Woolford et al., 1998) found significant reductions in CM with the use of an ITS alone or in conjunction with DCT. The current study did not find an effect of ITS on the reduction of CM. Possible explanations for this finding include: small sample size (303 cows), for this component of the study, different definitions of CM, or compliance of the owners to take and store milk samples. Although clinical trial protocol specifically requested that participating producers identify and collect milk samples in suspect CM cases, compliance was an issue in many (9) herds. Reminders and requests to collect CM samples were given on multiple occasions but this

objective of the study was not followed. The ensuing loss of power (from 16 to 7 herds) is certainly a valid explanation for the lack of significant results. Herd management, feeding, housing, and stocking density differences may account for dissimilar results. Differences may also be explained by seasonal influences. Parity was the only significant predictor of CM. Third and forth lactation cows were at higher risk of developing CM then 2nd lactation cows.

Although treatment was the parameter of interest in the current study, other parameters were found to increase the risk of the development of new IMI. When all pathogens were considered, right front quarters were at a significantly higher risk of developing new IMI. This quarter effect was not found in any of the other ITS trials (Woolford et al., 1998; Berry and Hillerton, 2002; Huxley et al., 2002; Godden et al., 2003). In support of our finding, Barkema et al. (1997) found that right front quarters were more susceptible to both IMI and high SCC than left front quarters of the same cow when evaluating milk samples from 150 herds.

Quarters infected with *C. bovis* at dry-off were at a significantly higher risk of developing a new IMI caused by gram-negative pathogens. Berry and Hillerton (2002) found a similar effect of *C. bovis* infection on the development of *S. uberis* IMI. It was interesting to observe that *C. bovis* was found more frequently in left front quarters as it is usually a pathogen isolated when inadequate post-milk teat dipping is practiced (Lam et al., 1997a). Because most milkers would be right handed and milking in a parallel parlor, it is plausible that left front quarters would be poorly dipped due to location. When minor pathogens have been evaluated, some

studies have demonstrated that IMI with these organisms decrease the risk of developing a new IMI caused by major pathogens (Rainard et al., 1988; Lam et al., 1997b). However, other studies have contradicted those findings when specific pathogens were considered (Lam et al., 1997b; Hogan et al., 1988; Berry et al., 2002). In support of our finding, Hogan et al., (1988) found that minor pathogens provided no protection against the development of coliform IMI. De Vliegher et al. (2004) demonstrated that *Staphylococcus chromogenes* (a CNS pathogen) inhibited growth of *S. aureus*, *S. dysgalactiae* and *S. uberis* in 20% of isolates, but no inhibition of *E. coli* occurred. Explanations for this finding stated that inhibition is more intensive against related species and genera, than inhibition against phylogenetically distant bacterial families (De Vliegher et al., 2004).

Cows in their 4th plus lactation (at calving) were at a significantly higher risk of developing new IMI caused by environmental pathogens than cows in their 2nd lactation. Cows starting their 3rd lactation were at no increased risk when compared with the younger age group. Increased parity resulting in greater susceptibility to IMI has been associated with streak canal laxity and is well documented in the literature (Oliver et al., 1983; Smith et al., 1985). The parity effect was observed in the environmental model only. A more open (older) teat orifice due to streak canal laxity may have caused higher IMI rates in this group.

The results of several studies, including the current trial, suggest that ITS works as well as DCT in prevention of all new IMI. Well-managed North American dairy herds that have implemented sound mastitis control programs, and therefore

have low bulk tank SCC, have greater problems controlling environmental pathogens than contagious organisms (Hogan and Smith, 2003). The possibility exists to establish an effective dry cow mastitis control program for these herds using ITS alone or in conjunction with a selective dry cow protocol. Adoption of selective DCT offers additional benefits. Concerns over the potential contribution of widespread DCT use to an emergence of antibiotic resistant organisms have been noted (Eberhart, 1986). Additionally, there has been recent interest in shorter dry periods (Bachman, 2004). Internal teat sealers used alone would eliminate the risk of antibiotic residues, if a cow was to have a shortened dry period or calve early. If an ITS is going to be used alone in a selective manner in herds, then attention to aseptic technique must be stressed because there is no antibiotic activity in the product and introduction of bacteria at the time of infusion could result in IMI. In the current study, no substantial post-treatment clinical disease was noted. However, it must be emphasized that trained technical support was used for ITS infusion.

Results from the current study must be interpreted carefully. Because of the trial design, the comparison of ITS alone versus DCT was only made in cows found uninfected in late lactation. All herds were on blanket DCT at the start of the trial and leaving infected cows untreated in the protocol was not possible. Furthermore, the comparison of ITS used in conjunction with DCT compared with DCT alone was only made in cows found infected in late lactation. The Minnesota study compared the use of both ITS and DCT with DCT in all cows, with promising results. Also, the current comparisons were made against one particular dry cow antibiotic product,

cloxacillin. Future studies should investigate differences between ITS and other dry cow antibiotic preparations.

In conclusion, under conditions typical of Canadian dairy farms, ITS provide enhanced protection in the dry period against the development of new IMI of all pathogens when used in conjunction with DCT. Herds which are interested in adding ITS into their existing dry cow management program should consult with their veterinarian to determine the most appropriate usage of ITS on their farm.

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Table 2-1 Exclusion criteria of cows and quarters from analysis by treatment group (Pre-Dry Uninfected or Pre-Dry Infected) and treatment within group (Internal Teat Sealer, Dry Cow Therapy, or Internal Teat Sealer and Dry Cow Therapy).

	Pre-Dry Uninfected (n=519)		Pre-Dry Infected (n=420)	
Cow-level exclusions				
Abortion	10		3	
Disease	1		0	
Death	7		4	
Sold	2		3	
Missed Sample	14		12	
Lost Sample	0		1	
Incorrect cow treatment	0		1	
Dry Period < 30 days	19		6	
Dry Period > 90 days	23		16	
Antibiotic Administration	6		9	
Contamination all samples	9		11	
Sample 3 > 8DIM	20		27	
Total Cow Exclusions	111		93	
Quarter-level Exclusions	ITS (n=999)	DCT (n=1031)	ITS+DCT (n=827)	DCT (n=827)
Contamination	26	30	35	31
Incorrect Treatment	29[a]	0	14[b]	0
Total Quarter Exclusions	55	30	49	31

a Error made due to decrease in CFU criteria (> 50 to > 10) of CNS IMI after first month of data collection.
b Although day -14 results allocated cow into Pre Dry-Uninfected, on-farm decision to treat with both ITS and DCT was made at farmer's request or high CMT score.

Table 2-2 Prevalence of IMI (quarters) in the pre-dry (Day -14) uninfected treatment group at dry-off and Day 1-8 postpartum, (Internal Teat Sealer (ITS) or Dry Cow Antibiotic (DCT)) (n=1562 qtrs).

	Dry-off		1-8 DIM	
	ITS (%)	DCT (%)	ITS (%)	DCT (%)
Total Quarters	774	788	774	788
Total IMI [a]	43 (5.6)	49 (6.2)	77 (9.9)	95 (12.1)
Staphylococcus aureus	1	2	11	13
Streptococcus uberis	1	1	4	3
Streptococcus dysgalactiae	2	1	1	3
Non-differentiated non-agalactiae Streptococci	10	5	16	18
Total non-agalactiae Streptococcus	13 (1.7)	7 (0.9)	21 (2.7)	24 (3.0)
Escherichia coli	2	5	6	14
Klebsiella spp.	-	-	1	1
Enterobacter spp.	-	-	2	3
Proteus spp.	-	-	-	1
Citrobacter spp.	-	-	-	1
Pseudomonas spp.	-	-	2	1
Serratia spp.	2	-	1	-
Salmonella spp.	-	-	-	-
Tatumella spp.	-	-	-	-
Non-differentiated gram-negatives	3	3	1	2
Total gram-negatives	7 (0.9)	8 (1.0)	13 (1.7)	23 (2.9)
Arcanobacterium pyogenes	-	1	2	2
Yeast	-	-	3	-
Mold	1	1	1	4
Mixed Major[b]	-	-	1	-
Total Major	22 (2.8)	19 (2.4)	52 (6.7)	66 (8.4)
≤10 CFUs Coagulase-negative staphylococci	165	162	195	209
>10 CFUs Coagulase-negative staphylococci	22	30	35	35
Total Coagulase-Negative staphylococci	187	192	230	244
Corynebacterium bovis	97	116	34	33
Mixed Minor	34	25	17	11

[a] IMI defined in Materials and Methods section, total major and ≥10 CFUs CNS do not add exactly if a quarter contained both

[b] Any quarter(s) with more than one major pathogen isolated

Table 2-3 Prevalence of IMI (quarters) in the pre-dry (Day -14) infected treatment group at dry-off and Day 1-8 postpartum, (Internal Teat Sealer and Dry Cow Antibiotic (DCT+ITS) or Dry Cow Antibiotic (DCT)) (n=1209 qtrs).

	Dry-off		1-8 DIM	
	ITS/DCT (%)	DCT (%)	ITS/DCT (%)	DCT (%)
Total Quarters	604	605	604	605
Total IMI [a]	149 (24.7)	163 (26.9)	90 (14.9)	114 (18.8)
Staphylococcus aureus	28	23	15	16
Streptococcus uberis	1	-	4	6
Streptococcus dysgalactiae	1	4	-	2
Non-differentiated non-agalactiae Streptococci	22	15	20	24
Total non-agalactiae Streptococcus	24 (4.0)	19 (3.1)	24 (4.0)	32 (5.3)
Escherichia coli	4	7	8	12
Klebsiella spp.	-	-	1	-
Enterobacter spp.	-	1	2	-
Proteus spp.	-	-	-	-
Citrobacter spp.	-	-	-	-
Pseudomonas spp.	-	1	1	1
Serratia spp.	-	1	-	1
Salmonella spp.	1	-	-	-
Tatumella spp.	-	1	-	-
Non-differentiated gram-negatives	4	3	2	7
Total gram-negatives	9 (1.5)	14 (2.3)	14 (2.3)	21 (3.5)
Arcanobacterium pyogenes	-	-	1	1
Yeast	1	2	1	-
Mold	-	1	-	-
Mixed Major[b]	2	3	2	5
Total Major	64 (10.6)	62 (10.2)	57 (9.4)	75 (12.4)
≤10 CFUs Coagulase-negative staphylococci	130	123	172	189
>10 CFUs Coagulase-negative staphylococci	100	107	39	48
Total Coagulase-Negative staphylococci	230	230	211	237
Corynebacterium bovis	99	88	20	19
Mixed Minor	41	33	11	12

See Table 2-2 for reminder of key.

Table 2-4 Prevalence of IMI by bacterial species from all quarters of all cows grouped by the third sample days in milk (≤3 DIM, ≤5 DIM, and ≤ 8 DIM).

	Days in milk		
	3	5	8
Total Quarters	1193	1989	2771
Staphylococcus aureus	27	47	55
Streptococcus uberis	11	15	17
Streptococcus dysgalactiae	2	6	6
Non-differentiated Streptococci non-agalactiae	35	55	78
Total Streptococcus non-agalactiae	48 (4.0)	76 (3.8)	101 (3.6)
Escherichia coli	18	27	40
Klebsiella spp.	2	1	3
Enterobacter spp.	5	6	7
Proteus spp.	1	1	1
Citrobacter spp.	-	-	1
Pseudomonas spp.	1	5	5
Serratia spp.	1	1	2
Salmonella spp.	-	-	2
Tatumella spp.	-	-	-
Non-differentiated gram-negatives	6	9	12
Total gram-negatives	34 (2.8)	50 (2.5)	73 (2.6)
Arcanobacterium bovis	3	4	6
Yeast	2	4	4
Mold	5	5	5
Mixed Major[b]	4	6	8
Total Major	123 (10.3)	193 (9.7)	252 (9.1)

Table 2-5 Partial results of the generalized linear mixed model (pathogen effects only) for the odds of developing a new IMI during the dry period between the treatments in the Pre-Dry Infected Treatment Group (Internal Teat Sealer and dry cow antibiotic or dry cow antibiotic). Other significant model variables are found in the text.

Model	ITS & DCT (n=604 qtrs)	DCT (n=605 qtrs)	Coefficient	Odds Ratio$_{tx}$ (95% CI)	*P* value
All pathogens	28 (4.6%)	49 (8.1%)	-0.67	0.51 (0.30,0.85)	0.01
Major pathogens	21 (3.5%)	38 (6.3%)	-0.71	0.49 (0.28, 0.89)	0.02
Environmental pathogens	14 (2.3%)	27 (4.5%)	-0.76	0.47 (0.23, 0.93)	0.03
Gram-negative pathogens	4 (0.7%)	8 (1.3%)	-0.75	0.47 (0.13, 1.66)	0.24
Non-agalactiae streptococci pathogens	10 (1.7%)	22 (3.6%)	-0.89	0.41 (0.18, 0.91)	0.03

Table 2-6 Partial results of the generalized linear mixed model (pathogen effects only) for the odds of developing a new IMI during the dry period between the treatments in the Pre-Dry Uninfected Treatment Group (Internal Teat Sealer or dry cow antibiotic). Other significant model variables are found in the text.

Model	ITS (n=774 qtrs)	DCT (n=788 qtrs)	Coefficient	Odds Ratio$_{tx}$ (95% CI)	*P* value
All pathogens	48 (6.2%)	54 (6.9%)	-0.13	0.88 (0.57,1.35)	0.55
Major pathogens	30 (3.9%)	42 (5.3%)	-0.40	0.67 (0.40, 1.12)	0.12
Environmental pathogens	18 (2.3%)	30 (3.8%)	-0.62	0.54 (0.29, 1.02)	0.06
Gram-negative pathogens	9 (1.2%)	15 (1.9%)	-0.70	0.50 (0.20, 1.26)	0.14
Non-agalactiae streptococci pathogens	10 (1.3%)	15 (1.9%)	-0.44	0.64 (0.28, 1.48)	0.30

Table 2-7 Logistic regression models to analyse the odds of having a case of clinical mastitis during the first 60 days of lactation.

Variables	Coefficient	Standard Error	Odds Ratio	95% Confidence Interval of odds ratios	*P* value
Clinical Mastitis caused by all pathogens(n=1195 qtrs)					
Constant	-5.48	0.81			
Treatment					
Dry Cow Antibiotics	ref.				
Teat Sealer	-0.40	0.52	0.67	0.24, 1.86	0.44
Teat Sealer/Dry Cow Antibiotic	-1.13	0.72	0.32	0.08, 1.31	0.11
Group					
Pre-Dry Uninfected	ref.			0.66, 6.19	
Pre-Dry Infected	0.70	0.57	2.01		0.22
Parity	ref.	0.63	4.20	1.22, 14.5	0.02
2^{nd} lactation	1.43	0.62	2.98	0.88, 10.2	0.08
3^{rd}-4^{th} lactation	1.09				
5^{th}+ lactation					
Clinical Mastitis Caused by Environmental Pathogens (n=1190 qtrs)					
Constant	-5.77	0.93			
Treatment					
Dry Cow Antibiotics	ref.				
Teat Sealer	-0.76	0.58	0.46	0.14, 1.44	0.19
Teat Sealer/Dry Cow Antibiotic	-1.27	0.86	0.28	0.05, 1.52	0.14
Group					
Pre-Dry Uninfected	ref.				
Pre-Dry Infected	0.39	0.61	1.48	0.44, 4.90	0.53
Parity	ref.	0.73	6.96	1.63, 29.4	0.01
2^{nd} lactation	1.94	0.75	3.60	0.83, 15.6	0.09
3^{rd}-4^{th} lactation	1.28				
5^{th}+ lactation					

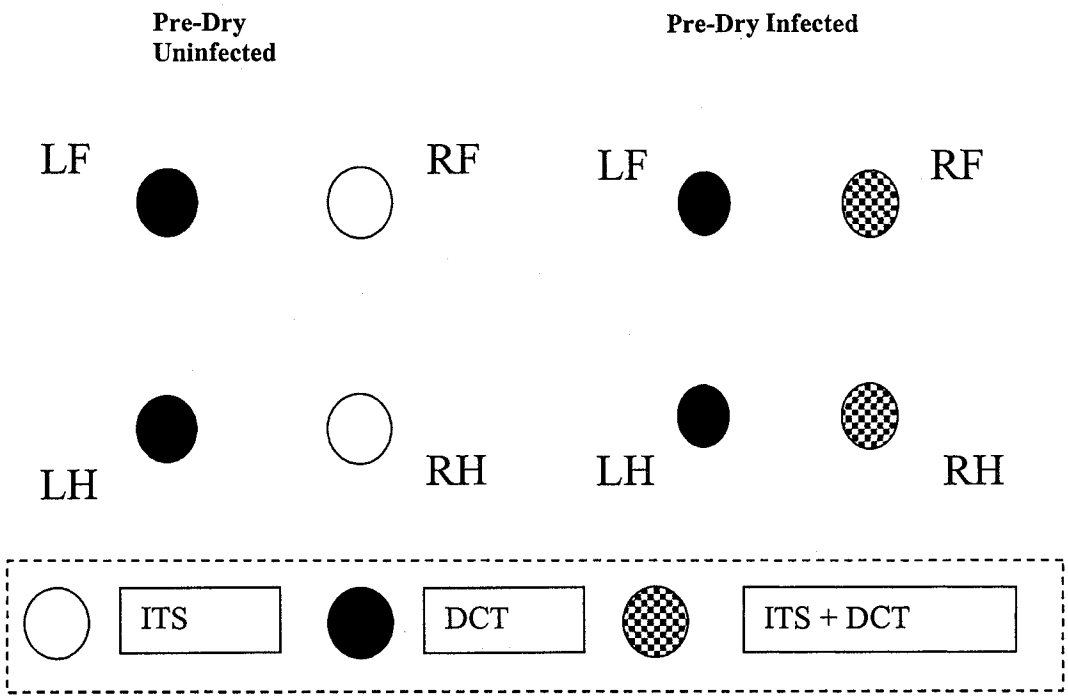

Figure 2-1 An example of treatment allocation (Internal Teat Sealer (ITS), Dry Cow Antibiotic (DCT), or ITS and DCT) by group.

CHAPTER 3 EFFICACY OF AN INTERNAL TEAT SEALER USED IN CONJUNCTION WITH INTRAMAMMARY ANTIBIOTICS FOR THE CURE OF INTRAMAMMARY INFECTIONS DURING THE DRY PERIOD

3.1. Abstract

The objective of this study was to determine if an internal teat sealer used in addition to dry cow antibiotics would have a beneficial effect on the elimination of intramammary pathogens during the dry period. In total, data from 425 culture-positive quarters from 270 Holstein-Friesian dairy cows were utilized in this trial. Milk samples were collected aseptically 2 weeks prior to the anticipated dry-off date, on the dry-off date and again at 1-8 days in milk. Infected quarters were randomly assigned to be treated with both an internal teat sealer and a dry cow antibiotic or a dry cow antibiotic alone. Quarters were defined as cured if the pathogen(s) that were isolated in the dry-off sample were not isolated in the postpartum sample. There was no difference in the overall bacteriological cure between the two treatment groups. Similarly, no significant differences were observed for cure of IMI with either major or minor pathogens during the dry period. Internal teat sealers should be utilized for the prevention of new IMI as they contribute no additional help for the cure of existing IMI.

3.2. Introduction

Dry cow management continues to be an active area of research and has been reviewed extensively (Dingwell et al., 2003). The current North American practice, recommended by the NMC, for the elimination of any existing infections and the prevention of new intramammary infections (IMI), during the dry period is blanket dry cow therapy. In other words, it is recommended to treat all quarters of all cows with an approved intramammary dry cow antibiotic (DCT) (Eberhart, 1986; National Mastitis Council, 1999). Blanket DCT has been successful in decreasing the incidence of new IMI during the dry period, particularly from gram-positive organisms, and also eliminates many existing IMI present at the end of lactation (Schukken et al., 1993; Smith et al., 1985). While dry cow therapy is effective, it has limitations. Therapeutic levels of antibiotics are only present in the mammary gland for a limited period of time, and commercially available DCT in North America is targeted specifically against gram-positive bacteria (Bradley and Green, 2001). As a result, quarters are left unprotected and susceptible to all types of new IMI in the latter part of the dry period, particularly to gram-negative infections.

Cure rates associated with DCT have been studied extensively and vary greatly (Dingwell et al., 2003). Dry cow antibiotic cure rates have been reported to be as high as 94-98% in the case of *Corynebacterium bovis* (Berry and Hillerton, 2002; Huxley et al., 2002) or as low as 20% in the case of resistant *Staphylococcus aureus* (Eberhart, 1986). There are large differences in cure rates when type or strain of pathogen (Sol et al., 2000), or parity (Browning et al., 1994) are considered.

Differences may also be attributed to herd-level factors, cow-level factors, management techniques, antibiotic selection, insertion techniques, and research trial protocols (Boddie and Nickerson, 1986; Dingwell et al., 2002).

Availability of internal teat sealers (ITS) has created a new area of research and has been the topic of multiple clinical trials worldwide over recent years (Woolford et al., 1998; Berry and Hillerton, 2002; Huxley et al., 2002; Godden et al., 2003; Sanford et al., submitted). Internal teat sealers function as inert physical barriers in the teat cistern, with the goal of preventing the penetration of bacteria from the environment into the udder. As a non-antibiotic device designed to seal and protect the teat canal, ITS offer a unique opportunity to dairy producers to modify their dry cow management. ITS can be used alone in non-infected cows as a means to prevent new IMI and decrease the use of DCT. Alternatively, they can be used in conjunction with DCT to provide protection beyond that achieved with DCT alone.

Internal teat sealers have no antibacterial properties. Therefore, ITS would not be expected to have any additional effect on cures of existing pathogens during the dry period. One study, however, found that ITS used alone had a spontaneous elimination rate of 63 and 59% on major and minor pathogens, respectively (Huxley et al., 2002). It is possible that the use of ITS in conjunction with DCT may have some additional benefit beyond the scope of prevention of new IMI. Many cows (and quarters) are dried off abruptly and leak milk for multiple days during the early dry period. This leakage may be caused from the increased pressure in the unmilked mammary gland (Eberhart, 1986). Clinical mastitis developed four times as often in cows that leaked milk after they were dried off when compared with cows that did not

leak (Schukken et al., 1993). One possible explanation of this finding is that leaking can cause the teat canal to be patent and allow entry of bacteria. Also, DCT may have leaked from the gland and have been less effective. The addition of ITS to these quarters possibly prevents or reduces the amount of milk leaking out of the mammary gland, reducing flushing out the DCT. Another hypothesis would be that a second intramammary infusion will induce a stronger local inflammatory response (higher numbers of leukocytes) in the mammary gland, which would secondarily assist the DCT in killing any existing organisms.

Neither Woolford et al. (1998) nor Godden et al. (2003) found a difference between cure rates of quarters treated with DCT versus those treated with both DCT and ITS. Both studies found high cures with the DCT alone (87.0 and 88.2%, respectively), and DCT used in conjunction with ITS (83.0% and 91.3%, respectively). However, there were many management differences between the previous studies examining cure rate and the current study. In New Zealand, three large herds with seasonal calving were used to assess cure rates, where the distribution of dry period pathogens was very different from what is typically seen in Canadian herds (Woolford et al., 1998). While the distribution of dry period pathogens is very similar between the current study and the Minnesota trial, the management practices between the two studies were vastly different. The cows in the latter trial were all housed in one large transition management facility through-out their dry period and had originated from two large dairies, which is different from what is typically seen in Canadian herds (Godden et al., 2003). The main objective of this study was to assess whether using ITS in conjunction with DCT would have any

additional benefit on the percentage of quarters cured during the dry period when compared with DCT alone.

3.3. Materials and Methods

3.3.1. Herd and Animal Selection

A total of 16 herds that were in close proximity to the participating veterinary schools in Canada (PEI, Quebec, and Ontario) and USA (Kansas) were selected. A total of 270 Holstein-Friesian cows provided data from 425 infected quarters (defined below). All milking cows in selected herds were eligible to participate if they had a positive culture result both two weeks prior to dry-off, and again on the day of dry-off. Study cows had to have at least three functional quarters, be confirmed pregnant, and be in good physical condition.

3.3.2. Treatment Allocation and Sample Collection Schedule

Treatment allocation, sampling schedule and bacteriological culture procedures have been previously described (Sanford et al., accepted). In brief, milk samples were taken aseptically from all quarters of all cows at three points in time: two weeks prior to dry-off, at dry-off and after calving (1-8 DIM). A California Mastitis Test (CMT) was performed on the day of dry-off. CMT scores were assigned immediately on a scale of 0 to 3. Cows were defined as infected if a mammary pathogen was isolated from one or more quarters from the milk samples

collected two weeks prior to dry-off. Quarters within these infected cows, were sequentially assigned to one of two treatments in a split udder design. Two ipsilateral quarters were assigned to receive an intramammary infusion of benzathine cloxacillin DCT (DryClox®, Ayerst Veterinary Laboratories, Guelph, Canada) and the remaining two ipsilateral quarters received the same DCT and were also infused with ITS OrbeSeal*, Pfizer Animal Health, Montreal, Canada). For quarters that received both DCT and ITS, the DCT was administered first. Teats were disinfected using a commercial teat dip and then the cows were moved to each farm's dry cow area.

3.3.3. Definitions

Intramammary Infection (IMI): Isolation of any major mastitis causing organism (i.e. *S. aureus, Streptococcus agalactiae*, other *Streptococcal* spp., coliforms, *Arcanobacterium pyogenes, Prototheca* spp., *Proteus* spp., *Citrobacter* spp., or yeast) or > 10 colony forming units (cfu) of a minor mastitis-causing organism (i.e. *C. bovis* or coagulase-negative staphylococci (CNS)), resulted in a quarter being defined as having an IMI. As noted above, cows were classified as infected (for the purpose of treatment allocation) if one or more quarters had IMI at the sampling performed two weeks prior to dry-off. For this study, only quarters which had IMI at the time of drying off were considered in the analysis.

Major pathogen cure: A quarter was defined as cured if all of the major bacteriological pathogens that were isolated at the dry-off sample were not present at the post-calving sample.

Minor pathogen cure: A quarter was defined as cured by isolation of none or ≤ 10 cfu of minor pathogen(s) in a quarter that had previously isolated > 10 cfu of the same organism.

Overall cure: A quarter was defined as cured if all of the pathogens isolated at the dry-off sample were not present at the post-calving sample. A quarter was considered to be cured if a new (different) pathogen was isolated post-calving.

Contaminated sample: A milk sample was considered contaminated if 3 or more different types of pathogens were isolated.

3.3.4. Statistical Analysis

Data were structured in a hierarchical manner. The primary experimental unit was the individual quarter, and quarters were nested within cows, cows were nested within herds, and herds were nested within regions. Only infected quarters (ie. IMI present at dry-off) were considered in this study. A generalized linear mixed model (Rabe-Hesketh et al., 2005) with a binomial family and a logit link was used to analyze risk factors for the probability of a quarter cure during the dry period (Dohoo et al., 2003). Three separate analyses were performed: cure of all pathogens, cure of major pathogens, and cure of minor pathogens. Factors controlled for and considered were quarter, parity, dry-period length, dry-period season, dry-off treatment, CMT score at dry-off, SCC at dry-off (log transformed, lnSCC/1000), housing type, herd

size and all biologically plausible first-order interactions. All analyses included terms to control for clustering of quarters within cow and cows within herd. Unconditional associations were performed to select predictors for each full model. All predictors with a $P < 0.25$ for an unconditional association with the outcome were retained for evaluation in the multivariable model. A backwards step-wise elimination process was utilized for model reduction, using a significance level of $P < 0.05$. The potential confounding effect of factors removed was evaluated by examining the change in the estimate of the treatment effect upon their removal.

3.4. Results

3.4.1. Descriptive Statistics

A total of 210 infected quarters were treated with DCT alone and 215 were treated with both DCT and ITS. After all exclusion criteria were considered (Table 3-1), data from 388 quarters (245 cows) was available for the analysis.

The infected quarters at dry-off were evenly distributed: 96, 101, 98, and 93 from left front, left hind, right front and right hind, respectively. There were three missing scores for the CMT, otherwise 43.3%, 28.4%, 16.6% and 11.6% of the quarters scored 0, 1, 2 and 3, respectively. The dry-off SCC values were missing from 60 of the quarters. Of the remaining 362, mean lnSCC was 5.3 (SD 1.7) ranging from 0-9.21.

Mean parity for the study cows after calving was 3.3 (median 3, range 2-9). Average dry period length was 60 days (standard deviation 17.4; range 28-150) and the dry periods studied were evenly distributed across seasons (winter 22.2%, spring 25.7%, summer 26.1%, fall 26.1%). Average herd size was 123 (median 100, range 40-380). Eight of the study herds used free-stall housing, and the remaining eight herds used tie-stall housing. Pathogens isolated at dry-off, along with their cure rates are summarized in Table 3-2. The total number of infections is higher than the number of quarters in each treatment group because a quarter could have multiple pathogens. Total pathogen cure rates across treatments were 88.7 and 85.3% from the DCT/ITS and the DCT, respectively. No differences were found between treatment groups. The average pathogen cure rates, across treatments, were slightly higher for minor versus major pathogens. Highest pathogen cures were seen for *S. agalactiae* and yeast (100%), although there were few IMI with these organisms at dry-off. The lowest cures were found for the *S. aureus* (76.7 and 75.9% in the DCT/ITS and the DCT treatment groups, respectively).

3.4.2. Quarter Cure of Major Pathogens

Only quarters containing a major IMI at dry-off were considered in this analysis. Quarters containing a mixed major IMI were only considered cured if both pathogens isolated at dry-off were not isolated at the post-calving sample. Unconditional associations between the following predictors and cure of a major pathogen gave values that allowed them to be utilized in model building: quarter (P =

0.06), dry period length (P = 0.04), dry period season (P = 0.03), CMT (P < 0.01), lnSCC (P = 0.09), herd size (P = 0.01), housing (P = 0.19) and parity (P = 0.24). Treatment was again forced into the model. The data from 151 quarters (112 cows) were utilized in the final model as only quarters containing major pathogens were considered. The full multivariable regression model is shown in Table 3-3. No treatment effect was found. Quarters treated with DCT and ITS had an 83.8% cure rate whereas quarters treated with DCT had an 87.9% cure rate. There was no evidence of a cow or herd effect on cure rate as variance estimates were non-significant.

3.4.3. Quarter Cure of Minor Pathogens

A total of 256 quarters from 183 cows were identified as having IMI caused by minor pathogens at dry-off and were considered in the analysis. Unconditional associations between the following predictors and cure of a minor pathogen gave values that allowed them to be utilized in model building: quarter (P = 0.12), dry period length (P = 0.07), and lnSCC (P=0.11). Treatment was forced into the model as well. The full multivariable regression model is shown in Table 3-4. No treatment effect was found. Quarters treated with DCT and ITS had a 92.2% cure rate compared with 87.5% cure rate in the DCT group. No cow or herd effect on cure rate was evident as the variance estimates were non-significant.

3.4.4. Quarter Cure of All Pathogens

Unconditional associations between the following predictors and cure of all pathogens during the dry period gave values that allowed them to be utilized in model building: dry period length (P = 0.19), and CMT (P = 0.25). Treatment was forced into the model as this was the predictor of interest. The data from 388 quarters (245 cows) was utilized in the final model. The full multivariable regression model is shown in Table 3-5. No treatment effect was found. Quarters treated with both DCT and ITS had a 79.6% cure rate compared to 78.9% of quarters treated with DCT alone. Overall quarter cure rates were lower than quarter cures for either major or minor pathogens. A quarter could contain up to two pathogens and both had to be eliminated to be considered in this category. There was no evidence of either a herd or cow effect on the overall cure rates (both variance estimates were non-significant).

3.5. Discussion

No additional benefit in percentage of quarters cured was found when using ITS in addition to DCT when compared with DCT used alone. Previous ITS studies also found no additional cure benefit to using both ITS and DCT (Woolford et al., 1998; Godden et al., 2003). The cure rates found in this study are consistent with previous ITS literature and were high (Woolford et al., 1998; Godden et al., 2003). Both major and minor pathogens had similar quarter cure rates. The overall quarter cure rate was lower than both the major and minor quarter cure. The cure of multiple pathogens in a quarter is less likely to occur than the cure of single pathogens.

Parity, dry period length, dry period season, quarter, housing, herd size or SCC at dry-off were not found to have any impact on cure rates, no matter whether all, major or minor pathogens were considered. Some of these variables had significant unconditional associations with cure, but these associations were not evident in the full model. Only one DCT was evaluated, so all estimations of cure are only relevant to this product.

Achieving the objectives of this study relied upon the determination of quarter IMI status at the time of calving. Quarter cure was defined by using a single milk bacteriological culture to isolate a pathogen from a quarter post-calving that was previously isolated at dry-off. Using one sample postpartum will overestimate cure rates because of the high false negative rate (i.e. quarters which were still infected but from which the organism was not isolated). This is particularly true when assessing *S. aureus*. Previous research has concluded that multiple cultures are required to specify

a cure of this pathogen (Dingwell et al., 2002). Therefore, the current study has overestimated the cure of *S. aureus*, although this exaggeration would not be expected to be different across treatment groups. Whilst multiple milk samples used in serial will increase the chance of detecting a pathogen (increase sensitivity) there will be an associated decrease in specificity (more false positives) due to the isolation of organisms from infections which developed after calving. Regardless of the number of samples taken errors will occur. Hence some misclassification bias, will be present. However, this bias would be expected to be the same in both of the treatment groups, and therefore it would be non-differential misclassification (Dohoo et al., 2003). When dichotomous (yes/no) outcomes are measured, non-differential misclassifications always biases the measure of association, in this case odds ratio, towards the null (Dohoo et al., 2003). Therefore, the estimates of treatment effects from our data would be conservative, but our estimates of quarter cure would be exaggerated. However, it is unlikely that the bias of treatment effect toward the null would have accounted for the complete lack of evidence of any treatment effect if even a modest effect had been present. Some previous ITS studies have also used one sample post-calving, and emphasized that care must be taken when interpreting results for cure (Huxley et al., 2002; Godden et al., 2003).

In conclusion, ITS used in conjunction with DCT did not have a beneficial effect on quarter cure when compared with using DCT alone. It should be emphasized that the primary use of ITS is for the prevention of new IMI during the dry period. The device has no antibacterial properties and therefore proper aseptic technique must be used when infusing this product into the mammary gland.

3.6. Acknowledgements

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Table 3-1 Description of exclusion criteria and number of quarters affected with treatment groups (Internal Teat Sealer (ITS) and Dry Cow Therapy (DCT) or DCT).

	ITS/DCT (n = 215 qtrs)	DCT (n = 210 qtrs)
No post-calving sample	13	9
Additional dry-off antibiotics	5	3
Additional post-calving antibiotics	4	3
Total remaining quarters	193	195

Table 3-2 Distribution of pathogens between treatment groups at dry-off and proportion of pathogen cures from 388 quarters.

	DCT / ITS		DCT	
	Dry	Cure (%)[1]	Dry	Cure (%)[1]
Major pathogens				
Staphylococcus aureus	34	26 (76.5)	29	22 (75.9)
Streptococcus agalactiae	0	-	2	2 (100.0)
Other streptococci	27	25 (92.6)	27	24 (88.9)
Gram negatives	17	14 (82.4)	19	18 (94.7)
Yeast	1	1 (100.0)	2	2 (100.0)
Mixed major[2]	2	1 (50.0)	6	2 (33.0)
Total major	79	66 (83.5)	79	68 (86.1)
Mixed major and minor	13	11 (84.6)	6	4 (66.7)
Minor pathogens				
Coagulase-negative staphylococci[3]	124	113 (91.1)	127	108 (85.0)
Corynebacterium bovis[3]	9	9 (100.0)	5	4 (80.0)
Mixed minor	4	4 (100.0)	5	4 (80.0)
Total minor	133	122 (91.7)	132	112 (84.8)
Overall total pathogens[4]	212	188 (88.7)	211	180 (85.3)

[1]Cure percentages based on a single milk sample, therefore overestimated.
[2]Quarter containing 2 major pathogens
[3]> 10 cfu
[4]Pathogen cures calculated differently from quarter cure rates (in text)

Table 3-3 Logistic regression model analysis of odds of curing an intramammary infection caused by major pathogens during the dry period (n = 151 quarters) in 112 cows.

Variables	Coefficient	Standard Error	Odds Ratio	95% Confidence Interval of odds ratios	*P* value
Fixed effects					
Constant	1.99	0.47			
Treatment					
Dry Cow Antibiotic					
Internal Teat Sealer/Dry Cow Antibiotic	ref.				
	-0.35	0.50	0.71	0.27, 1.86	0.48
	Variance	Standard Error[a]			
Random effects					
Herd	0.71	0.63			
Cow	<0.01	<0.01			

[a] Standard error of estimate of variance component

Table 3-4 Logistic regression model analysis of odds of curing an intramammary infection caused by minor pathogens during the dry period (n = 256 quarters) in 183 cows.

Variables	Coefficient	Standard Error	Odds Ratio	95% Confidence Interval of odds ratios	*P* value
Fixed effects					
Constant	1.95	0.44			
Treatment					
Dry Cow Antibiotic	ref.				
Internal Teat Sealer/Dry Cow Antibiotic	0.52	0.42	1.68	0.74, 3.83	0.22
	Variance	Standard Error[a]			
Random effects					
Herd	<0.01	<0.01			
Cow	0.21	0.94			

[a] Standard error of estimate of variance component

Table 3-5 Logistic regression model analysis of odds of curing an intramammary infection caused by all pathogens during the dry period (n = 388 quarters) in 245 cows.

Variables	Coefficient	Standard Error	Odds Ratio	95% Confidence Interval of odds ratios	*P* value
Fixed effects					
Constant	1.32	0.25			
Treatment					
Dry Cow Antibiotic	ref.				
Internal Teat Sealer/DryCow Antibiotic	0.04	0.26	1.04	0.62, 1.75	0.87
	Variance	Standard Error[a]			
Random effects					
Herd	<0.01	<0.01			
Cow	0.85	0.63			

[a] Standard error of estimate of variance component

CHAPTER 4 THE IMPACT OF ADMINISTRATION OF AN INTERNAL TEAT SEALER ON TEAT CLOSURE, SOMATIC CELL COUNT AND PARTICLE RECOVERY IN EARLY LACTATION

4.1. Abstract

The objectives of this study were to determine the impact of an internal teat sealer (ITS) applied at dry-off on teat closure mid-dry period, and on post-calving somatic cell counts (SCC) and particle recovery in milk for seven days postpartum. Thirty Holstein cows were enrolled in this trial. Milk samples were taken aseptically starting at the first morning milking post-calving and continuing for seven morning milkings postpartum. The SCC was lower in quarters that were treated with an ITS, either alone or in combination with dry cow antibiotics, than those treated with dry cow antibiotic therapy alone. This difference was marginally significant on the first two days post-calving. Teat ends classified as closed had significantly lower SCC than open teats on the fourth day postpartum. More particles were recovered from the quarters treated with an ITS than those treated with dry cow antibiotics. This difference was significant on the first day post-calving, but subsequently, the level of particle recovery was similar between the treatment groups. In conclusion, there are no significant effects of ITS on dry period teat-closure and early lactation SCC and particle recovery.

4.2. Introduction

Somatic cell count (SCC) has been accepted as a standard method to monitor udder health status in dairy cattle for many years and is the most common indirect measure of mammary gland inflammation. In addition to inflammation, SCC is affected by various factors including: parity, days in milk (DIM), season, stress, diurnal variation, day to day variation, as well as management (Dohoo and Meek, 1982).

With respect to DIM, SCCs are elevated immediately after calving. The period of time post-calving before SCCs return to normal has been estimated to be from 5 to 35 days depending on the study (Dohoo and Meek, 1982; Dohoo, 1993; Barkema et al, 1999; Sargeant et al, 2001). Uninfected quarters have a much more rapid decrease in SCC than those quarters with intramammary infections (IMI) (Dohoo and Morris, 1993; Barkema et al, 1999; Sargeant et al, 2001).

An internal teat sealer (ITS) made of 25% bismuth subnitrate was studied in Ireland in 1977 in several small research trials to test the efficacy of the product for the prevention of new IMI during the dry period (Meaney, 1977). This paste-like product has since been modified to its current formulation, OrbeSeal* (Pfizer Animal Health, Montreal, Quebec) containing 65% bismuth subnitrate. This product has been the subject of many research trials worldwide over the last five years (Woolford et al., 1998; Huxley et al., 2002; Berry and Hillerton, 2002; Godden et al., 2003; Sanford et al., submitted). The ITS acts as a physical barrier in the lower teat cistern during the

dry period, and remains in place until it is either stripped out by hand, by the calf or by the milking machine.

A natural physical barrier composed of a teat-canal keratin plug forms in the non-lactating cow. This coupled with mammary gland involution provides enhanced protection against microorganism invasion, thereby making the udder resistant to dry period IMI (Capuco et al., 1992; Williamson et al., 1995). Partial removal of teat-canal keratin increased quarter susceptibility to IMI with bacterial challenges of *Streptococcus agalactiae* (Capuco et al., 1992). Several studies have highlighted some limitations of the dry period keratin plug formation (Williamson et al., 1995; Dingwell et al., 2004). The vast majority (97%) of clinical mastitis cases identified while cows were dry occurred in open teats and, in total, between 3-5% of all quarters did not close up to 90 days during the dry period (Williamson et al., 1995). The early formation of a keratin plug was associated with a lower incidence of new IMI. One study found that higher milk production at dry-off had a negative impact on teat closure and that at six weeks dry 23% of quarters had still not closed (Dingwell et al., 2004). With the evidence of delayed or even non-existent teat end closure, the question arises as to whether ITS mimics the keratin plug and mediates its effects through this manner.

While there is an increasing volume of literature on the efficacy of ITS for the prevention of new IMI during the dry period, there is limited information available on their impact on SCC in the immediate postpartum period. Godden et al. (2003) measured the impact of ITS on cow-level linear scores (LS) at 1-3 and 6-8 days in milk. The treatment groups (DCT alone and ITS and DCT) had similar LS at dry-off,

but the ITS and DCT quarters had significantly lower LS than DCT at 1-3 and 6-8 days post-calving.

A valid concern of many dairy producers and veterinarians regarding the use of an ITS is that particulate material may be found in the milk following calving. Both Woolford et al. (1998) and Berry and Hillerton (2002) reported that the sealer was found in the foremilk of all treated cows following calving. Although neither study reported particle observation as a serious or widespread concern, flecks of sealer were observed from several days (Woolford et al., 1998) to three weeks (Berry and Hillerton, 2002) post-calving. Particles of residual sealer would be a concern because of the potential for them to cause problems by fouling sensitive milking equipment. To date, no research has been conducted to quantify the length of time that particles are recovered during the postpartum period.

The objectives in this study were three-fold. The first objective was to evaluate the impact of administration of an ITS on udder health status, as measured by quarter-level SCC following calving in the first four days postpartum, since milk becomes available for commercial sale on the fourth day. The second objective of our research was to assess the particle recovery pattern, and to determine whether there was a difference in particle recovery in the first four days postpartum, between quarters that had received an ITS at dry-off and quarters that were treated with a standard DCT. As recent research has highlighted some limitations of keratin plug formation, the third and final objective of this study was to measure teat closure midway through the dry period and assess whether administration of ITS had an impact on closure, and in turn whether closure had any impact on SCC postpartum.

4.3. Materials and Methods

4.3.1. Animal Selection

A total of 30 lactating Holsteins-Friesian cows in good health and with at least three functional quarters were selected from the Elora Research herd at the University of Guelph, Ontario. Cows were enrolled as their dry-off date approached until 30 animals were recruited. Two weeks prior to the anticipated dry-off date, quarter milk samples were taken aseptically before milking for bacteriological culture and SCC. All samples were taken following the routine pre-milking udder preparation, and before the milking units were applied. A determination of the infection status of the animal was made based on the most current guidelines set forth by the National Mastitis Council (NMC, 1987).

4.3.2. Treatment Allocation and Administration

Cows were assigned to one of two groups based on milk sample bacteriology results taken two weeks prior to dry-off. Milk bacteriology technique has been previously described in detail (Sanford et al., accepted). Cows with no quarter IMI were assigned to the Pre-Dry Uninfected Group (PD-Uninf). Quarters of cows in the PD-Uninf were randomly assigned to receive an ITS (OrbeSeal*, Pfizer Animal Health, Montreal, Canada) in two ipsilateral quarters and DCT (DryClox®, Ayerst Laboratories, Montreal, Canada) in the other two ipsilateral quarters, forming two

treatment groups (PD-Uninf ITS and PD-Uninf DCT). Cows with one or more quarters that cultured positive at the milk sample taken two weeks prior to dry-off were assigned to the PD-Infected Group (PD-Inf). Quarters in cows in the PD-Inf were randomly assigned to receive the ITS in two ipsilateral quarters following infusion of all quarters with DCT, forming an additional two treatment groups (PD-Inf ITS/DCT and PD-Inf DCT). All treatments were administered after the final milking.

At the day of dry-off, quarter milk samples were taken aseptically before milking and sent to the laboratory for SCC determination. A Bentley SomaCount 300 (Bentley Instruments Inc., Chaska, MN) was used for SCC determination. After the cow was milked, and following aseptic preparation of the teat ends, the treatments were administered. The DCT was infused and massaged up into the mammary gland in a routine manner, whereas the ITS was infused without subsequent massage. Post-milking teat disinfectant was applied, as per the usual farm practice, and the cow was moved into the dry-cow housing area.

4.3.3. Dry Period Evaluation

Approximately midway through the dry period (35 days dry) quarters of enrolled cows were evaluated for teat closure using the manual technique described by Williamson et al. (1995). The teat sinus was gently grasped using the index and third finger and a slight pressure was applied simulating a slow milking action. A teat was defined as closed if no secretion appeared at the teat end in response to this

manual manipulation. All these assessments were performed by the same individual, who was blinded to the treatments and who had been involved with a previous study assessing teat closure (Dingwell et al., 2004)

4.3.4. Post-Calving Sampling

Beginning at the first morning milking post-calving and proceeding through the seventh morning milking, quarter milk samples were taken aseptically and sent to the laboratory for SCC analysis and particle recovery determination. All samples were taken following the routine pre-milking udder preparation and before the milking units were applied. Particle recovery determination was performed at the laboratory by pouring a 15mL milk sample into a stainless steel mesh screen of 21 gauge (0.83mm x 0.83mm) pores. The laboratory personnel were blinded to the treatment assignment. The strainer was rinsed with cold water three times. After the final wash, a visual assessment of the strainer was made and a dichotomous (presence or absence) score was assigned for particle observation.

4.3.5. Statistical Analysis

Teat closure data consisted of measures taken at the quarter level which were nested within sides (ipsilateral groups) and cows. A generalized linear mixed model with a binomial family and a logit link was used to assess if treatment with ITS had an affect on teat closure using cow as a random effect (Rabe-Hesketh and Skrondal, 2005).

Both the SCC and particle recovery data consisted of repeated measures performed over seven days on the same quarters, which furthermore were hierarchically nested within sides (ipsilateral groups) and cows. Initial descriptive analyses showed the variations to differ considerably between the test days. Accounting for such heteroscedasticity in a combined analysis for all test days would require complex statistical modeling, and a simpler approach was preferred in which data for each test day were analyzed separately. To account for the multiple analyses, the p-values were multiplied by 4 (Bonferroni correction), corresponding to the primary hypotheses involving days 1-4 post-calving. This approach to repeated measures data does not lead to bias in the treatment estimates, but does imply some loss of power.

At each test day, the SCC data were analyzed using a linear mixed model for repeated measures and the particle recovery data were analyzed using a generalized estimating equation (Dohoo et al., 2003). Somatic cell counts were converted to LS for the analyses (National Mastitis Council, 1996). These models contained random effects for the two hierarchical levels (side and cow), as well as fixed effects of treatment, quarter, teat closure and parity; the analysis of particle recovery data did not include the teat closure data, but also included LS at test day as a covariate. The four treatments (PD-Uninf ITS, PD-Uninf DCT, PD-Inf ITS/DCT, PD-Inf DCT) were expanded into main effects of ITS treatment (yes/no), pre-dry infection group and their interaction. Three parity groups (2^{nd}, 3^{rd}, and 4^{th} plus) were formed. Furthermore, all first-order interactions were included. A backwards model selection was performed, using a significance level of $P < 0.05$. The LS values at dry-off were

analyzed along similar lines as above using a linear mixed model with cow random effects and treatment groups as the sole fixed effect. For presentation purposes, the particle recovery data were combined into two treatment groups (ITS and non-ITS). The analyses of SCC and particle recovery data were carried out using commercial statistical software (proc mixed in SAS 8.02, and the xtgee in Stata 8.0, respectively).

4.4. Results

4.4.1. Teat Closure Data

Teat closure data were available from 111 quarters in 28 study cows. One cow had a blind right front quarter. In total, 83.8% of all quarters were closed by day 35 dry. There was no significant difference between the percentage of quarters that closed in those treated with an ITS (87.5%) and quarters treated with DCT (80.0%).

4.4.2. Somatic Cell Counts

A total of 30 cows were initially used for this study. One cow was excluded from the analyses as the proper treatment protocol was not followed. The mean lactation number for the study cows was 2.8 (median = 3) ranging from 2 to 6. Fifteen of the study cows were PD-Uninf and fourteen cows were PD-inf. A total of thirty quarters were included in each of the PD-uninf treatment groups (PD-Uninf ITS and PD-Uninf DCT). A total of twenty-eight quarters were included in each of the

PD-inf treatment groups (PD-Inf ITS/DCT and PD-Inf DCT). During each of the seven collection days, there were some missing samples as a result of cows or quarters that were missed or SCCs that could not be read by the machine. Least square means of the LS by treatment group and teat closure can be found in Tables 4-1 and 4-2, respectively. Both the PD-Inf ITS/DCT and the PD-Inf DCT quarters had significantly higher SCC at dry-off than the uninfected cows. There were no other significant differences in the means at the time of enrollment.

The LS data are shown graphically in Figure 4-1 and 4-2, respectively. While all groups experienced a similar decline in SCC over the time period studied, a marginally significant treatment effect of ITS was seen on the first two days post-calving (Bonferroni-corrected $P = 0.08$). On the fourth day post-calving, quarters that were classified as closed during the dry period had significantly lower LS than open teats (Bonferroni-corrected $P < 0.01$).

4.4.3. Particle Recovery

Figure 4-3 shows the daily proportion of quarters from which particles were recovered by treatment group (ITS or non-ITS). The quarters treated with an ITS had a steep decline in the proportion from which particles were recovered over the first three days post-calving. The proportion for the non-ITS treated quarters remained relatively constant over the time period. Table 4-3 provides the results of the separate day analysis for particle recovery. There was a significant difference in the proportion of quarters from which particles were recovered in ITS (40%) and in non-

ITS (21%) treated quarters on the first day (Bonferroni-corrected $P = 0.04$). Dry-off infection status and other variables were not significant. On day two there was a significant difference in the proportion of quarters from which particles were recovered between infected (7%) and uninfected (40%) quarters (Bonferroni-corrected $P = 0.04$). There were no other significant effects or interactions including ITS use on day two.

4.5. Discussion

This is the first study to assess the impact of administration of an ITS on teat closure in the dry period, and on SCC and particle recovery in the early postpartum period. Somatic cell counts are elevated during the immediate postpartum period and then steadily decline over the first 10 days of lactation (Sargeant et al., 2001). Our results are consistent with these findings. The quarter LS was elevated immediately postpartum, followed by a steep decline over subsequent days (Figure 4-1). We are unable to comment on whether further declines occur beyond the first week postpartum.

There were differences in LS in the four treatment groups at dry-off. The quarters from the PD-Inf cows had significantly higher SCC than those cows in the PD-Uninf. This finding was expected. The treatment groups from infected cows consisted of a portion of culture negative and culture positive quarters, whereas the treatment groups from uninfected cows consisted solely of culture negative quarters at the time of treatment allocation.

As milk is not available for sale commercially until day four postpartum, the study period was divided into two times of interest, days 1-4 and days 5-7. The focus was to determine whether the SCC and particle recovery would be comparable across all treatment groups, by day four postpartum. Following calving, the PD-Inf ITS/DCT group had lower SCC than the other three treatment groups. This finding is supported by the results of our large clinical trial that determined that quarters treated with both an ITS and DCT acquired significantly fewer new IMI during the dry period than did quarters treated with either ITS or DCT alone (Sanford et al., accepted). In this small dataset, while the trend is consistent throughout the postpartum period, the difference between ITS-treated and control quarters were significant only on the first two days after calving. Somatic cell counts taken five to seven days post-calving seemed to level off compared to the steep decline seen from day one to five. The pattern of declining SCC appeared to remain the same no matter what the dry-off treatment entailed.

There was no significant treatment effect on teat closure during the dry period although the quarters treated with the ITS tended to have better closure rates. However, with the relatively small sample size, there was not enough power to detect a difference between the treatments. The method utilized to assess the closure status of the teat end involved applied gentle downward pressure on the teat in an attempt to visualize any secretions. Due to the physical nature of the ITS material, it was possible to expose some of this material at the teat end which could have been mistaken for secretory material. Therefore, the teat closure rate in the ITS treated quarters may have been underestimated.

Closed teats consistently had lower LS post-calving than open teats but this difference was only significant on the fourth day postpartum. Closed quarters may have been better protected from new IMI than open teats which may have accounted for their lower LS. Between 16-26% of quarters treated with DCT were not closed at 35 days dry which is consistent with Dingwell et al. (2004) who found that 23% of quarters treated with DCT did not close. In contrast, our findings were not consistent with Williamson et al. (1995) who found that treating quarters with DCT facilitated teat closure, although this study compared DCT with negative controls.

Particle recovery was significantly higher in quarters treated with an ITS than in quarters treated with DCT on the first day post-calving. This makes biological sense because the inert paste-like material is not absorbed and must be removed from the teat cistern following calving. The majority of the ITS appears to be discharged by the initial hand stripping. However, there remain flecks of material which are removed in subsequent milkings. Figure 4-2 shows the rapid decline in the particle recovered in ITS-treated quarters over the first three days postpartum. Subsequently, the level of particle recovered in both treatment groups seemed to remain fairly constant throughout the remainder of the week. This trend is not seen in the DCT treated quarters which appear to remain fairly constant through out the first week postpartum. All fresh quarters had some amount of particles recovered. Further analysis on the particles recovered from DCT treated quarters was not performed, however it is plausible that milk clots or colostrums may have caused the particulate matter.

The milk samples for the study were taken pre-milking, immediately after udder preparation. As such, they should contain the greatest amount of particulate matter. However, to decrease the amount of particles entering the milk line, producers using an ITS are encouraged to strip out the sealer prior to first milking. In the current study, there were no problems observed with interference with milking machine function.

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Table 4-1 Least square means of linear score values between treatment groups at dry-off and in the first seven days post-calving.

	Pre-Dry Uninfected				Pre-Dry Infected				
Day	Internal Teat Sealer		Dry Cow Antibiotic		Internal Teat Sealer/ Dry Cow Antibiotic		Dry Cow Antibiotic		
	Mean	SE[1]	Mean	SE	Mean	SE	Mean	SE	*P*-value for ITS[2]
Dry-off	0.72	0.49	0.56	0.48	2.17	0.50	2.33	0.50	
1	4.92	0.52	5.54	0.48	4.45	0.53	5.80	0.50	0.02
2	5.02	0.56	5.44	0.52	4.16	0.54	4.80	0.51	0.02
3	3.56	0.72	4.84	0.67	2.52	0.69	3.26	0.65	>0.50
4	4.45	0.70	4.42	0.66	2.51	0.70	3.15	0.66	0.34
5	1.99	0.74	2.86	0.67	1.64	0.69	2.93	0.63	0.29
6	2.20	0.78	2.99	0.71	1.43	0.76	2.65	0.72	0.11
7	1.59	0.72	1.70	0.66	2.44	0.75	3.33	0.71	>0.50

[1] Standard Error

[2] Uncorrected for multiple day analyses, see text for corrected *P*-values

Table 4-2 Least square means of linear score values between closed and open teats (at day 35 of dry period) during the first seven days post-calving.

	Teat Closure				
	Closed		Open		
Day	Mean	SE[1]	Mean	SE	*P*-value[2]
1	4.97	0.32	5.56	0.43	0.10
2	4.69	0.34	5.00	0.45	0.39
3	3.59	0.41	3.86	0.57	>0.50
4	2.80	0.42	4.45	0.58	0.001
5	1.83	0.39	3.18	0.59	0.02
6	1.90	0.46	2.90	0.65	0.08
7	2.39	0.43	2.36	0.60	>0.50

[1] Standard Error

[2] Uncorrected for multiple day analyses, see text for corrected *P*-values

Table 4-3 Generalized estimating equation model coefficients for particulate matter recovery from quarters treated with Internal Teat Sealer for the first seven days post-calving (reference values were non-ITS treated quarters).

	Internal Teat Sealer Treated Quarters		
Day	Coefficient	Standard Error	*P*-value[1]
1	1.82	0.70	0.01
2[2]	0.94	0.64	0.14
3	1.25	0.57	0.03
4	0.35	0.66	0.59
5	1.58	1.01	0.12
6	0.89	0.83	0.29
7	-0.14	0.56	0.80

[1] Uncorrected for multiple day analyses, see text for corrected *P*-values
[2] Uninfected quarters (Coefficient -3.33, SE 1.29, *P*-value 0.01)

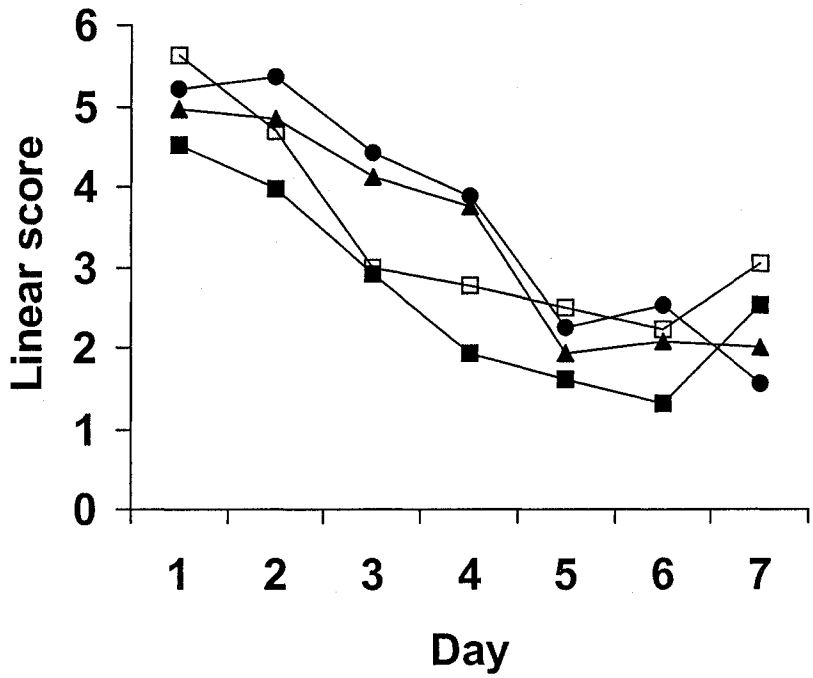

Figure 4-1 Graphical comparison of Linear Score in PD-Inf ITS/DCT (■), PD-Inf DCT (□), PD-Uninf ITS (▲) and PD-Uninf DCT(●) quarters for the first seven days post-calving.

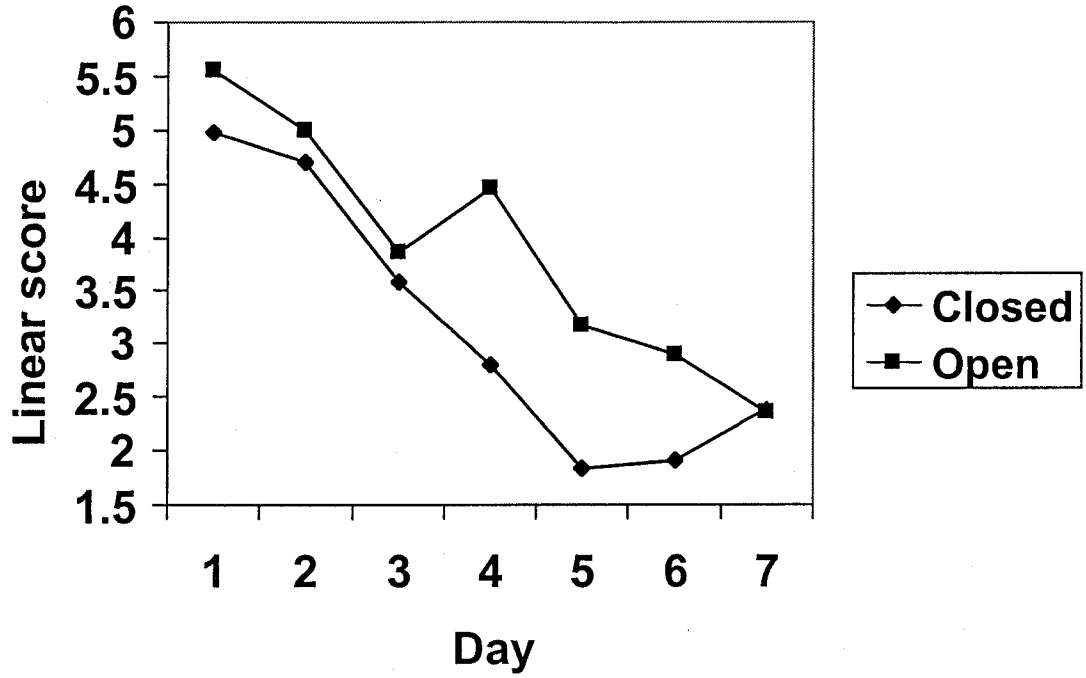

Figure 4-2 Graphical comparison of Linear Score in closed(♦) and open (■) teats during the first seven days post-calving.

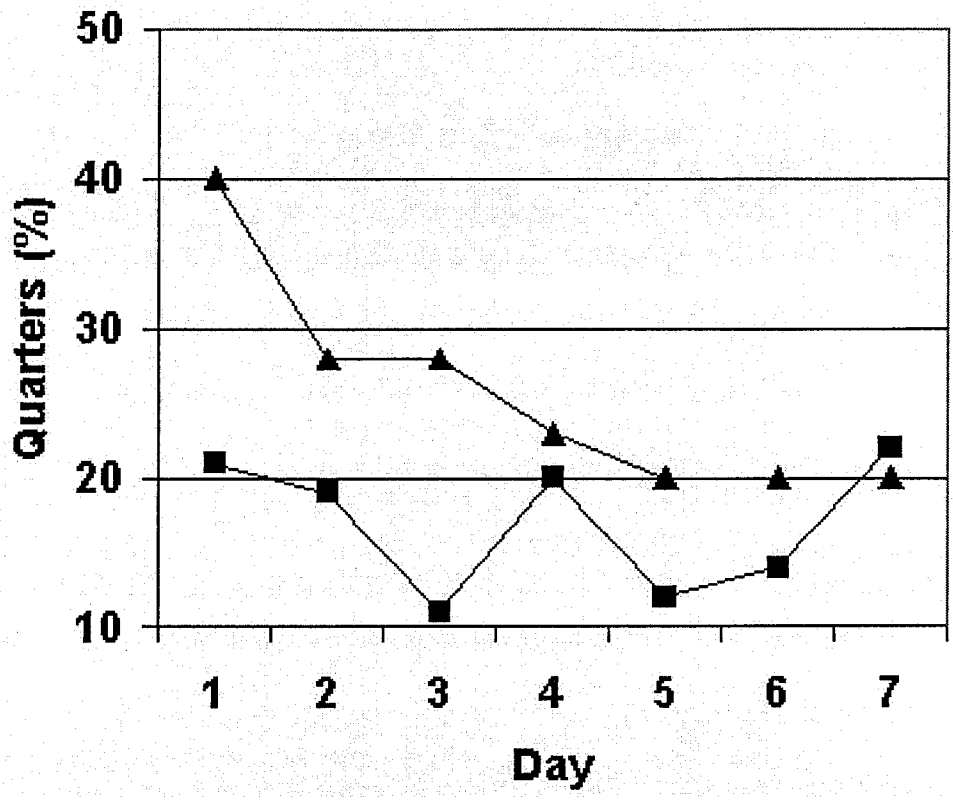

Figure 4-3 Percentage of quarter milk samples in the ITS(▲) and non-ITS(■) treated groups from which particulate material were recovered during the first seven day post-calving.

CHAPTER 5 EFFECT OF AN INTERNAL TEAT SEALER ON MILK PRODUCTION, SOMATIC CELL COUNT, AND CLINICAL MASTITIS IN DAIRY COWS IN CANADA

5.1. Abstract

A minimally monitored study measuring the effects of internal teat sealers (ITS) on milk production, somatic cell count (SCC) and clinical mastitis (CM) was conducted across Canada. A total of 1296 Holstein-Friesian cows were enrolled in the trial. The study protocol, with instructions for treatment allocation was distributed to herd veterinarians. Together with the producers, veterinarians randomized cows into four different treatment groups, "Group 1-DCT", "Group 1-ITS", "Group 2 -DCT", and "Group 2 -DCT/ITS" based on a two-level decision. Group allocation (1 or 2) was based on SCC and CM history (ie low SCC and no recent history of CM were assigned to Group 1), and within-group intramammary treatment was based on ear tag numbers (odd or even). Producers recorded the presence or absence of CM during the first 30 days post-calving. Dairy Herd Improvement records were used to monitor milk production and SCC for the first 180 days of lactation. Cows from both groups 1 and 2, treated with ITS produced significantly less milk (-1.53kg/day) during the first 6 months of lactation. Cows in group 2 had higher SCC during the first 6 months of lactation than cows in group 1. However, there were no additional differences attributed to ITS. Cows in group 2 had significantly more cases of CM in early lactation than cows in group 1, but there were no additional differences attributed to ITS. Results of the current study were unexpected. There appears to be no obvious explanation for the decrease in milk production associated with ITS administration.

5.2. Introduction

Mastitis continues to be the most costly disease in the dairy industry (Fetrow et al., 2000; Dingwell et al., 2003). Mastitis (subclinical or clinical) in early lactation results in larger production losses than intramammary infections (IMI) in later stages (Fetrow et al., 2000; Wilson et al., 2004). It has been estimated that the bulk of this loss (70%) is due to decreased milk production from subclinically infected animals. The remaining economic losses (30%) are a composition of treatment costs, culling, and decreased productivity due to clinical mastitis (CM) (Fetrow et al., 2000). One study estimated that second lactation and older cows, lose 598 kg of milk due to a CM event (Wilson et al., 2004). Therefore, cows with IMI generate economic losses that are generally underestimated because it is difficult to calculate losses that can not be readily seen.

The dry period is a high risk period for the development of new IMI (Eberhart, 1986). These IMI are often carried forward into the subsequent lactation and result in decreased milk production (Beck et al., 1992). In addition, IMI established in the dry period, are unlikely to become clinical at that time, but there is an increased chance that these infections will become clinical in early lactation (Green et al., 2002). One study used DNA fingerprinting to determine that CM in early lactation was the result of IMI that were established during the dry period (Bradley & Green, 2000). Clinical mastitis occurring in early lactation is extremely frustrating and costly to the dairy producer, as it can have many negative effects throughout the remainder of the lactation.

Several studies have now shown that internal teat sealers (ITS) can help protect the udder from the development of new IMI during the dry period (Woolford et al., 1998; Berry and Hillerton, 2002; Huxley et al., 2002; Godden et al., 2003; Sanford et al., submitted). One study found that the addition of ITS to dry cow antibiotic therapy (DCT) decreased the incidence of new IMI from 29.1% to 22.8% (Godden et al., 2003). Another study found that quarters treated with ITS when compared with DCT acquired significantly fewer IMI caused by all major pathogens combined (Huxley et al., 2002). As IMI negatively impact milk production, the administration of ITS, mediated through decreased new IMI, would be expected to reduce this loss, resulting in higher yields in treated quarters (compared to non-treated quarters).

No large clinical trials have been published to date to examine the effects of ITS on milk production. Hassfurther et al. (2003) conducted a small trial of 30 cows which were treated with ITS either alone or in conjunction with DCT. When milk production was measured for 20 days postpartum, there was no treatment effect. Another study compared the arithmetic mean yield production between 82 cows treated with ITS and 81 negative-control cows (Berry et al., 2004). Although the cows treated with ITS had higher milk yields (7845 liters) than the negative control cows (7636 liters), this difference was not significant. Results of both these studies are interesting, but a large-scale study to measure the effects of ITS on milk production is warranted. Milk production comparisons between cows treated with ITS and cows treated with DCT would have greater external validity in North America, where blanket DCT is commonly practiced.

Little has been published regarding the effect of ITS on SCC in the subsequent lactation. Godden et al. (2003) measured the impact of ITS on cow-level linear scores (LS) at 1-3 and 6-8 days in milk. Milk from both treatments (DCT alone and DCT/ITS) had similar LS at dry-off, however milk from DCT/ITS treated quarters had significantly lower LS on days 1-3 and 6-8 post-calving when compared to milk from DCT-treated quarters. Another study measured the effect of ITS on SCC during the first week post-calving and found that quarters treated with ITS had marginally lower SCC during the first two days postpartum, than quarters treated with DCT (Sanford et al., accepted). Both previous studies measured the effect of ITS on SCC over a short time period. Investigations into the effects of ITS on SCC in the subsequent lactation, on a longer time scale, are warranted.

The impact of ITS on CM in the subsequent lactation has also been documented (Woolford et al., 1998; Berry and Hillerton, 2002; Huxley et al., 2002; Godden et al., 2003; Sanford et al., accepted). The results of these studies are inconsistent. In one study, when ITS used alone or in conjunction with DCT, were compared with a negative control, significant reductions in the incidence of CM were found (Woolford et al., 1998). However, a similar study comparing ITS with negative-control quarters found no difference (Berry and Hillerton, 2002). Furthermore, when ITS alone were compared with DCT, no difference in the incidence of CM was detected (Woolford et al., 1998; Huxley et al., 2002; Sanford et al., accepted). In studies that compared the efficacy of ITS and DCT used in combination, with DCT alone, one study found a significant reduction in the

development of CM (Godden et al., 2003), whereas others found no difference between treatments (Cook et al., 2004; Sanford et al., accepted).

Due to the lack of milk production and SCC data in the literature, and to clarify the effect of ITS on clinical mastitis, a large Canadian minimally monitored study was designed. The objectives of this study were to assess the effects of producer-administered ITS, used alone and in conjunction with DCT, on milk production and SCC during the first six months (180 days) of lactation, and to measure the incidence of CM during the first 30 days post-calving. In addition, the efficacy of a minimal intervention clinical trial was assessed.

5.3. Materials and Methods

5.3.1. Herd and Cow Selection

The study coordinator and industry representatives conducted half-day presentations to area veterinarians in multiple locations in an attempt to recruit veterinarians to participate. Veterinarians from across Canada recruited eligible herds from their active client list. The selected sites were typical dairy operations representing all major dairy areas in Canada. In order to participate, herds had to have bulk-tank SCCs of < 250,000cells/ml, over the last 3 months. In addition, herds had to have regular supervised herd health checks, subscribe to Dairy Herd Improvement (DHI) services, and have monthly individual cow SCC. In order to be eligible, dairy cows had to be at the end of their lactation, confirmed pregnant, in

good physical condition, and have clear individual identification numbers. Cows with expected dry periods < 30 or > 90 days were excluded. The study coordinator attempted to recruit 25 veterinarians to enroll a total of 1000 cows in the study as this was the sample size deemed necessary to find biologically important differences in a previous clinical trial (Sanford et al., accepted).

5.3.2. Study Procedures

A binder containing all information pertinent to the study was distributed to participating veterinarians. The study protocol was explained and reviewed by the study monitor at regional meetings which were attended by participating veterinarians. The data sheets required for proper enrollment and recording were also distributed. The herd veterinarian, together with the producer, identified the next cows (five in small herds, 10 in large herds) due to dry-off. Once these animals were identified, a two-step treatment assignment process was followed to ensure that cows were classified into the correct treatment group (Table 5-1). In summary, cows with no history of CM and a SCC < 200,000cells/ml on the previous three milk tests were assigned into group 1. Cows within this group were treated with either ITS or DCT depending on their identification number. Cows with a history of CM or a SCC > 200,000cells/ml on any of the three previous milk tests were assigned into group 2. Cows within this group were treated with either ITS/DCT or DCT depending on their identification number.

Enrolled cows were observed for signs of CM, identified by the dairy producer until 30 days post-calving. Herd veterinarians were responsible for reviewing the definition of CM. Presence or absence of CM was recorded by the producer, on the data sheets provided.

5.3.3. Statistical Analysis

There were three outcomes of interest: milk production and SCC for the first 180 days of lactation, and incidence of CM during the first 30 days post-calving. For the production and SCC analysis, data consisted of repeated measures (6) per individual cow over time. Furthermore, cows were clustered within herds. Due to the hierarchical structure of these data, a linear mixed model for repeated measures was used (PROC MIXED in SAS 8.02, The SAS Institute, Cary, NC). There were four treatment groups (group 1- DCT, group 1- ITS, group 2- DCT, and group 2- ITS/DCT) which were truly an outcome of two different factors (group and ITS). To account for this in the analysis, main effects for group and ITS, and an interaction between the two factors were assessed. The DHI SCC (/1000) was log-transformed to create a new variable "lnSCC". Univariable analysis was performed using a P-value < 0.25 to identify predictors for assessment in the multivariable model. Predictors used included: ITS, group, parity, test-month, and days in milk (DIM). Parity was reduced into three categorical predictors: 2^{nd} lactation, 3^{rd} lactation, and $\geq 4^{th}$ lactation. A second DIM variable ($DIM^{-0.05}$) was created using the Wilmink's function to model the standard lactation curve (Schaeffer et al., 2000). Once all

predictors were identified, a backwards step-wise elimination process was used for model reduction. A significant P-value of 0.05 was assigned. All plausible first order interactions were assessed. Different correlation structures were assessed to find the model with the best fit. Autoregressive moving average (ARMA) correlation was utilized in the final model as it gave the best model fit. A locally weighted regression smoothed scatterplot of mean milk production and SCC by DIM were generated for each treatment group using a bandwidth of 0.8 (Stata 8.2, StataCorp, College Station, TX).

To analyze the CM data, a generalized linear mixed models was used. Univariable analysis was performed using a P-value < 0.25 to identify predictors for the multivariable models. Predictors assessed included: ITS, group, DHI-recorded SCC on last test prior to dry-off, parity, dry-off season, last recorded milk production before dry-off, DIM at dry-off, and 305 milk production at dry-off. Parity was categorized as above. Dry-off date was utilized to calculate season. SCC was log-transformed as described above. Once predictors were identified for the multivariable analysis, a backwards step-wise elimination was used as above. All plausible first order interactions were assessed. Due to the hierarchical structure of the data, province and herd were utilized in the model as random effects to account for the hierarchical nature of the data. Generalized linear mixed models were fit using MLwiN 2.0 (University of Bristol) and Stata 8.2 (Rabe-Hesketh and Skrondal, 2005).

5.4. Results

5.4.1. Descriptive Statistics

A total of 92 herds, selected by 17 veterinarians, in six provinces were initially recruited for this project. For unspecified reasons, 16 of these herds did not participate in the trial. One enrolled herd lost their data sheets prior to submission. Therefore, data was available from 75 herds, totaling 1633 individual cow records. Of these cows, 79% (1296) were assigned into the appropriate treatment group, and had properly completed data sheets. The remaining 21% (337) of cows were assigned to an incorrect treatment group, were treated with something different than the trial protocol (eg. external teat sealants), or had missing values for essential information on the data sheets. Of the 1296 cows, 67% (870) were assigned to group 1, and the remaining 32.9% (426) were assigned to group 2. The treatments were evenly distributed across groups (Figure 5-1). Cases of CM were recorded for 87% (1131) of cows from 72 herds, and DHI data was accessible from 81% (1047) of cows from 64 herds. In total 3 cows died during the study. All these animals were treated with ITS alone.

A summary of descriptive statistics is shown in Table 5-2 and Table 5-3. Significant between- and within-group differences are indicated. The average parity at calving, was 3.2 (median 3) and this ranged from 2 to13. When parity was grouped into categories, second, third, and four-plus lactation cows accounted for 45.7, 26.1, and 28.2% of the animals, respectively. The average 24 hour milk production on the

last milk test taken prior to dry-off was 20.6 kg (standard deviation 7.7). The average DIM at the last test taken prior to dry-off was 324 days (standard deviation 67). The mean composite lnSCC at the last test taken prior to dry-off was 4.56 (equivalent to 95,000 cells/ml) (standard deviation 1.25). Average 305-day milk production from the previous lactation was 10,224 kg (standard deviation 2020).

Clinical mastitis during the last 3 months of lactation was reported in 7.6% (98) of the total study cows. Due to the study design all these cases were from cows in the group 2. A SCC > 200,000 cells/ml during at least one of the last 3 DHI tests, was recorded in 30.8% of the total study cows. Due to the study design, all these cows were in the group 2.

Cases of CM, during the first 30 days of lactation, were reported in 9.7% (110) of the 1131 cows in which data was available. The average projected 305-day milk production was 10,317 kg (standard deviation 2124).

5.4.2. Multivariable Analysis

A graph showing the average test day milk production, by treatment is presented in Figure 5-2. Initially, all groups appeared to produce a similar amount of milk, with the exception of the group 1- DCT, which began at the highest production. Over time, cows treated with ITS in both groups, had lower peak milk production than the DCT animals. This difference remains consistent until mid-lactation. Results of the linear mixed model for repeated measures on milk production are shown in Table 5-4. Cows treated with ITS, whether alone or in conjunction with

DCT, produced significantly less milk (-1.53 kg/day) during the first six months of lactation than cows treated solely with DCT. No significant group and ITS interaction was found. When 2nd lactation cows were used as a reference, 3rd lactation animals produced 1.72 kg more milk/day, and 4th lactation and older cows produced 0.03 kg less milk/day. Milk test month was also a significant predictor of milk production. Highest milk production occurred in May, whereas lowest milk production occurred in July.

A graph showing the average test day lnSCC, by treatment is presented in Figure 5-3. The pattern of falling and rising lnSCC appears to be similar amongst all cows. Significant predictors of SCC are summarized in Table 5-5. Cows in group 2 had significantly higher SCC (0.50 log units/day) than cows in group 1 during the first 180 days of lactation. There was no significant effect of ITS on lnSCC although there was a slight indication of lower SCC in ITS treated cows in group 1.

When 2nd lactation cows were used as a reference, older animals had significantly higher SCC. Milk test month was also a significant predictor of SCC. The SCC peaked in September, whereas lowest SCC occurred in February.

When all study cows were considered, being in group 2 increased the odds of developing CM post-calving 2.6 times ($P < 0.01$) (Table 5-6). There were no other significant predictors of CM.

5.5. Discussion

A minimally monitored research design is useful if resources are very limited. Participation from Canadian veterinarians and producers was excellent. The study was unique as it expected the herd veterinarian and producer to follow the study protocol without any supervision from the study monitor. This involved allocating cows into various treatment groups, administering dry-off treatments, and recording cow data. It was unfortunate that some herds dropped out of the study, but the sample size in the trial was still adequate. Approximately one quarter of the data was not useable, because cows were allocated into the wrong treatment group. This loss of data, was inevitable in this trial and was expected by the researchers. On a positive note, approximately 75% of the data were useable and represented many more herds across Canada than could have been recruited if a typical clinical trial had been performed.

The results showed that cows treated with ITS, whether used alone or in conjunction with DCT, produced less milk during the first 6 months of lactation. This was an unexpected result. Østerås & Sandvik (1996) have shown that treatment with DCT increases milk production. This cannot explain the effect in the current study, as cows that were treated with both ITS and DCT still had significantly lower milk production. When the projected 305-day milk production was assessed, no difference was found between the treatments in group 1. Hwever, a trend of lower projected 305-day production was found in cows treated with ITS and DCT when compared to animals treated with DCT alone. None of the previously published ITS research trials

had measured milk production as an outcome (Woolford et al., 1998; Berry and Hillerton, 2002; Huxley et al., 2002; Godden et al., 2003), so it is impossible to compare the results of this trial with previous work. Two studies have looked briefly at ITS production and found no differences between treatments (Hassfurther et al., 2003; Berry et al., 2004).

Other risk factors have negative impacts on milk production, such as dystocia, milk fever, retained placenta, metritis, ketosis, displaced abomasums, lameness, and cystic ovarian disease (Wilson et al., 2004). The current study did not collect information regarding any other disease process that may have occurred during lactation. Therefore, no conclusions can be made about the effects of other risk factors on milk production, and whether the occurrence of other diseases was different between treatment groups.

Bismuth subnitrate absorption has been studied in the stomachs of humans and while virtually none of it is absorbed, a small amount may be absorbed in the presence of citrate (Committee for Vet Med Products: Bismuth Subnitrate, 1999). Citrate concentrations are elevated at both dry-off and colostrogenesis (Hurley, 1989). Because ITS remains in the mammary gland for the entire dry period, the question arises as to whether any of it is absorbed. This remains to be elucidated, as does whether this could potentially impact milk production for a period of time during early lactation or longer. Internal teat sealers are very viscous substances, and difficult to remove from hands and surfaces. It is known that ITS moves around the mammary gland somewhat with gravity (Jim Allison, personal communication). Therefore, it is possible that some of the sealer ascended into the mammary gland,

and may be coating or blocking some milk producing cells. Additionally, in this study, the producers were responsible for the administration of the ITS. Although the instructions state not to massage the udder post-infusion, due to a long-standing habit of intramammary infusion techniques, it is possible that the ITS was massaged up into the gland. Furthermore, there is some air in the ITS infusion tubes. If the product was infused rapidly, this air could potentially aerosolize particles, sending them higher into the gland than originally thought. Further studies involving the effect of ITS on milk cell histology and physiology may be warranted.

It is certainly perplexing as to why cows treated with ITS would have lower peak milk production than their comparison groups. One explanation could be that lower SCC over lactation have a strong effect on milk projections in later lactation, but this is not substantiated when the SCC for the first 180 days of lactation were compared. The SCC in ITS treated cows were not significantly different than cows that were not treated with ITS.

Other findings in the current study were expected and are well documented in the literature. Increased days in milk reduced milk production (Carlén et al., 2004). Third lactation cows produced the most milk. Also, month of test had a significant impact on milk production. The effects of season and parity on lactation curves, has been described (Macciotta et al., 2004). That study reported that that third and fourth parity cows had the highest peak milk production, whereas first parity cows had the lowest. Also, cows that calved in spring had the highest peak milk.

A proportion of dairy producers involved in the current study did not complete the data sheets and monitor CM. This issue is certainly not a new one, as compliance

to collect CM data can be poor. One review emphasizes the need to record clinical diseases, including CM in dairy cattle, and introduces recommendations, standards and guidelines to follow (Kelton et al., 1998).

Cows in group 2 were more likely to have a case of CM during the first 30 days post-calving than cows in group 1. This finding adds strength to previous research that there are cow-level and quarter-level risk factors making certain animals and/or quarters more susceptible to IMI (Dingwell et al., 2003). Despite the treatment randomization in this study, a significantly higher proportion of ITS/DCT treated cows had a case of CM during the last 3 months of their previous lactation than did DCT treated cows in group 2. There appears to be no obvious explanation for this difference as the treatment assignment was based on identification numbers. One study found that milk yield losses due to CM are pathogen-specific (Gröhn et al., 2004). The study also concluded that there is an interaction between parity and pathogen. Therefore, the specific CM pathogen-causing production losses for one age group were different than for other age groups. The current study had parity information but did not have milk bacteriological cultures for the CM cases. Both the CM and the milk production results of the current study would have been strengthened if bacteriology had been performed on all clinical cases. Culture would have allowed classification of CM organisms as contagious or environmental. If the majority of the cases of CM were contagious in etiology, then ITS would not be expected to have any effect. Conversely, if the etiological agents were environmental, then ITS would be expected to have a stronger protective effect on the udder. Cows in group 1 were younger than cows in group 2. This parity difference

between groups is expected, as older cows are more commonly affected by IMI and tend to have higher SCC (Dingwell et al., 2003).

The deaths of 3 cows treated with ITS alone were investigated. All deaths occurred within a few days post-treatment and all occurred during summer months. Infusion techniques utilized by producers were reviewed and found to be acceptable. Milk bacteriological culture revealed *Klebsiella* spp. pathogens in 2 of the 3 cases, whereas the third cow had a negative culture result. Internal teat sealers are currently marketed in North America for use in conjunction with DCT. These 3 losses in this study reinforced concerns regarding the infusion of a non-antibiotic only at dry-off if it is possible that an IMI exists in the quarter. In addition, concern over the use of ITS alone during the hot and humid weather has been raised. Further studies to demonstrate efficient and economical means to identify IMI at dry-off are needed.

In conclusion, there appears to be no obvious explanation for the unexpected findings of lower milk production in ITS-treated cows. While much research has shown that ITS decrease the incidence of new intramammary infections during the dry period (Woolford et al., 1998; Berry and Hillerton, 2002; Huxley et al., 2002; Godden et al., 2003; Cook et al., 2005; Sanford et al., accepted) this study suggests that treatment with ITS may have a negative impact on milk production during early lactation. The SCC were not significantly differently when ITS-treated cows were compared with animals that were not treated with ITS.

5.6. References

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Table 5-1 Group assignment based on three-month history of somatic cell count (SCC) and clinical mastitis (CM), and treatment allocation (internal teat sealer (ITS), dry cow antibiotic (DCT), or ITS and DCT), based on cow identification (ID) numbers.

History of SCC & CM	Group	Cow ID	Treatment
< 200,000 & no CM	1	odd	DCT
< 200,000 & no CM	1	even	ITS
> 200,000 or CM	2	odd	DCT
> 200,000 or CM	2	even	ITS/DCT

Table 5-2 Descriptive statistics between and within-groups 1 and 2 from the previous lactation before treatment administration, listed by treatment (internal teat sealer (ITS), dry cow antibiotic (DCT), and ITS/DCT).

	Between groups		Within groups			
			Group 1		Group 2	
	Group 1	Group 2	ITS	DCT	ITS/DCT	DCT
Parity	3.0[a]	3.7[a]	3.0	2.9	3.7	3.6
24h milk production (kg)	21.9[a]	17.9[a]	22.2	21.6	17.2[b]	18.5[b]
Days in milk	323[b]	328[b]	320	325	331	326
SCC (ln)	4.10[a]	5.64[a]	4.07	4.13	5.65	5.62
305d milk production (kg)	10161[b]	10356[b]	10208	10117	10185[b]	10530[b]
Clinical mastitis prior to dry-off (n)	-	98	-	-	60[a]	38[a]
SCC > 200,000 prior to dry-off (n)	-	399	-	-	193	206

[a] $p < 0.05$
[b] $p < 0.10$

Table 5-3 Descriptive statistics between and within-groups 1 and 2 listed by treatment (internal teat sealer (ITS), dry cow antibiotic (DCT), and ITS/DCT).

	Between groups		Within groups			
			Group 1		Group 2	
	Group 1	Group 2	ITS	DCT	ITS/DCT	DCT
Projected 305d. milk production (kg)[a]	10355	10231	10266	10438	9971[b]	10408[b]
Clinical mastitis post-calving (n)	58	52	34	24	28	24

[a] projection at last test
[b] $p < 0.10$

Table 5-4 Linear mixed model for repeated measures for milk production over the first 180 days of lactation (n=4673 milk tests).

	Coefficient	Standard Error	P-value
Fixed Effects			
Intercept	215.90	5.84	
Treatment			
Dry cow antibiotic	reference		
Internal teat sealer	-1.53	0.61	0.01
Group			
1	reference		
2	-0.33	0.64	0.60
Interaction term			
Treatment * Group	0.15	0.89	0.87
Days in milk	-0.17	0.01	<0.01
Days in milk$^{-.05}$	-199.82	6.73	<0.01
Interaction term			
Dry cow antibiotic * Days in milk	reference		
Internal teat sealer * Days in milk	0.01	0.004	0.06
Parity			
2nd lactation	reference		
3rd lactation	1.72	0.45	<0.01
$\geq$ 4th lactation	-0.03	0.64	
Month			
January	reference		
February	0.78	0.30	
March	0.87	0.34	
April	1.55	0.39	
May	2.73	0.46	
June	1.74	0.54	<0.01
July	-0.12	0.59	
August	1.19	0.78	
September	0.98	0.64	
October	0.56	0.50	
November	0.44	0.39	
December	-0.08	0.31	
Random Effects			
Herd	19.80	4.15	
Error	63.10	1.96	

Table 5-5 Linear mixed model for repeated measures for somatic cell count (ln) over the first 180 days of lactation (n=4679 milk tests)

	Coefficient	Standard Error	P-value
Fixed Effects			
Intercept	3.31	0.11	
Treatment			
Dry cow antibiotic	reference		
Internal teat sealer	0.04	0.09	0.65
Group			
1	reference		
2	0.50	0.11	<0.01
Interaction term			
Treatment * Group	-0.20	0.16	0.20
Days in milk	0.002	0.0005	<0.01
Parity			
2nd lactation	reference		
3rd lactation	0.30	0.08	<0.01
≥ 4th lactation	0.72	0.11	
Month			
January	reference		
February	-0.10	0.06	
March	-0.09	0.06	
April	-0.07	0.07	
May	0.08	0.08	
June	0.07	0.10	<0.01
July	0.21	0.11	
August	0.37	0.14	
September	0.54	0.11	
October	0.37	0.09	
November	0.02	0.07	
December	-0.01	0.06	
Random Effects			
Herd	0.21	0.05	
Error	2.08	0.06	

Table 5-6 Generalized linear mixed model analysis of odds of the incidence of clinical mastitis among all study cows during the first 30 days of lactation (n = 1131).

Variable	Coefficient	Standard Error	Odds Ratio	95% Confidence Interval	*P*-value
Fixed Effects					
Intercept	-3.80	.60			
Treatment					
Dry cow antibiotic	reference				
Internal teat sealer	0.69	0.70	1.99	0.49, 8.08	0.32
Group					
1	reference				
2	0.95	0.34	2.59	1.84, 3.63	<0.01
Interaction					
Treatment * Group	-0.21	0.45	0.81	0.51, 1.27	0.64
Random Effects					
Province	0.26	0.28			
Herd	0.78	0.33			

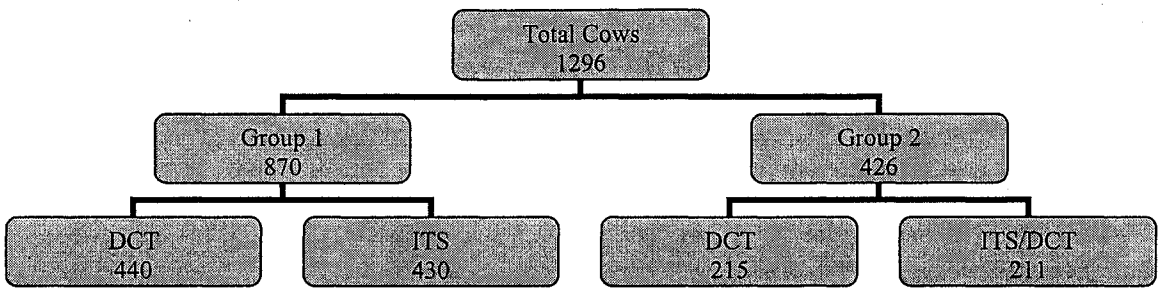

Figure 5-1 Number of cows within each group (1 or 2) and treatment category (internal teat sealer (ITS), dry cow antibiotic (DCT), and ITS/DCT).

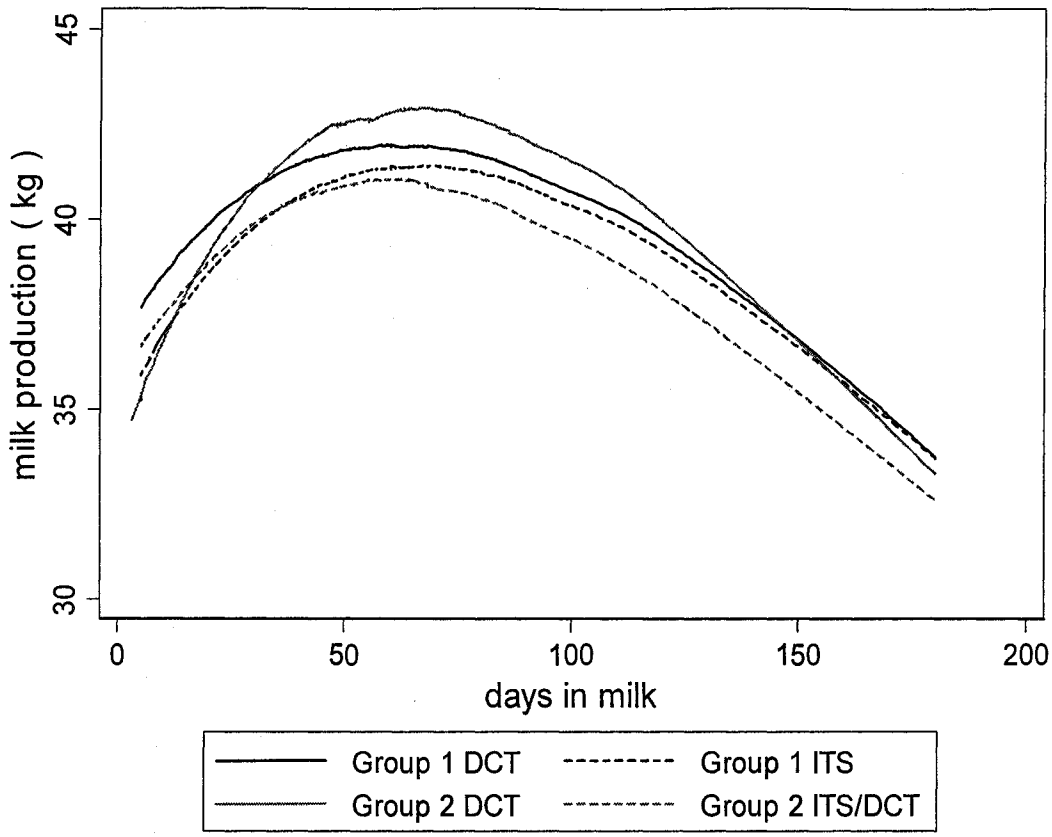

Figure 5-2 Graph of mean milk production by treatment group over first six months of lactation.

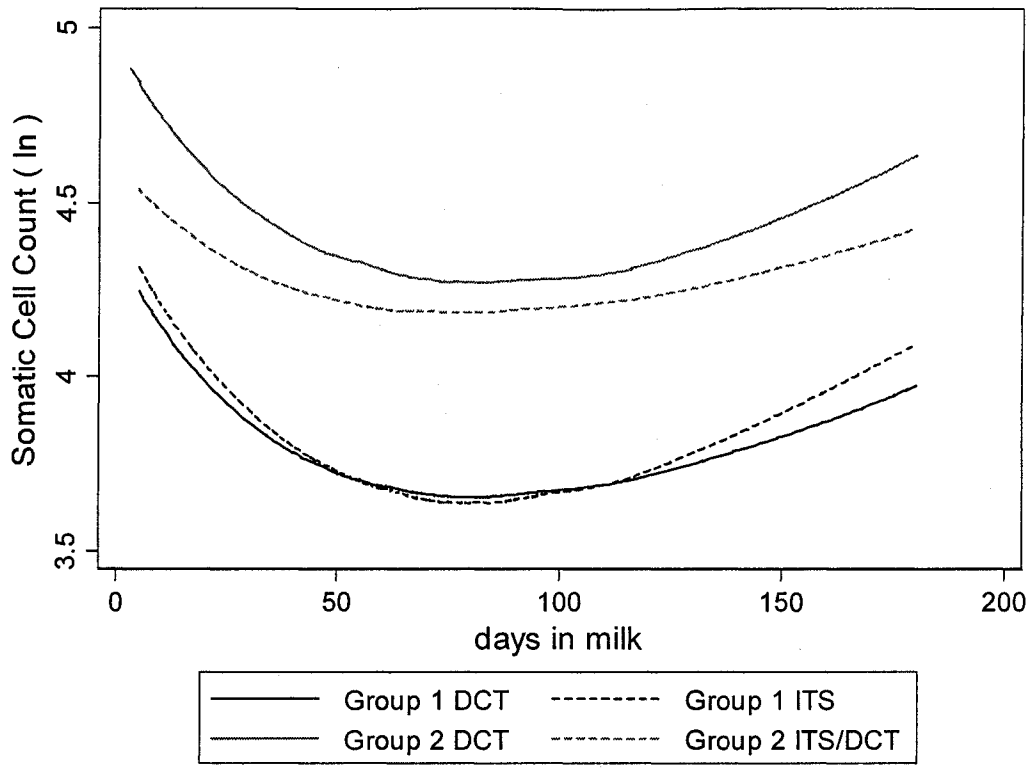

Figure 5-3 Graph of mean somatic cell count (log transformed) across treatment groups for the first 180 days of lactation.

CHAPTER 6 ASSESSMENT OF CLOXACILLIN MOVEMENT BETWEEN QUARTERS OF THE MAMMARY GLAND IN NON-LACTATING DAIRY COWS, USING HIGH PRESSURE LIQUID CHROMATOGRAPHY

6.1. Abstract

The objective of this study was to determine if cloxacillin infused intramammarily, moved from treated to untreated quarters, and if so, in what concentrations. An additional objective was to determine if antibiotic movement affects the selection of ipsilateral vs contralateral split udder treatment designs. A total of 80 quarters from 20 Holstein-Friesian cows were sampled from one herd. A split udder design was used to treat half of the udder (ipsilaterally or contralaterally) with cloxacillin on the day of dry-off. Three days later, milk samples were taken from all untreated quarters and high pressure liquid chromatography was used to detect and quantify cloxacillin concentrations. Cloxacillin was detected in 25% of all untreated quarters. The average concentration of cloxacillin in sampled quarters was below minimum inhibitory concentrations for targeted mastitis pathogens. No significant difference in cloxacillin levels was found been the ipsilateral or contralateral treatment groups. Therefore, split udder designs are valid for cloxacillin dry cow antibiotic trials although transfer of antibiotic does occur in trace concentrations. Ipsilateral or contralateral treatment designs perform similarly. This type of design is more economical for researchers, although care must be taken to account for clustering of quarters within cows in the analyses.

6.2. Introduction

Historically, clinical trials involving dry cow antibiotics (DCT), and their effect on cure rates or development of new intramammary infections (IMI) have randomized treatment at the cow-level, meaning the same treatment is applied to all four quarters of cow. Treatment differences were then analyzed between cows in the study. More recently, with advances in statistical techniques for handling multilevel data, it has become possible to correctly analyze results from split udder design trials. These involve the treatment of half the udder (two quarters) with one treatment (test article) and the other half with a different treatment (positive or negative control). This can be done in an ipsilateral manner, where the udder is split into right and left sides and both quarters of the same side receive one treatment, or in a contralateral manner, where diagonal quarters (e.g. right front and left rear) receive one treatment (Fig. 6-1). Statistical techniques can be used to control for the clustering of quarters within cows in the analysis, because failure to control for the quarter interdependencies can lead to errors in the results (Barkema et al., 1997; Berry et al., 2003; Dohoo et al., 2003).

Clinical trials using udder halves or quarter as the level for treatment randomization have many advantages. A smaller number of cows are required to find statistically significant differences, thereby decreasing the cost of the study by reducing the number of animals and quantity of therapeutics used, samples collected, and research time required. Additionally, the animal serves as its own control for cow-level factors such as parity, milk production, nutritional status, and udder

conformation. Such factors could have substantial impacts on the research results, if not controlled for adequately in the analysis (Dohoo et al., 2003). Split udder designs can also control for quarter-level factors and their relationship to the development of IMI. Factors such as quarter location, teat-end integrity, and teat-canal keratin plug formation in the dry period, also have a significant effect on the outcomes (Barkema et al., 1997; Neijenhuis et al., 2001; Dingwell et al., 2004). However, in order to validate this type of research design, it must be ascertained that DCT infused in two quarters of the udder are not able to move into untreated quarters in any therapeutic quantity.

The mammary gland is well supplied with blood. The external pudendal (mammary) artery supplies the majority of the udder, but it is assisted by both the subcutaneous abdominal and perineal arteries, cranially and caudally, respectively (Garrett, 1988; Prescott et al., 2000). A venous circle is formed by the anastomoses of the veins of the external pudendal with the subcutaneous abdominal, cranially, and the perineal, caudally. The ring is completed when the right and left subcutaneous abdominal and perineal veins anastomose with each other (Garrett, 1988). The venous system provides ample opportunity for antibiotics to pass from milk in the mammary gland into the systemic circulation, and travel to other quarters.

Multiple factors affect antibiotic movement, and these can be attributed to specific drug factors and cow factors. The protein binding capability, pH, lipid solubility and elimination formulation all affect the ability of the drug to cross the milk-blood barrier (Smith, 2005). Similarly, milk pH, milk production and IMI status of the mammary gland also play important roles in drug absorption (Smith, 2005).

It has been established that antibiotics infused into one quarter can appear in untreated quarters in lactating cows (Ziv and Schultze, 1982). However, there are no data available on the movement of DCT between quarters within cows. Therefore, the objectives of the current study were threefold: to assess if DCT is detectable in untreated quarters at three days post-infusion; if detectable, to quantify the concentrations in untreated quarters to determine if they reach minimum inhibitory concentrations (MIC) for pathogens of interest; and lastly, to compare contralateral and ipsilateral split udder designs to see if one is preferable.

6.3. Materials and Methods

6.3.1. Cow Selection

All Holstein-Friesian cows from one dairy herd in Prince Edward Island, Canada, at the end of their lactation, with four functional quarters, were eligible to participate in the study. Herds consisted of 120 milking cows housed in a free-stall barn. Cows were required to have a composite somatic cell count (SCC) < 250,000 cells/ml on the last three recorded milk tests, and no evidence of clinical mastitis during the last 30 days. Cows were ineligible to participate if they were administered systemic or local antibiotics in the two weeks prior to dry-off.

6.3.2. Day of Dry-Off

All quarters of all participating cows were prepared for milking as per usual farm procedures. Each teat was scrubbed with alcohol, and after the first few fore-strips of milk were discarded, milk samples were collected using an aseptic technique for both bacteriology and SCC. Cows were milked and randomly allocated to one of two treatment groups, ipsilateral or contralateral, by drawing numbers from a hat. In the ipsilateral treatment group, two quarters of a randomly selected side (right front and right rear or left front and left rear) were infused with 500 mg of intramammary benthazine cloxacillin.[a] In the contralateral treatment group, two diagonal quarters (right front and left rear or left front and right rear) were randomly selected for intramammary infusion of benthazine cloxacillin. Partial insertion technique was used and all treated quarters were subsequently massaged. All quarters were dipped with a post-milking teat disinfectant and cows were moved into the dry cow area.

6.3.3. Day Three After Dry-off

Three days after dry-off, untreated quarters of study cows were scrubbed with alcohol and prepared for milk sample collection. Quarter milk samples were collected aseptically for high pressure liquid chromatography (HPLC) cloxacillin analysis. In a subset of cows (4), samples were taken from all four quarters (treated and untreated). After milk collection, all sampled quarters were infused with intramammary benzathine cloxacillin and teat-dipped. Milk was frozen at -80C until use for HPLC analysis.

6.3.4. Bacteriological Culture, SCC and HPLC Procedures

All bacteriological laboratory procedures were performed in accordance with NMC recommendations (National Mastitis Council, 1999). Laboratory personnel were blinded to treatments. An inoculum of 0.01 ml of milk was plated onto a Columbia base agar containing 5% sheep blood. Plates were incubated at 37°C and examined for growth at 24 and 48 h. Colonies were tentatively identified as staphylococci, streptococci, coliform, or other pathogens based on colony growth, morphology and appearance, pattern of hemolysis, catalase reaction, and Gram staining. Staphylococci were tested for coagulase production with the tube coagulase test. Streptococci were further subcultured with agar containing esculin. Gram-negative bacteria were plated on MacConkey agar for identification. Gross appearance and reaction to citrate were used to differentiate *Escherichia coli* and *Klebsiella* sp. For each positive quarter, the number of colony forming units per 0.01 ml milk was reported in one of four categories: 1 to 5, 6 to 10, 11 to 50, or >50 cfu. A sample was considered contaminated if three or more colony types were present on a plate. Quarter milk SCC were analyzed using fresh milk[b] (Provincial Dairy Laboratory, Prince Edward Island). A liquid chromatographic method (Laboratory Services Division, University of Guelph, Ontario) using a dual wavelength absorbance detector system[c] with detection at 325nm was used for determination of cloxacillin in milk using techniques previously described (Boison et al., 1994). Briefly, milk samples were thawed and diluted in a multi-step process with untreated control milk to ensure concentration of sample was within the previously validated

concentration range of 5-60ppb. A standard curve was run for quantitation and positive and negative control samples were tested to ensure the method was correct.

6.3.5. Statistical Analysis

Descriptive statistics were calculated using Stata 8.0 (Statacorp, College Station, TX). The data structure consisted of quarters clustered within cows. The outcome of interest was cloxacillin in untreated quarters. Cloxacillin was dichotomized as yes or no, depending on whether it was detectable by HPLC. Factors affecting the detection of cloxacillin in untreated quarters were investigated using a generalized estimating equation with robust SEs and an exchangeable correlation structure to account for within-cow clustering of quarter data. The main predictor of interest was treatment group: ipsilateral or contralateral. Other variables in the model included: quarter, weighted average of composite SCC prior to dry-off, quarter SCC at dry-off, 305 day milk production, parity (1^{st} lactation or greater), overall IMI, major IMI , and minor IMI for both treated and untreated quarters. The composite SCC variable was created by calculating a weighted average of the last three recorded SCC ([(3^{rd} last SCC * 1) + (2^{nd} last SCC * 2) + (last SCC * 3)]/6). This variable was then log transformed to create lnSCC. Parity was divided into two lactation groups by 1^{st} lactation or $>1^{st}$ lactation (at dry-off).

Unconditional associations were tested using a simple logistic regression which accounted for quarters clustered within cow to identify predictors for consideration in the multivariable mixed model (P value < 0.20). Unconditional

associations with liberal p-values less than 0.20 were retained for use in the full model. A backwards step-wise elimination process was performed using significance at $P < 0.05$. All plausible interactions were assessed. Additionally, a likelihood-ratio test was performed to determine if there was any difference between the ipsilateral and contralateral treatment groups on cloxacillin movement.

6.4. Results

6.4.1. Descriptive Statistics

A total of 20 Holstein-Friesian cows from one herd were utilized in this trial. Average parity of the study cows at dry-off was 2.9 (median 1, range 1-10). Average 305-day milk production was 9,026 kg ranging from 6,181 to 12,079 kg. At dry-off, IMI were detected in 9 quarters (11.25%), and major pathogens, specifically *S. aureus* and *Streptococci* spp. (non-agalactiae) accounted for 3 of these IMI. The remaining 6 IMI were attributed to coagulase-negative Staphylococci.

Cloxacillin was detected in 25% of the untreated quarters (10 of 40). The mean cloxacillin concentration from untreated quarters was 0.006 ppm (median 0, range 0-0.19). The mean concentration of cloxacillin quantified in the 8 positive-control quarters that were tested was 6.83 ppm (median 5.25, range 1.4-22). In the ipsilateral treatment group, cloxacillin was detected in 32% (7 or 22) of the untreated quarters and had an average value of 0.01 ppm (median 0, range 0-0.19) (Fig. 6-2). One large outlying value of 0.19 ppm was found the ipsilateral group and this had a

strong effect on the group mean as well as the overall mean. In the contralateral treatment group, cloxacillin was detected in 17% (3 of 18) of untreated quarters and had an average value of 0.001 ppm (median 0, range 0-0.016).

6.4.2. Factors Affecting Transfer

The following predictors had unconditional association values that allowed them to be utilized for the full multivariable regression model: quarter ($p = 0.11$), weighted composite lnSCC ($p = 0.04$), lnSCC of treated quarters ($p = 0.15$), and lnSCC of untreated quarters ($p = 0.10$). Treatment group was forced into the full model although the unconditional association was weak ($p = 0.41$).

A weighted average of the last 3 composite lnSCC was the only significant predictor of cloxacillin movement (odds ratio 0.26, $P = 0.04$). For every unit increase in lnSCC, the odds of cloxacillin movement into untreated quarters, was 74% less likely (Figure 6-3). For example, if a cow's composite SCC increased from 100,000 to 200,000 cells/ml (0.69 units on log scale), then it would be 60% less likely to have cloxacillin movement into untreated quarters.

Finally, analysis of cloxacillin movement (yes/no) and treatment group (ipsilateral/contralateral) found no significant difference between groups (likelihood-ratio test = 0.22, $p = 0.64$).

6.5. Discussion

Three days after dry-off and administration of a benzathine cloxacillin dry cow intramammary product, cloxacillin was detected in 25% of untreated quarters. In 39/40 milk samples tested, the concentration of antibiotic detected was well below 10ppb, which is cited as the milk residue screening tolerance level of cloxacillin in North America (Food and Drug Administration, 2005). The exception to this was the outlying value which is discussed below. Concentrations were also well below the MIC for labeled pathogens which are discussed below. This movement of DCT was presumably facilitated through the systemic circulation. Researchers chose to sample untreated quarters at three days after dry-off, because this should have allowed adequate time for movement to occur while DCT concentrations in milk were still high. Benzathine cloxacillin is a weak acid with low solubility, which is formulated in a slow-release oil-gel base. Cloxacillin should remain in an unionized state in milk, which has an approximate pH of 6.8, which would promote is solubility into other compartments, including plasma (Prescott et al., 2000). The minimum detection level for cloxacillin using the HPLC was 0.002 ppm. This screening technology was able to detect cloxacillin at 20 percent of the milk residue screening tolerance level and well below effective treatment dosages. Using a technology calibrated to the screening tolerance level, would have detected only one untreated quarter.

A weighted average of the composite SCC was the only significant predictor of cloxacillin movement. As composite SCC increased, cloxacillin movement between quarters decreased. This finding is contrary to the assumption that inflammation disrupts endothelial junctions, thereby leading to increased vascular permeability and enhanced systemic absorption (Smith, 2005). In previous studies,

mastitis has been found to promote the absorption of antibiotics from the mammary gland into the blood stream (Ziv and Schultze, 1982; Sweeney et al., 1996). Intramammary infections in treated quarters was not a significant predictor of cloxacillin movement. However, cows were selected based on the presumption that they had a low probability of IMI. There were very few IMI detected at dry-off in the study cows, and the majority of these infections were caused by coagulase-negative staphylococci. Of the three quarters where major IMI were detected, only one was from a treated quarter (left front). The outlying value with the elevated cloxacillin concentration in an untreated quarter was found in the right front quarter of the same cow. It is possible that the IMI enhanced absorption in this case and that IMI in treated quarters may be a predictor of cloxacillin transfer. However, due to the selection criteria of the current study, there were not enough infected quarters to detect this effect.

Because it was determined that cloxacillin moved from one quarter to another, it was necessary to quantify the concentration of DCT in untreated quarters. The overall mean concentration of cloxacillin in these quarters was 2.4% and 1.2% of the MIC_{50} and MIC_{90} for *Staphylococcus aureus*, respectively (MIC_{50} 0.25 ppm, MIC_{90} 0.5 ppm) and less than 1% of the MIC_{50} and MIC_{90} for *Streptococcus agalactiae* (MIC_{50} 1.0 ppm, MIC_{90} 2.0 ppm) (Owens et al., 1997; Schlegelová et al., 2002). Cloxacillin has a label claim against *S. aureus* and *S. agalactiae*.[a] If other gram-positive pathogens such as *Streptococcus uberis*, *Streptococcus dysgalactiae*, coagulase-negative staphylococcus are considered, the mean concentration of cloxacillin in untreated quarters was well below all MIC (Owens et al., 1997;

Schlegelová et al., 2002). Even the large outlying value observed in one quarter (0.19) is still below all the MIC_{50} for all pathogens, with the exception of *S. dysgalactiae*, which has a value of 0.125 ppm (Schlegelová et al., 2002). The presence of a low dose of antibiotic in untreated quarters needs to be investigated further, to determine if the inhibition of bacterial growth can occur. The current study simply compared HPLC concentrations with published MIC values but did not plate milk and measure zones of inhibition.

The third objective of the current study was to determine if there was any difference in cloxacillin movement when quarters were treated in an ipsilateral or contralateral split udder design. No statistically significant difference was found between the treatment groups, although a numerically larger proportion of untreated quarters contained detectable levels of cloxacillin in the ipsilateral group. The abundance of vasculature and venous ring located at the base of the udder make cloxacillin movement possible between all quarters. Decisions based on which split udder design (ipsilateral or contralateral) should be left to researcher preference. Yet it remains necessary to ensure that treatment randomization is performed in an appropriate manner.

Split udder designs for DCT trials using benzathine cloxacillin are valid. Only a small amount of cloxacillin moves between quarters, and the levels detected in untreated quarters are below residue tolerance levels and well below MIC for labeled pathogens. Split udder designs are more economical to the researcher. However, care must be taken to account for the clustering of quarters within cows in the analysis (Barkema et al., 1997; Berry et al., 2003). One study which examined selective dry

cow therapy trials found that quarters which were left untreated showed interdependence and were more likely to develop new IMI, but positive control quarters were independent and no more likely to develop IMI when from the same udder (Berry et al., 2003). This finding is in contrast to other studies which found more general interdependence among quarters (Barkema et al., 1997; Lam et al., 1996). Even if interdependence is not significant, it is possible that it exists but was not found due to Type I or Type II error. Modern statistical software programs have simplified controlling for data clustering and it is advisable to account for multiple levels in all analyses (Dohoo et al., 2003).

Results of the current study may not be generalizable to all types of DCT. Variation in product pharmacokinetics means that further split udder design studies need to be performed to assess the movement of different dry cow antibiotics among quarters of the same cow. Cloxacillin was chosen for the present study because it is a commonly used DCT in Canada and represents a substantial proportion of DCT sales (Olde Riekerink et al., 2005). Future studies should also be conducted to assess DCT movement in cows and quarters with IMI. Previous trials have found mastitis to enhance the systemic absorption of antibiotics (Ziv and Schultze, 1982; Sweeney et al., 1996), and cows in the current study were based on a history of low SCC and no evidence of clinical mastitis.

In conclusion, split udder designs using cloxacillin DCT are valid, although some transfer of antibiotics into untreated quarters does occur but at trace levels. Minimum inhibitory concentrations of the pathogens of interest and the residue tolerance level of cloxacillin are much higher than the average concentration of

cloxacillin detected in these quarters. No difference was found in either ipsilateral or contralateral split udder treatment designs.

6.7. Sources and Manufacturers

[a]DryClox®, Ayerst Veterinary Laboratories, Division of Wyeth-Ayerst Canada Inc., Guelph, ON

[b]400 Series Fossomatic, Hillerod, Denmark

[c]Waters Alliance System, Waters, Milford, MA

6.8. Acknowledgements

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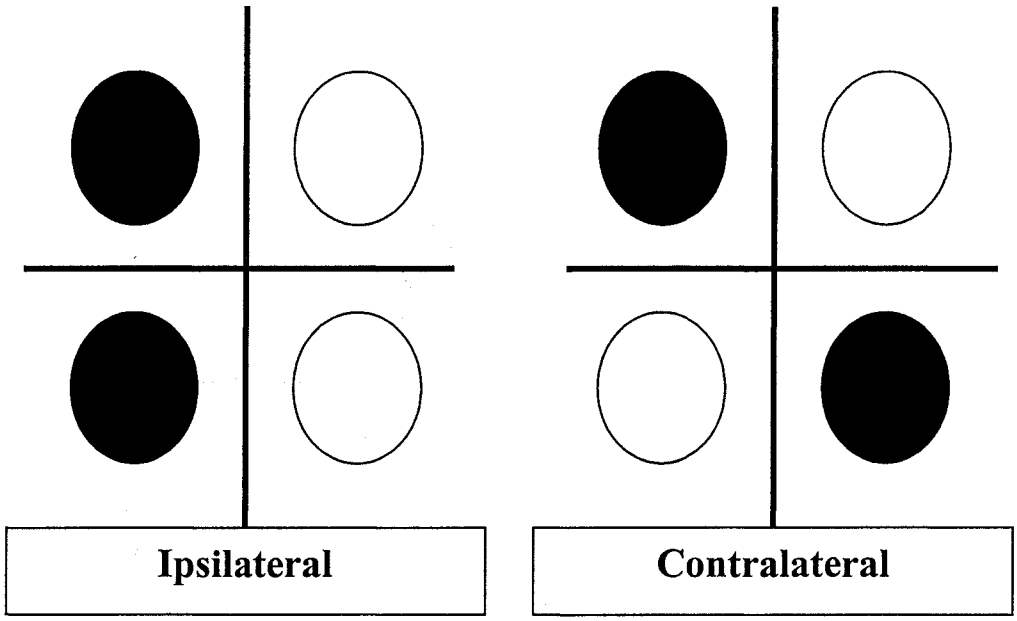

Figure 6-1 Ipsilateral and contralateral treatment design.

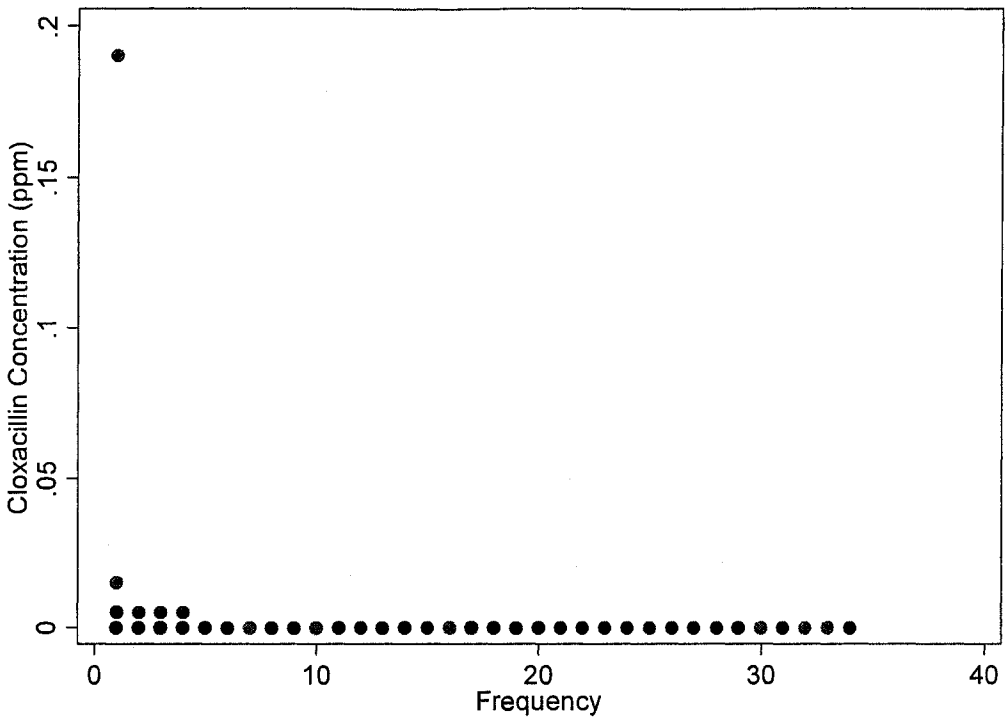

Figure 6-2 Dotplot showing cloxacillin concentrations in untreated quarters. Each dot represents one quarter sample.

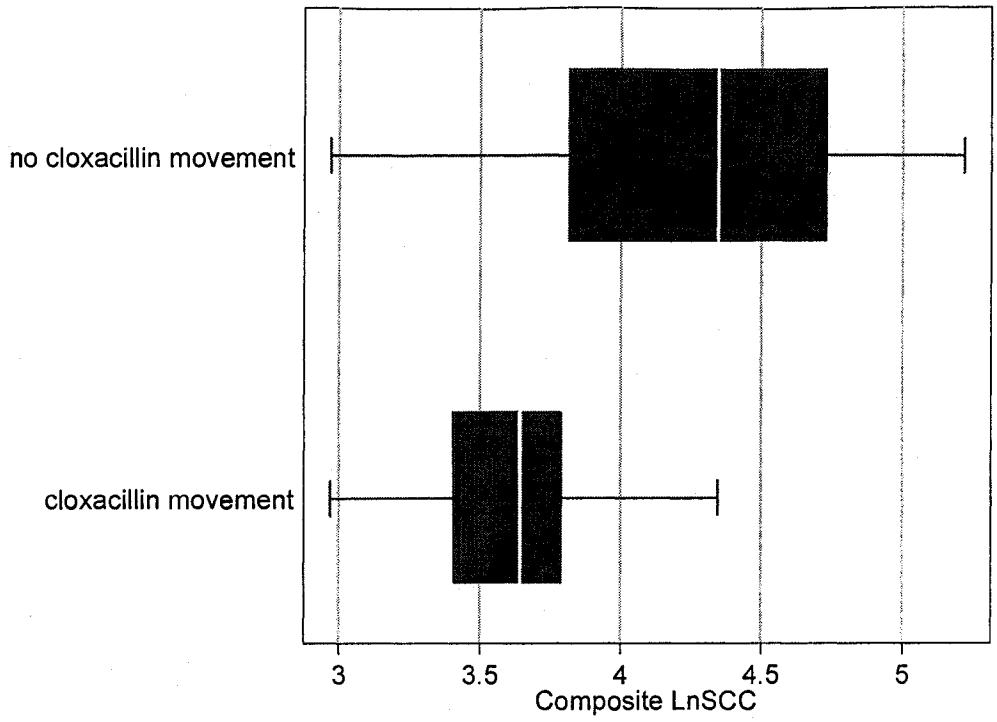

Figure 6-3 Boxplot showing difference in composite (ln)SCC between untreated quarters which had no cloxacillin detected, and quarters which had cloxacillin detected. Boxplot demonstrates, median, 25th to 75th interquartile range, and adjacent values.

CHAPTER 7 ESTIMATION OF THE TEST CHARACTERISTICS OF THE CALIFORNIA MASTITIS TEST AND SOMATIC CELL COUNT FOR IDENTIFICATION OF INFECTED QUARTERS AND COWS AT DRY-OFF USING LATENT CLASS MODELS

7.1. Abstract

The ability of the California Mastitis Test (CMT), somatic cell count (SCC), and bacteriological culture, to identify quarters and cows with intramammary infections on the day of dry-off was evaluated using latent class models. A total of 2921 quarters from 752 Holstein-Friesian cows from 11 herds were used for this investigation. Milk samples were collected for bacteriology and SCC, and the CMT was performed cow-side, prior to milking on the day of dry-off. At the quarter-level, the CMT performed poorly (sensitivity ~ 53%, specificity ~ 71%) in identifying quarters classified as infected by the latent class model. At the cow-level, the sensitivity and specificity of the CMT for identifying all pathogens was estimated at 70 and 48%, respectively. In both populations, the negative predictive value of both CMT and SCC, evaluating the presence of major pathogens at cow-level, was high enough (97%) to identify cows that did not require DCT. This finding could be very useful in helping low-prevalence herds adopt a selective dry cow program.

7.2. Introduction

In North America, one of the cornerstones of mastitis control programs is the use of dry cow therapy (DCT) for every quarter of every cow (Eberhart, 1986). This practice has been very effective in the reduction of intramammary infections (IMI) with contagious mastitis organisms and in the prevention of new IMI. However, blanket DCT results in uninfected quarters and cows being treated with dry cow antibiotics (Dingwell et al., 2003a). Consumers have paid increasing attention to the use of prophylactic antibiotics in food producing animals. Concern regarding the potential emergence of antibiotic-resistant strains of bacteria (Radostits et al., 1994) is one of the major arguments against continued use of blanket DCT (Berry and Hillerton, 2002b). This concern, coupled with a desire to shift away from antibiotic use, is enticing some producers to consider a selective DCT approach. In addition, with the advent of internal teat sealers (ITS), there is a viable alternative to DCT for the prevention of new IMI (Woolford et al., 1998; Berry and Hillerton, 2002a; Huxley et al., 2002; Sanford et al., submitted).

Selective dry cow strategies have been extensively investigated. In previous studies, untreated quarters consistently had higher rates of IMI and clinical mastitis, both during and after the dry period (Robinson et al., 1983; Browning et al., 1990; Schukken et al., 1993; Browning et al., 1994; Berry and Hillerton, 2002b). Several studies have determined that decisions regarding selective DCT should be made at the cow-level, rather than the quarter-level (Browning et al., 1990, Browning et al., 1994; Østerås et al., 1999; Berry et al., 2003). Some studies have highlighted the

interdependencies between quarters of a cow (ie. quarters within a cow are not independent in their risk for IMI) (Lam et al., 1996; Barkema et al., 1997; Berry et al., 2003). These findings, coupled with previous selective DCT research, further support cow-level DCT decisions on-farm. However, none of these studies have been performed in North America. Guidelines have been proposed for protocols to direct selective DCT programs. These protocols should emphasize simplicity and low cost for the producer, as well as adequate sensitivity and specificity of the diagnostic test(s) used (Poutrel and Rainard, 1981).

There is currently no quick and inexpensive method to reliably identify IMI. Bacteriological culture is often accepted as the gold standard for the identification of IMI. However, culture has not been adopted for widespread use by the dairy industry for identifying IMI due to logistic and financial considerations (Sargeant et al., 2001). Furthermore, agreement between duplicate quarter milk samples is good for contagious organisms, but poor for coliform pathogens (Erskine and Eberhart, 1988). Somatic cell counts (SCC) can be good predictors of IMI, and are much more economical for the producer (Sargeant et al., 2001). Yet, the Dairy Herd Improvement (DHI) test date interval is too long to adopt this for identification of quarters or cows for DCT at the time of drying-off (Wallace et al., 2001). New on-farm technologies such as the PortaSCC™ (PortaScience Inc., Moorestown, NJ) test and the DeLaval DCC (DeLaval, Tumba, Sweden) have also been investigated. While the PortaSCC test was found to be a fast and accurate estimator of SCC, it was not a reliable indicator of IMI (Barratt et al., 2004). The DeLaval DCC has been evaluated for its ability to predict IMI. Using a SCC threshold of 250,000 cells/ml,

one study reported a sensitivity and specificity of 74 and 58%, respectively, when compared with culture (van Werven et al., 2005). However, the equipment and individual test cassettes may be too expensive to permit a widespread adoption of the test.

The California Mastitis Test (CMT) was developed in 1957 to fulfill a need for a cow-side test to quickly and reliably detect abnormal milk (Schalm and Noorlander, 1957). The CMT is an inexpensive, fast, cow-side test that can be performed by any individual with minimal training. The CMT reaction is an indirect measure of SCC in milk (Barnum and Newbould, 1961). With increasing SCC (or total leukocyte count), CMT score also increases (Schalm and Noorlander, 1957; Barnum and Newbould, 1961; Dohoo and Meek, 1982). Over the past 50 years, many studies have been conducted in attempts to validate the CMT as a predictor of IMI. The CMT showed some promise as a test to identify *Streptococcus agalactiae* infected cows (Brookbanks, 1966). However, the CMT could not predict herd infection through the use of bulk tank samples (Barnum and Newbould, 1961). Positive CMT scores, 8 and 4 weeks prior to dry-off, detected 80% of all major IMI (Poutrel and Rainard, 1981). The sensitivity of the CMT to detect IMI post-calving was only fair and ranged from 55-69%; the corresponding specificities were slightly better, ranging from 57-86% (Sargeant et al., 2001; Dingwell et al., 2003b; Wallace et al., 2004). All studies reported that the CMT was not sensitive enough to be used as a screening test for IMI (Cole et al., 1965; Middleton et al., 2004; Ruegg and Sekito, 2004).

All previous studies describing the test characteristics of the CMT have compared it to a "gold standard" in the analysis. This approach has limitations as there is no perfect gold standard for the diagnosis of IMI. Therefore, previous estimates of sensitivity and specificity may be biased. This bias will undoubtedly occur, if classification errors in reference tests are ignored (Enøe et al., 2000). It is possible to estimate diagnostic test parameters and disease prevalence in the absence of a gold standard using latent class models, which are fit using maximum likelihood procedures or Bayesian inference (Hui and Walter, 1980; Joseph et al., 1995; Georgiadis et al., 1998; Enøe et al., 2000; Orr et al., 2003). These methods may be more valid and give more reasonable estimates than procedures based on flawed "gold standards". Latent class models utilize data which are cross-classified by test outcomes in a common 2 X 2 table. Two populations with different prevalences are required. This provides enough degrees of freedom to estimate a total of 6 parameters: the sensitivity and the specificity of both tests, as well as 2 population prevalences. However, specific assumptions that have been adopted from Hui and Walter (1980) must be considered and validated (Toft et al., 2005). To summarize, the prevalence of disease must differ between the two populations, each test must have the same operating characteristics (sensitivity and specificity) across populations, and tests must be conditionally independent (Hui and Walter, 1980; Georgiadis et al., 1998; Enøe et al., 2000; Branscum et al., 2005; Toft et al., 2005). Conditional independence means that the results of one test would have no impact on the results of another test. Bayesian methods involve the incorporation of prior information, expressed through probability distributions, with current data, to elicit a

posterior estimation of the test parameters and population prevalence of disease (Branscum et al., 2005). Prior information may be elicited from previous research or expert opinion. Because of the ability to incorporate prior information for population prevalences and test characteristics, Bayesian methods are well suited for diagnostic test evaluation (Branscum et al., 2005).

While a study has been performed to evaluate the value of CMT to select quarters for DCT, these milk samples were taken eight and four weeks prior to the actual dry off (Poutrel and Rainard, 1981). Furthermore, the CMT was characterized using bacteriological culture as the gold standard. Additionally, it must be acknowledged that IMI status can change quickly, and that bacteriological culture is not a perfect test (Erskine and Eberhart, 1988; Bradley et al., 2005). Therefore, the primary objective of this study was to determine whether the CMT performed cow-side on the day of dry off would be a useful method to detect IMI. Given the absence of a gold standard, latent class models should be used for this evaluation. Furthermore, use of CMT and SCC values aggregated to the cow-level, were evaluated to determine their test characteristics and their predictive abilities to identify cows for selective DCT.

7.3. Materials and Methods

7.3.1. Herd Selection

Data was collected from 2921 quarters in 752 Holstein-Friesian cows in 11 dairy herds from July 2002 until June 2004. These herds were selected out of convenience for their close proximity to the Atlantic Veterinary College (Charlottetown, PEI, Canada) (n = 6), Ontario Veterinary College (Guelph, ON, Canada) (n = 4), and Kansas State University (Manhattan, Kansas, USA) (n = 1).

7.3.2. Sampling Procedures

Single quarter milk samples were taken two weeks prior to dry-off and on the day of dry-off for culture and SCC. Pre-milking udder preparation was performed as per the farm's usual practice. Teat ends were scrubbed with alcohol and allowed to dry. Foremilk was stripped from each quarter prior to the sampling. On the day of dry-off, immediately following the quarter milk sampling, a CMT (scored as 0, 1, 2, or 3, based on gel formation) was performed on each quarter of each cow by either a study investigator or a trained technician. Somatic cell counts were dichotomized at cut-points of 150,000 and 200,000.

7.3.3. Bacteriological Culture

All laboratory procedures were performed by the same individual, in accordance with NMC recommendations (National Mastitis Council, 1999). All milk plating followed the same standardized approach (Dingwell et al., 2002). Quarters were considered infected if either a major mastitis pathogen (ie. *Staphylococcus aureus, Streptococcus agalactiae,* non-agalactiae Streptococci, *Aeromonas* sp., *Citrobacter* sp., *Escherichia coli, Enterobacter* sp., *Klebsiella* sp., *Proteus* sp., *Pseudomonas* sp., *Serratia* sp., *Salmonella* sp., *Tatumella* sp., *Arcanobacterium pyogenes,* Yeast, *Prototheca* sp.) or a minor mastitis pathogens (ie. > 10 colony forming units (CFU) of either coagulase-negative staphylococci (CNS) or *Corynebacterium bovis*) were isolated.

7.3.4. Population Classification

The bacteriological culture results of milk samples taken two weeks prior to dry-off were used to identify two populations of cows. When no major mastitis causing pathogen (listed above), or ≤ 10 CFU of CNS were isolated from any quarter of a cow, the cow was assigned to Population 1. This population was expected to have a low prevalence of IMI at the time of dry-off. When a major mastitis causing pathogen (listed above) or >10 CFU of CNS were isolated from one or more quarters of a cow, that cow was assigned to Population 2. This population was expected to have a high prevalence of IMI at the time of dry-off. The isolation of *C. bovis* from a milk sample taken two weeks prior to dry-off did not have any influence on the population classification of cows. A kappa statistic to measure the level of agreement

was performed between the culture results two weeks prior to dry-off and at dry-off.

7.3.5. Latent Class Models Using Bayesian Methods

7.3.5.1. Quarter-level Models

Bayesian estimation using a Gibbs sampler in WinBUGs (MRC Biostatistics Unit, Cambridge, UK) was utilized to estimate test parameters and population prevalence (Spiegelhalter et al., 1996). Models with a CMT cut-point >0 were assessed for all pathogens, major pathogens and minor pathogens. When the minor pathogen models were assessed, quarters containing major pathogens were excluded from the analysis.

Priors in the form of beta probability distributions for the sensitivity and specificity of the CMT at various cut points and pathogen types were compiled using data from CMT studies published since 2000 (Sargeant et al., 2001; Dingwell et al., 2003b; Middleton et al., 2004; Ruegg and Sekito, 2004; Wallace et al., 2004). Priors (also beta probability distributions) for bacteriological culture sensitivity and specificity were compiled using data from previously published studies (Erskine and Eberhart, 1988; Dinsmore et al., 1991; Davidson et al., 1992; Buelow and Nordlund, 1999). Priors for the two populations were estimated based on subjective estimates of low prevalence and high prevalence IMI populations. Beta distributions were determined by specifying the mode and either the 5^{th} or 95^{th} percentile (depending on whether the mode was ≥ 0.50 or < 0.50) as input values for the pooled prevalence calculator website (**www.ausvet.com.au**). A complete listing of priors, showing the mode and either 5^{th} or 95^{th} percentile (the two values required to specify the beta distribution) are presented in Table 1.

A total of 20,000 iterations were run using the Gibbs sampler and the first 1000 were discarded as the burn-in phase. Three chains with different initial values were used to ensure model convergence and this was assessed by viewing chain paths and by using the Gelman-Rubin statistic. The Gelman-Rubin statistic compares pooled chained variance to within chain variance using ANOVA and converges around 1. All models were fit using informative priors, and then repeated using uninformative priors (beta (1, 1)). Finally, conditional independence of the tests was evaluated using a model which allowed for dependency of the tests and the estimates were compared (Georgiadis et al., 2003; Branscum et al., 2005).

7.3.5.2. Cow-level Models

Quarter results for the CMT test, SCC and bacteriological culture were aggregated to the cow-level to give each animal a single score for each test by interpreting the four quarter-level results in parallel. For example, if a cow had at least one quarter with a positive (eg. >0) CMT score, the cow was considered test-positive for CMT. Similarly, if a cow had at least one quarter with a SCC higher than the threshold chosen (eg. >150,000), the cow was considered test-positive for SCC. The same method was applied to the bacteriological culture results, using all, major and minor pathogen categories.

Two sets of models were fit, one examining CMT and culture and another examining SCC and culture. Both models were fit at the cow-level. Uninformative priors (beta (1, 1)) were used to fit the cow-level models, as no previous studies have reported sensitivities and specificities of testing in this manner. Exceptions to this were the cow-level dependency models. Because of the need to estimate eight parameters, prior information was required for at least two estimates, otherwise, the model would be non-identifiable. Priors for the populations were estimated based on subjective estimates of low prevalence and high prevalence IMI populations and are listed in Table 1. Model diagnostics were performed in the same manner as explained above.

7.3.5.3. Predictive Values

Positive and negative predictive values (PPV and NPV, respectively) were estimated for each test by using the sensitivity (Se), specificity (Sp) estimates for each test and prevalence (*P*) estimates ranging from 1-100%. For example, the PPV for CMT was estimated as

$$PPV = \frac{(P * Se_{CMT})}{((P * Se_{CMT}) + ((1 - P) * (1 - Sp_{CMT})))}$$

The same method was employed to estimate all PPVs, with substitutions of values for the SCC. The NPV for CMT was estimated as

$$NPV = \frac{((1 - P) * Sp_{CMT})}{(((P * (1 - Se_{CMT})) + ((1 - P) * Sp_{CMT}))}$$

Again, the same method was employed to estimate all NPVs.

7.4. Results

A total of 752 cows (2921 quarters) were included in the study. Average herd size was 144 cows (median 110), and this ranged from 40-380 lactating cows. Of the 11 herds included, 6 were free-stall and 5 were tie-stall operations. The average parity at dry-off of study cows was 2.2 (SD 1.4), ranging from 1-9. The largest percent of cows were dried off in the summer (29%), followed by winter (25%), spring and fall (23%).

The quarter- and cow-level prevalence of IMI two weeks prior to dry-off was 16 and 41%, respectively. At this time point, the distribution of pathogens at quarter-

and cow-level were: 2 and 6.7% for S. aureus, 2.2 and 7.2% for non-agalactiae streptococci, 2.3 and 6.6% for Gram-negatives, and 10.6 and 27.7% for CNS (> 10 CFU), respectively.

The quarter- and cow-level prevalence of SCC > 200,000 two weeks prior to dry-off was 29 and 48%, respectively. The distribution of quarters at the various CMT scores were 1884 (65%), 745 (26%), 214 (7%), and 78 (3%) for 0, 1, 2, or 3, respectively. A total of 419 cows (1627 quarters) were included in population 1, and 333 cows (1294 quarters) in population 2.

Results of the kappa statistic used to measure the level of agreement between 2 culture results were very low and showed only slight agreement.

7.4.1. Quarter-level Models

The quarter-level prevalence estimates of IMI for all pathogens in population 1 and 2, were 12 and 46%, respectively. Sensitivity and specificity of both the quarter-level CMT (using cut-point > 0) and bacteriological culture along with the estimated prevalence of IMI in both populations are summarized in Table 7-2. Few differences were found between the informed prior models and the uninformed prior models. A 9% decrease in the culture sensitivity for major pathogens was found when uninformed priors were used compared to informed priors. Small decreases in population prevalence for all pathogens and small increases in population prevalence for major pathogens were noted when the uninformed priors were used. The coefficients and confidence intervals did not change when the models were assessed for conditional test dependence. Estimates of between-test correlations were very small and their probability intervals included zero.

Specific pathogen groups (all, major, or minor) are represented in each section of the table. Sensitivity of the CMT at a cut-point > 0 had the highest value (64%) when major pathogens were considered, compared to all and minor pathogens. The specificity of CMT was similar across pathogens. Sensitivity of bacteriological culture was highest (75%) when all pathogens were considered. In contrast, culture specificity was highest (98%) when major pathogens were assessed.

7.4.2. Cow-level Models

7.4.2.1. CMT and Culture

The cow-level prevalence of estimates of IMI for all pathogens in population 1 and 2, were 11 and 80%, respectively, when the CMT model was performed. Cow-level sensitivity and specificity estimates, along with the population prevalence estimates are presented in Table 7-3. The population 1 prevalence estimate increased in the dependency models which utilized an informative prior for population prevalence. Also, the sensitivity of CMT decreased in the dependency model. In addition, the specificity of culture increased in the all and minor pathogen dependence models. However, there was no evidence of dependence between the cow-level CMT and culture (ie. all estimates of correlations between-tests were low and all confidence intervals included zero). Consequently, the results from the model based on the assumption of conditional independence (and uninformative priors) were used for all subsequent analyses.

The CMT cow-level sensitivity for identifying cows with a major pathogen IMI in one or more quarters was high (86%) while for all pathogens it was moderate (70%). Regardless of pathogen group consideration, the CMT cow-level specificity was poor (< 50%). The sensitivity of bacteriological culture at the cow-level was high (≥ 89%) with the exception of major pathogens (62%). In contrast, the culture specificity was highest for major IMI (91%).

7.4.2.2. CMT Predictive Values

The PPV and NPV of CMT (cut-point > 0) for major pathogens across all possible prevalence values are shown in Figure 7-1. The NPV for identifying cows with major pathogens with the CMT was high when the prevalence of major pathogens is low. For example, in a herd with a cow-level major pathogen prevalence of 15%, the NPV was 95%. The chance of a truly positive cow being identified falsely as negative (1-NPV) in this situation was low (5%).

7.4.2.3. SCC and Culture

The cow-level population 1 and 2 prevalence estimates were 31 and 86%, respectively, when the SCC model was used. The estimate of prevalence in population 1 was much higher with the SCC models than in the CMT models. Cow-level SCC (cut-point > 150,000) sensitivity and specificity estimates, along with the population prevalence estimates are presented in Table 7-4. Overall, SCC cow-level sensitivity and specificity were fair. Sensitivities improved approximately 10% if SCC positive threshold, in at least one quarter, was lowered from >200,000 cells/ml to >150,000 cells/ml. The sensitivity was highest (87%) when identification of cows with major pathogen IMI were considered. Test characteristics for bacteriological culture were very similar to the CMT model described above. Again, no dependency between tests was detected.

7.4.2.4. SCC Predictive Values

The PPV and NPV of SCC (cut-point > 150,000) for major pathogens across all possible prevalence values are shown in Figure 7-1. As with the CMT, the NPV for identifying cows with major pathogens with the SCC was high when the prevalence of major pathogens was low.

7.5. Discussion

7.5.1. Evaluation of Model Assumptions

Latent class models are a valuable tool in epidemiology because of their capability to evaluate test characteristics in the absence of a gold standard. These methods reduce estimation bias which will undoubtedly occur if errors in the reference tests are ignored (Enøe et al., 2000). In order to use Bayesian methods to characterize test performance when no gold standard is available, 3 assumptions must be met: different population prevalence of disease, test independence, and tests must perform the same across populations (Hui and Walter, 1980).

The resulting estimates of population prevalences were very different and seemed to support the fact that we were successful in creating two different prevalence populations using our culture results two weeks prior to dry off. Questions may arise as to the validity of using culture results to generate two populations, when culture was used as one of the reference tests. Bacteriological culture results can change quickly, especially when environmental pathogens are considered (Bradley et al., 2005). This, coupled with a slight change in culture criteria

used at the two time points, support this method to create the two populations of different prevalences. Additionally, using a microbial method to define the population may affect the estimates of sensitivity and specificity for culture, but the current studies main interest was CMT.

The CMT relies on a reaction of reagent with nuclear DNA in SCC, whereas bacteriological culture relies on the isolation of the pathogen. Therefore, the tests have a different biological basis and therefore, should be independent. However, biological independence of tests may not be adequate to meet this assumption (Nerette et al., 2005). Therefore, the assumption of dependence was tested using a model to account for conditional dependence (Georgiadis et al., 2003). As coefficients of test parameters and population prevalence did not differ much between the independence and the dependence models and the correlations between tests were low, the assumption of independence appeared to be satisfied.

The third assumption of equal test accuracy across different populations was not evaluated in the current study. It might be assumed that sensitivity of CMT increased in population 2. This assumption can be checked by conducting separate analysis of each population. If results are different across populations, they can still be interpreted as average estimates for the population rather than population-specific values, because populations were derived from one population (Branscum et al., 2005).

One distinct feature of Bayesian methods for fitting latent class models is the use of prior information to generate test parameter estimates. A literature search was undertaken to incorporate as much previous data as possible to elicit prior

information. Prior information appeared to have an impact on the estimates of culture sensitivity of major pathogen IMI, as well as the population prevalences. Estimate of major pathogen CMT and culture sensitivity was higher, when the informed prior was used in comparison to the uninformative prior (64 vs 59%, 36 vs 26%) respectively. It is possible that previous studies have overestimated major CMT sensitivity due to errors in the reference test which were overlooked (Enøe et al., 2000). Population prevalences for major pathogens were lower in the presence of prior information. Again, it is possible that the infection proportion with major pathogens was underestimated in our subjective opinion, which could explain the differences. No other parameter estimates appeared to be affected by the use of prior information in the quarter-level models.

No previous data were available to formulate priors for the cow-level test. However, the cow-level dependency models had to use some prior information in order to be identifiable (ie. have enough degrees of freedom to estimate all the parameters). Experts were reluctant to give opinions regarding the population prevalences because they had no data to support their suggestions. Because of this hesitation, the population prevalence estimates were generated using culture results from the current data taken at a previous time point. In the cow-level CMT model, impacts of the prior information were seen affecting the population 1 estimates. Prevalence estimates were higher in the presence of prior information. As the current data was utilized to estimate prevalence priors from a different time point, this may demonstrate that infection status within a cow is ever-changing. There was virtually

no impact of prior information in the SCC and culture model, although the sensitivity of both SCC and culture were lower in the presence of informed prevalence priors.

7.5.2. CMT Performance at the Quarter-level

Overall quarter-level CMT performed poorly at predicting IMI caused by all, major or minor organisms at dry off. The sensitivity was moderate (64%) for major pathogens, only if the informative prior model was used. This reflects the impact of the priors based on previous literature which had major pathogen sensitivity estimates ranging from 55-76% (Sargeant et al., 2001; Middleton et al., 2004; Ruegg and Sekito, 2004; Wallace et al., 2004). In general low sensitivity of the CMT were observed which is consistent to results from previous studies using culture-based gold standard in terms of quarter-level CMT sensitivity and specificity.

Choosing the best cut-point should rely on the relative severity of false-negatives and false-positives (Dohoo et al., 2003). When utilizing the CMT for a selective DCT program, false-negatives would have the worst consequences, as truly infected quarters would be left untreated. Therefore, the lowest cut point (>0) should be used, which gave the highest sensitivity and NPV. The result would be that uninfected quarters would be treated with DCT. However, this would ensure that the majority of truly infected quarters were correctly identified. Furthermore, there would still be fewer quarters treated with DCT when compared with a blanket DCT program.

7.5.3. CMT Performance at the Cow-level

Improved sensitivities were found when the quarter-level data were collapsed to form a cow-level test result, specifically when major pathogens were considered. Cow-level CMT specificity was poor in all models. Therefore, many false positive test results would be obtained.

Culture sensitivities of all and minor pathogens were excellent, but the culture sensitivity of major pathogens was moderate, although the confidence interval was very large. Previous studies have shown that the sensitivity of culture is dependent on the specific pathogen, and also on the number and timing of samples (Erskine and Eberhart, 1988; Dinsmore et al., 1991; Sears et al., 1991; Buelow and Nordlund, 1999). Our tabulation of sensitivity and specificity was based on one bacteriological culture per quarter, which was then collapsed down to make a cow-level decision. This method would have low sensitivity for detecting pathogens such as *S. aureus* which require multiple milk sampling for improved detection (Godden et al., 2002). Culture specificity in the independent all pathogen model was poor and did not agree with the dependence and SCC model estimates. As the estimates for culture were not of primary interest and the CMT estimates were not affected the independence model was used, but this discrepancy between culture specificity should be noted.

One of the primary interests when designing this study was to assess the usefulness of the CMT at dry-off to identify quarters and cows requiring DCT. This study was instigated as a part of a larger clinical trial involving the use of ITS during

the dry period. Interest in selective DCT programs is growing as reasonable alternatives to blanket DCT exist. Decisions regarding the use of selective DCT programs must be made initially at a herd-level, and next at the cow-level. Positive and negative predictive values reflect the predictive ability of a test in a particular population because they are affected by disease prevalence (Dohoo et al., 2003). Therefore, the current study tabulated the PPV and NPV across all possible prevalences. The priority in a selective DCT program is to ensure that infected animals are not falsely identified as negative. The NPV of a test is the proportion of test-negative animals that are truly negative. Therefore, greatest interest is in the percentage of CMT-negative cows that are not truly negative (ie. 1-NPV). In herds with low major pathogen prevalences (eg. < 20%), this proportion was very small ($\leq$ 5%). This means that a very small proportion of truly infected cows (with major pathogens) would not receive DCT.

Because there are no studies that report cow-level prevalence of IMI, the current study tabulated prevalences for all pathogens and major pathogens for participating herds. In this instance, over half of the study herds had true prevalence estimates of IMI with major pathogens equal to or below 20%. Therefore, these seven herds could successfully utilize the CMT at dry-off in a selective DCT program.

The NPV for all pathogens was slightly lower than that of major IMI, depending on the proportion of minor IMI. This means that the chance of missing a truly infected animal (with all pathogens) was slightly larger, but still acceptable in low prevalence herds. The value of treating quarters with minor IMI pathogens is

still uncertain. When minor pathogens have been evaluated, some studies have demonstrated that IMI with these organisms decrease the risk of developing a new IMI caused major pathogens (Rainard and Poutrel, 1988; Lam et al., 1997). However, other studies have contradicted those findings when specific pathogens were considered (Hogan et al., 1988; Lam et al., 1997; Berry et al., 2002). Therefore, whether missing minor IMI treatment with DCT is an important issue in the success of selective DCT programs, remains to be elucidated. However, most importantly, if results of the CMT were used to decide treatment, then few major pathogens would be missed.

As prevalence increases, NPV decreases. As such, high IMI prevalence herds should be cautious employing this use of CMT for use in a selective DCT program. Herds with high prevalence of IMI should probably not consider selective DCT programs at all, and should continue to blanket treat all cows as per NMC recommendations (National Mastitis Council, 1999). The PPV were of little value, unless herds had cow-level prevalences of 100%. And again in this instance, selective DCT programs should not be considered.

The CMT although relatively simple to use, can be difficult to read due to the subjectivity of the scoring. In this study, CMT was performed by trained technicians and/or veterinarians, so poor operator performance should have been minimized and the results should be interpreted with that in mind. However, no methods were utilized to compare operator performance when interpreting the CMT. Results have good validity in that the test was performed cow-side instead of in a laboratory setting, but in a real world scenario the producer themselves will be responsible for

conducting the test and interpreting the results. Sensitivity of the CMT was low for detection of IMI postpartum when the producer performed the test (Wallace et al., 2002). It was also noted that as herd prevalence of IMI increased so did the producer's sensitivity in detecting IMI (Wallace et al., 2002). This has implications for the latent class models used in this study, as it would violate the assumption of equal test performance across populations. However, as was previously discussed, the estimates from this study are an average across populations and not a population-specific estimate.

7.5.4. SCC Performance at the Cow-level

As with the cow-level CMT, the cow-level SCC was a parallel combination of individual quarter test results, not a test result based on a composite sample. The cow-level sensitivities for SCC to identify infected animals with major IMI were good and followed the same trend as did the CMT test performance. When the threshold to identify positive cows was reduced from 200,000 to 150,000 cells/ml, a slight gain in sensitivity was achieved. Many would argue that classifying an animal as infected, if at least one quarter has a SCC greater than 150,000, is too stringent a classification but this cut-point did perform better in this study. The specificity of SCC was slightly better than that of the CMT. The predictive values of the SCC were also similar to those of the CMT, providing another option to identify truly negative animals at a small risk of missing major IMI (3%).

There was a large difference in the population 1 prevalence estimates for all and minor pathogens between the CMT and SCC models. The SCC models gave much higher prevalences than the CMT models. This difference is most likely explained by the SCC threshold > 150,000 cells/mL. Identifying a positive CMT at a SCC of 150,000 cells/mL would be difficult as the gel formation at this level would be minimal. It is possible that minor pathogen IMI are responsible for this difference as minimal population prevalence differences were noted between the CMT and SCC models when major pathogen IMI were considered.

7.5.5. Conclusions

In conclusion, given the convenience and low-cost of the CMT, low-prevalence herds, could use the CMT on quarter milk samples and then combine these test results (in parallel) to the cow-level to identify animals that do not require DCT. Quarter SCC, interpreted in parallel, also works well to identify animals that do not require DCT. However, use of SCC would have a greater cost and require more planning for the producer, unless on-farm cell counting technology was used (Ruegg, 2005).

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Table 7-1 Prior information, shown by mode and 5th or 95th percentile (perc) for population prevalence (*P*), sensitivity (Se) and specificity (Sp) of California mastitis test and culture for all, major and minor pathogens at the quarter- (qtr-) and cow-level

	All pathogens		Major pathogens		Minor pathogens	
	Mode	5/95th perc	Mode	5/95th perc	Mode	5/95th perc
Qtr-level						
Se_{CMT}	0.55	0.40	0.70	0.50	0.50	0.25
Sp_{CMT}	0.71	0.60	0.75	0.60	0.70	0.50
Se_{Cul}	0.80	0.40	0.83	0.45	0.68	0.45
Sp_{Cul}	0.91	0.80	0.98	0.85	0.83	0.65
P_1	0.25	0.45	0.01	0.25	0.20	0.50
P_2	0.55	0.80	0.20	0.60	0.45	0.80
Cow-level						
P_1	0.40	0.90	0.05	0.50	0.35	0.60
P_2	0.95	0.50	0.50	0.99	0.80	0.99

Table 7-2 Estimates of infection prevalence (P), sensitivity (Se) and specificity (Sp) of quarter-level CMT (cut-point >0) and culture for detection of all, major and minor pathogens using latent class models

		Informed Prior Independence		Uninformed Prior Independence		Informed Prior Dependence[a]	
		Est.[b]	95% PI[c]	Est.	95% PI	Est.	95% PI
All	Se_{CMT}	0.53	0.48-0.59	0.55	0.49-0.62	0.52	0.45-0.59
	Sp_{CMT}	0.71	0.68-0.74	0.70	0.68-0.73	0.71	0.68-0.74
	Se_{Cul}	0.75	0.61-0.91	0.81	0.64-0.98	0.73	0.52-0.92
	Sp_{Cul}	0.90	0.87-0.95	0.88	0.84-0.94	0.90	0.86-0.95
	P_1	0.12	0.07-0.20	0.08	0.02-0.18	0.13	0.07-0.21
	P_2	0.46	0.36-0.59	0.40	0.30-0.55	0.47	0.36-0.69
Major	Se_{CMT}	0.64	0.55-0.75	0.59	0.48-0.72	0.69	0.57-0.79
	Sp_{CMT}	0.69	0.66-0.72	0.70	0.67-0.73	0.69	0.66-0.72
	Se_{Cul}	0.35	0.23-0.65	0.26	0.16-0.44	0.39	0.25-0.66
	Sp_{Cul}	0.98	0.96-0.99	0.98	0.96-1.00	0.98	0.96-0.99
	P_1	0.03	0.00-0.09	0.06	0.01-0.17	0.03	0.00-0.08
	P_2	0.24	0.12-0.38	0.33	0.18-0.57	0.22	0.13-0.35
Minor	Se_{CMT}	0.53	0.46-0.60	0.53	0.45-0.62	0.50	0.41-0.60
	Sp_{CMT}	0.71	0.68-0.73	0.70	0.68-0.74	0.70	0.67-0.73
	Se_{Cul}	0.68	0.54-0.84	0.73	0.53-0.97	0.65	0.45-0.84
	Sp_{Cul}	0.90	0.87-0.95	0.90	0.86-0.97	0.90	0.86-0.95
	P_1	0.09	0.03-0.17	0.08	0.02-0.20	0.09	0.03-0.18
	P_2	0.39	0.29-0.53	0.36	0.24-0.56	0.41	0.30-0.65

[a]Covariance (All) Sensitivity[b(c)] 0.04 (-0.11,0.19), Specificity[b(c)] -0.02 (-0.07,0.05)
Covariance (Major) Sensitivity[b(c)] -0.10 (-0.31,0.10), Specificity[b(c)] -0.01 (-0.04,0.04)
Covariance (Minor) Sensitivity[b(c)] 0.08 (-0.15,0.25), Specificity[b(c)] -0.00 (-0.09,0.08)
[b] estimate
[c] probability interval

Table 7-3 Estimates of infection prevalence (P), sensitivity (Se) and specificity (Sp) of cow-level California mastitis test (cut-point >0) and culture for detection of all, major and minor pathogens using latent class models

		Independence		Dependence[a,b]	
		Est.[c]	95% PI[d]	Est.	95% PI
All	Se_{CMT}	0.70	0.64-0.76	0.69	0.62-0.76
	Sp_{CMT}	0.48	0.42-0.53	0.49	0.42-0.56
	Se_{Cul}	0.95	0.84-1.00	0.93	0.81-1.00
	Sp_{Cul}	0.70	0.61-0.89	0.81	0.64-0.94
	P_1	0.11	0.01-0.32	0.25	0.04-0.38
	P_2	0.80	0.70-0.94	0.85	0.74-0.98
Major	Se_{CMT}	0.86	0.73-0.97	0.79	0.65-0.95
	Sp_{CMT}	0.46	0.41-0.51	0.46	0.41-0.52
	Se_{Cul}	0.62	0.41-0.94	0.56	0.31-0.96
	Sp_{Cul}	0.91	0.88-0.96	0.93	0.89-0.97
	P_1	0.03	0.00-0.13	0.07	0.01-0.19
	P_2	0.38	0.22-0.63	0.45	0.24-0.89
Minor	Se_{CMT}	0.63	0.55-0.70	0.61	0.54-0.69
	Sp_{CMT}	0.47	0.41-0.53	0.47	0.40-0.54
	Se_{Cul}	0.89	0.74-0.99	0.90	0.74-0.99
	Sp_{Cul}	0.82	0.69-0.99	0.93	0.79-1.00
	P_1	0.17	0.01-0.36	0.28	0.14-0.38
	P_2	0.79	0.64-0.98	0.81	0.69-0.98

[a] informed priors were only available for population prevalence estimates
[b]Covariance (All) Sensitivity[b(c)] 0.13 (-0.07,0.32), Specificity[b(c)] -0.11 (-0.35,0.08)
Covariance (Major) Sensitivity[b(c)] 0.21 (-0.14,0.60), Specificity[b(c)] -0.11 (-0.25,0.03)
Covariance (Minor) Sensitivity[b(c)] 0.11 (-0.12,0.32), Specificity[b(c)] -0.03 (-0.25,0.16)
[c]estimate (median)
[d]probability interval

Table 7-4 Estimates of infection prevalence (*P*), sensitivity (Se) and specificity (Sp) of the cow-level somatic cell count (cut-point >150,000) for all, major and minor pathogens using latent class models

		Independence		Dependence[a,b]	
		Est.[c]	95% PI[d]	Est.	PI
All pathogens	Se_{SCC}	0.68	0.62-0.74	0.66	0.60-0.73
	Sp_{SCC}	0.62	0.55-0.68	0.59	0.51-0.68
	Se_{Cul}	0.94	0.85-1.00	0.92	0.82-1.00
	Sp_{Cul}	0.89	0.76-0.99	0.87	0.66-0.99
	*P*1	0.31	0.18-0.42	0.30	0.05-0.43
	*P*2	0.86	0.77-0.95	0.87	0.76-0.99
Major pathogens	Se_{SCC}	0.87	0.77-0.97	0.79	0.63-0.93
	Sp_{SCC}	0.56	0.50-0.64	0.55	0.49-0.63
	Se_{Cul}	0.59	0.42-0.87	0.50	0.30-0.86
	Sp_{Cul}	0.96	0.91-0.99	0.95	0.90-0.99
	*P*1	0.09	0.03-0.20	0.10	0.01-0.24
	*P*2	0.45	0.28-0.63	0.53	0.29-0.91
Minor pathogens	Se_{SCC}	0.60	0.53-0.67	0.58	0.50-0.65
	Sp_{SCC}	0.61	0.55-0.68	0.60	0.52-0.67
	Se_{Cul}	0.88	0.75-0.99	0.89	0.75-0.99
	Sp_{Cul}	0.93	0.78-1.00	0.93	0.78-1.00
	*P*1	0.29	0.14-0.40	0.29	0.15-0.39
	*P*2	0.84	0.70-0.98	0.82	0.70-0.98

[a] informed priors were only available for population prevalence estimates
[b]Covariance (All) Sensitivity[b(c)] 0.06 (-0.12,0.25), Specificity[b(c)] 0.10 (-0.18,0.30)
Covariance (Major) Sensitivity[b(c)] 0.20 (-0.19,0.41), Specificity[b(c)] 0.02 (-0.14,0.19)
Covariance (Minor) Sensitivity[b(c)] 0.02 (-0.20,0.23), Specificity[b(c)] 0.14 (-0.11,0.33)
[c]estimate (median)
[d]probability interval

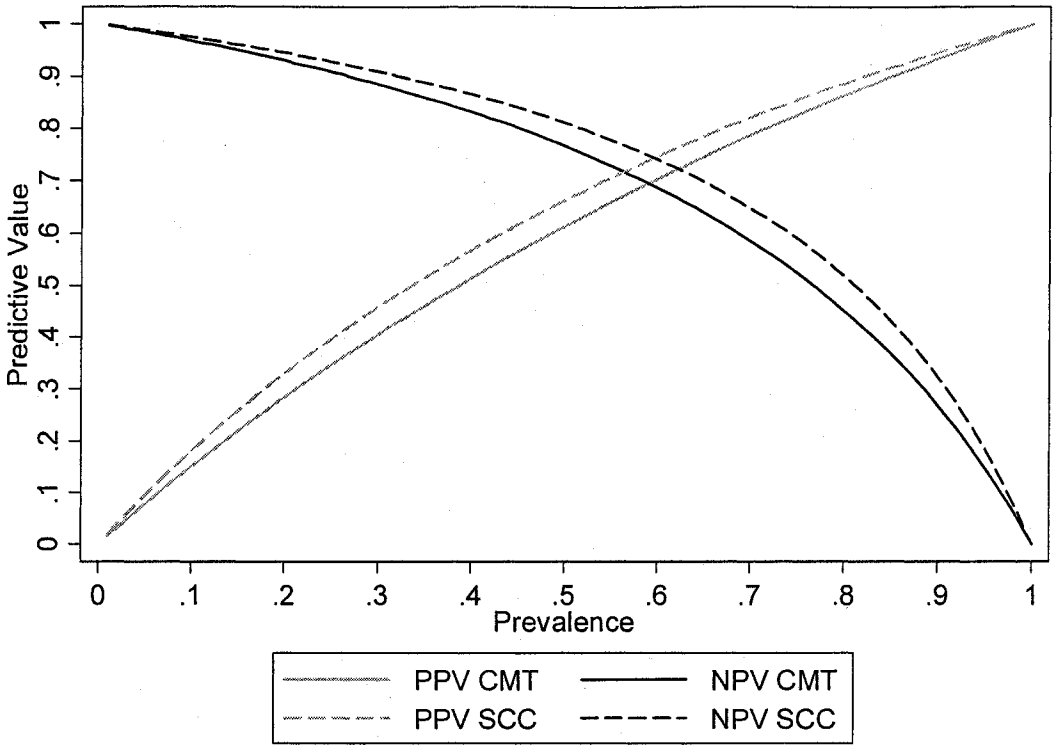

Figure 7-1 Line graph of the positive predictive values (PPV) and negative predictive values (NPV) for both the California mastitis test (CMT) and somatic cell count (SCC) when major pathogens are considered.

CHAPTER 8 SUMMARY AND GENERAL DISCUSSION

8.1. General Discussion

8.1.1. Rationale for Study

Several clinical trials examining the effects of internal teat sealers (ITS) have been conducted in other countries. In general, ITS have been found to be effective in preventing new intramammary infections (IMI) when used alone or in conjunction with dry cow therapy (DCT). In summary, quarters infused with ITS at dry-off were less likely to develop new IMI during the dry period than negative control quarters (Woolford et al., 1998; Berry and Hillerton, 2002). The ITS provided significantly better protection from the development of specific pathogen IMI (ie. *Escherichia coli* and all Enterobacteriaceae combined), than DCT during the dry period (Huxley et al., 2002). Furthermore, using ITS in conjunction with DCT significantly decreased the development of new IMI during the dry period when compared with DCT alone (Godden et al., 2003). Therefore, the beneficial effects of using ITS at dry-off to prevent the development of new IMI during the dry period have been well established. Previous studies have also measured the effect of ITS on the development of clinical mastitis (CM) during the subsequent lactation. However, results from previous studies on the effect of ITS on CM are inconsistent, making a general conclusion difficult.

Despite the publication of several clinical trials, there remained no information regarding the effect of ITS in Canadian dairy herds. In New Zealand, the study measuring the effect of ITS used cows which were seasonally calved on pasture

and grazed year-round. The Canadian dairy industry, while considered similar to the US, has features which make it unique, such as the milk marketing system, dairy housing design, and widely varying climatic changes. Because of the quota system, year-round calvings are necessary. In addition, because of the northern climate in Canada, year-round grazing is not an option. In the UK, herds with summer grazing and winter housing with low bulk-tank somatic cell count (SCC) (often organic), and cows with low SCC were chosen for participation in their clinical trials (Berry and Hillerton, 2002; Huxley et al., 2002). In the US, large commercial dairies were utilized for the ITS studies (Godden et al., 2003; Cook et al., 2005). In Canada, there are still a high proportion of small herds with different housing and management compared to the UK systems and the large commercial operations found in the US. All of the differences from previous studies make it difficult to conclude that ITS used in Canadian dairy herds would work in the same manner. Therefore, an investigation into the efficacy of ITS for the prevention of new IMI during the dry period in Canadian dairy herds was needed.

8.1.2. Study Design

In previous studies, with the exception of the New Zealand trial, ITS were either used in conjunction with DCT and compared with DCT alone, or used alone and compared with negative controls or DCT alone. The treatment randomization in these studies was performed at the cow- or split-udder level. In the New Zealand study, four different quarter-level treatments were compared (ie. negative control,

DCT, ITS and ITS and DCT). The current study design consisted of a two-level treatment assignment. This approach was chosen because the researchers wanted to utilize the product in the same manner that may be employed on-farm. Because of the drive to decrease prophylactic antibiotic usage in food-producing animals, an interest in selective DCT programs is developing. Our two-level treatment assignment and split-udder design made it necessary to seek out potential diagnostic tools that could predict IMI status on or near the time of dry-off, as culturing two weeks prior to dry-off is not practical nor economical for producers. To accomplish this, a California Mastitis Test (CMT) was performed and quarter milk samples for SCC were collected prior to milking on the day of dry-off. The CMT, SCC, and culture were used and then evaluated for their ability to predict IMI at dry-off using latent class models. The results and summary of the diagnostic test evaluation are presented in section 8.3.6. Because a split-udder treatment allocation was utilized, it was necessary to ensure that DCT, specifically cloxacillin, administered in one quarter, could not have any effect on an ITS treated quarter. This was also validated using a contralateral and ipsilateral split-udder designs that were compared to see if either method had much cloxacillin movement to untreated quarters. The results and summary of the validation study are presented in section 8.3.5.

In the current study, there were 16 dairy herds (15 in Canada and 1 in the the US) that participated. Herds were selected out of convenience for their proximity to participating institutions, because weekly visits during milking were necessary. Regions of herd participation represent greater than 70% of milk sales and farms in Canada (Dairy Farmers of Canada, 2002). A study of this size, measuring the

effectiveness of ITS either at the herd-level or cow-level, had not been previously reported and should have good external validity to the Canadian dairy industry. With the exception of the Kansas State University and University of Guelph research herds, all herds in the study were commercial operations. Other previous ITS studies were performed using either small numbers of farms, specific herd- or cow-level inclusion criteria, or sampled from one geographic location.

8.2. General Thesis Overview

This thesis examined the use of ITS, administered at dry-off, on the prevention of new IMI and quarters cure rates during the dry period when compared to a standard DCT. In addition, the incidence of CM, particulate matter recovery, milk production, and SCC were monitored during the subsequent lactation, after the ITS were administered. Since the majority of research included in this thesis was performed using split-udder-designed clinical trials, this research method was validated by performing a small clinical trial to determine if between-quarter movement of cloxacillin DCT occurred. Finally, in an attempt to identify a reliable and feasible diagnostic tool for the identification of IMI at dry-off, the CMT and quarter SCC were evaluated from their test characteristics using latent class models.

8.3. Summary of Results

8.3.1. Prevention of New IMI and CM

A total of 519 Pre-Dry Uninfected cows and 420 Pre-Dry Infected cows were studied to examine the efficacy of ITS used alone, and in conjunction with DCT for the prevention of new IMI during the dry period, when compared with DCT. When Pre-Dry Infected cows were considered, ITS used in combination with DCT worked significantly better than DCT alone. New IMI caused by all, major, environmental and non-agalactiae streptococci pathogens were 49, 51, 53, and 59% less likely to develop in quarters receiving both treatments, respectively. This finding was similar to a Minnesota study (Godden et al., 2003).

When Pre-Dry Uninfected cows were considered, quarters treated with ITS were 46% less likely to develop a new environmental IMI, than DCT treated quarters. When all other pathogen groups were assessed, ITS alone worked no differently than DCT. This finding is similar to a UK study that found that ITS alone were significantly better at preventing new IMI caused by *Escherichia coli* and all Enterobacteriaceae combined (Huxley et al., 2002).

Right front quarters were 47% more likely to develop new IMI caused by all pathogens when compared with left front quarters but the reason for this difference is not obvious. Fourth parity and older cows were 69% more likely to develop new IMI caused by environmental pathogens than second parity animals. Quarters infected with *Corynebacterium bovis* were 96% more likely to develop new IMI caused by Gram-negative pathogens than non-infected quarters.

The only significant predictor of CM during the first 60 days of lactation was parity. Third and fourth parity cows, were 4.2 and 7.0 times more likely to develop CM when all and environmental pathogens were considered, respectively, when compared to second parity animals. A similar trend was seen when fifth parity and older cows were compared with two year olds. The treatment effect of ITS on the incidence of CM was inconclusive. Trends for the reduction of CM when ITS were used were observed. Lack of participation from all study herds for collecting CM samples, decreased the sample size of this component of the research trial. Therefore, the power of the study was decreased.

8.3.2. Cure Rates During the Dry Period

A total of 425 infected quarters at dry-off, from 270 cows were assessed to compare the cure rates of ITS used in combination with DCT and DCT used alone. No additional benefit was seen in the cure of IMI present at dry-off, when ITS were used in addition to DCT. Quarter cure rates when ITS were added to DCT compared with DCT alone were 83.8 and 87.9% for major pathogens, 92.2 and 87.5% for minor pathogens, 79.6 and 78.9% for all pathogens, respectively. Because the current study relied on only one sample taken post-calving to classify a cure, an overestimation in the rates exist due to the imperfect sensitivity of bacteriological culture. Because this overestimation would not be different between treatment groups, the coefficients would be biased towards the null. However, there was clearly no effect of ITS on quarter cure during the dry period.

8.3.3. SCC and Particle Recovery

A subset of 30 cows (120 quarters) in one herd, from the large clinical trial, was used to monitor the affect of ITS on SCC and particle recovery during the first week of lactation. Quarters that were treated with ITS, whether alone or in conjunction with DCT, had marginally lower SCC than DCT treated quarters, on the first two days postpartum. No differences in SCC between treatment groups were seen after this. The US trial in Minnesota also found significant reduction at both 1 to 3 days in milk (DIM) and 6 to 8 DIM, in SCC from quarters treated with ITS (Godden et al., 2003).

Results of the current study found that there were more particles present in quarters treated with ITS for the first three days postpartum, when compared with DCT treated quarters, however this difference was only significant on the first day. There were no significant differences detected for the remainder of the week. One interesting finding in this study was that 20% of quarters treated with DCT had particles present for the first seven days postpartum. Composition of these particles was not investigated. Producers' concerns that particulate matter from quarters treated with ITS will foul expensive milking equipment is valid. Internal teat sealer material should be stripped from treated quarters prior to milking, in an attempt to remove the bulk of the particulate matter. Particles in milk should not be a problem, when the milk is ready to be shipped (four days in milk).

8.3.4. ITS on Milk Production and Clinical Mastitis

A total of 75 herds with bulk-tank SCC < 250,000 cells/ml on their last three DHI tests participated in this observational trial. Using individual cow history of CM and SCC, cows were allocated into Group1 or 2. The individual cow ear tag number was then utilized to further randomize cows within groups into treatments groups. Producers recorded CM events for the first 30 days post-calving and DHI milk production and SCC data was made available.

Overall, the compliance in this study was very good considering that the researcher involvement was limited to the study design, veterinarian training and data analysis. When data sheets were collected, 75 of 92 herds (82%) had complied. From these data sheets, 79% (1296) of cows had been correctly allocated into treatment groups by following the study protocol. Clinical mastitis records were recorded from 87% (1131) of the correctly classified study cows in 72 herds, and DHI data was accessible from 81% (1047) of the correctly classified study cows.

The study was conducted with the assistance of local veterinarians and producers. These compliance results hold promise for future studies of this type to be undertaken to include a large numbers of herds across all regions with minimal intervention from the research coordinators specifically when the outcomes needed are routinely recorded parameters such as milk production and SCC.

The cow was utilized as the level of treatment allocation. Cows treated with ITS, whether alone or in combination with DCT, produced significantly less milk (-1.53 kg/day), during the first 180 days of lactation than their DCT treated

counterparts. There was a marginally significant interaction between treatment with ITS and DIM on milk production (ie. apparent detrimental effect of ITS on production was limited to early lactation). Effect of ITS on milk production has not been published elsewhere to date.

As expected, many other factors had a significant effect on milk production during the first 180 days of lactation. When compared with second lactation animals, third lactation cows produced 1.72 kg/day more milk, whereas fourth lactation and older animals produced -0.03 kg/day less milk. Test day month was also found to be a significant predictor of milk production.

A total of 87.3% of participating producers recorded CM events for the first 30 days postpartum, totaling 1131 cows. There was no significant treatment effect. A concern of this study, was the significantly higher proportion of cows that were treated with ITS and DCT, had experienced an episode of CM in the 3 months prior to dry-off. There was no formal training in the definition of CM for the producers. It is possible that particulate matter from ITS treated quarters was mistaken and recorded as CM.

8.3.5. Cloxacillin Transfer

Given that the entire clinical trial was based on a split-udder design, it was important to ensure that this method of treatment allocation did not introduce any potential bias into the study. A bias could have been introduced if cloxacillin from treated quarters was able to move into untreated quarters at a therapeutic level. The

current researchers were also interested in comparing cloxacillin movement between quarters in contralateral and ipsilateral treatment designs. A total of 20 cows from 1 herd were utilized to determine that although 25% of untreated quarters had trace levels of cloxacillin detected by HPLC, the mean concentration (0.006ppm) of the cloxacillin, was well below both the minimum inhibitory concentration (MIC) for labeled pathogens and the residue safe tolerance levels. The only significant predictor of cloxacillin movement from treated to untreated quarters was a weighted average of three composite SCC during the preceding three milk tests. For every unit increase in SCC (log transformed), the movement of cloxacillin from treated to untreated quarters was 74% less likely. This finding was counter intuitive, as previous studies have shown that mastitis has been found to promote the absorption of antibiotics from the mammary gland into the blood stream (Ziv and Schultze, 1982; Sweeney et al., 1996). The significant predictor in the current study was composite SCC and not individual quarter SCC. Increases in composite SCC in late lactation, can often be a reflection of low milk production. The 305 day milk production from the previous lactation was utilized in the model, and no effect was found, but test day milk production at dry-off was not known and therefore not used in the model. Dry-off day milk production could have explained the composite SCC effect. However, the effects of milk production on intramammary antibiotic absorption have not been investigated.

There was no significant difference between the ipsilateral and contralateral treatment designs. Thus, the decision of which design to use should be left to personal preference. Finally, researchers can feel confident regarding the use of split udder

designs when developing clinical trials, as long as the clustering of quarters within cows are accounted for in the analysis.

8.3.6. CMT Selective Dry Cow Therapy

A total of 752 cows from 11 herds, were used to assess the performance of the CMT as a predictor of IMI at dry-off. Latent class models using Bayesian methods were used to evaluate the test characteristics of CMT in the absence of a gold standard. Resultant quarter level sensitivities (53-64%) and specificities (69-71%) of all, major and minor pathogens, were similar to previous CMT studies that had used bacteriological culture as a gold standard for comparison. These values were all too low to allow for the use of CMT at the quarter-level to predict IMI status.

Interpreting the quarter CMT scores in parallel to formulate a cow-level test result (ie. positive in any quarter meant the cow was classified as positive), resulted in better sensitivities for the CMT. When major pathogens were considered the sensitivity of the CMT was 86% and the specificity of 46%. In a selective dry cow program, it is critical that all infected animals are identified and treated with DCT. The number of truly positive animals that would be falsely identified as negative can be determined by using the negative predictive value (NPV). The NPV is the number of test negative animals that are truly negative. Therefore, 1-NPV equals the number of test negative animals that are truly positive. The NPV of the CMT in a group of low prevalence cows was excellent when all and major pathogens were considered.

Having a 99% NPV for major pathogens in these animals means that the chance of missing a truly infected animal with a major pathogen IMI was 1%.

Using quarter-level SCC in parallel to formulate a cow-level SCC result, gave similar promising results as did the CMT. The NPV of the cow-level SCC was a better at ensuring that cows infected with minor pathogens would not be identified as negative. Therefore, quarter-level SCC used in parallel could also be effectively utilized in a selective dry cow program as a means for identifying non-infected animals.

8.4. Conclusions

It was determined that ITS were effective in Canadian dairy herds for preventing new IMI during the dry period (Chapter 2). Using ITS in conjunction with DCT in cows thought to be infected at dry-off, was superior to DCT alone to prevent new IMI. In cows that have a low probability of being infected at dry-off, ITS work with equal efficacy as DCT for preventing new IMI (considering all pathogens). However, ITS alone were better than DCT for preventing new environmental IMI during the dry period. It was determined that ITS alone could be used effectively as a dry cow IMI preventative in uninfected cows in Canadian herds, although the product label specifies that ITS should be used in conjunction with DCT. Therefore, using ITS in this manner would be strictly off-label in Canada. It is unknown how many producers will attempt to use ITS as sole preventatives during the dry period, but

assuming that the device is infused in the proper manner, ITS will provide adequate protection from new IMI.

In the controlled clinical trial, the effects of ITS on the reduction of CM in early lactation were not substantiated. There was no obvious reduction in CM when ITS were used either alone or in conjunction with DCT. When all previous clinical trials regarding ITS are reviewed, no clear effect of ITS were found. The Minnesota trial found a reduction in CM in quarters treated with ITS (Godden et al., 2003). The Wisconsin study found a significant reduction in CM in quarters treated with ITS when a time-to-event analysis was performed (Cook et al., 2005). Other studies did not find any significant differences with the exception of the New Zealand study, which found that any treatment (DCT, ITS or ITS and DCT) was better at reducing CM than no treatment (negative control), but there was no difference across treatments (Woolford et al., 1998). One explanation for the finding in the Minnesota study is that these two large dairies were previously using an on-farm triplate culture system for CM samples prior to the start of the study, and therefore, those herds were actively monitoring CM. Furthermore, herd personnel were previously trained in the detection and sampling of CM cases which may have led to an overall increase in the sensitivity of detecting CM in the Minnesota study. Other studies, including the current study relied on producers and their employees to identify and sample CM. These producers may or may not have had an interest in detecting CM in their herds which could have affected the results.

It was determined that ITS used in conjunction with DCT did not enhance cure rates for existing pathogens during the dry period (Chapter 3). This finding makes intuitive sense as there are no antibacterial properties in ITS.

Early postpartum SCC was decreased in quarters treated with ITS (Chapter 4), but this effect was short-lived. The decrease in SCC at this point is most likely a direct reflection of the decrease in new IMI that developed during the dry period, as was found in Chapter 2. There were more particles recovered from quarters treated with ITS for the first three days postpartum (Chapter 4). Producers should be made aware of this phenomenon, so that particulate matter is not mistaken for CM. A quick CMT can be used to differentiate between bismuth subnitrate and CM if there is a concern. There should be no concern feeding calves the colostrum and early milk from cows treated with ITS. A safety study feeding calves 16 grams of bismuth subnitrate (each tube contains 4 grams) found no adverse effects on these animals (McHardy and Meaney, 1999). When milk is available for sale (4 days in milk) there was no particle difference between quarters treated with ITS or DCT, therefore, it should not be a concern to dairy producers.

The minimally monitored study was an excellent hands-off method to collect data from a large number of herds from varying locations (Chapter 5). Recently, three large scale minimal intervention clinical trials have been conducted by the Atlantic Veterinary College where the producers were asked to administer treatment and record it (Sanchez et al., 2002; Sithole, 2005). In all instances utilization of DHI records has been the main source of data from these herds. The compliance in all these studies was excellent (82-100%) and decreased as the number of herds

increased and the amount of work required from the producer increased (Sanchez et al., 2002; Sithole, 2005). Compliance from participating producers and veterinarians in my study was very good, as 82% of the recruited herds returned completed data sheets. This study required that herd-veterinarians and producers followed the study protocol to assign cows into the correct treatment groups, administer the appropriate dry-cow treatment, and record CM data for each cow for 30 days post-calving. Therefore, a lot was asked of these individuals. It is possible to design similar studies of this manner where outcomes (eg. milk production, SCC) are routinely measured and are available to researchers with permission from producers, with the expectation that compliance will be less than 100%, and to account for this in the sample size calculation. These minimal intervention studies will provide an excellent way to perform studies on a much larger scale than was previously considered.

The negative effect of ITS on milk production during the first six months of lactation (Chapter 5) was surprising and needs to be investigated further. Since ITS reduce the number of new IMI acquired during the dry period, rationally, it should have a positive effect on milk production. Therefore, there must be an explanation for this finding. Without further investigations into the histopathology of the ITS in the mammary gland, conclusions can not be made.

Due to the low cost and simplicity of the CMT, herds with low prevalence of IMI could effectively utilize this test in a cow-level manner to identify cows that do not require DCT (Chapter 7). This finding should allow for greater adoption of selective DCT programs in North America.

8.5. Future Research

Many previous studies to evaluate the ability of the CMT have been performed, but none of the previous studies used latent class model techniques. Furthermore, none of the previous studies used quarter-level results in parallel to form a cow-level test result decision. These methods provided promising results for the use of CMT in a selective dry cow program. Another study to examine the ability of the CMT to predict infected cows at dry-off needs to be performed using latent class models to ensure that results are consistent with that of the current study. In addition, future studies need to investigate specific criteria to develop an accurate and safe tool for use in selective DCT programs. Studies enrolling low bulk-tank SCC herds should be used to evaluate: cow-level historical parameters, (ie SCC, milk production, CM) and dry-off data (ie.CMT results, teat-end conditions, parity, dry-cow housing, and dry-period season), to determine if a cow needs DCT. Bacteriological cultures taken at dry-off can be used to determine if the diagnostic criteria for selective DCT is adequate. The development of a selective dry cow therapy tool for use by herds would be a substantial benefit to the Canadian dairy industry.

There are many effects of ITS that need to be explored. Additional research, in the form of a controlled cow-level clinical trial, needs to be performed to investigate the effects of ITS on milk production. Following random assignment of treatments, cows should be followed for the entire lactation post-administration of ITS to assess production, and shape and persistency of the lactational curve in

animals treated with ITS. Other production limiting disease parameters should be recorded for their potential confounding effects. Further studies will help to substantiate, or contradict, the current negative finding of ITS on milk production.

It is unknown whether the milk production effect of ITS was physical or physiological in nature. A pathological and histopathological examination of mammary glands, that have been infused with ITS should be conducted. Non-pregnant cows that are going to be culled when they dry off could be used for this study. Participating cows should be infused with either ITS or DCT in a split-udder design. Trial design of this manner would allow a histo- and gross pathological comparison of mammary tissue, particularly the alveolar region, between treatments. It has been previously demonstrated that ITS can be recovered from alveolar tissue (Meaney, 1977).

The effects of ITS on CM are inconclusive. There have been no consistent findings when previous studies are examined (Woolford et al., 1998; Berry and Hillerton, 2002; Huxley et al., 2002; Godden et al., 2003). The current study examined the effect of ITS on CM and again had inconsistent findings. A future study with strict definitions of CM, bacteriological culture of CM cases, and a specific time period at risk needs to be addressed. Sample size calculations should be performed prior to commencing the study. If the 2% difference that was found in the Minnesota study is utilized, then at least 2400 quarters will be needed to have a power of 80% for detecting a significant difference.

Economic investigations into the cost-benefit of using ITS either alone, or in conjunction with DCT should be explored. Now that there are clinical trial results

from Canadian herds, discussing the impacts of ITS on IMI, CM and milk production, the economics of use in the Canadian dairy industry can be properly determined.

When the current primary research trial was developed, the primary concern was that quarters treated with ITS would send particles into milking machines and block filters. At present, given the product's full year in the marketplace, a more urgent issue has arisen. It appears that ITS leave a film in the milk line, even after the milking system has been washed. There are concerns that this foreign film could provide a favorable habitat for bacterial growth. Currently, several different cleaning solutions are being recommended to remedy this problem. Producer testimonials to the cleaner's efficacy are available but there are no hard data to assess this issue. Therefore, a future study should be developed to identify the presence of absence of films in milking lines, perhaps by simple swabbing techniques, in herds using ITS. Furthermore, several cleaners need to be tested for their ability to remove these films. Finally, the frequency of cleaner usage should be established to prevent films from establishing in milking systems.

Further selective DCT trials using ITS alone in comparison to DCT should be conducted. The current study identified "Pre-Dry Uninfected" cows on the basis of culture two weeks prior to dry-off, for treatment with ITS or DCT alone. The proportion of these "Pre-Dry Uninfected" cows in a low bulk-tank SCC herd needs to be determined to help establish specific herd criteria for possible selective DCT programs.

The evaluation of cloxacillin DCT movement between quarters has not been previously documented in the literature. Future studies examining the movement of

different DCT formulations should be performed. Potential differences between these trials would be expected due to the pharmacokinetics of various DCT. In addition, other studies should be performed which sample untreated quarters at 1,2,4 and 7 days-dry, to ensure that cloxacillin concentrations detected at 3 days-dry were peak levels.

In conclusion, findings of this thesis help elucidate the effects of ITS on prevention of new IMI, cure rates, milk production, SCC, particle recovery and CM, but much more research remains to be conducted.

8.6. References

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APPENDIX A

Generalized linear mixed model for the odds of a quarter developing an intramammary infection by all pathogens.

Variables	Coefficient	Standard Error	Odds Ratio	95% Confidence Interval of Odds Ratios	*P* value
Fixed effects					
Constant	-3.23	0.28			
Treatment					
Dry Cow Antibiotic	Reference				
Internal Teat Sealer	-0.13	0.22	0.88	0.57, 1.35	0.55
Teat Sealer/Dry Cow Antibiotic	-0.67	0.26	0.67	0.30, 0.85	0.01
Pre-Dry Group					
Uninfected	Reference				
Infected	0.22	0.25	1.24	0.77, 2.05	0.36
Quarter					
Left front	Reference				
Left hind	-0.04	0.24	0.96	0.60, 1.54	0.86
Right front	0.47	0.22	1.60	1.04, 2.49	0.03
Right hind	-0.15	0.25	0.86	0.53, 1.40	0.54
	Variance	Standard Error[a]			
Random effects					
Herd	0.09	0.09			
Cow	1.55	0.43			

[a] Standard error of estimate of variance component

Generalized linear mixed model for the odds of a quarter developing an intramammary infection by major pathogens.

Variables	Coefficient	Standard Error	Odds Ratio	95% Confidence Interval of Odds Ratios	*P* value
Fixed effects					
Constant	-3.62	0.30			
Treatment					
Dry Cow Antibiotic	Reference				
Internal Teat Sealer	-0.40	0.26	0.67	0.40, 1.12	0.12
Teat Sealer/Dry Cow Antibiotic	-0.71	0.30	0.49	0.28, 0.89	0.02
Pre-Dry Group					
Uninfected	Reference				
Infected	0.25	0.29	1.28	0.73, 2.24	0.39
	Variance	Standard Error[a]			
Random effects					
Herd	0.18	0.15			
Cow	1.94	0.58			

[a] Standard error of estimate of variance component

Generalized linear mixed model for the odds of a quarter developing an intramammary infection by environmental pathogens.

Variables	Coefficient	Standard Error	Odds Ratio	95% Confidence Interval of Odds Ratios	*P* value
Fixed effects					
Constant	-4.38	0.41			
Treatment					
Dry Cow Antibiotic	Reference				
Internal Teat Sealer	-0.62	0.32	0.54	0.29, 1.02	0.06
Teat Sealer/Dry Cow Antibiotic	-0.76	0.36	0.47	0.23, 0.93	0.03
Pre-Dry Group					
Uninfected	Reference				
Infected	0.13	0.33	1.14	0.60, 2.18	0.68
Parity					
2nd lactation	Reference				
3rd lactation	-0.05	0.36	0.95	0.47, 1.91	0.88
≥ 4th lactation	0.69	0.33	2.00	1.05, 3.82	0.03
	Variance	Standard Error[a]			
Random effects					
Herd	<0.01	<0.01			
Cow	2.32	0.78			

[a] Standard error of estimate of variance component

Generalized linear mixed model for the odds of a quarter developing an intramammary infection by gram-negative pathogens.

Variables	Coefficient	Standard Error	Odds Ratio	95% Confidence Interval of Odds Ratios	*P* value
Fixed effects					
Constant	-5.67	0.65			
Treatment					
Dry Cow Antibiotic	Reference				
Internal Teat Sealer	-0.70	0.47	0.50	0.20, 1.26	0.14
Teat Sealer/Dry Cow Antibiotic	-0.75	0.64	0.47	0.13, 1.66	0.24
Pre-Dry Group					
Uninfected	Reference				
Infected	-0.44	0.53	0.64	0.23, 1.26	0.40
Corynebacterium bovis IMI dry off	0.96	0.43	2.60	1.11, 6.11	0.03
	Variance	Standard Error[a]			
Random effects					
Herd	<0.01	<0.01			
Cow	3.51	1.56			

[a] Standard error of estimate of variance component

Generalized linear mixed model for the odds of a quarter developing an intramammary infection by Streptococcal pathogens.

Variables	Coefficient	Standard Error	Odds Ratio	95% Confidence Interval of Odds Ratios	*P* value
Fixed effects					
Constant	-4.85	0.53			
Treatment					
Dry Cow Antibiotic	Reference				
Internal Teat Sealer	-0.44	0.43	0.64	0.28, 1.48	0.30
Teat Sealer/Dry Cow Antibiotic	-0.89	0.41	0.41	0.18, 0.91	0.03
Pre-Dry Group					
Uninfected	Reference				
Infected	0.91	0.40	2.49	1.14, 5.42	0.02
	Variance	Standard Error[a]			
Random effects					
Herd	0.89	0.53			
Cow	1.61	0.83			

[a] Standard error of estimate of variance component

APPENDIX B

TITLE:

An observational study on the effect of OrbeSeal® dry cow internal teat sealer on SCC, clinical mastitis and milk production in dairy cows in Canada.

OBJECTIVE:

To assess the effect of OrbeSeal® used alone and in conjunction with dry cow antibiotics on somatic cells counts and milk production in dairy cows in the field.
To assess the effect of OrbeSeal® used alone and in conjunction with dry cow antibiotics on the incidence of clinical mastitis until thirty days post calving on dairy cows in the field.
To acquire producer and dairy veterinarian feedback on the characteristics and concept of OrbeSeal®, as well as attributes about selective dry cow antibiotic therapy.

LOCAL INFORMATION

This will be a multi-centric study using dairy farms located in Western Canada, Ontario, Quebec and the Maritimes. A description of each farm will be included with the final data to be collected.

DATES: **Start: May 2003** **Finish: TBD**

Investigator: Dairy Veterinary Practitioners from approx 20-25 practices

Study Director: Bruce I. Groves, DVM, MSc
Address/Telephone: Pfizer Animal Health
17300 Trans Canada Highway
Kirkland Quebec H9J 2M5

Voice: 800-461-0917 (Toll Free) or (514) 693-4235
Fax: 877-878-4555 (Toll Free) or (514) 693-4272

Contract Monitor: Carolyn Sanford, DVM
Atlantic Veterinary College
Department of Health Management
Charlottetown PEI C1A 4P3

Voice: (902) 566-0968
Fax: (902) 566-0823

DRUG FORMULATION:

Generic Name:

Trade Name: OrbeSeal

Formulation: off-white paste, 4g per syringe

Concentration: **65% w/w bismuth subnitrate in a mineral oil vehicle**

Lot No. and Expiry Dates: **TBD**

TRIAL ANIMALS:

Animal:	Bovine	**Origin:**	Dairy
Breed/Strain:	Commercial Dairy Cows	**Sex:**	female
Inclusion Criteria:	Cows at the end of their first lactation or greater in one of four geographic regions of Canada (Western Provinces, Ontario, Quebec, and Maritimes). Participating herds must have a Bulk Tank Somatic Cell Count (SCC) of less than 250,000 cells/mL on the last three Dairy Herd Improvement (DHI) tests. Selected herds must have regular supervised herd health checks, subscribed to DHI services and have monthly individual cow SCC. Cows enrolled must be confirmed pregnant, be in good physical condition and have clear individual I.D. numbers.		
Exclusion Criteria:	Cows with expected dry periods less than 30 days or greater than 90 days.		

STUDY DESIGN:

The veterinarians will recruit eligible study herds from their active client list. Herds with 40-79 milking cows will receive 20 tubes (enough to enroll five treatment cows and five positive controls). Herds with greater than 80 milking cows will receive 40 tubes (enough to enroll 10 treatment cows and 10 positive controls). Tubes will be packaged together in groups of 20 for convenience of allocation to participants.

A total of 7000 tubes of the test article OrbeSeal® will be made available for this trial. These tubes will be evenly distributed to participating veterinarians.

Treatment Group A
Intramammary infusion of approved dry cow antibiotic product (Positive Control)
On the day of dry off, cows with odd numbered (1,3,5,...) identification tags will be treated with the standard intramammary infusion of dry cow antibiotic used in the heard in all four quarters following their final milking. Cows with a SCC less than 200,000 cells/mL on all the 3 previous DHI test dates, and no case of clinical mastitis during the last 3 months will become part of Treatment Group A.

Treatment Group B
Intramammary infusion of approved dry cow antibiotic product (Positive Control)

On the day of dry off, cows with odd numbered (1,3,5,...) identification tags will be treated with the standard intramammary infusion of dry cow antibiotic used in the herd in all four quarters following their final milking. Cows with a SCC greater than 200,000 on any of the previous three milk tests or who experienced a case(s) of clinical mastitis during the past three months will become part of Treatment Group B.

Treatment Group C
Intramammary infusion of OrbeSeal® (Test Article)
On the day of dry off cows with even numbered (2,4,6,...) identification tags will be treated based on their SCC history. Cows with a SCC less than 200,000 cells/mL on all of the three previous DHI test dates, and no case of clinical mastitis during the last three months, will be dried off using an intramammary infusion of OrbeSeal® in each of the four quarters following their final milking.

Treatment Group D
Intramammary infusion of approved dry cow antibiotic product and OrbeSeal® (Test Article)
Cows with a SCC greater than 200,000 on any of the previous three milk tests or who experienced a case(s) of clinical mastitis during the past three months will be treated first with the standard herd intramammary infusion of dry cow antibiotic in all four quarters followed by intramammary infusion of OrbeSeal®, both following their final milking.

PRETREATMENT:

Selected herds must have regular supervised herd health checks, subscribe to DHI services and have monthly individual cow SCC. Cows enrolled must be confirmed pregnant and be in good physical condition.

MANAGEMENT:

The cows will be subjected to normal management procedures for the farm except for drying off treatments when OrbeSeal® will be used as per the Study Design.

SITE DESCRIPTION:

The selected sites will be typical dairy operations representing all major dairy areas in Canada.

PROCEDURES:

1. It is the responsibility of the clinic veterinarians to get consent/release forms signed, and to forward a list of names, addresses and phone numbers of all herds recruited to the contract monitor. When the OrbeSeal® tubes are provided, the producer will sign the study consent form and DHI data release form.
2. The veterinarian together with the producer will then identify the next cows that are due to dry off in the herd. The odd numbered cows will be assigned to Treatment Groups A and B and put on the data sheet. The even numbered cows will have their last three milk tests assessed and will be assigned by the veterinarian and producer into the appropriate treatment groups (C or D) and recorded on the data sheet. Odd numbered cows will be dried off using routine dry cow antibiotic. Even numbered cows will be dried off using either

OrbeSeal® alone (Group C) or routine dry cow antibiotic and OrbeSeal® internal teat sealant (Group D), as in the criteria described above.

3. Enrolled study cows will be observed for signs of clinical mastitis until 30 days post-calving. This event will be recorded as either yes or no on the data sheets provided. Whenever possible, pre-treatment milk samples will be collected from the affected quarter for submission to the University of Guelph, Mastitis Research Laboratory for standard milk bacteriological culture.

OBSERVATIONS / SAMPLES:

All data entries should be legibly recorded on the appropriate forms provided by Pfizer's Animal Health Group. All forms must be signed and dated by the individual recording the data. Data entries requiring dates should be recorded using the format DDMMMYY (e.g., 18APR99).

1. DHI Data:
   DHI data will be collected electronically from participating herds in order to assess somatic cell counts and milk production data.
2. Clinical Mastitis:
   Producers will be asked to record any episode of clinical mastitis occurring in the study cows up to 30 days post-calving. This information will be collected on the Herd Data Sheets. Distribution of clinical cases by pathogen will be evaluated.

Forms

1. Data collection sheet 5 cows/page (Appendix 1)
2. Enrolment/DHI Release/Herd Identification (to follow)
3. Veterinary Clinic Instruction Sheet (to follow)

ANALYSIS:

Completed forms will be returned to Dr Carolyn Sanford at AVC for analysis.

_____ _____

Carolyn Sanford, Contract Monitor Date (DDMMMYY)

_____ _____

Bruce I. Groves, Study Director Date (DDMMMYY)

COW NUMBER	**COW DHI** NUMBER	**CLINICAL MASTITIS** 3 months before dryoff (Please circle one)	**SCC** <200,000 for 3 months before dryoff (Please circle one)	**DRY DATE** (dd/mm/yy)	**TREATMENT** Dry Cow Antibiotic (DC) OrbeSeal (OS) (Please circle one)			**CLINICAL MASTITIS** Within 30 days post-calving? (Please circle one)	**COMMENTS**
		YES or **NO**	**YES** or **NO**	/ /03	**DC**	**OS**	**DC+ OS**	**YES** or **NO**	
		YES or **NO**	**YES** or **NO**	/ /03	**DC**	**OS**	**DC+ OS**	**YES** or **NO**	
		YES or **NO**	**YES** or **NO**	/ /03	**DC**	**OS**	**DC+ OS**	**YES** or **NO**	
		YES or **NO**	**YES** or **NO**	/ /03	**DC**	**OS**	**DC+ OS**	**YES** or **NO**	
		YES or **NO**	**YES** or **NO**	/ /03	**DC**	**OS**	**DC+ OS**	**YES** or **NO**	
		YES or **NO**	**YES** or **NO**	/ /03	**DC**	**OS**	**DC+ OS**	**YES** or **NO**	
		YES or **NO**	**YES** or **NO**	/ /03	**DC**	**OS**	**DC+ OS**	**YES** or **NO**	

FARM NAME ______________________ **DHI NUMBER** ______________________

Veterinarian Name: ______________________ ______________________ ____________

Signature Date