Randomized Non-inferiority Clinical Trial Evaluating Three Commercial Dry Cow Mastitis Preparations

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CHAPTER 1

LITERATURE REVIEW

Chapter 1

Dry Cow Mastitis- A Literature Review

Part 1- Mastitis Incidence and Impact in Dairy Herds

This review will discuss the importance of mastitis in dairy herds, focusing on dry cow mastitis epidemiology and control. An overview of the incidence and impact of mastitis will be presented in Part I. Part II will discuss dry cow mastitis incidence, risk factors for the disease, common pathogens and consequences, while Part III will cover strategies for prevention of dry cow mastitis. Finally, Part IV will describe dry cow therapy products currently approved for use and efficacy of those products, and Part V will summarize this review and discuss the proposed research.

Mastitis is defined as an inflammatory reaction of the mammary gland that can be caused by infection with microorganisms (such as bacteria or fungi) or physical injury. It is characterized by physical and chemical alterations of the milk and possible pathological changes in the mammary tissue. Clinical mastitis is associated with visible changes in the appearance of the milk and/or inflamed quarters, while in subclinical mastitis there are no external changes in milk or quarters even though infection is present. Consequences of clinical mastitis affecting the farmers directly include discarded milk, treatment drugs and veterinarian services, while indirect costs include reduced milk yield after disease occurrence, reduced genetic improvement, penalties for high somatic cell count (**SCC**), extra labor for treatment of sick animals, culling and deaths. Additionally, subclinical cases cause decrease in desirable milk components such as total protein, casein, lactose, butterfat, calcium, phosphorus and potassium and an increase in the undesirable

components such as plasmin, lipase, sodium and immunoglobulin (Blowey and Edmonson, 2010).

It is not easy to quantify the incidence of mastitis in the United States (**US**) dairy herd. Different rates for prevalence and incidence have been reported for both clinical and subclinical cases. Hortet and Seegers (1998) reviewed studies having at least 250 lactations and reported that 7 to 64% of lactations experience at least one case of clinical mastitis. The National Animal Health Monitoring System estimate the disease affected approximately 16.5% of the dairy cattle in 2007 (USDA- NAHMS, 2010), while in the United Kingdom, this estimation was between 40 to 50 cases (10-150) per 100 cows per year for the year of 2009 (Blowey and Edmonson, 2010). Fetrow et al. (2010, personal communication) suggested that it is not uncommon for dairy veterinarians to face herds that experience a mastitis incidence of over 100% in a year.

Although calculating costs of mastitis is difficult, it is generally recognized as the most costly infectious disease in dairy production. The cost of a clinical mastitis case was estimated to range between 106 to 867 dollars according to Pinzon-Sanchez et al. (2011). Factors such as costs of drugs, labor, discarded milk, milk losses due to clinical and subclinicaml mastitis, culling and recurrences were considered in the calculations of this estimate (Pinzon-Sanchez et al., 2011). It is agreed that up to 70% of the losses from subclinical mastitis cases can be attributed to reduced milk production (Gill et al., 1990). It has been estimated that fat and protein yield are reduced by 1.5-7.5% and 0-8.5%, respectively (Hortet and Seegers, 1998).

For the near future, consumers and dairy processing companies will require high quality milk. As an example, in the European Union producers with a SCC over 400,000 cells/ml

are not able to sell their milk. This threshold is currently at 750,000 cells/ml in the US, but the pressure on lowering this SCC limit in the near future is already underway.

Part 2- Dry Period Mastitis

Incidence in Dairy Herds

Dry cow mastitis refers to new intramammary infections (**IMI**) that were acquired during the dry period, or alternatively it could refer to infections that persisted from the previous lactation (therefore, were never cured).

The importance of dry cow mastitis has been explored by several authors (Ward and Schultz, 1974; Natzke et al., 1975; Oliver and Mitchell, 1983; Eberhart, 1986; Erskine, 2001). However, estimates of dry cow mastitis incidence vary between studies. This is in part due to regional and herd differences, and differences in definitions of IMI. The proportion of quarters developing a new infection during the dry period was reported to be between 8 to 12% by Eberhart (1986), 25% by Godden et al. (2003) and between 8 to 16.7% by Cook et al. (2004). The majority of new infections is subclinical in the udder during the dry period, but can flare up as clinical mastitis, usually in early lactation (Green et al., 2002). Smith et al. (1985) reported that the risk for new infections from environmental pathogens can be ten times higher in the dry period than during the lactation period.

Mammary Gland Physiology and Immune Defenses during the Dry Period

Physiological factors are known to affect the susceptibility for occurrence of new IMI (Oliver and Sordillo, 1989). During the dry period, there are two transitions or three

phases for the mammary gland: active involution phase (first transition), steady-state involution (the gland is involuted) and colostrogenesis (second transition; Bradley and Green, 2004).

The active involution starts with cessation of milk flushing and increase of intramammary pressure. During this period, the alveolar and lobar structures are disrupted. The steady-state phase starts approximately 21 to 30 days into the dry period and the gland is totally involuted (Oliver and Sordillo, 1989). At the end of the dry period, the secretory cells regenerate and differentiate to start the production of colostrum.

During this time, the mammary gland has several innate and aquired defense mechanisms. An example of the innate immune defense arm is the presence of lactoferrin, an iron-binding protein that prevents bacteria from binding iron, therefore inhibiting growth. Citrate is another compound that also binds to iron. Another example of the innate immune system is that the mammary secretions also include phagocytic cells: polymorphonuclear neutrophils (**PMNs**) and macrophages. PMNs are characterized by the presence of granules in the cytoplasm containing defensins, myeloperoxidase, lysozyme, lacteroferin, etc. They are recruited from the blood to the site of infection and kill bacteria during the phagocytosis process. Macrophages also have the role of promoting bacteria phagocytosis, and they are also able to help initiate acquired immune responses through stimulation of lymphocytes through antigen process and presentation.

Another major factor in the innate immune defense during the dry period is timely formation of the teat canal keratin plug. This physical barrier prevents entry of potential mastitis-causing organisms and its lipid composition is known to have bacteriostatic activity (Hogan et al., 1986; Hogan et al., 1987).

Lymphocytes T and B are responsible for the aquired immune response of the mammary gland. These are activated when there is contact with bacteria and macrophages followed by antigen presentation that result in the production of antibodies and chemicals called cytokines. These cytokines activate other cells to enhance bacterial killing. Some cytokines known to be involved with mastitis control include interleukin (**IL**)-1 and IL-2, interferon and colony stimulating factors (Erskine, 2001). The role of antibodies against mastitis is unclear and their main function might be opsonization of bacteria to be phagocytized (Blowey and Edmonson, 2010).

Risk Factors for Mastitis Occurrence during the dry period

Physiological factors: The mammary gland is more susceptible to new IMI during the two transition periods of the dry period: the first 2-3 weeks after dry off (active phase) and the last 2 weeks before calving (colostrogenesis; Bradley and Green, 2004). There are several factors related to mammary gland physiology and immune defenses that contribute to these two high risk periods.

During the first risk phase, active involution phase, the intramammary pressure increases because the cow is no longer milked. At the same time, bacteria are not flushed out of the streak canal, the practice of teat dipping with antibacterial solutions stops and the keratin plug is not yet formed in the end of the streak canal to prevent new infections. Dingwell et al. (2004) reported that approximately 50% and 23% of teat streak canals were still open within the first week after dry off, and 6 weeks after dry off, respectively. According to that study, quarters that formed a complete keratin plug within 6 weeks after dry off were 40% less likely to develop a new IMI during the dry period. During

active involution the mammary secretion changes, becoming a secretion with higher levels of lactoferrin, serum albumin, somatic cells and pH and lower levels of fat, casein and citrate. High concentrations of fat, casein, lactose and citrate can interfere with natural defense mechanisms and enhance bacterial growth according to Oliver and Sordillo (1989).

Once the gland is fully involuted, it is very resistant to new infections. Phagocytosis is more efficient in the involuted gland because phagocytic cells are in higher concentrations and fat and casein are in lower concentrations (Oliver and Sordillo, 1989). This resistance lasts until approximately 2 weeks before calving.

During the second risk phase, colostrogenesis, the mammary tissues grow and the secretory function is increased. The components of milk also change: concentrations of fat, citrate, lactose and α-lactalbumin increase while somatic cell counts decrease. Additionally, the intramammary pressure increases, which can lead to break down of the keratin plug and leaking of colostrum.

Close to parturition, hormonal changes and increased cortisol levels affect the expression of adhesion molecules from PMNs (such as L-selectin and CD18). This leads to impaired function and reduced production of reactive oxygen species to kill bacteria (Pyorala, 2008; Hoeben et al., 2000). Futhermore, milk secretion during the late dry period may have an inhibitory effect on phagocytosis and protective factors in the milk such as lactoferrin, imunoglobulins and phagocytic cells, which are known to be in lower concentrations (Paape et al., 1992). Finally, levels of antibiotic from the dry cow therapy (**DCT**), infused at dry off, drops below the minimum inhibitory concentration (**MIC**).

The combination of all of these physiological factors increases the cow's susceptibility for new infections during the dry period.

Metabolic state: Some common diseases that occur after calving include hypocalcemia, ketosis, and abomasal displacement. Concomitant infectious diseases can also occur, such as endometritis and mastitis. When an animal is hypocalcemic, the teat sphincter can be at a greater risk for pathogen penetration due to reduced contraction of the teat sphincter (Goff and Kimura, 2004). In addition, cows experience a negative energy balance, which occurs when energy requirements exceed energy intake during this time and lipolysis is triggered as a compensatory mechanism. As a result, the concentration of nonesterified fatty acids (**NEFA**) increases and triacyl glycerol (**TAG**) accumulates in the liver. Zerbe et al. (2000) demonstrated that blood-derived neutrophils from postpartum cows with high liver TAG content displayed altered phenotypical and functional characteristics compared to those of low or normal liver TAG cows. The immunossupressive condition described above plays an important role on the high risk of mastitis development during the late dry period and also on the high clinical mastitis incidence during the first 30 days in milk (**DIM**) (Erskine, 2001).

Infections present at dry off: Pathogens isolated at dry off can be classified as major or minor. Major pathogens generally are of greater concern to the dairy farmer since they are considered more virulent and damaging to the udder (Reyher et al., 2012) and responsible for most of the clinical cases (Hassan, 2009). Infections caused by major pathogens are usually associated with a higher chance for the quarter to develop a new infection after the dry period. In a study conducted by Osteras et al. (1991), cows with a major pathogen present at dry off or that had at least one case of acute mastitis during the

previous lactation were more likely to have a major pathogen infection in the next lactation (calving and 30 ± 17 DIM), while cows diagnosed with Coagulase Negative *Staphylococcus* (**CNS**) present before entering the dry period, were less likely (OR = 0.34) to have a major pathogen IMI after calving.

Subclinical IMI present at dry off caused by minor pathogens have shown to have both protective and detrimental associations with subsequent risk for new infections during the dry period according to different studies. Berry and Hillerton (2007) found that infections caused by CNS or *Corynebacterium spp*. at dry off increased the risk of new infection by *Streptococcus uberis* or coliforms when no dry cow therapy was used. According to a study conducted in 2012 using a large dataset with samples collected pre-dry-off, postcalving and in midlactation, the presence of CNS was a significant risk factor for development of a new infection with *Staphylococcus aureus* (OR = 1.4) when all samples were grouped together (Reyher et al., 2012). In this study, a new infection was defined as a quarter that did not have previous infection but was diagnosed with one in the subsequent sampling (interval of 1-3 weeks). When analyzed alone, pre-dry-off samples and midlactation samples from quarters where CNS was isolated were at increased risk for a new *Staphylococcus aureus* infection. (Reyher et al., 2012). In contrast, a study conducted in the UK reported that the risk of isolating either *Streptococcus uberis* or Coagulase Positive *Staphylococci* was significantly lower when *Corynebacterium spp.* were isolated from a milk sample, showing a possible protective relationship (Green et al., 2005).

<u>The environment</u>- The environment affects the amount and type of bacteria that are in contact with the cow's teat ends during the dry period. For this reason, the environment

should be clean, dry, cool and comfortable (Bradley and Green, 2004), since some of the requirements for bacteria growth includes moisture, warmth and organic material (bedding, manure, milk, soil).

Bedding material is as an important determinant: inorganic material such as sand is inert and does not support bacterial growth, while organic materials such as straw, sawdust, shavings and recycled manure are organic and support bacterial growth. A study conducted in 2008 (Godden et al.) involved the collection of 4 different types of bedding from 49 dairy farms followed by in vitro inoculation of *Klebsiella pneumonia* and *Enterococcus faecium* (representing the 2 large groups of environmental bacteria). Bacterial growth in shavings, clean sand, recycled sand and manure solids were investigated. Digested manure solids promoted the greatest amounts of growth of coliform bacteria, followed by recycled sand, shavings and, least of all, clean sand. Regarding growth of environmental streptococci, recycled sand promoted a mild increase in bacteria numbers, manure solids showed no change in bacteria count, and clean sand and shavings promoted a decline in bacteria counts over the 72 hour incubation period. Total carbon content and pH were important in determining bacterial survival and multiplication. Amount of bedding and frequency of renewal are also important determinants of bacterial growth. The amount provided depends on cubicle design, presence of mattresses and bedding type.

Attention should be given to properly manage ventilation, stocking density, cubicle comfort and manure. Pen density and alley width determine the degree of cow to cow contact: the higher the density, the more facilitated the fecal contamination, bacterial multiplication and risk for acquiring an intramammary infection. Comfort should be

assured since cows otherwise tend to lie on concrete, increasing the risk for mastitis (Blowey and Edmonson, 2010).

The cleanliness and dryness of the environment may be evaluated, in part, by assessing cow cleanliness when using an udder hygiene score. A worse udder and leg hygiene score was associated with subclinical mastitis according to a study conducted in 2003, with a higher risk of IMI caused by major pathogens for cows with udders characterized as dirty compared to udders characterized as clean (Schreiner and Ruegg, 2003). A study by de Pinho Manzi et al. (2011) concluded that a one unit change in udder score hygiene (in a scale from 1-3) was associated with a 47% increase in the chances of the quarter developing an IMI.

Teat end integrity: It has been found that teat end integrity is associated with the probability of developing an IMI. A study conducted in 2011 by de Pinho Manzi et al. with lactating cows found a clear relationship between presence of subclinical IMI and teat end condition. Using a scale from 1-4 to classify teat end score, they reported that the increase of one unit for teat end condition score increased the chances of developing an IMI by 30%. These results are similar to those reported by Dingwell et al. (2004): quarters that had a teat end crack entering the dry period had a 1.7 times higher odds of having a new infection present at freshening as compared to quarters that did not have a crack. Other studies however, found that a small amount of teat-end callosity does not increase the risk of IMI, and may be considered a physiological response of the teat to the milking machine (Neijenhuis et al., 2001). Sieber and Farnsworth (1981) reported that prevalence of infection in quarters with normal teat ends and those with chronic ring

lesions was not significantly different. This contradiction could be due to the lack of standardization on teat end classification.

Parity: It is well documented that the parity of the cow at dry off has a relationship with the risk for development of new infections. Oliver and Mitchell (1983) found a positive association between risk for having an IMI and parity, with 2.6% of quarters infected at calving for cows from second and third parity and 23.8% of quarters infected for cows from fourth or more parities. Smith et al. (1985) stated that coliform infections during the dry period should be more common in "older" herds compared to "younger" herds. Another study reported that the age of cows was associated with the rate of environmental streptococcal infections during early dry period and also during lactation, with older cows (dry period greater than 4^{th}) having significantly higher rates of new infections (Todhunter et al., 1995). This agrees with a previous study published in 1973 by Ward and Schultz, where they suggested that dry cow therapy might be used selectively with older cows. Finally, Sol et al. (1994) reported that cure rates for subclinical staphylococcal mastitis decreased as age increased. Some reasons that might explain the relationship between age and risk for new IMI in the dry period may include teat-streak canal characteristics, rate of udder involution and milk production across different age groups (Dingwell et al., 2003).

Dry period length: Natzke et al. (1975) reported that cows with dry period of 30 days or less had fewer new infections and also responded better to dry cow therapy in 36 dairy farms located in New York. It is important to mention, however, that this study was conducted many years ago, and the animals' production had changed, so these results should be reexamined. In a more recent study that investigated the effect of a dry period

length of 30 days versus 45 days and 30 days versus 60 days on udder health parameters, there was no effect of dry period length on percentage of cured IMI or prevention of new IMI (Church et al., 2008).

A recent retrospective cohort study (Pinedo et al., 2011) examined dry period length and subclinical mastitis occurrence in early lactation, and they concluded that extended dry periods (143 to 250 days) were associated with increased odds of subclinical mastitis in early lactation when compared to a reference dry period of 53 to 76 days.

Milk production and methods of milk cessation at dry off: Due to increased internal udder pressure, high producing cows are more likely to leak after dry off. Natzke et al. (1975) enrolled more than 9000 quarters to investigate effects of drying off practices on mastitis. Intermittent and abrupt dry off methods were compared, and as long as infused with a long acting antibiotic formulation, the method of dry off did not influence the rate of new infections (Natzke et al., 1975). Level of production at dry off had little effect on number of new infections. However, Dingwell et al. (2004) reported that cows producing above 21 kg of milk on the day before drying off decreased significantly the chances of teat closure compared to cows producing less than 21 kg. For both cow and quarter level analysis, teat ends closed by week 6 of the dry period had lower odds of developing new infections during the dry period.

Milk leaking was strongly associated with clinical mastitis and new IMI caused by major pathogens in the dry period according to Schukken et al. (1993). In this study, cows that leaked in the dry off period had a four times greater risk of having a clinical mastitis event during the dry period. Recent comparisons between abrupt or intermittent cessation

of milk for drying off cows are lacking and further studies should be conducted in this area to improve management strategies.

Somatic Cell Count and previous clinical mastitis cases: It is accepted that SCC can be an indirect indicator of presence of subclinical mastitis, and monthly values obtained from the Dairy Herd Improvement (**DHI**) can be used to monitor for subclinical mastitis and estimate the prevalence and incidence of disease (Pantoja et al., 2009). In a study conducted in 2007 involving 52 facilities, cow level variables significantly associated with risk for clinical mastitis cases in the first 30 DIM were SCC≥ 200,000 in the last 90 days of the previous lactation and increased parity (Green et al., 2007).

Another study showed that monitoring SCC across the dry period can allow identification of infected quarters and prediction of clinical events in early lactation. The sensitivity and specificity of that method is poorer when compared to the bacteriological culture gold standard, but it can be cost-effective and practical (Pantoja et al., 2009). Quarters and cows with SCC ≥ 200,000 cells/ml (samples taken at dry off and postcalving) had a greater risk of being subclinically infected by a major pathogen at the first DHI test and also were at greater risk of developing a clinical mastitis flare up until 120 days in milk compared to quarters with SCC < 200,000 cells/ml at both sampling periods (Pantoja et al., 2009). The occurrence of clinical mastitis in the previous lactation was strongly associated with SCC status at dry off and after calving (Pantoja et al., 2009). In this same study, it was concluded that quarters that experienced at least one clinical case of mastitis in the previous lactation were 4.2 times more likely to experience a clinical mastitis case in the first 120 days in milk in the next lactation.

In a German study conducted by Gundelach et al. (2010), number of lactations and health status of the quarter at dry off (based on quarter SCC, microbiology and udder tissue status) were the main determinants of new infections and clinical events during the dry period and early lactation for quarters treated with antibiotic DCT.

Pathogens Associated with Mastitis Development during the Dry Period

There are different ways commonly used to classify mastitis pathogens: major and minor, contagious and environmental, Gram-positive and Gram-negative. All the pathogens presented in this review are considered major pathogens, except for *Corynebacterium spp* and Coagulase Negative *Staphylococcus spp*. The categories contagious and environmental will be used to briefly characterize clinically important pathogens.

Environmental Pathogens:

Most of the new infections acquired during the dry period are caused by environmental bacteria due to the fact that cows are continuously exposed to these organisms in their environments (Dingwell et al., 2003).

The environment is the reservoir for these pathogens, and teat ends are exposed between milking or during the dry period (constant environmental exposure). Up to 95% of all IMI caused by major pathogens during the dry period were shown to be caused by environmental pathogens (Oliver and Mitchell, 1983). These findings are potentially due to management strategies such as total confinement housing, increased cow density and use of bedding materials that facilitate growth of these microorganisms.

Environmental streptococci play an important role in causing new IMI during the dry period. A study conducted by Todhunter et al. (1995) concluded that the rate of new IMI

caused by these pathogens during the dry period was 5.5-fold greater than the rate of infection during lactation. A review article (Bradley and Green, 2004) suggests the majority of new IMIs during the dry period are caused by major environmental pathogens such as *E. coli* and *S. uberis*, as well as minor pathogens such as CNS. For control, adequate bedding, ventilation and manure management, teat disinfection before and after milking, vaccination and maintenance of adequate stocking density are considered important.

Streptococcus dysgalactiae: This environmental organism is a Gram-positive cocci that can be found colonizing the teat skin, especially when it is damaged. It is also common in tonsils of calves, making suckling a possible transmission pathway (Blowey and Edmondson, 2010).

Streptococcus uberis: This is a non-hemolytic Gram-positive cocci that splits esculin. It is primarily considered an environmental pathogen and it has been incriminated in causing some chronic infections, possibly due to the fact that it has the ability to enter and survive inside neutrophils and macrophages, where it can remain dormant for a certain period. Its main reservoir is the bedding of the cows, with a particular preference for straw. It can also be found in feces as well as body parts such as mouth and vulva (Blowey and Edmondson, 2010).

Escherichia coli: This Gram-negative coliform bacillus can be hemolytic or non-hemolytic. A high percentage of *E. coli* associated intramammary infections self-cures, although a study conducted by Bradley and Green (2001) found that 23.9% of all clinical mastitis cases caused by *E. coli* were recurrent and of those, 85.7% showed the exact same DNA fingerprint pattern. This pathogen does not adhere to the epithelium lining of

the teat and udder, but releases toxins common to all Gram-negative bacteria called lipopolysaccharides (**LPS**) that induces inflammatory reactions and can lead to toxic shock. It is known that the bacteria can remain in the udder during the dry period and cause new clinical cases after the cow calves (Blowey and Edmonson, 2010).

Klebsiella pneumoniae: These Gram-negative coliform bacteria can cause severe mastitis cases and are known for its poor antibiotic response and rapid progression to shock and death (Paulin-Curlee et al., 2007).

Coagulase Negative *Staphylococcus* (**CNS**): These Gram-positive cocci do not coagulate rabbit serum and may be hemolytic. They commonly colonize the teat-end and streak canal, so it is controversial whether they are acting as a potential pathogen or if they are part of the animal's normal flora. CNS could be responsible for 3-10% of clinical infections (Ruegg, 2011). In a study conducted in Wisconsin (Pantoja et al., 2009), CNS was the most common pathogen found in both dry off and postcalving milk samples (63 and 44% of all pathogens recovered, respectively) from multiparous cows in one dairy herd. Green et al. (2005) found that the prevalence of this pathogen can be higher during the dry period than at drying off or after calving.

Bacillus spp: These Gram-positive bacteria usually appear as large, rough, dry appearing colonies on blood agar plates. They can be hemolytic or not. They are commonly found on the teat skin and canal, possibly even having a protective effect against Gram-positive organisms (Al-Qumber and Tagg, 2006). However, pure cultures of this pathogen have been isolated from clinical mastitis cases, suggesting it can also be highly pathogenic (Parkinson et al., 1999).

Corynebacterium spp: This is a Gram-positive, rod-shaped bacterium commonly considered to be a minor pathogen. In a study conducted by Green et al. (2005), this pathogen was the most commonly found in dry off milk samples (41.4% of all pathogens recovered), and this percentage decreased between dry off and the late dry to calving period. This same trend has also been previously described by Oliver (1988).

Contagious mastitis pathogens:

Although the importance of environmental pathogens is evident, the control of contagious pathogens should also be mentioned, since the dry period can also be considered a chance to eliminate subclinical infections present at dry off.

The primary reservoir for these pathogens is the mammary gland. Transmission occurs from the carrier quarter to the teats of non-infected quarters during the milking process, so the dry period is not an important time for new infections caused by these pathogens. Control procedures for contagious pathogens include careful parlor routine (teat disinfection, use of gloves), good overall parlor hygiene, good equipment disinfection and maintenance, dry cow therapy, treatment and recording of clinical cases and culling or segregation of cows with chronic mastitis cases.

Staphylococcus aureus: These Gram-positive bacteria are hemolytic cocci. They grow as white/yellow colonies on blood agar and are mostly coagulase positive. Chronic infections are relatively difficult to treat, since the organism is able to live within epithelial cells, PMNs, macrophages and abscesses, becoming unreachable by most antibiotics.

Streptococcus agalactiae: This Gram-positive pathogen has the udder as its primary reservoir but it can also be found colonizing the teat end or the teat streak canal. They are alpha-hemolytic cocci and are mostly susceptible to commonly used antibiotics.

Mycoplasma: Having no cell wall, this pathogen is neither a Gram-positive nor a Gram-negative organism. It requires specific growth conditions and a longer incubation time to be detected by culture. It has always been described as a highly contagious pathogen, but recent work found that it might have an important environmental source as well (Fox, 2012). Cure is difficult, therefore culling of infected cows or separation in a particular pen to be milked last is highly recommended.

Other less common agents causing mastitis include *Arcanobacterium pyogenes, Nocardia spp, Serratia spp, Pasteurella spp, Enterobacter spp, Citrobacter spp, Pseudomonas spp,* Yeast, *Prothotheca spp* and *Proteus spp.*

Consequences of Dry Cow Mastitis

Persistence of preexisting IMIs during the dry period and development of new IMIs during the dry period are two of the main factors that influence the risk for manifestation of clinical mastitis in the next lactation. It is known that 55% of environmental infections established early in the dry period persist into the next lactation and can possibly cause clinical flare ups, and that 52% of all clinical coliform mastitis cases in the first 100 days of lactation may originate in the dry period (Todhunter et al., 1995).

The effect of mastitis on reproductive parameters has also been investigated. Moore et al. (1991) reported that for one California dairy herd with predominantly coliform mastitis cases, cows that had a mastitis episode were 1.6 times more likely to have an altered

inter-estrus interval (less than 18 days or more than 24 days) compared to cows that did not experience mastitis. However, infected cows from another herd in the same state where contagious pathogens were predominant (*S. aureus* and *S. agalactiae*) were at the same risk for an altered interval as uninfected cows. Barker et al. (1998) compared mastitis episodes before postpartum artificial insemination (**FPAI**), between FPAI and pregnancy diagnosis and after pregnancy diagnosis. The number of days to first AI was longer for cows experiencing a clinical case before AI compared to the other groups. Cows that had mastitis after FPAI had a greater calving to conception interval compared to cows that had mastitis after pregnancy diagnosis and cows that did not have mastitis. Finally, another study (Chebel et al., 2004) reported that cows that had clinical mastitis between AI and pregnancy confirmation were 2.8 times more likely to experience pregnancy loss between 31 to 45 days after AI compared to cows that did not have mastitis. Some factors that could be involved in decreased reproductive performance would be fever and production of cytokines that could interfere in hormone regulation (such as prostaglandin F2α and cortisol), compromising follicle and oocyte development (Chebel, 2007).

Other important consequences of dry cow mastitis relate to culling and deaths. Studies work conducted by Green et al. (2005) identified that cows with a major pathogen isolated from one or more quarters during the late dry to calving period were more likely to be culled in the next lactation than those which were uninfected. Lescourret and Coulon (1994) reported that approximately 7% of mastitis cases occurring in the first 5 weeks of lactation needed to be culled or dried-off.

Finally, an important consequence of dry cow mastitis is reduction of future milk production. A review published in 1998 by Hortet and Seegers compiled information from 20 different studies and focused on the economic impact of milk yield and milk composition changes due to clinical cases. It was estimated that losses were between 0 and 200 kg for animals that had a clinical case before the expected peak of the lactation curve and between 0 and 100 kg of milk for cows with mid-to-late lactation occurrences. Another study found that for 36% of the cases of mastitis that occurred in early lactation, milk production was affected for an extended period (Lescourret and Coulon, 1994).

Part III- Prevention Strategies

Maximize Cow's Immune Defense

Maximizing the cow's immune defenses can result in benefits such as prevention and elimination of new infections more rapidly and decreased frequency and severity of clinical cases (Godden et al., 2006). One way of enhancing innate immunity is to design transition diets that contain nutrient levels of protein, energy, vitamins and minerals (vitamin A, D, E, selenium, copper, zinc) recommended by the Nutrient Requirements of Dairy Cattle (**NRC**) 2001, and also provide adequate water and dry matter intake (Godden et al., 2006).

Avoiding stress is another way of enhancing cow's immunity. In stressful situations, cortisol is produced. This hormone is immunosuppressive and can cause deleterious effects on neutrophil and lymphocyte function. Importance should be given to cow comfort, which can be maximized by maintenance of low stocking density, bedding level

and cleanliness, correct design of stalls, gentle handling of animals, heat abatement (adequate ventilation, use of sprinkler systems), etc.

Vaccination to stimulate the humoral arm of the acquired immune system is another point that should be addressed. Currently, there are vaccines available to help control clinical signs associated with *E. coli* mastitis: J-Vac (Merial®, Athens, GA) and J5 *E. coli* vaccine (Pfizer Animal Health, New York, NY). J-Vac is made with an *E. coli* bacterin toxoid and it is labeled for control of clinical signs due to *E. coli* and the effects of endotoxemia caused by *E. coli* and *Salmonella typhimurium*. The J-5 vaccine is made with a rough mutant of *E. coli* (O:111:B4) that produces LPS with a fragment common to all LPS produced by other strains and it is labeled to help controlling clinical signs associated with *E. coli* mastitis. The first study to elucidate efficacy of this vaccine in natural challenge conditions was completed in 1989 by Gonzalez et al. using approximately 480 animals from 2 different dairies in a double-blind randomized clinical trial. The authors reported that cows vaccinated with the *E. coli* J5 vaccine were at a five times lower risk of having a clinical mastitis event until 90 DIM compared to unvaccinated cows. This vaccine was shown not to reduce the number of coliform clinical cases, but to decrease their severity. According to a recent economic consideration, vaccination against coliform mastitis seems to be one of the recommended "best practices", profitable across essentially all herds (Fetrow et al., 2010).

Strategies to Reduce Environmental Exposure

When distributed in the stalls, bedding can be already contaminated with bacteria or it can be later contaminated with manure. Once bacteria are present, bedding materials can

then support and enhance bacteria growth, as long as requirements such as nutrients availability, humidity and temperature are met. Therefore, in order to reduce environmental bacterial load, attention should be taken regarding ventilation, frequent removal of contaminated bedding and replacement with new, clean dry bedding. Bedding material in use is also an important consideration since organic material is a supply of nutrients for bacteria, while inorganic materials do not support bacterial growth. According to Godden et al. (2008), clean new sand appears to be the bedding material supporting less growth of coliforms and environmental streptococci species. This study suggests that for farms using shavings, digested solids or recycled sand, a way of reducing bacteria multiplication would be to adopt other management practices such as maintenance of adequate bedding amount in stalls, frequent addition of new bedding to stalls, avoid overcrowding and improve parlor hygiene.

Green et al. (2007) focused on management factors during the dry period regarding farm facilities, cow characteristics and herd management strategies and the rate of new clinical infections until 30 days in milk. In the herd management model, factors associated with hygiene such as management of early and late dry-period housing, calving area hygiene and hygiene during DCT administration were associated with rate of clinical mastitis within 30 days of calving. Finally, cows on pasture and vaccinated against bovine leptospirosis were at a lower risk for a clinical mastitis event, as well as farms that implement policies limiting negative energy status (body condition scoring and diet concerns) and farms foremilking cows within 6 hours of calving.

Teat Sealants

A management strategy that has been investigated for the last 30 decades is the use of formulations that would work as physical barriers in order to prevent the development of new intramammary infections during the dry period. The use of these products has the main objective of protecting the teat from pathogen's invasions before and while the keratin plug is formed, providing protection to the teat end throughout the entire dry period.

External teat sealants: The idea of an external teat sealant resembles the post-dipping routine for lactating cows. In 1980, Farnsworth et al. published an article addressing the importance of prevention of subclinical intramammary infections caused by environmental pathogens, and a clinical trial for testing a teat dip indicated particular effectiveness in prevention of infections caused by coliform microrganisms for the lactating cow. The difference from those products to the dry cow external dip formulation is that the product should be able to adhere more persistently in order to induce protection during the dry period. A recent study reported the impact of the use of an external teat sealant at dry off on IMI status at calving and adherence of the product and its association with new infections incidence (Lim et al., 2007). In this study, 2 quarters were randomly assigned to receive either DCT alone (DryClox, Wyeth Animal Health, Guelph, Ontario, Canada) and the other 2 quarters received either a single sealant application (Stronghold, DeLaval, Kansas City, MO) or a double sealant application of the same product (with 3-5 minutes between applications). Quarters receiving external sealant applications were dipped again approximately 3 days before expected calving. The results showed that duration of sealant adherence should be considered when evaluating the treatment at

drying off and infections after calving (Lim et al., 2007). No association was found between treatment and new infections caused by coliforms or environmental pathogens. Teat length was associated with sealant adherence duration, and teats covered with a double application of the product showed lower mean linear score after calving. The efficacy of external teat sealants is still controversial and highly dependent on herd production and management practices.

Internal teat sealants: An internal teat sealant is an inert viscous paste with no antibacterial properties that is infused into the teat canal at drying off. It has the objective to mimic the keratin plug and work as a physical barrier to prevent pathogen entry in the mammary gland.

A UK study (Huxley et al., 2002) compared the use of an internal teat sealant (TeatSeal, Cross Vetpharm Group Ltd, Ireland) to the use of an intramammary antibiotic formulation (Cepravin Dry Cow, Shering-Plough Ltd, UK) in 16 different herds and over 1000 animals. Animals presenting clinical signs of mastitis in the previous lactation and animals with SCC > 200,000 cells/mL in all tests during the previous lactation were not included in this study. The use of the internal sealant showed a significant higher protective effect to prevent new infections caused by all major pathogens, *E. coli* and *Enterobacteriaceae*. Results of a North American study demonstrated that the use of an internal sealant (Orbeseal, Pfizer Animal Health, New York, NY) in combination with a DCT therapy (Orbenin-DC, Shering-Plough Corp, Kenilworth, NJ) reduced the risk of acquisition of new infections during the dry period and the risk of a clinical mastitis event between dry off and 60 DIM as compared to DCT alone (Godden et al., 2003).

It is important to mention that this technique is at some point invasive, therefore infusion should be carefully and aseptically conducted. Some of the issues that could potentially limit the use of this technique would be related to persistency of the product in the mammary gland (Woolford et al., 1998) and risk of introducing pathogens if product application is not totally aseptic (Schiefer et al., 1976).

Blanket Dry Cow Therapy

Dry cow therapy is a procedure recommended by the National Mastitis Council (**NMC**) for mastitis control. This is the intramammary infusion of long-acting antibiotic formulations after the last milking of the cows, immediately prior to dry off, for the purpose of curing existing subclinical infections and preventing new infections that could be acquired during the early dry period. This procedure has a high adoption rate within the US (Dingwell et al., 2003).

Because it is administered at dry off, advantages of using DCT include avoidance of milk discarded, use of larger doses of antibiotic (so that concentrations can stay above MIC for longer periods of time) and reduction in risk for antibiotic residues in the milk. North American studies estimate that the proportion of quarters infected subclinically at dry off varies between 13 - 35% (Oliver and Mitchell, 1983; Godden et al., 2003; Pantoja et al., 2009). As stated by Dingwell et al. (2003), due to the lack of efficient and practical tests to guide selective treatment, infusion of all quarters is probably the best option, especially considering benefits related to DCT's prophylactic effect. A quantitative meta-analysis of published data regarding incidence of IMI during the dry period and use of DCT was published by Robert et al. (2006). The authors subdivided 36 world-wide published

papers based on study design and one of the conclusions was that the incidence of new IMI was reduced when selective therapy was applied to high new IMI risk herds, but the impact was limited when the risk of new IMI without DCT is low. The overall reduction of IMI during the dry period with the use of a DCT was estimated between 33 to 53%, but this varied according to pathogen.

One of the limitations of DCT is that for most of the antibiotic formulations commercially available in the North America, the spectrum of action against gram negatives is poor or unknown. Also, DCT may not be protective against new IMI in the late dry period (colostrogenesis phase) because drug concentration in the gland falls below MIC. According to Fetrow et al. (2010), DCT will not be fully effective in a herd whose main mastitis problem is environmental mastitis resulting from manure in calving areas, dirty freestalls and bad udder preparation. Other important considerations when using DCT include administration technique and observation of labeled milk withdrawal periods.

Part IV- Availability and Efficacy of Blanket Dry Cow Therapy Products

According to the "Milk and Dairy Beef Drug Residue Prevention Manual" of 2011, the current Food and Drug Administration (**FDA**) approved antibiotic formulations for dry cows in the US include cephapirin benzathine, ceftiofur hydrochloride, cloxacillin benzathine, novobiocin, penicillin G procaine, penicillin G/novobiocin and penicillin G/dihydrostreptomycin. The milk withholding time for these commercially available drugs varies according to the drug from zero to 96 hours post-calving.

Cephapirin benzathine is a first generation cephalosporin, a β-lactam antibiotic that acts inhibiting cell-wall synthesis by interacting with enzymes responsible for peptidoglycan synthesis. It is usually effective against Gram-positive bacteria and some Gram-negatives. The commercial dry cow product Tomorrow® Dry Cow (Boehringer Ingelheim Vetmedica, Inc., St Joseph, MO) contains 300 mg of cephapirin and it is claimed to be effective on the treatment of mastitis caused by *Streptococcus agalactiae* and *Staphylococcus aureus,* including penicillin-resistant strains. Milk and meat withholding time are 3 days after calving and 42 days after treatment, respectively. The dry period length required is 30 days.

Ceftiofur hydrochloride is a third generation cephalosporin that acts in a similar manner as cephapirin. Second and third-generation cephalosporins have a broader spectrum of action compared to first generation cephalosporins, especially against Gram-negative pathogens. The antibiotic formulation Spectramast® DC (Pfizer Animal Health, New York, NY) is composed by 500mg of ceftiofur hydrochloride and it is indicated for treatment of subclinical mastitis at time of dry off associated with *Staphylococcus aureus, Streptococcus dysgalactiae* and *Streptococcus uberis.* There is no milk withholding time and the meat withdrawal period is 16 days provided a minimum dry period length of 30 days is met.

Cloxacillin benzathine is a semisynthetic penicillin resistant to penicillinases. There are two products commercially available containing this active drug: Dry-Clox® (Boehringer Ingelheim Vetmedica, Inc, St Joseph, MO) and Orbenin®-DC (Merck Animal Health, Summit, NJ). These antimicrobial products contain 500 mg of cloxacillin and are indicated for treatment of mastitis caused by penicillinase-producing *Staphylococcus*

aureus and *Streptococcus agalactiae*. The product Orbenin®-DC is also labeled for prophylaxis of mastitis caused by the previous cited pathogens. Meat withhold period is 30 and 28 days for Dry-Clox® and Orbenin®-DC respectively. There is no milk withhold period for either of the two products, provided a minimum dry period of 30 and 28 days respectively is met.

Novobiocin is an antibiotic that interferes with bacterial protein and nucleic acid synthesis. BioDry® (Pfizer Animal Health, New York, NY) is the commercial product, claimed to be effective for treatment of mastitis caused by *Staphylococcus aureus* and *Streptococcus agalactiae*. It contains 400 mg of novobiocin sodium, meat withholding time is 30 days, milk withholding time is 72 hours after calving and minimum dry period required is 30 days.

Penicillins are generally effective against Gram-positive pathogens but not against gram negatives. This is a β-lactam antibiotic that acts on bacteria cell wall synthesis and it is commonly used associated with other products. There are currently three products commercially available containing penicillin. The product containing 100,000 international units (**IU**) of procaine penicillin G, US Vet Go-Dry® (G. C. Hanford Mfg. Co., Syracuse, NY) claims to be effective for treatment of mastitis caused by *Streptococcus agalactiae*. Milk and meat withholding time is 72 hours post calving and 14 days post administration, respectively, and a 14 day minimum dry period length is required. Combinations with other products include penicillin G/novobiocin, available in a product called Albadry® Plus Suspension (Pfizer Animal Health, New York, NY), which contains 400 mg of novobiocin and 200,000 IU of procaine penicillin G and claims to be effective on treatment of mastitis caused by *Staphylococcus aureus, Streptococcus*

agalactiae, Streptocuccus dysgalactiae and *Streptococcus uberis.* Milk withholding time is 72 hours after calving, meat withholding time is 30 days after drug administration and dry period length is 30 days.

Aminoglycosides such as streptomycin are effective against β-lactamase producing *Staphylococci* and some coliforms, but they have poor penetration in the udder tissue (Blowey and Edmonson, 2010). For that reason, they are commonly used combined with penicillins. Penicillin G/dihydrostreptomycin, as in the product Quartermaster® (Pfizer Animal Health, New York, NY), which claims to be effective in terms of reducing the frequency of existing infections and preventing new infections with *Staphylococcus aureus*. It is composed of 100,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin. Milk withholding time is 96 hours postcalving and meat withholding time is 60 days. Dry period length should be at least 42 days.

Efficacy of commercial DCT products as compared to negative controls (untreated quarters) has been demonstrated in order to receive FDA approval. However, peer reviewed studies comparing efficacy among available DCT products in field conditions in the US are extremely limited. Such studies would be helpful in terms of providing information to the producer to promote judicious drug selection.

A study conducted in Kentucky by Harmon et al. (1986) investigated the efficacy of three different antibiotic formulations (novobiocin, cephapirin benzathine and penicillin/dihydrostreptomycin) against a negative control (no treatment) on the cure of minor pathogens such as *Corynebacterium bovis* and Coagulase Negative Staphylococci. All three antibiotics eliminated significantly more *C. bovis* infections than spontaneous elimination in the negative control group. For CNS only, the combination

penicillin/streptomycin eliminated the pathogen significantly more than controls. Cephapirin was the only treatment that significantly reduced the number of new infections when both outcomes compared to untreated controls. Results from this study suggest that DCT has a great impact in controlling minor pathogens.

A drug registration trial that was conducted in 2006 (Hallberg et al.) compared the efficacy of different ceftiofur hydrochloride doses (125, 250 or 500mg), a positive control (300mg cephapirin benzathine) and a negative control (no DCT). In this study, the authors reported that a single intramammary infusion of 500 mg of ceftiofur was the only treatment statistically effective for treatment of existing IMI and prevention of new IMI during the dry period (Hallberg et al., 2006). However, this study had some limitations: the sample population was not representative of the target population, since only cows with a SCC over 400,000 or linear score over 5 were included. In the field, producers routinely use the blanket dry cow therapy, with no selection to treat only high SCC animals. The second issue was the limited sample size, which reduces the power of detecting significant differences if differences are truly present. Also, there were no between-product comparisons: all the statistical analysis was done comparing one of the four therapies to the negative control group. Finally, the authors did not report long-term outcomes in the subsequent lactation such as risk for clinical mastitis events, risk for culling, milk production, besides other factors of great importance to the producer, nor was an economic analysis completed.

A recent study (Pinedo et al., 2012) compared intramammary treatment with ceftiofur hydrochrolide and treatment with penicillin/ dihydrostreptomycin at the cow level regarding risk for a clinical mastitis event and risk for a high somatic cell count in the

first 30 and 60 DIM. Results from this study showed that cows treated with ceftiofur had lower odds of having a high SCC and of experiencing a clinical case in the first 30 and 60 DIM compared to cows treated with penicillin. However, it is important to notice that this study was limited to two herds in the state of Florida, it had a limited sample size and finally, the authors did not report short-level outcomes or the magnitude of difference detected for neither outcome.

Part V- Summary and Objectives

In summary, it is clear that a great deal of research has been completed during the last 5 decades to develop programs to control and reduce damages caused by mastitis pathogens. The dry period deserves a great deal of attention, and many studies were devoted to find effective management practices that would lead to prevention and treatment of this costly infectious disease during the dry period. The practice of blanket DCT is one such management practice that has been highly successful.

Although dry cow antibiotic formulations are widely used in dairy herds across the US, the efficacy of these products was typically established many years ago, and well-designed head-to-head studies comparing efficacy among DCT products are largely lacking. It is clear that the comparative efficacy of available DCT formulations deserves to be investigated so that producers can make informed science-based decisions when selecting DCT products for use in their herds. The main goal of this multi-herd, multi-state study is to promote judicious drug use while providing producers with needed information to guide the selection of efficacious DCT products, thus promoting cow health and welfare, as well as economic sustainability of the dairy farm. This study will

also provide valuable information on pathogen prevalence and types of pathogens causing subclinical mastitis during the dry period in different states.

Objective 1- Conduct a multi-state, multi-herd non-inferiority randomized clinical trial to describe the efficacy of three different mastitis preparations to cure of existing IMI and to prevent new infections during the dry period. The major quarter level outcomes to be evaluated will include the risk for presence of an IMI after calving, risk for cure of an IMI during the dry period, risk for development of a new IMI during the dry period and risk of a clinical mastitis event between calving and 100 days in milk.

Objective 2- Use data collected in the same non-inferiority clinical trial to describe the effects of treatment group on cow level health and production parameters for the first 100 DIM including milk production, linear score, risk of culling and death, risk for a clinical mastitis case and days to pregnancy.

CHAPTER 2

RANDOMIZED NON-INFERIORITY CLINICAL TRIAL EVALUATING THREE COMMERCIAL DRY COW MASTITIS PREPARATIONS: I. QUARTER LEVEL OUTCOMES

Chapter 2

Randomized Non-Inferiority Clinical Trial Evaluating Three Commercial Dry Cow Mastitis Preparations: I. Quarter Level Outcomes

Overview

The objective of the current study was to conduct a non-inferiority study to compare the efficacy of three commercial dry cow mastitis formulations regarding quarter level prevalence of intramammary infections (**IMI**) postcalving, cure of preexisting infections over the dry period, prevention of new infections during the dry period and risk for a clinical mastitis case between calving and 100 days in milk (**DIM**). A total of 1,091 cows (4,364 quarters) from 6 commercial dairy herds in 4 different states (CA, IA, MN and WI) were enrolled and randomized to one of the three treatments at dry off: Quartermaster (**QT**, 100,000 IU procaine penicillin G and 1 g dihydrostreptomycin, Pfizer Animal Health, New York, NY), Spectramast DC (**SP**, 500 mg ceftiofur hydrochloride, Pfizer Animal Health, New York, NY) or Tomorrow Dry Cow (**TM**, 300 mg cephapirin benzathine, Boehringer Ingelheim Vetmedica, Inc., St Joseph, MO). Quarter milk samples were collected for routine bacteriological culture prior to dry cow therapy treatment at dry off, at 0 to 6 DIM, at 7 to 13 DIM and in the event of a clinical mastitis case until 100 DIM. Teat end condition and udder hygiene scoring were conducted at dry off, 0 to 6 DIM and 7 to 13 DIM. An on-farm record keeping system and Dairy Herd Improvement Association electronic records were used to retrieve data describing parity, previous lactation somatic cell count, previous lactation milk production, and clinical mastitis episodes. Non-inferiority analysis was used to evaluate

the effect of treatment on the primary outcome, risk for a bacteriological cure during the dry period. Additionally, multivariable logistic regression techniques were used to describe the effect of treatment on presence of IMI postcalving, risk for experiencing a cure and risk for development of new IMI during the dry period. Cox proportional hazards regression was used to describe the effect of treatment on the risk and time for quarters to experience an episode of clinical mastitis between calving and 100 DIM. Overall quarter level prevalence of infection at dry off was 19.2%. The most common pathogen isolated from milk samples at dry off was coagulase negative *Staphylococcus*, representing 53.9% of all isolates recovered, followed by *Aerococcus* spp. and other *Streptococcus* spp. with 12.3% and 7.4% of isolates recovered, respectively. Non-inferiority analyses showed no effect of treatment on risk for a quarter to experience a cure. Multivariate logistic regression and Cox proportional regression analyses showed there was no effect of treatment on risk for presence of an IMI in a quarter at 0 to 6 DIM (LSM: QT = 16.5%, SP = 14.1% and TM = 16.0%), risk for presence of an IMI in a quarter at 7 to 13 DIM (LSM: QT = 19.4%, SP = 18.7% and TM = 16.8%), risk for a quarter to experience a cure between dry off and calving (LSM: QT = 93.3%, SP = 92.6% and TM = 94.0%), risk for a quarter to develop a new IMI between dry off and 0 to 6 DIM (LSM: QT = 14.8%, SP = 12.3% and TM = 14.2%), risk for a quarter to develop a new IMI between dry off and 7 to 13 DIM (LSM: QT = 17.9%, SP = 17.2% and TM = 16.0%) or risk for a quarter to experience a clinical mastitis event between calving and 100 DIM (QT = 5.3%, SP = 3.8% and TM = 4.1%). In conclusion, there was no difference in efficacy between the three products evaluated when assessing the quarter level outcomes of interest.

Introduction

The importance of mastitis during the dry period has been explored by several authors (Ward and Schultz, 1974; Natzke et al. 1975; Oliver and Mitchell, 1983; Eberhart, 1986; Erskine, 2001). The dry period corresponds to a crucial period when lactating cows go through physiological changes in order to prepare their mammary gland for the next lactation. Persistence of preexisting intramammary infections (**IMI**) through the dry period and development of new IMI (**NIMI**) during the dry period are two important factors that increase the risk for manifestation of clinical mastitis in the next lactation. Estimates of dry cow mastitis incidence rates vary among studies, in part due to differences in definitions of IMI, regional differences and herd differences. North American studies have estimated that the proportion of quarters developing a NIMI during the dry period ranges between 8 and 25% (Eberhart, 1986; Godden et al., 2003; Cook et al., 2004). The majority of new infections are subclinical during the dry period, but can flare up as clinical mastitis, usually in early lactation (Green et al., 2002). It has been estimated that 55% of environmental infections established early in the dry period persist into the next lactation and can possibly cause clinical flare ups, and that 52% of all clinical coliform mastitis cases occurring in the first 100 days of lactation may originate during the previous dry period (Todhunter et al., 1995). Smith et al. (1985) reported that the risk for NIMI from environmental pathogens can be ten times higher during the dry period than during the lactation period.

Blanket dry cow therapy (**DCT**) is a procedure recommended by the National Mastitis Council (**NMC**) as a mastitis control practice, for the purpose of curing existing subclinical infections and preventing new infections that could be acquired during the

early dry period. North American studies estimate the proportion of quarters infected subclinically at dry off to vary between 13 and 35% (Oliver and Mitchell, 1983; Godden et al., 2003; Pantoja et al., 2009). Because it is administered at dry off, advantages of using DCT include avoidance of milk discarded during the lactation period, use of larger doses of antibiotic (so that concentrations can stay above minimum inhibitory concentration for longer periods of time) and reduction in risk for antibiotic residues in the milk. It has been estimated that 72.3% of the US dairy operations use blanket DCT, which corresponds to 81.7% of US dairy cows (NAHMS, 2008).

According to the "Milk and Dairy Beef Drug Residue Prevention Manual" of 2011, there are currently 7 commercial dry cow mastitis products approved by the Food and Drug Administration (**FDA**) for use in United States dairy herds. Milk and meat withhold period, dry period length, claimed spectrum of action and cost for these products vary considerably. The products evaluated in this study were Quartermaster (**QT**, Pfizer Animal Health, New York, NY), Spectramast DC (**SP**, Pfizer Animal Health, New York, NY) and Tomorrow Dry Cow (**TM**, Boehringer Ingelheim Vetmedica, Inc., St Joseph, MO). The product QT is composed of 100,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin. It is labeled to reduce the frequency of existing infections and prevent new infections with *Staphylococcus aureus*. Milk and meat withholding times are 96 hours postcalving, and 60 days post infusion, respectively. Dry period length is required to be at least 42 days. The product SP is composed of 500 mg of ceftiofur hydrochloride and labeled for subclinical mastitis associated with *Staphylococcus aureus, Streptococcus dysgalactiae* and *Streptococcus uberis.* There is no milk withholding time and the meat withdrawal period is 16 days post infusion for this product. Required dry

period length is 30 days. Finally, TM contains 300 mg of cephapirin and is labeled to be effective on the treatment of mastitis caused by *Streptococcus agalactiae* and *Staphylococcus aureus*, including penicillin-resistant strains. Milk and meat withholding times are 3 days after calving and 42 days after treatment, respectively, and the required dry period length is 30 days.

Efficacy of commercial DCT products as compared to negative controls (untreated quarters) has been previously demonstrated for each of these products in order to receive FDA approval. However, studies comparing efficacy among available DCT products in field conditions in North America have been previously lacking. Hallberg et al. (2006) conducted a study to evaluate the efficacy of ceftiofur hydrochloride for the treatment of existing IMI at dry off and prevention of NIMI during the dry period using a negative control and a positive control (cephapirin benzathine), but this study was not designed with an appropriate sample size to compare efficacy between the two antimicrobial formulations used, nor did the authors complete and report statistical analysis comparing the two antimicrobial treatments. Furthermore, that study only enrolled cows with an elevated SCC (> 400,000 cells/ mL), and so results may not be generalizable to commercial dairy herds wherein blanket DCT is usually applied to all cows. A recent study (Pinedo et al., 2012) conducted in Florida compared treatment with SP versus treatment with QT, but the authors did not report quarter level outcomes such as risk for new IMI or risk for cure of preexisting IMI. The comparative efficacy of available DCT formulations deserves to be investigated so that producers can make informed science-based decisions when selecting DCT products for use in their herds. The ultimate goal of this non-inferiority multi-herd multi-state study is to promote judicious drug use while

providing producers with needed information to guide the selection of efficacious DCT products, thus promoting cow health and welfare, as well as economic sustainability of the dairy farm.

The study objective was to compare the efficacy of three commercial DCT as measured by quarter level risk for presence of an IMI postcalving, risk for cure of an IMI during the dry period, risk for development of NIMI over the dry period and risk for experiencing a clinical mastitis event between calving and 100 days in milk (**DIM**). The hypothesis tested was that quarters infused with cephapirin benzathine at the time of dry off would have a non-inferior proportion of quarters cured from preexisting IMI, and would have similar prevalence of IMI postcalving, incidence of NIMI over the dry period and incidence of clinical mastitis until 100 DIM as compared to quarters infused with either ceftiofur hydrochloride or penicillin/ dihydrostreptomycin.

Materials and Methods

Herd Selection

A non-inferiority randomized clinical trial was conducted under IACUC approval between February 2011 and November 2011 in six commercial dairy herds located in California (n = 2), Iowa (n = 1), Minnesota (n = 1) and Wisconsin (n = 2). To be included, study herds must be located within 300 km from the respective collaborating institution, be on a regular Dairy Herd Improvement Association (**DHIA**) testing program, and comply with the study protocol. This convenience sample of herds averaged 2,230 lactating cows (range 1,050 to 3,600), with an average bulk tank somatic milk somatic cell count (**SCC**) of 242,170 cells/ mL (range 148,000 cells/ mL to 330,000

cells/ mL), and a rolling herd average of 12,360 kg (range 10,610 kg to 13,550 kg; Table 2.1). Bulk tank samples were negative on culture for *Mycoplasma* spp. for all 6 herds prior to initiating the study. All herds routinely used an internal teat sealant at dry off (Orbeseal^{TM}, Pfizer Animal Health, New York, NY), commercial coliform mastitis vaccines and blanket dry cow therapy.

Cow Enrollment

To be eligible for enrollment, cows had to be in good general health, have four functioning quarters, have not received parenteral or intramammary treatment with an antibacterial or anti-inflammatory medication during a 30-day period immediately prior to dry off and show no clinical signs of mastitis on the day of dry off.

All study enrollment and sampling activities were conducted by study (University) technicians who visited the herd on dry off day each week. Cows due to be dried off were brought into the parlor for their last milking and routine DCT. Cow identification numbers were previously assessed and animals were checked for previous medication. Animals were identified while entering the parlor and visually inspected for clinical signs of illness such as very low body condition score (< 2.0) or moderate to severe lameness. The udder and milk were inspected for signs of clinical mastitis. Eligible cows were randomly allocated to treat all four quarters with one of the three treatments (QT, SP or TM) according to a previously prepared randomized spreadsheet created on Excel. Randomization was blocked within farms on the day of enrollment.

Routine parlor udder preparation was performed by the farm personnel while study investigators recorded teat end and udder hygiene scores. Teat end scores ranged from 1

(no teat end crack or callosity) to 4 (cracked teat end) (Falkenberg et al., 2003) and udder hygiene scores ranged from 1 (clean) to 4 (dirty) (Schreiner and Ruegg, 2003). Following routine udder preparation, study technicians cleaned and disinfected the teat ends using gauze squares soaked in 70% isopropyl alcohol. Three strippings of fore milk were discarded and duplicate quarter samples were carefully taken into sterile milk vials previously identified with herd, cow number, quarter and date. After sample collection (S1 = Sample 1), routine milking procedure took place and milk sample vials were placed into a chilled cooler on ice. Immediately following the final milking, all four quarters were again scrubbed with alcohol-soaked gauze, the assigned treatment was infused into each of the four quarters and the udder was massaged. The internal teat sealant was then infused and no massage was applied. Blue leg bands were attached to the animal's legs, according to the farm protocol, in order to facilitate identification of study animals postcalving. All cows were post-dipped and moved to their freestalls, where usual farm dry cow husbandry practices were undertaken.

Postcalving Sampling and Follow-up.

Study technicians visited the herds once per week and postcalving duplicate quarter milk samples were collected at two different time periods: 0 to 6 DIM (S2 = Sample 2) and 7 to 13 DIM (S3 = Sample 3). The procedure for sample collection was the same as previously described for S1 samples collected at dry off. All clinical mastitis events occurring in the first 100 DIM were recorded by farm staff using an on-farm electronic record keeping system (DairyComp305, Valley Agricultural Software, Tulare, CA) and farm personnel were asked to collect and freeze an aseptic milk sample from the affected

quarter at time of detection of a clinical case. Clinical mastitis was defined as visibly abnormal milk accompanied or not by changes in the quarter. Samples were kept frozen (-20°C) at the farm until the next investigator's visit. DairyComp305 software was utilized to capture electronic DHIA records for all study cows throughout the 100 DIM observation period in order to provide test day measures of SCC and milk production, clinical mastitis events, death and culling events (Note: long term cow-level outcomes will be reported in Chapter 3).

Laboratory Methods

Milk samples collected on farms were placed on ice and transported back to the local participating laboratory (Laboratory for Udder Health, St Paul, MN; Veterinary Diagnostic Laboratory, Ames, IA; or Dairy Food Safety Laboratory, Tulare, CA), where they were immediately frozen at -20°C until they could undergo bacterial culture. Bacteriological milk culture procedures were standardized as much as possible amongst the three participating laboratories and followed published procedures recognized by the NMC for bovine mastitis (NMC, 1999). Milk samples were kept frozen and out of the duplicate samples, only one was routinely subjected to microbiological investigation. The second sample was kept in reserve and only examined in cases where the first sample was contaminated. Samples were thawed to room temperature and 0.01 mL of milk was plated into MacConkey agar and Factor (for those samples submitted to the MN laboratory) or Blood agar (for samples submitted to CA or IA laboratories) agar using calibrated loops (Note: The Factor agar is routinely used at the Laboratory for Udder Health from the University of Minnesota, St Paul, MN, for selective growth of Gram-

positive pathogens). Inoculated plates were incubated at 37°C for 48 hours and then observed for bacterial growth. For plates with bacterial growth, the number of colonies was recorded for each species isolated, and colonies were reisolated in Blood agar for further characterization. Colony morphology and hemolysis pattern were determined. Gram-positive organisms were Gram-stained and the catalase test reaction was conducted to differentiate *Staphylococcus* and *Streptococcus* species. Organisms that were catalase positive and coagulase negative were reported as coagulase negative *Staphylococcus* (**CNS**), while catalase and coagulase positive organisms were reported as *Staphylococcus aureus*. Catalase negative organisms had their identity confirmed by the API *Streptococcus* identification system (bioMeriux-Vitek, Inc. Hazelwood, MO). Pathogens reported as 'other *Streptococcus* spp.' corresponded to subspecies of *Streptococcus* that are less commonly reported in literature or to pathogens that could not be identified by the API *Streptococcus* system. Gram-positive organisms that were in very low prevalence and pathogens that grew in Factor but not in MacConkey agar and could not be identified were reported as 'other Gram-positives'.

Gram-negative organisms were initially identified based on colony morphology and motility test, with identity confirmed using the API20E test (bioMeriux-Vitek, Inc. Hazelwood, MO). Organisms that could not be identified by the API system were reported as 'other Gram-negatives'. Finally, non bacterial pathogens such as yeast were reported as 'others'.

In case three or more pathogens were present in a single sample, this was considered contamination and the duplicate sample was cultured. If the duplicate sample also yielded three or more bacterial pathogens, the quarter sample was reported as contaminated.

Blinding of the study technician or producer at the time of treatment was not possible. However, laboratory personnel were blinded to treatment.

Definitions

Presence of an IMI. An IMI was defined as one or more colonies isolated from a 0.01-mL milk sample for all pathogens except for CNS and *Bacillus* spp. For CNS, two or more colonies isolated from a 0.01-mL milk sample were needed to establish presence of an IMI (Dohoo et al., 2011). For *Bacillus* spp., an IMI was defined as five colonies isolated from a 0.01-mL milk sample. Since there are no peer-reviewed studies determining a cut-off point for the latter organism, the definition for IMI for *Bacillus* spp. was established by an informal discussion among mastitis experts conducted during the 2011 Mastitis Research Workers' Conference (Nov 1, 2011, Chicago, IL). A single IMI was defined as the presence of only one pathogen in the sample, while mixed infections corresponded to the presence of two different bacterial species.

Bacteriological Cure: A cure was defined as the disappearance of one or two of the pathogens originally present at the dry off sample (Godden et al., 2003) in both postcalving samples (S2 and S3). Quarters with contaminated or missing samples were not included in the analysis.

New IMI. A NIMI was defined as either a) quarters from which no pathogens were recovered at dry off (S1) but growth was later detected in the postcalving samples (S2 or S3) or b) quarters from which a different pathogen was isolated in the postcalving samples (S2 or S3) as compared to pathogens isolated in the dry off sample (S1). New infections were examined separately for the periods: from dry off to 0 to 6 DIM and from

dry off to 7 to 13 DIM. The decision to analyze risk for acquiring a NIMI as two separate intervals was made due to the fact that the sampling interval that would best capture this outcome is still contradictory. In theory, an IMI might go undetected if using the sample collected in the first week after calving (0 to 6 DIM) if antibiotic residues were still present in the mammary gland. On the other hand, the sample collected in the second week after calving (7 to 13 DIM) could also be more likely to include NIMI acquired between calving and sampling. Quarters that had one or both samples contaminated or missing were not included in the analysis. It was possible for the same quarter to have both a cure and a NIMI.

Statistical Analysis

For a priori sample size calculation, the primary outcome was risk for a cure. The maximum difference to declare non-inferiority was pre-stated at 10%. To demonstrate non-inferiority, a total of 339 cows (1356 quarters) per group were estimated to be required assuming $\alpha = 0.025$, $\beta = 0.2$, 10% losses to follow-up and that 30% of the quarters would be infected at dry off, and therefore at risk for a cure (non-inferiority tests for two proportions, Pass 2008; NCSS, Kaysville, UT, USA).

All statistical analyses were conducted using the intent-to-treat approach at the quarter level on SAS version 9.2 (SAS Inst. Inc., Cary, NC). Initially descriptive statistics and plots were generated for exploratory data analysis. Basic diagnostic techniques were used to evaluate normality and the presence of outliers. Characteristics of cows and quarters assigned to the three treatment groups were initially compared at baseline using chi-square test and ANOVA.

A logistic multivariable regression model approach was used for outcomes that involved binary response variables such as risk for presence of IMI, risk for cure and risk for new IMI between dry off and calving. The effect of treatment effect on the dependent variables listed above were analyzed by the Generalized Linear Mixed Models for Dichotomous Variables (PROC GLIMMIX), with region included as a fixed effect and herd and cow included as random effects in the model, to account for the clustering effects of herds within regions, cows within herds and quarters within cows. Covariates offered to the model included DCT treatment group (forced), cow parity, previous lactation linear score (**LS**), previous lactation total milk production, dry period length, teat end score at dry off and postcalving; and udder hygiene score at dry off and postcalving (Table 2.2). The variables previous lactation LS, previous lactation total milk production and dry period length were offered as continuous variables. Udder hygiene score was offered as a categorical variable in four levels and cow parity was dichotomized in two categories: second parity and third or greater parities. Teat end score was initially captured in four categories but two categories were considered in the model: categories 1 and 2 and categories 3 and 4, for the reason that there were relatively few teat ends that scored 4. Univariate analysis was initially conducted between each of the aforementioned variables and the dependent variable of interest, and variables with a $P < 0.2$ were then carried forward to offer in the full model. Non-significant variables were then removed one at a time in a backward stepwise approach with final significance declared at $P < 0.05$. First-order interactions between DCT treatment group and other remaining main effects were tested and included in the model if significant. Models were compared during the model building process using the -2 Log Likelihood statistics and

the final model fit was assessed using PROC LOGISTIC with the Hosmer and Lemeshow Goodness-of-Fit test.

Non-inferiority analysis of the effect of treatment on risk for a bacteriological cure was completed by constructing a figure containing the confidence intervals for the treatment relative to both null (reference treatment) and the margins of equivalence (Piaggio et al., 2006).

Cox proportional hazards regression (PROC PHREG) was used to describe the effect of DCT treatment on the survival distribution function of quarters experiencing a case of clinical mastitis between calving and 100 DIM (Note: no clinical mastitis events were reported by the farm personnel during the dry period). Quarters were considered to be at risk between calving and 100 DIM, with the failure date defined as the date when the quarter was first reported to be affected by a clinical mastitis event. Quarters not reported to experience a clinical mastitis event were classified as censored either at the cow's culling or death date (if before 100 DIM) or at 100 DIM. Clustering at the herd level was controlled for with a Covsandwich statement. Covariates offered to the model included DCT treatment group (forced), region (forced), cow parity, previous lactation LS, previous lactation total milk production, dry period length, teat end score at dry off and udder hygiene score at dry off.

Results

A total of 4,364 quarters (1,091 cows) were enrolled into the study between February and April of 2011. Of those, 1,492, 1,396 and 1, 476 quarters were allocated to treatment groups QT, SP and TM, respectively. The treatment groups did not differ at enrollment

regarding the following cow level parameters: parity, previous LS, previous total milk, dry period length and udder hygiene score at dry off (Table 2.3). At the quarter level, treatment groups did not differ regarding teat end scores at dry off (Table 2.3). Missing information included 11 cows with missing previous LS and previous total milk (QT = 7, SP = 2 and TM = 2), 27 cows with missing dry period lengths (QT = 10, SP = 10 and TM = 10) and 15 quarters with missing teat end scores (QT = 9, SP = 0 and TM = 6). Previous total milk was excluded for one cow due to an unrealistic reported value of 117 kg. Table 2.4 summarizes the overall results from this study. However details of these results are provided in the next sections.

IMI status at dry off

A total of 4,260 quarters were used for analysis of risk for presence of infection at dry off, due to 13 missed samples (QT = 5, SP = 5 and TM = 3) and 91 contaminated samples (QT = 28, SP = 33 and TM = 30). The overall crude prevalence of IMI at dry off was 19.2% (Table 2.5) and was not different between treatments (LSM: QT = 0.22 (95% CI: 0.18, 0.26), SP = 0.21 (95% CI: 0.18, 0.26) and TM = 0.23 (95% CI: 0.19, 0.27); P = 0.73). Significant covariates in the model predicting presence of IMI at dry off included region ($P < 0.01$), previous LS ($P < 0.01$), teat end score at dry off ($P < 0.01$), udder hygiene score at dry off ($P = 0.04$) and parity ($P < 0.01$; Table 2.6). The most common pathogen isolated from milk samples at dry off was CNS, representing 53.9% of all isolates recovered, followed by *Aerococcus* spp. (12.3%) and *Streptococcus* spp. (7.4%). Gram-positive organisms, Gram-negatives and 'others' represented 94.4%, 4.9% and 0.7% of all organisms isolated, respectively (Table 2.7).

Effect of treatment on risk for presence of an IMI at 0 to 6 DIM (S2) and 7 to 13 DIM (S3)

A total of 4,058 quarters were used in the analysis of risk for presence of infection at 0 to 6 DIM (S2). From the 4,364 quarters initially enrolled, 108 quarters (27 cows) were either culled or died during the dry period (QT = 40, SP = 40 and TM = 28), 99 quarters did not have their first postcalving sample collected (QT = 20, SP = 47 and TM = 32), 16 quarters were from cows that died between calving and their first postcalving sample (QT = 4, SP = 4 and TM = 8), 8 quarters were from cows that were culled between calving and their first postcalving sample (QT = 4, SP = 4 and TM = 0) and 75 samples were contaminated (QT = 26, SP = 27 and TM = 22).

The overall crude proportion of quarters with an IMI present at S2 (0 to 6 DIM) was 14.7%, with no difference among the three treatments (LSM: QT = 0.16 (95% CI: 0.14, 0.19), SP = 0.14 (95% CI: 0.12, 0.17) and TM = 0.16 (95% CI: 0.14, 0.19); $P = 0.34$; Table 2.5). The variables describing region ($P < 0.01$), previous LS ($P < 0.01$) and teat end score at dry off ($P = 0.01$) were kept in the final model (Table 2.8).

The most common pathogen isolated at the first sampling postcalving (S2) was CNS (44.6% of all isolates recovered), followed by *Aerococcus* spp. (15.5%) and *Bacillus* spp. (10.0%). Gram- positives, Gram-negatives and 'others' represented 89.7%, 7.0% and 3.3% of all pathogens recovered, respectively (Table 2.7). Separate analyses were performed after stratifying the dataset by Gram-negatives and Gram-positives. There was no effect of treatment on risk for presence of a Gram-positive IMI (crude proportions: QT = 14.5%, SP = 12.8% and TM = 13.6%, $P = 0.44$) or a Gram-negative IMI (crude proportions: QT = 1.2%, SP = 1.0% and TM = 1.3%, $P = 0.79$) at 0 to 6 DIM (Table 2.9).

A total of 3,974 quarters were used in the analysis for presence of infection at 7 to 13 DIM (S3). Out of the 4,232 quarters remaining in the study at this point (eliminating quarters lost from cows that were culled or died during the dry period and between calving and 0 to 6 DIM), 97 quarters did not have their postcalving sample collected at 7 to 13 DIM (QT = 25, SP = 52 and TM = 20), 28 quarters were from cows that died between calving and their second postcalving sample (QT = 8, SP = 4 and TM = 16), 60 quarters were missed due to cows that were culled between calving and their second postcalving sample was taken (QT = 12, SP = 24 and TM = 24) and 73 samples were contaminated (QT = 31, SP = 20 and TM = 22). The overall crude proportion of quarters with an IMI at 7 to 13 DIM was 14.7%, with no difference among the three treatments (LSM: QT = 0.19 (95% CI: 0.16, 0.24), SP = 0.19 (95% CI: 0.15, 0.23) and TM = 0.17 (95% CI: 0.14, 0.21); $P = 0.37$; Table 2.5). Covariates associated with the presence of an IMI between 7 and 13 DIM in the final model included region ($P < 0.01$), previous LS ($P < 0.01$), and both teat end score ($P = 0.03$) and udder hygiene score at 7 to 13 DIM ($P = 0.04$; Table 2.8).

The pathogens isolated most frequently at 7 to 13 DIM included CNS (45.5% of all isolates), *Aerococcus* spp. (16.7%) and other *Streptococcus* spp. (9.1%). Gram-positive organisms, Gram-negatives and 'others' represented 91.1%, 6.4% and 2.5% of all pathogens isolated, respectively (Table 2.7). Separate subgroup analysis showed there was no effect of treatment on risk for Gram-positive IMI (crude proportions: QT = 14.5%, SP = 14.3% and TM = 12.6%; $P = 0.30$) or Gram- negative IMI (crude proportions: QT = 0.9%, SP = 1.0% and TM = 1.4%; $P = 0.59$) at 7 to 13 DIM (Table 2.10).

Effect of treatment on risk for experiencing a cure between dry off and postcalving

A total of 835 quarters had an IMI present at dry off and so were at risk for a cure. However, quarters from which samples were contaminated or missing for any one of the two samples postcalving (S2 or S3) could not be assigned a cure status and therefore were not included in the analysis. Out of the initially eligible quarters, there were a total of 11 contaminated samples (QT = 5, SP = 2 and TM = 4) and 41 missing samples (QT = 10, SP = 17 and TM = 14) for samples collected at 0 to 6 DIM, and 15 contaminated samples (QT = 8, SP = 5 and TM = 2) and 27 missing samples (QT = 11, SP = 12 and TM = 4) for samples collected at 7 to 13 DIM. Therefore, 741 quarters were included in the final analysis. Overall, the crude proportion of quarters experiencing a cure between dry off and postcalving was 88.9%, with no difference among the three treatment groups (LSM: QT = 0.93 (95% CI: 0.87, 0.97), SP = 0.93 (95% CI: 0.86, 0.96) and TM = 0.94 (95% CI: 0.89, 0.97); $P = 0.79$; Table 2.11). The effect of treatment on this primary outcome was also evaluated using non-inferiority analysis by constructing a figure containing the confidence interval for the treatment effect and both the margins of inferiority and the null effect (Piaggio et al., 2006). Because the confidence interval is wholly between the margins of inferiority and includes zero, therefore we confirm that treatment with TM is non-inferior to both treatments with QT and SP (Figures 2.2 and 2.3). Teat end score at dry off ($P < 0.01$) and teat end score at S3 ($P = 0.04$) were associated with risk for cure (Table 2.11). While this final model defined cure as the disappearance of bacterial pathogens in both samples postcalving, separate models that defined cure as the disappearance of a bacterial pathogen between dry off and either the

first or the second sampling postcalving found similar results (models and results not shown).

Separate subgroup analysis showed that risk for cure was not different among the three products for IMI caused either by Gram-positive pathogens (crude proportions: QT = 87.3%, SP = 88.0% and TM = 89.5%, *P* = 0.70) or for IMI caused by Gram-negative pathogens (crude proportions: QT = 100.0%, SP = 77.8% and TM = 81.2%; *P* = 0.62; Table 2.11). Due to the very small number of Gram-negative pathogens (n = 42) considered, this last model could not include the random effects of quarters clustered within cows or cows clustered within herds (Table 2.13).

Effect of treatment on risk for developing a new IMI between dry off and 0 to 6 DIM (S2) or between dry off and 7 to 13 DIM (S3)

All the quarters enrolled were considered at risk for developing a NIMI over the dry period. However, quarters that had contaminated or missing samples at dry off (S1) or at the first or second sampling postcalving (S2 or S3) were not assigned a new infection status and were therefore excluded from the respective analysis. There were a total of 3,962 and 3,883 quarters used for analysis of effect of treatment on new infections between dry off and 0 to 6 DIM and between dry off and 7 to 13 DIM, respectively. A total of 402 quarters were not eligible for the analysis of NIMI between dry off and 0 to 6 DIM. Of these, 165 quarters were excluded due to contaminated samples (QT = 55, SP = 60 and TM = 50) and 237 quarters were excluded due to missing samples (QT = 71, SP = 97 and TM = 69). For the analysis of NIMI between dry off and 7 to 13 DIM, 481

quarters were excluded: 157 contaminated samples (QT = 59, SP = 49 and TM = 49) and 234 missing samples (QT = 95, SP = 134 and TM = 95).

The crude proportions of eligible quarters developing a NIMI between dry off and 0 to 6 DIM and between dry off and 7 to 13 DIM was 13.3% and 13.4%, respectively. There was no effect of treatment on risk for developing a NIMI between dry off and 0 to 6 DIM (LSM: QT = 0.15 (95% CI: 0.12, 0.18), SP = 0.12 (95% CI: 0.10, 0.15) and TM = 0.14 (95% CI: 0.12, 0.17); $P = 0.27$) or between dry off and 7 to 13 DIM (LSM: QT = 0.18 (95% CI: 0.15, 0.22), SP = 0.17 (95% CI: 0.14, 0.21) and TM = 0.16 (95% CI: 0.13, 0.20); $P = 0.60$; Table 2.14). Region ($P < 0.01$), previous LS ($P = 0.02$), teat end score at dry off ($P = 0.02$) and parity ($P = 0.03$) were associated with risk for developing a NIMI between dry off and 0 to 6 DIM (Table 2.14). Region ($P < 0.01$), teat end score at S3 ($P = 0.02$), udder hygiene score at S3 ($P = 0.04$) and parity ($P < 0.01$) were associated with risk for developing a NIMI between dry off and 7 to 13 DIM (Table 2.14).

Separate subgroup analysis showed no effect of treatment on risk for developing a new Gram- positive IMI (crude proportions: QT = 13.1%, SP = 11.1% and TM = 11.9%, $P = 0.29$) or a new Gram-negative IMI (crude proportions: QT = 1.2%, SP = 1.0% and TM = 0.9%, $P = 0.77$) between dry off and 0 to 6 DIM (Tables 2.15, 2.16). Similarly, there was no effect of treatment on NIMI caused by Gram-positive organisms (crude proportions: QT = 12.9%, SP = 12.8% and TM = 11.5%, $P = 0.59$) or NIMI caused by Gram-negative organisms (crude proportions: QT = 1.0%, SP = 0.8% and TM = 1.0%, $P = 0.89$) between dry off and 7 to 13 DIM (Tables 2.15, 2.17).

Effect of treatment on risk for experiencing a clinical mastitis event between calving and 100 DIM

For the survival analysis using the Cox Proportional Hazards Regression Model, 4,232 quarters were used, with a total of 24 quarters excluded from analysis due to missing previous LS information (QT = 12, SP = 4 and TM = 8). Overall, 4.4% of the quarters experienced a clinical mastitis event from calving to 100 DIM. This analysis showed no effect of treatment on risk or days to a clinical mastitis event by 100 DIM (crude proportions: QT = 5.3%, SP = 3.8% and TM = 4.1%, $P = 0.27$; Figure 2.1). Other covariates significant in the multivariate model included region ($P < 0.01$), previous LS ($P < 0.01$) and parity ($P = 0.03$; Table 2.18).

Out of the total numbers of quarters that experienced a clinical case from calving to 100 DIM, 51.6% of the milk samples were missing (i.e. not collected by herd staff), 23.1% yielded no growth, 2.7% corresponded to mixed infections and 1.6% corresponded to contaminated samples. Of the milk samples from which bacteria were isolated, the majority of IMI were caused by *E. coli* (22.4%), followed by *Streptococcus uberis* (12.2%) and other *Streptococcus* spp. (12.2%; Table 2.19).

Discussion

The current study found that TM was non-inferior in effecting the primary outcome of bacteriological cure as compared to QT and SP. The study also found no difference in efficacy between the three DCT treatments when evaluated at the quarter level for risk for presence of IMI at 0 to 6 DIM and at 7 to 13 DIM, risk for cure of an IMI during the dry

period, risk for development of NIMI between dry off and 0 to 6 DIM or between dry off and 7 to 13 DIM and risk for a clinical mastitis event between calving and 100 DIM. This is the first prospective multi-state multi-herd non-inferiority North American study specifically designed to compare efficacy among three commonly used commercial DCT products. It is difficult to compare results from this study to previous research because peer-reviewed publications directly comparing those three or any other DCT products are almost entirely lacking. A randomized trial conducted in 2006 evaluated the efficacy of both cephapirin benzathine and ceftiofur hydrochloride to treat existing IMI and prevent new IMI during the dry period (Hallberg et al., 2006). However, the latter study was designed to compare these antimicrobial treatments against a negative control group, and not each other, and lacked a sufficient sample size to compare outcomes between the two antimicrobial agents. Appropriately, Hallberg et al. (2006) did not complete a statistical analysis comparing the two drug products for that study. Furthermore, the study enrolled only cows with a SCC > 400,000 cells/mL, which is not necessarily representative of the target population, considering that most dairy producers apply blanket DCT to all cows at dry off. A non-inferiority study was recently conducted in New Zealand with the aim of comparing efficacy of two cephalonium products (McDougall, 2011). However, because that study had geographical differences and used different drug formulations, it does not allow for comparison with the present US study.

A strength of the present study is that it was conducted in commercial dairy herds from four different states, and using different dry cow housing and management strategies. It must be acknowledged that the herds used in this study were larger than average (2,230 lactating cows) when compared to the average number of lactating cows in US dairy

herds (167 lactating cows, NAHMS, 2010) and compared to herds that are enrolled on DHIA (129 lactating cows, DHIA Annual Summary, 2011). Study herds also had higher rolling herd average (**RHA**) and lower average somatic cell count (RHA = 12,360 kg, SCC = 242,170 cells/mL) as compared to DHIA herds (RHA = 9,600 kg, SCC = 304,000 cells/mL; DHIA Annual Summary, 2011). Despite these differences, the types and frequencies of pathogens recovered were very similar to those reported in other North American dry cow mastitis studies.

IMI status at dry off and effect of treatment on risk for presence of an IMI at 0 to 6 DIM (S2) and 7 to 13 DIM (S3)

The current study found no effect of treatment on risk for presence of an IMI at dry off or after calving. Prevalence of infection at dry off reported in this study (19.2%) was within the range commonly reported in other North American dry cow studies, even though some difference may be expected partly due to differences in IMI definitions or sampling methodology among studies. In a study of two Wisconsin dairies, a prevalence of IMI at dry off of approximately 32% was reported when an IMI was defined as the presence of one colony in 0.1 mL of milk for any pathogen (Godden et al., 2003), while 12.8% was reported in another study of one Wisconsin herd where the threshold of presence of 3 or more colonies in 0.01 mL of milk was required to define an IMI (Pantoja et al., 2009). Postcalving prevalence of IMI reported in recent dry cow mastitis studies is highly variable. Prevalence of infection from 2 to 9 DIM for quarters from cows treated with penicillin/dihydrostreptomycin has been reported as 6.9% (Pantoja et al., 2009), while a different study reported a postcalving prevalence of IMI of 40.4% for quarters treated

with ceftiofur hydrochloride and 44.5% for quarters treated with cephapirin benzathine (Hallberg et al., 2006). However, the latter study enrolled only high somatic cell cows at dry off, and therefore one might expect quarters to be more likely to have subclinical infections present after calving. Postcalving prevalence of IMI in the current study (14.7%) is relatively similar to that reported by Godden et al. (2003), wherein IMI prevalence rates of 22.8% and 20.6% were reported at 1 to 3 and 6 to 8 DIM, respectively.

Similar to previous dry cow mastitis studies, the pathogen most commonly isolated for all sampling events in the current study was CNS. Hallberg et al. (2006) reported that 62.6% of the pathogens isolated at dry off were CNS, and Pantoja et al. (2009) reported that CNS was responsible for 63% and 44% of the infections at dry off and postcalving, respectively. The high frequency of environmental *Streptococcus* spp. found in the current study differs from some other dry cow studies, wherein lower frequencies were reported (Pantoja et al., 2009; Gundelach et al., 2011), but it is similar to what was reported by Godden et al. (2003). Interestingly, *Bacillus* spp. was isolated consistently in the S1, S2 and S3 samples from all herds in all regions. The role of *Bacillus* spp. in subclinical IMI is not well established in the literature. *Bacillus* spp. is known to be incriminated as a cause of clinical mastitis (Parkinson et al., 1999; Nieminem, 2007; Hawkes et al., 2008) but is also reported to be found in the normal bovine teat microflora (Al-Qumber and Tagg, 2006). The authors speculate that this pathogen might be considered a common contaminant by many microbiology laboratories and is therefore underreported in routine laboratory work results. An alternative is that the prevalence of this pathogen may be increasing. The latter hypothesis may be supported by findings

from a recent study wherein 4% of clinical mastitis cases were caused by *Bacillus* spp. (Lago et al., 2011). Further research is required to investigate the relationship between *Bacillus* spp. and udder health and disease.

In the current study, relatively few Gram-negative pathogens and very few contagious pathogens (*S. aureus* and *S. agalactiae*) were reported. Two previous studies reported the proportion of IMI caused by Gram-negative pathogens in dry off samples to be approximately 0.25% (Pantoja et al., 2009), 1.5% (Hallberg et al., 2006), and 22% (Godden et al., 2003), while the proportion of IMI postcalving caused by Gram-negative pathogens were reported as 0.86% (Pantoja et al., 2009) and 30% (Godden et al., 2003). Despite these highly variable rates of subclinical IMI, studies consistently report that the rate of subclinical coliform IMI during lactation is highest at calving and tends to decrease as days in milk advances (Hogan and Smith, 1998). The low number of *S. aureus* and *S. agalactiae* common to all study herds in the present study very likely reflects good overall mastitis control programs, including the implementation of the Five-Point Program, a program developed at the National Institute for Research in Dairying in England that was adopted by progressive herds in order to control and prevent IMI caused by contagious pathogens. Adoption of such methods has resulted in great progress over the years, and in this scenario other pathogens such as CNS and environmental *Streptococcus* spp. have become relatively more important (Ruegg, 2011). The current study did not report any *S. agalactiae*, which is similar to findings from other North American dry cow mastitis studies (Godden et al., 2003; Hallberg et al., 2006; Pantoja et al., 2009).

Effect of treatment on risk for experiencing a cure between dry off and postcalving

The current study found that TM was non-inferior to QT and SP on risk for experiencing cure of an IMI during the dry period. The crude proportion of quarters experiencing a cure in this study (88.9%) was similar to that reported in recent dry cow studies (Godden et al., 2003; Pantoja et al., 2009; Gundelach et al., 2011). In a North American study where two of the antibimicrobials tested were ceftiofur hydrochloride and cephapirin benzathine, cure rates from dry off to 3 and 5 DIM were lower than those reported in the present study (61.8% and 56.3% versus 88.0% and 89.7% for ceftiofur and cephapirin respectively). However, the latter study only enrolled cows with a high SCC, which may have represented more chronic infections and so might be less likely to cure (Hallberg et al., 2006).

Effect of treatment on risk for developing a new IMI between dry off and 0 to 6 DIM (S2) or between dry off and 7 to 13 DIM (S3)

The current study found no effect of treatment on risk for development of NIMI postcalving. New crude IMI rates reported in the current study (13.3% and 13.4% for NIMI developed between dry off and 0 to 6 DIM and between dry off and 7 to 13 DIM, respectively) are within the range reported in other recent studies; which have reported that NIMI incidence vary between 6 to 36% (Godden et al., 2003; Cook et al., 2005; Hallberg et al., 2006; Pantoja et al., 2009; Gundelach et al., 2011). The majority of NIMI was caused by CNS and environmental Streptococci in the current study and similar findings were reported by Godden et al. (2003) and Pantoja et al. (2009). However, both

of the two latter mentioned studies had Gram-negative pathogens among the three most common organisms causing NIMI, which was not observed in the current study.

Effect of treatment on risk for experiencing a clinical mastitis event between calving and 100 DIM

The crude incidence of clinical mastitis in early lactation in the current study (4.4%) agrees with incidence rates reported in previous North American studies, which ranged between 3 to 6% (Godden et al., 2003; Gundelach et al., 2011). The authors were not able to characterize pathogens causing all of these cases because herd staff did not consistently collect and submit milk samples from clinical cases.

Secondary Findings

Previous Linear Score. The current study detected interesting associations between other covariates and the dependent variables of interest. As an example, the previous LS from the last DHIA test day before dry off was positively associated with risk for presence of an IMI at dry off, at 0 to 6 DIM and at 7 to 13 DIM, and with risk for a clinical mastitis event between calving and 100 DIM. This is consistent with a previous study that reported that cows with a SCC $\geq$ 200,000 at dry off and postcalving were 2.7 times more likely to experience a first case of mastitis in the first 120 DIM than quarters with SCC < 200,000 cells/mL (Pantoja et al., 2009). In the current study, a one unit increase in LS before dying off was associated with a 23% increased odds of developing a clinical case between calving and 100 DIM. Previous LS was also positively associated with risk for development of a NIMI between dry off and postcalving, which has also been previously

reported by several authors (Godden et al., 2003; McDougall, 2011). Somatic cell count measure is commonly used as an indicator of udder infection status, since if reflects the amount of leucocytes moving from the bloodstream to the cow's mammary gland in order to fight infection. Pre-existing inflammation (high SCC) and potential subclinical infection might facilitate bacteria colonization for a quarter at greater risk for development of NIMI or clinical flare ups, especially during periods when the cow is immunosuppressed such as during the transition time.

Teat End Score. Previous reports on the nature of the association between teat end score and risk for presence or incidence of IMI is somewhat contradictory, and the relationship between teat end score and cure risk has never been previously described in the literature. Some studies found that risk for IMI in quarters with normal teat ends was not different from quarters with chronic ring lesions on teat ends (Sieber and Farnsworth, 1981). However, findings from the current study are consistent with other studies reporting that worse teat end condition is positively associated with risk for presence of subclinical infections (Pinho de Manzi et al., 2012), and that rough or cracked teat ends are a risk factor for the development of new IMI during the dry period (Dingwell et al., 2004). Counterintuitively, quarters with a teat end scored 1 or 2 at dry off were 85% less likely to experience a cure compared to teat ends scored 3 or 4. The authors have no immediate explanation for this observed relationship. Quarters with teat ends that have lesions or that are keratinized could potentially be at a higher risk for development of new IMI due to the fact that pathogens may colonize these cracks and crevices, putting them in close proximity to the streak canal, and so predisposing the quarter to infection by ascending bacteria (Timms et al., 1998). It has been recently reported that teats with a calloused end

and hyperkeratosis are characterized by a higher environmental microbial load (Paduch et al., 2012).

Udder Hygiene Score. Udder hygiene score at dry off was positively associated with presence of an IMI at dry off, and hygiene score at 7 to 13 DIM was positively associated with presence of IMI at 7 to 13 DIM. These observations are in agreement with findings from a previous study wherein a positive association was reported between subclinical mastitis and measurements of animal hygiene (Schreiner and Ruegg, 2003). It has been proposed that udder and leg hygiene scores of cows provide evidence on the degree that teat ends are exposed to environmental mastitis pathogens, which is correlated to risk for presence of subclinical IMI (Ruegg, 2003).

Parity. The current study found that increasing parity was positively associated with risk for development of NIMI during the dry period and also with risk for a clinical mastitis event from calving until 100 DIM. Increasing parity has been previously reported as a risk factor for presence of IMI (Green et al., 2005), new infections (Dingwell et al., 2004; Cook et al., 2005; McDougall, 2011), and clinical cases until 120 DIM (Pantoja et al., 2009). Anatomical and intramammary defense mechanisms of cows may deteriorate with age, an example being the reduced function and increased diameter of the streak canal (Dingwell et al., 2004). Older cows are also more likely to have been previously exposed or infected with mastitis pathogens, which has been discussed as potential risk factor that can contribute to susceptibility for new infections (Pantoja et al., 2009).

Summary

The current study found that TM was non-inferior in effecting a bacteriological cure as compared to SP or QT, and there was no difference in efficacy between the three commercial DCT products tested when considering all quarter level outcomes. Despite the fact that sample size calculations were not initially done in order to allow for subgroup analysis, the effect of treatment on most outcomes was also modeled separately for IMI caused by Gram-positive and Gram-negative organisms. In these analyses there was no difference in efficacy among treatments for either pathogen group. However, because a priori sample sizes were not calculated to allow for analysis by individual pathogens or groups, the results of the subgroup analysis should be interpreted with caution, particularly for Gram-negative infections, for which relatively low frequencies of IMI were detected.

All products evaluated in the current study are labeled to be effective against one or more Gram-positive organisms. Considering this, and considering that the majority of IMI (94.4% of all pathogens recovered at dry off, 89.7% at 0 to 6 DIM and 91.1% at 7 to 13 DIM) in the current study were caused by Gram-positive organisms, the authors are not surprised that the study found all three DCT products to have equivalent efficacy. None of the three DCT products evaluated are labeled against Gram-negative organisms, even though they are all recognized to have varying degrees of gram negative activity in in vitro tests (Salmon et al., 1996; Constable and Morin, 2002; Oliver and Murinda, 2007). However, gram negative IMI made up relatively few IMI cases in the current study.

Conclusions

Results from this non-inferiority study demonstrate that there was no difference in efficacy between the products QT, SP and TM, regarding risk for presence of IMI at 0 to 6 DIM and 7 to 13 DIM, risk for experiencing a cure during the dry period, risk for developing a NIMI between dry off and 0 to 6 DIM and between dry off and 7 to 13 DIM and risk for experiencing a clinical mastitis event between calving and 100 DIM. Specifically, TM was non-inferior in effecting a bacteriological cure as compared to QT or SP. As such, dairy producers could potentially put aside concerns about differences in efficacy, and instead base their selection decision among these three products on other characteristics such as milk and meat withholding time, targeted dry period length and cost.

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Table 2.1. Herd descriptors

	Herd A	Herd B	Herd C	Herd D	Herd E	Herd F
State	Wisconsin	Wisconsin	Minnesota	Iowa	California	California
Size (lactating cows)	1,550	1,050	1,650	3,030	2,500	3,600
Housing during dry period	Freestall, pasture	Freestall	Freestall	Open lot	Open lot	Freestall, open lot
Bedding during dry period	Sand	Bio solids	Sand	Corn stalk	Bio solids	Bio solids
Housing during lactation	Freestall	Freestall	Freestall	Freestall	Open lot	Freestall
Bedding during lactation	Sand	Bio solids	Sand	Sand	Bio solids	Bio Solids
RHA[1] (kg)	13,170	12,690	13,550	12,310	10,610	11,790
SCC[2] (cells/mL)	284,000	275,000	236,000	148,000	330,000	180,000

[1]Milk production annual rolling herd average
[2]Bulk tank milk somatic cell count average

Table 2.2. Description of variables offered as fixed effects to the models

Variable	Levels	Type (unit)
Region	California	Categorical
	Iowa	
	Minnesota[1]	
Parity	2	Categorical
	> 2	
Udder Hygiene Score[2]	1 (free of dirt)	Categorical
	2 (slightly dirty)	
	3 (moderately dirty)	
	4 (extremely dirty)	
Teat End Score[3]	1 and 2 (no ring or smooth ring)	Categorical
	3 and 4 (rough or cracked ring)	
Previous Lactation Total Milk (kg)		Continuous
Previous Lactation Linear Score		Continuous
Dry period length (days)		Continuous

[1]Herds from Minnesota and Wisconsin were included in the 'MN' region

[2]Score 2: dirt from udder covers between 2-10% of surface area; Score 3: dirt from udder covers between 10-30% of surface area; Score 4: dirt from udder covers more than 30% of surface area (Schreiner and Ruegg, 2003)

[3]Scores 1 and 2: teat end with no ring or smooth ring; Scores 3 and 4: teat end with rough or very rough, cracked ring (Falkenberg et al., 2003)

Table 2.3. Baseline characteristics from study cows and quarters, by treatment group and overall, as mean and standard deviation of the mean

	QT[1]	SP[2]	TM[3]	Overall
Dry period length (days)	53.9 (10.8)	54.4 (13.1)	54.3 (10.7)	54.2 (11.5)
N[4]	362	339	363	1,064
Parity	2.9 (1.2)	2.9 (1.1)	2.9 (1.2)	2.9 (1.2)
N[4]	372	349	370	1,091
Udder hygiene score at dry off	1.7 (0.7)	1.7 (0.7)	1.6 (0.7)	1.7 (0.7)
N[4]	372	349	370	1,091
Previous linear score	2.8 (1.6)	2.9 (1.6)	3.1 (1.6)	3.0 (1.6)
N[4]	365	347	368	1,080
Previous lactation milk production (kg)	12,577 (3,162)	12,648 (3,755)	12,435 (3,256)	12,551 (3,392)
N[4]	365	346	368	1,079
Teat end score at dry off	1.6 (0.8)	1.7 (0.8)	1.7 (0.8)	1.7 (0.8)
N[4]	1,483	1,396	1,470	4,349

[1]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[2]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[3]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[4]Total number of observations considered

Table 2.4. Crude quarter level prevalence of intramammary infection (IMI), incidence of new IMI, cure of IMI and incidence of clinical mastitis by treatment group for all pathogens, Gram-positives and Gram-negatives

Variable[1]	QT[3] [% (N)][2]	SP[4] [% (N)][2]	TM[5] [% (N)][2]	Total [% (N)][2]
Presence of IMI at 0-6 DIM[6]	15.7 (1398)	13.7 (1274)	15.6 (1386)	15.0 (4058)
Gram-positives	14.5 (1398)	12.8 (1274)	13.6 (1386)	13.6 (4058)
Gram-negatives	1.2 (1398)	1.0 (1274)	1.3 (1386)	1.2 (4058)
Presence of IMI at 7-13 DIM[6]	15.8 (1368)	15.2 (1244)	13.9 (1362)	14.9 (3974)
Gram-positives	14.5 (1368)	14.3 (1244)	12.6 (1362)	13.8 (3974)
Gram-negatives	0.9 (1368)	1.0 (1244)	1.4 (1362)	1.1 (3974)
New IMI (dry off to 0-6 DIM)[6]	13.7 (1366)	11.9 (1239)	13.8 (1357)	13.3 (3962)
Gram-positives	13.1 (1366)	11.1 (1239)	11.9 (1357)	12.1 (3962)
Gram-negatives	1.2 (1366)	1.0 (1239)	0.9 (1357)	1.0 (3962)
New IMI (dry off to 7-13 DIM)[6]	14.2 (1338)	13.5 (1213)	12.6 (1332)	13.4 (3883)
Gram-positives	12.9 (1338)	12.8 (1213)	11.5 (1332)	12.4 (3883)
Gram-negatives	1.0 (1338)	0.8 (1213)	1.0 (1332)	0.9 (3883)
Cure of IMI	88.9 (243)	88.0 (217)	89.7 (281)	88.9 (741)
Gram-positives	87.3 (228)	88.0 (208)	89.5 (268)	88.3 (704)
Gram-negatives	100.0 (17)	77.8 (9)	81.2 (16)	88.1 (42)
Clinical Mastitis until 100 DIM[6]	5.3 (1440)	3.8 (1352)	4.1 (1440)	4.4 (4232)

[1]The sum of the fractions of Gram-positives and Gram-negatives does not equal total fraction due to mixed infections
[2]The fraction numerator was the number of quarters experiencing the event, the denominator (N) was the total number of quarters at risk for each outcome
[3]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin), [4]SPECTRAMAST DC (500 mg ceftiofur hydrochloride),
[5]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[6]Days in milk

Table 2.5. Crude prevalence of intramammary infections (IMI) for quarters at dry off, 0 to 6 days in milk (DIM) and 7 to 13 DIM, by treatment group and overall

		IMI present at dry off				IMI present at 0 to 6 DIM				IMI present at 7 to 13 DIM			
		QT[1]	SP[2]	TM[3]	Total	QT[1]	SP[2]	TM[3]	Total	QT[1]	SP[2]	TM[3]	Total
No growth	n	1,180	1,105	1,140	3,425	1,179	1,100	1,170	3,449	1,152	1,055	1,173	3,380
	%	79.4	79.4	77.4	78.7	82.8	84.6	83.1	83.5	82.3	83.5	84.8	83.5
Total IMI	n	279	253	305	835	219	174	216	609	216	189	189	594
	%	18.8	18.2	20.7	19.2	15.4	13.4	15.3	14.7	15.4	15.0	13.7	14.7
Single IMI	n	256	226	275	753	189	146	194	529	193	158	166	517
	%	17.2	16.5	18.7	17.3	13.3	11.2	13.8	12.8	13.8	12.5	12.0	12.8
Mixed IMI	n	23	27	30	82	30	28	22	80	23	31	23	77
	%	1.6	1.9	2.0	1.9	2.1	2.1	1.6	1.9	1.6	2.4	1.7	1.9
Contaminated	n	28	33	30	91	26	27	22	75	31	20	22	73
	%	1.9	2.4	2.0	2.1	1.8	2.1	1.6	1.8	2.2	1.6	1.6	1.8
Total quarters	n	1,487	1,391	1,473	4,351	1,424	1,301	1,408	4,133	1,399	1,264	1,384	4,047

[1,2,3]Correspond to the three treatment groups: Quartermaster (penicillin and dihydrostreptomycin), Spectramast (ceftiofur hydrochloride) and Tomorrow (cephapirin benzathine)

Table 2.6. Final multivariate logistic regression model for the analysis of risk for presence of an intramammary infection at dry off

					95% CI[1]		
Variable		Coefficient	SE[2]	OR[3]	LCL	UCL	*P*-value
Intercept		-1.52	0.35				
Treatment	QT[4]	-0.07	0.12	0.93	0.73	1.18	0.73
	SP[5]	-0.09	0.12	0.91	0.72	1.16	
	TM[6]	Referent		1.00			
Region	CA	-0.28	0.12	0.76	0.60	0.95	<0.01
	IA	-0.69	0.15	0.50	0.37	0.67	
	MN	Referent		1.00			
Previous LS[7]		0.29	0.03	1.33	1.25	1.42	<0.01
Udder hygiene	1	-0.71	0.30	0.49	0.27	0.89	0.04
score at S1[8]	2	-0.75	0.31	0.47	0.26	0.86	
	3	-0.46	0.33	0.63	0.33	1.21	
	4	Referent		1.00			
Teat end score	1 and 2	-0.35	0.13	0.71	0.55	0.91	0.01
at S1[8]	3 and 4	Referent		1.00			
Parity	2	0.90	0.11	2.46	1.99	3.04	<0.01
	> 2	Referent		1.00			

[1]Confidence Interval for the odds ratio, lower (LCL) and upper (UCL) confidence limits.
[2]Standard Error
[3]Odds for presence of IMI
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]Last linear score before dry off
[8]S1 corresponds to the dry off event

Table 2.7. Description and crude frequency of bacterial species isolated from quarters that had an infection present at dry off and at 0-6 days in milk

			Intramammary infections present at dry off				Intramammary infections present at 0-6 DIM[1]			
			QT[2]	SP[3]	TM[4]	Total	QT[2]	SP[3]	TM[4]	Total
Gram positives	*Aerococcus* spp.	n	33	40	40	113	45	29	33	107
		%	10.9	14.3	11.9	12.3	18.1	14.4	13.9	15.5
	Bacillus spp.	n	21	25	17	63	31	23	15	69
		%	7.0	8.9	5.1	6.9	12.4	11.4	6.3	10.0
	Coagulase Negative	n	175	135	184	494	108	94	105	307
	Staphylococcus (CNS)	%	57.9	48.2	54.9	53.9	43.4	46.5	44.1	44.6
	Corynebacterium spp.	n	20	18	26	64	6	7	9	22
		%	6.6	6.4	7.8	7.0	2.4	3.5	3.8	3.2
	Enterococcus spp.	n	4	8	6	18	9	6	5	20
		%	1.3	2.9	1.8	2.0	3.6	3.0	2.1	2.9
	Other gram positive	n	1	4	3	8	0	1	6	7
		%	0.3	1.4	0.9	0.9	0.0	0.5	2.5	1.0
	Other *Strep* spp.	n	19	23	26	68	18	13	23	54
		%	6.3	8.2	7.8	7.4	7.2	6.4	9.7	7.8
	Staphylococcus aureus	n	5	9	9	23	4	4	1	9
		%	1.7	3.2	2.7	2.5	1.6	2.0	0.4	1.3
	Streptococcus dysgalactiae	n	1	6	4	11	2	6	6	14
		%	0.3	2.1	1.2	1.2	0.8	3.0	2.5	2.0
	Streptococcus uberis	n	2	0	2	4	3	3	3	9
		%	0.7	0.0	0.6	0.4	1.2	1.5	1.3	1.3
	Total Gram positives	n	281	268	317	866	226	186	206	618
		%	93.0	95.7	94.6	94.4	90.8	92.1	86.6	89.7
Gram negatives	*Escherichia coli*	n	2	1	2	5	9	7	3	19
		%	0.7	0.4	0.6	0.5	3.6	3.5	1.3	2.8
	Enterobacter spp.	n	1	0	4	5	0	0	2	2
		%	0.3	0.0	1.2	0.5	0.0	0.0	0.8	0.3
	Klebsiella spp.	n	5	1	5	11	0	1	1	2
		%	1.7	0.4	1.5	1.2	0.0	0.5	0.4	0.3
	Other gram negative	n	7	5	3	15	7	5	9	21
		%	2.3	1.8	0.9	1.6	2.8	2.5	3.8	3.0
	Serratia spp.	n	3	3	3	9	1	0	3	4
		%	1.0	1.1	0.9	1.0	0.4	0.0	1.3	0.6
	Total Gram negatives	n	18	10	17	45	17	13	18	48
		%	6.0	3.6	5.1	4.9	6.8	6.4	7.6	7.0
Other organisms (Yeast)		n	3	2	1	6	6	3	14	23
		%	1.0	0.7	0.3	0.7	2.4	1.5	5.9	3.3
Total		n	302	280	335	917	249	202	238	689

[1]Days in milk, [2]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin), [3]SPECTRAMAST DC (500 mg ceftiofur hydrochloride), [4]ToMORROW Dry Cow (300 mg cephapirin benzathine)

Table 2.8. Final multivariate logistic regression models for the analysis of risk for presence of an intramammary infection at 0 to 6 days in milk (DIM) (Model 1) and 7 to 13 DIM (Model 2)

Variable		Coefficient	SE[2]	OR[3]	95% CI[1]		*P*-value
					LCL	UCL	
Model 1							
Intercept		-1.67	0.18				
Treatment	QT[4]	0.04	0.12	1.04	0.81	1.32	0.34
	SP[5]	-0.15	0.13	0.86	0.67	1.12	
	TM[6]	Referent		1.00			
Region	CA	-0.46	0.12	0.63	0.50	0.81	< 0.01
	IA	-0.21	0.13	0.81	0.63	1.05	
	MN	Referent		1.00			
Teat end score at S1[7]	1 and 2	-0.33	0.13	0.72	0.56	0.93	0.01
	3 and 4	Referent		1.00			
Previous LS[8]		0.14	0.03	1.15	1.08	1.22	< 0.01
Model 2							
Intercept		-1.13	0.36				
Treatment	QT[4]	0.18	0.13	1.19	0.93	1.53	0.37
	SP[5]	0.13	0.13	1.14	0.88	1.47	
	TM[6]	Referent		1.00			
Region	CA	-0.54	0.13	0.58	0.45	0.75	< 0.01
	IA	-0.29	0.14	0.74	0.56	0.98	
	MN	Referent		1.00			
Udder hygiene score at S3[9]	1	-0.67	0.32	0.51	0.27	0.95	0.04
	2	-0.64	0.32	0.53	0.28	0.98	
	3	-0.24	0.36	0.78	0.39	1.59	
	4	Referent		1.00			
Teat end score at S3[9]	1 and 2	-0.28	0.13	0.75	0.59	0.97	0.03
	3 and 4	Referent		1.00			

[1]Confidence Interval for the odds ratio, lower (LCL) and upper (UCL) confidence limits.
[2]Standard Error
[3]Odds for presence of IMI
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]S1 corresponds to the dry off event
[8]Last linear score before dry off
[9]S3 corresponds to the postcalving sampling collected between 7 and 13 DIM

Table 2.9. Final multivariate logistic regression models for the analysis of risk of presence of an intramammary infection at 0 to 6 days in milk caused by Gram-positive organisms (Model 1) or Gram-negative organisms (Model 2)

					95% CI[1]		
Variable		Coefficient	SE[2]	OR[3]	LCL	UCL	*P*-value
Model 1							
Intercept		-1.74	0.19				
Treatment	QT[4]	0.10	0.13	1.11	0.86	1.43	0.44
	SP[5]	-0.07	0.13	0.94	0.72	1.22	
	TM[6]	Referent		1.00			
Region	CA	-0.55	0.13	0.58	0.45	0.74	<0.01
	IA	-0.31	0.14	0.73	0.56	0.95	
	MN	Referent		1.00			
Teat end score	1 and 2	-0.31	0.14	0.73	0.56	0.96	0.02
at S1[7]	3 and 4	Referent		1.00			
Previous LS[8]		0.12	0.03	1.13	1.06	1.20	<0.01
Model 2							
Intercept		-4.67	0.35				
Treatment	QT[4]	-0.07	0.35	0.93	0.47	1.85	0.80
	SP[5]	-0.25	0.37	0.78	0.38	1.63	
	TM[6]	Referent		1.00			
Region	CA	0.38	0.37	1.46	0.70	3.04	0.38
	IA	0.53	0.39	1.71	0.79	3.69	
	MN	Referent		1.00			

[1]Confidence Interval for the odds ratio, lower (LCL) and upper (UCL) confidence limits.
[2]Standard Error
[3]Odds of having an IMI
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]Teat end score at dry off
[8]Last linear score before dry off

Table 2.10. Final multivariate logistic regression models for the analysis of risk of presence of an intramammary infection at 7 to 13 days in milk caused by Gram-positive organisms (Model 1) or Gram-negative organisms (Model 2)

					95% CI[1]		
Variable		Coefficient	SE[2]	OR[3]	LCL	UCL	*P*-value
Model 1							
Intercept		-2.02	0.15				
Treatment	QT[4]	0.19	0.13	1.21	0.93	1.57	0.30
	SP[5]	0.17	0.14	1.18	0.91	1.54	
	TM[6]	Referent		1.00			
Region	CA	-0.65	0.13	0.52	0.41	0.67	<0.01
	IA	-0.44	0.14	0.65	0.49	0.84	
	MN	Referent		1.00			
Previous LS[7]		0.12	0.03	1.13	1.05	1.20	<0.01
Model 2							
Intercept		-5.10	0.46				
Treatment	QT[4]	-0.28	0.38	0.75	0.36	1.58	0.66
	SP[5]	-0.30	0.39	0.74	0.35	1.58	
	TM[6]	Referent		1.00			
Region	CA	-0.39	0.38	0.68	0.32	1.44	0.47
	IA	0.06	0.38	1.06	0.51	2.23	
	MN	Referent		1.00			
Previous LS[7]		0.26	0.09	1.30	1.09	1.55	< 0.01

[1]Confidence Interval for the odds ratio, lower (LCL) and upper (UCL) confidence limits.
[2]Standard Error
[3]Odds of presence of IMI
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]Last linear score before dry off

Table 2.11. Final multivariate logistic regression models for the analysis of odds for experiencing a cure between dry off and calving

					95% CI[1]		
Variable		Coefficient	SE[2]	OR[3]	LCL	UCL	*P*-value
Intercept		3.62	0.66				
Treatment	QT[4]	-0.10	0.30	0.90	0.50	1.63	0.79
	SP[5]	-0.21	0.31	0.81	0.44	1.47	
	TM[6]	Referent		1.00			
Region	CA	-0.41	0.28	0.67	0.38	1.16	0.30
	IA	-0.41	0.35	0.66	0.33	1.31	
	MN	Referent		1.00			
Teat Score	1 and 2	-1.87	0.61	0.15	0.05	0.51	< 0.01
at S1[7]	3 and 4	Referent		1.00			
Teat Score	1 and 2	0.65	0.32	1.92	1.03	3.60	0.04
at S3[8]	3 and 4	Referent		1.00			

[1]Confidence Interval for the odds ratio, lower (LCL) and upper (UCL) confidence limits.
[2]Standard Error
[3]Odds of experiencing a cure
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]S1 corresponds to the dry off event
[8]S3 corresponds to the postcalving sampling collected between 7 and 13 DIM

Table 2.12. Crude quarter level bacteriological cures by pathogen group

	Cure			
	QT[1]	SP[2]	TM[3]	Total
Quarters at risk for a cure [N][4]	243	217	281	741
Quarters experiencing a cure [% (n)][5]	88.9 (216)	88.0 (191)	89.7 (252)	88.9 (659)
Gram-positives [% (N)][6]				
Aerococcus spp.	93.3 (30)	88.9 (36)	97.4 (39)	93.3 (105)
Bacillus spp.	94.7 (19)	87.0 (23)	88.2 (17)	89.8 (59)
CNS	81.7 (153)	82.0 (111)	84.5 (168)	82.9 (432)
Corynebacterium spp.	100.0 (13)	100.0 (18)	100.0 (24)	100.0 (55)
Enterococcus spp.	100.0 (4)	100.0 (8)	100.0 (5)	100.0 (17)
Other Gram-positive	100.0 (1)	100.0 (4)	100.0 (3)	100.0 (8)
Other *Strep* spp.	100.0 (16)	84.2 (19)	77.3 (22)	86.0 (57)
S. aureus	80.0 (5)	42.9 (7)	88.9 (9)	71.4 (21)
S. dysgalactiae	-	60.0 (5)	75.0 (4)	66.7 (9)
S. uberis	100.0 (1)	-	100.0 (2)	100.0 (3)
Gram-negatives [% (N)][6]				
E. coli	100.0 (2)	100.0 (1)	100.0 (1)	100.0 (4)
Enterobacter spp.	100.0 (1)	-	100.0 (4)	100.0 (5)
Klebsiella spp.	100.0 (5)	-	80.0 (5)	90.0 (10)
Other Gram-negative	100.0 (7)	100.0 (5)	66.7 (3)	93.3 (14)
Serratia spp.	100.0 (2)	33.3 (3)	66.7 (3)	62.5 (8)
Others [% (N)][6]	100.0 (2)	100.0 (1)	0.0 (1)	75.0 (4)

[1]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin), [2]SPECTRAMAST DC (500 mg ceftiofur hydrochloride), [3]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[4]N corresponds to the total number of quarters at risk for the event
[5]The fraction corresponds to the percentage of quarters with the event and n corresponds to the total of quarters experiencing the event
[6]The fraction numerator corresponds to the number of quarters experiencing a cure; the denominator corresponds to N, the number of quarters at risk for each category

Table 2.13. Final multivariate logistic regression models for analysis of odds of experiencing a cure for Gram-positive organisms (Model 1) or Gram-negative organisms (Model 2)

					95% CI[1]		
Variable		Coefficient	SE[2]	OR[3]	LCL	UCL	*P*-value
Model 1							
Intercept		3.00	0.55				
Treatment	QT[4]	-0.24	0.29	0.79	0.44	1.40	0.70
	SP[5]	-0.19	0.30	0.83	0.46	1.50	
	TM[6]	Referent		1.00			
Region	CA	-0.37	0.27	0.69	0.40	1.18	0.40
	IA	-0.25	0.35	0.78	0.39	1.56	
	MN	Referent		1.00			
Teat end score at S1[7]	1 and 2	-1.31	0.49	0.27	0.10	0.71	0.01
	3 and 4	Referent		1.00			
Teat end score at S3[8]	1 and 2	0.67	0.31	1.96	1.07	3.59	0.03
	3 and 4	Referent		1.00			
Model 2[9]							
Intercept		6.53	2.81				
Treatment	QT[4]	13.15	510	-	0.00	-	0.62
	SP[5]	-1.37	1.38	0.26	0.02	3.80	
	TM[6]	Referent		1.00			
Region	CA	0.00	1.55	1.00	0.05	20.75	0.81
	IA	-0.84	1.41	0.43	0.03	6.86	
	MN	Referent		1.00			
Previous LS[10]		-0.93	0.44	0.40	0.17	0.93	0.04

[1]Confidence Interval for the odds ratio, lower (LCL) and upper (UCL) confidence limits.
[2]Standard Error
[3]Odds of experiencing a cure
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]S1 corresponds to the dry off event
[8]S3 corresponds to the postcalving sampling, collected between 7 and 13 DIM
[9]Model does not control for clustering of quarters within cows or clustering of cows within herds
[10]Last linear score before dry off

Table 2.14. Final multivariate logistic regression models for the analysis of effect of treatment on odds for acquiring a new intramammary infection between dry off and 0 to 6 days in milk (DIM) (Model 1) and between dry off and 7 to 13 DIM (Model 2)

					95% CI[1]		
Variable		Coefficient	SE[2]	OR[3]	LCL	UCL	*P*-value
Model 1							
Intercept		-1.46	0.21				
Treatment	QT[4]	0.05	0.13	1.05	0.81	1.35	0.27
	SP[5]	-0.17	0.13	0.85	0.65	1.11	
	TM[6]	Referent		1.00			
Region	CA	-0.65	0.13	0.52	0.41	0.68	<0.01
	IA	-0.25	0.13	0.78	0.60	1.01	
	MN	Referent		1.00			
Parity	2	-0.25	0.12	0.78	0.62	0.98	0.03
	> 2	Referent		1.00			
Teat Score	1 and 2	-0.31	0.14	0.73	0.56	0.96	0.02
at S1[7]	3 and 4	Referent		1.00			
Previous LS[8]		0.08	0.04	1.09	1.01	1.17	0.02
Model 2							
Intercept		-0.54	0.33				
Treatment	QT[4]	0.13	0.13	1.14	0.88	1.47	0.60
	SP[5]	0.08	0.13	1.08	0.83	1.41	
	TM[6]	Referent		1.00			
Region	CA	-0.60	0.13	0.55	0.42	0.71	< 0.01
	IA	-0.32	0.14	0.73	0.55	0.96	
	MN	Referent		1.00			
Parity	2	-0.32	0.11	0.72	0.58	0.90	< 0.01
	> 2	Referent		1.00			
Udder Hygiene	1	-0.80	0.31	0.45	0.24	0.83	0.04
Score at S3[9]	2	-0.75	0.31	0.47	0.26	0.88	
	3	-0.43	0.36	1.43	0.32	1.31	
	4	Referent		1.00			
Teat Score	1 and 2	-0.30	0.13	0.74	0.57	0.96	0.02
at S3[9]	3 and 4	Referent		1.00			

[1]Confidence Interval for the odds ratio, lower (LCL) and upper (UCL) confidence limits.
[2]Standard Error
[3]Odds of acquiring a new infection
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]S1 corresponds to the dry off event
[8]Last linear score before dry off
[9]S3 corresponds to the postcalving sampling collected between 7 and 13 DIM

Table 2.15. Crude quarter level risk for new infections between dry off and 0 to 6 days in milk (DIM) and dry off and 7 to 13 DIM

	New Infections between dry off and 0-6 DIM				New Infections between dry off and 7-13 DIM			
	QT[1]	SP[2]	TM[3]	Total	QT[1]	SP[2]	TM[3]	Total
Quarters at risk for the event [N][4]	1366	1239	1357	3962	1338	1213	1332	3883
Quarters with new IMI [n (%)][5]	14.1 (192)	11.9 (148)	13.8 (187)	13.3 (527)	14.2 (190)	13.5 (164)	12.6 (168)	13.4 (522)
Gram-positives [% (N)][6]								
Aerococcus spp.	2.8 (1334)	2.9 (1201)	2.4 (1318)	2.7 (3853)	3.3 (1308)	2.2 (1176)	2.3 (1292)	2.6 (3776)
Bacillus spp.	1.3 (1346)	0.8 (1215)	0.5 (1340)	0.9 (3901)	2.1 (1318)	1.5 (1190)	1.1 (1315)	1.6 (3823)
CNS	7.2 (1201)	7.8 (1115)	6.8 (1185)	7.2 (3501)	7.2 (1182)	6.7 (1100)	7.1 (1161)	7.0 (3443)
Corynebacterium spp.	0.7 (1349)	0.8 (1221)	1.1 (1332)	0.8 (3902)	0.4 (1325)	0.6 (1195)	0.7 (1308)	0.5 (3828)
Enterococcus spp.	0.4 (1362)	0.2 (1231)	0.2 (1352)	0.3 (3945)	0.7 (1334)	0.5 (1205)	0.3 (1327)	0.5 (3866)
Other Gram-positive	0.1 (1365)	0.2 (1235)	0.2 (1354)	0.2 (3954)	0.0 (1337)	0.1 (1209)	0.4 (1329)	0.2 (3875)
Other *Strep* spp.	1.7 (1348)	1.5 (1226)	1.2 (1339)	1.5 (3913)	1.2 (1322)	0.9 (1200)	1.7 (1315)	1.3 (3837)
S. aureus	0.1 (1361)	0.2 (1232)	0.1 (1348)	0.1 (3941)	0.2 (1333)	0.1 (1205)	0.0 (1323)	0.1 (3861)
S. dysgalactiae	0.2 (1366)	0.3 (1234)	0.2 (1353)	0.3 (3953)	0.1 (1338)	0.5 (1208)	0.4 (1328)	0.3 (3874)
S. uberis	0.3 (1365)	0.3 (1239)	0.3 (1355)	0.3 (3959)	0.2 (1337)	0.2 (1213)	0.2 (1330)	0.2 (3880)
Gram-negatives [% (N)][6]								
E. coli	0.4 (1364)	0.2 (1238)	0.4 (1356)	0.4 (3958)	0.6 (1336)	0.6 (1212)	0.2 (1330)	0.5 (3878)
Enterobacter spp.	0.0 (1365)	0.0 (1239)	0.0 (1353)	0.0 (3957)	0.0 (1337)	0.0 (1213)	0.2 (1328)	0.1 (3878)
Klebsiella spp.	0.0 (1364)	0.0 (1236)	0.1 (1352)	0.0 (3952)	0.0 (1333)	0.1 (1212)	0.0 (1327)	0.0 (3872)
Other Gram-negative	0.4 (1364)	0.5 (1236)	0.4 (1355)	0.5 (3955)	0.5 (1336)	0.4 (1210)	0.6 (1330)	0.5 (3876)
Serratia spp.	0.1 (1363)	0.2 (1236)	0.1 (1354)	0.1 (3953)	0.1 (1336)	0.0 (1210)	0.2 (1329)	0.1 (3875)
Other organisms [% (N)][6]	0.5 (1365)	0.2 (1237)	0.4 (1356)	0.4 (3958)	0.3 (1337)	0.2 (1213)	1.0 (1332)	0.5 (3882)

[1]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin), [2]SPECTRAMAST DC (500 mg ceftiofur hydrochloride), [3]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[4]N corresponds to the total number of quarters at risk for a new IMI, [5]n corresponds to the total of quarters experiencing a new IMI and the fraction corresponds to the percentage of quarters developing a new IMI
[6]The fraction numerator was the number of quarters developing a new IMI and the denominator was the number of quarters at risk to acquire a new IMI for each pathogen. The total number of quarters at risk is represented by N and excluded quarters that already had the specific pathogen present at dry off, [7]Coagulase negative *Staphlylococcus*

Table 2.16. Final multivariate logistic regression models for the analysis of effect of treatment on odds for acquiring a new Gram-positive intramammary infection (IMI) (Model 1) or a new Gram-negative IMI (Model 2) between dry off and 0 to 6 days in milk

					95% CI[1]		
Variable		Coefficient	SE[2]	OR[3]	LCL	UCL	*P*-value
Model 1							
Intercept		-1.76	0.19				
Treatment	QT[4]	0.13	0.13	0.87	0.87	1.47	0.29
	SP[5]	-0.09	0.14	0.69	0.69	1.21	
	TM[6]	Referent		1.00			
Region	CA	-0.76	0.13	0.47	0.36	0.61	<0.01
	IA	-0.33	0.14	0.72	0.55	0.94	
	MN	Referent		1.00			
Teat end score at S1[7]	1 and 2	-0.32	0.14	0.73	0.55	0.96	0.03
	3 and 4	Referent		1.00			
Previous LS[8]		0.11	0.04	1.11	1.04	1.19	<0.01
Model 2							
Intercept		-4.94	0.39				
Treatment	QT[4]	0.27	0.39	1.32	0.61	2.83	0.77
	SP[5]	0.09	0.42	1.09	0.48	2.48	
	TM[6]	Referent		1.00			
Region	CA	0.27	0.40	1.32	0.60	2.88	0.67
	IA	0.38	0.42	1.46	0.64	3.32	
	MN	Referent		1.00			

[1]Confidence Interval for the odds ratio, lower (LCL) and upper (UCL) confidence limits.
[2]Standard Error
[3]Odds of acquiring a new infection
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin), [5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride), [6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]S1 corresponds to the dry off event, [8]Last linear score before dry off

Table 2.17. Final multivariate logistic regression models for the analysis of odds of acquiring a new Gram-positive intramammary infection (IMI) (Model 1) or a new Gram-negative IMI (Model 2) between dry off and 7 to 13 days in milk

					95% CI[1]		
Variable		Coefficient	SE[2]	OR[3]	LCL	UCL	*P*-value
Model 1							
Intercept		-0.53	0.33				
Treatment	QT[4]	0.12	0.13	1.13	0.87	1.47	0.59
	SP[5]	0.12	0.14	1.13	0.86	1.48	
	TM[6]	Referent		1.00			
Region	CA	-0.70	0.14	0.50	0.38	0.65	<0.01
	IA	-0.37	0.15	0.69	0.52	0.92	
	MN	Referent		1.00			
Parity	2	-0.35	0.11	0.71	0.56	0.89	<0.01
	> 2	Referent		1.00			
Teat end score at S3[7]	1 and 2	-0.37	0.14	0.68	0.53	0.90	0.01
	3 and 4	Referent		1.00			
Udder hygiene score at S3[7]	1	-0.81	0.32	0.45	0.24	0.83	0.03
	2	-0.77	0.32	0.46	0.25	0.86	
	3	-0.43	0.36	0.65	0.32	1.32	
	4	Referent		1.00			
Model 2							
Intercept		-3.94	0.45				
Treatment	QT[4]	0.00	0.40	1.00	0.45	2.20	0.89
	SP[5]	-0.19	0.43	0.83	0.36	1.94	
	TM[6]	Referent		1.00			
Region	CA	-0.26	0.42	0.77	0.34	1.76	0.65
	IA	-0.14	0.41	0.87	0.39	1.95	
	MN	Referent		1.00			
Teat end score at S1[8]	1 and 2	-0.82	0.39	0.44	0.21	0.94	0.03
	3 and 4	Referent		1.00			

[1]Confidence Interval for the odds ratio, lower (LCL) and upper (UCL) confidence limits.
[2]Standard Error
[3]Odds of acquiring a new infection
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]S3 corresponds to the second sampling postcalving (7-13 DIM)
[8]S1 corresponds to the dry off event

Table 2.18. Final Cox Proportional Hazards Regression model for the analysis of effect of treatment on risk for experiencing a clinical mastitis event between calving and 100 days in milk

					95% CI[1]		
Variable		Coefficient	SE[2]	Hazards Ratio[3]	LCL	UCL	*P*-value
Treatment	QT[4]	0.27	0.21	1.31	0.87	1.947	0.27
	SP[5]	-0.05	0.21	0.95	0.63	1.44	
	TM[6]	Referent		1.00			
Region	MN	1.13	0.20	3.10	2.09	4.60	<0.01
	IA	-1.26	0.38	0.28	0.13	0.60	
	CA	Referent		1.00			
Previous LS[7]		0.17	0.05	1.19	1.08	1.30	<0.01
Parity	2	-0.39	0.18	0.68	0.48	0.96	0.03
	>2	Referent		1.00			

[1]Confidence Interval for the odds ratio, lower (LCL) and upper (UCL) confidence limits.
[2]Standard Error
[3]Hazards of having a clinical mastitis event
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]Last linear score before dry off

Table 2.19. Description and crude frequency of bacteria isolated from those clinical mastitis samples that were collected between calving and 100 DIM

Pathogen	Number of quarters affected	Frequency (%)
Aerococcus spp.	1	2.0
CNS[1]	5	10.2
Arcanobacterium pyogenes	1	2.0
Streptococcus dysgalactiae	5	10.2
Staphylococcus aureus	1	2.0
Other *Streptococcus* spp.	6	12.2
Streptococcus uberis	6	12.2
Escherichia coli	11	22.4
Klebsiella spp.	4	8.2
Other Gram-negative	5	10.2
Serratia spp.	2	4.1
Yeast	2	4.1
Total Pathogens[2]	49	100.0

[1]Coagulase negative *Staphylococcus* spp.

[2]Total excluded quarters for which samples were not collected (n = 96) and quarters that yielded contaminated results (n = 3), total does not reflect total of quarters affected due to mixed infections (n = 5)

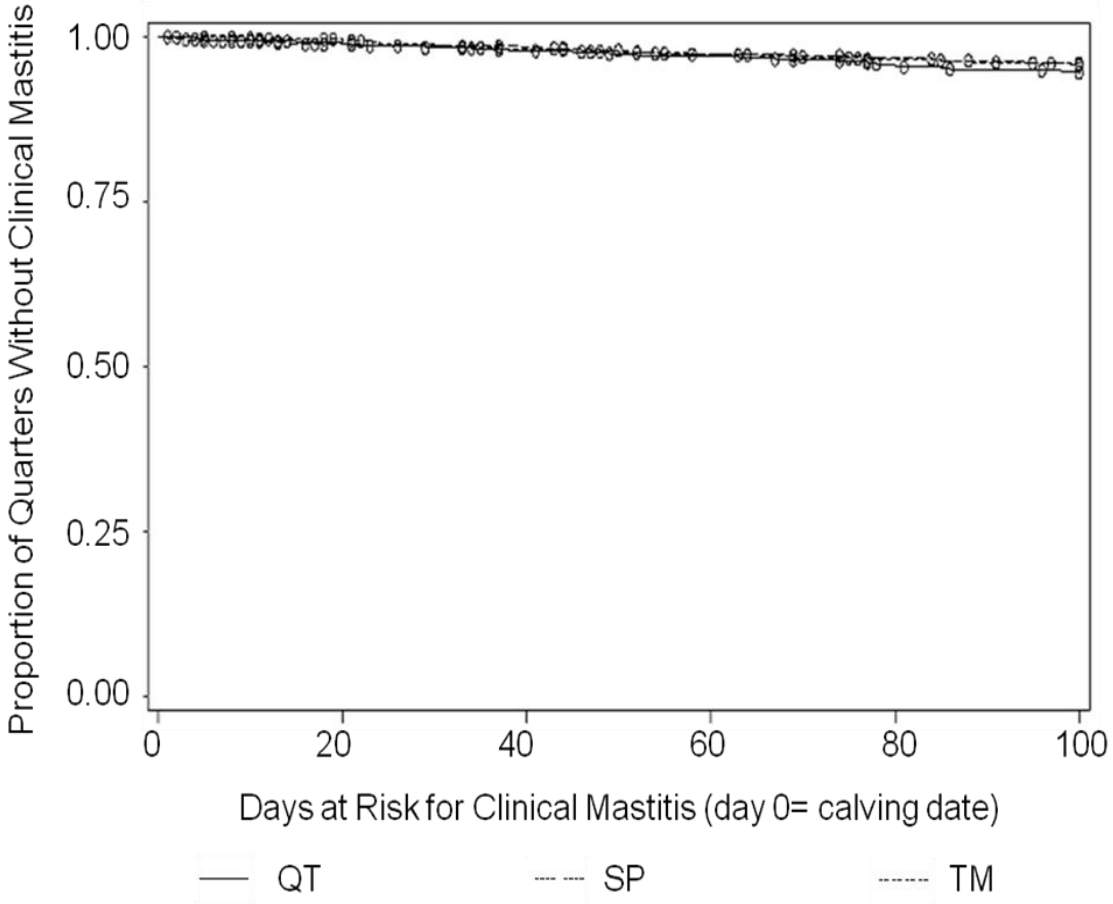

Figure 2.1. Survival distribution function for effect of dry cow therapy treatment on risk and days to a clinical mastitis event between calving (day 0) and 100 days in milk (unadjusted, QUARTERMASTER = 5.3%, SPECTRAMAST DC = 3.8% and ToMORROW Dry Cow = 4.1%)

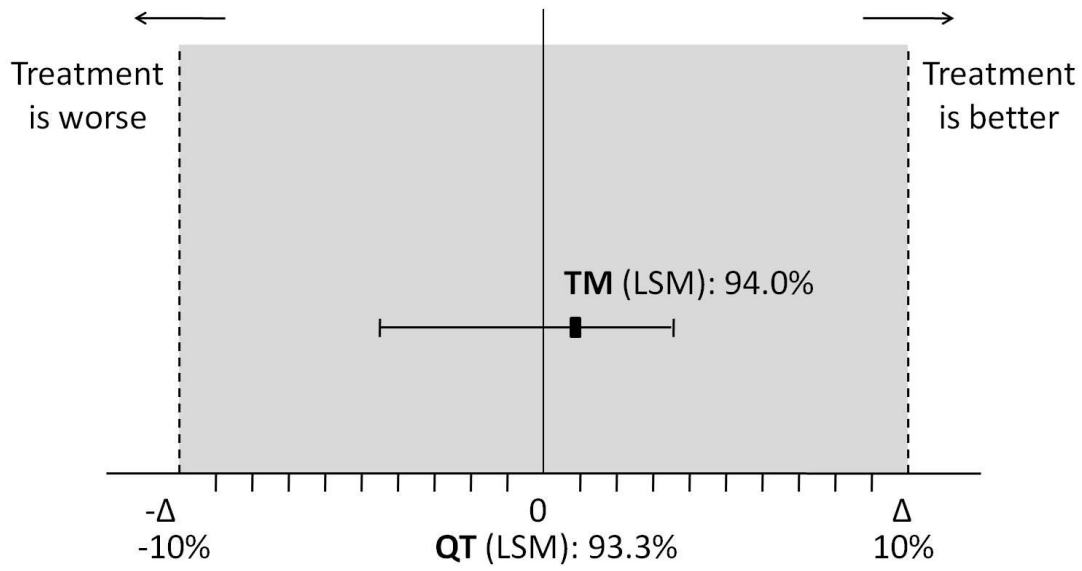

Figure 2.2. Non-inferiority analysis of risk for cure for quarters from cows treated with ToMORROW Dry Cow (TM; LSM = 0.94; 95% CI: 0.89 to 0.97) compared to cows treated with QUARTERMASTER (QT; LSM = 0.93; 95% CI: 0.87 to 0.97). The error bars indicate 2-sided 95% confidence intervals and the shaded area indicates zone of non-inferiority. Delta (Δ) represents the margin of non-inferiority, pre-established at 10%

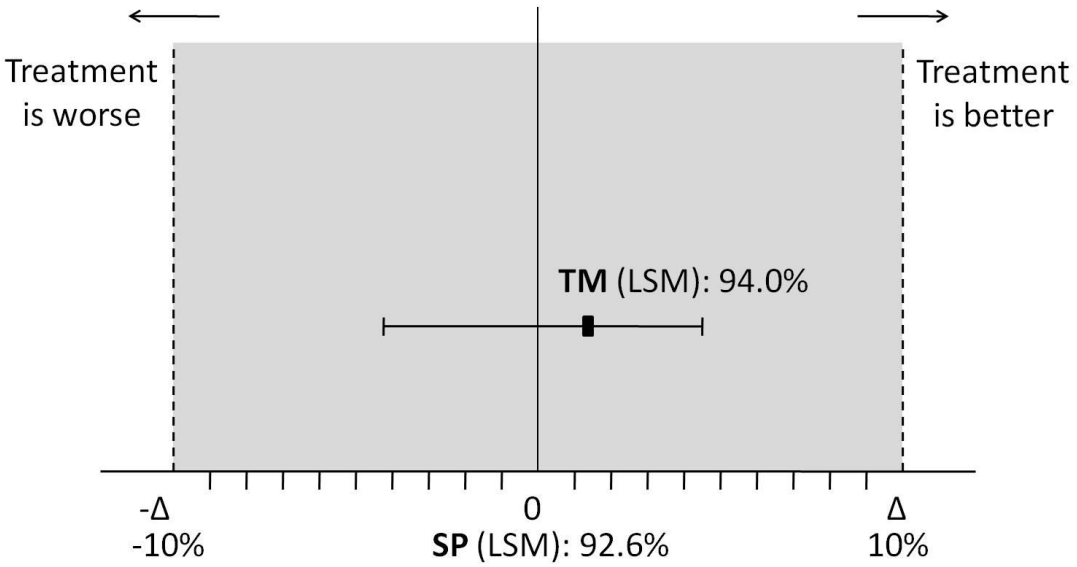

Figure 2.3. Non-inferiority analysis of risk for cure for quarters from cows treated with ToMORROW Dry Cow (TM; LSM = 0.94; 95% CI: 0.89 to 0.97) compared to cows treated with SPECTRAMAST DC (SP; LSM = 0.93; 95% CI: 0.86 to 0.96). The error bars indicate 2-sided 95% confidence intervals and the shaded area indicates zone of non-inferiority. Delta (Δ) represents the margin of non-inferiority, pre-established at 10%

CHAPTER 3

RANDOMIZED NON-INFERIORITY CLINICAL TRIAL EVALUATING THREE COMMERCIAL DRY COW MASTITIS PREPARATIONS: II. COW HEALTH AND PERFORMANCE IN EARLY LACTATION

Chapter 3

Randomized Non-Inferiority Clinical Trial Evaluating Three Commercial Dry Cow Mastitis Preparations: II. Cow Health and Performance in Early Lactation

Overview

The objective of this multi-state multi-herd non-inferiority clinical trial was to describe the effects of treatment with three different dry cow therapy formulations at dry off on cow level health and production parameters in the first 100 days in milk (**DIM**) in the subsequent lactation. These parameters included milk production, linear score (**LS**), risk for experiencing a clinical mastitis case, risk for culling or death and risk for pregnancy by 100 DIM. A total of 1,091 cows from 6 commercial dairy herds in 4 different states (CA, IA, MN and WI) were randomly assigned at dry off to receive treatment with one of the three commercial products: QUARTERMASTER (**QT**, Pfizer Animal Health, New York, NY), SPECTRAMAST DC (**SP**, Pfizer Animal Health, New York, NY) or ToMORROW Dry Cow (**TM**, Boehringer Ingelheim Vetmedica, Inc., St Joseph, MO). All clinical mastitis, pregnancy, culling and death events occurring in the first 100 DIM were recorded by farm staff using an on-farm electronic record keeping system. Dairy Herd Information Association electronic records were used to retrieve consecutive test data regarding milk production, milk composition and LS. The effect of treatment on the dependent variables energy corrected milk (**ECM**) production, fat corrected milk (**FCM**) production, milk production per day, fat production per day, protein production per day,

305 mature equivalent (**ME**) and LS from calving to 100 DIM were analyzed by a mixed linear model. Cox proportional hazards regression was used to describe the effect of treatment on the survival distribution function of cows experiencing a case of clinical mastitis, cows leaving the herd and cows getting pregnant between calving and 100 DIM. There was no effect of treatment on milk production per day (least square means [**LSM**]: QT = 42.9 kg, SP = 42.1 kg and SP = 42.8 kg, $P = 0.14$), protein production per day (LSM: QT = 1.21, SP = 1.18 and TM = 1.19, $P = 0.18$), 305 ME (LSM: QT = 11,587 kg, SP = 11,463 kg and TM = 11,540 kg, $P = 0.31$), linear score (LSM: QT = 1.9, SP = 2.0 and TM = 1.7, $P = 0.12$), risk for a clinical mastitis episode (QT = 14.8%, SP = 12.7% and TM = 15.0%, $P = 0.80$), risk for leaving the herd (QT = 7.5%, SP = 9.2% and TM = 10.3%, $P = 0.55$) or risk for pregnancy by 100 DIM (QT = 31.5%, SP = 26.1% and TM = 26.9%, $P = 0.26$). There was an effect of treatment on ECM production (LSM: QT = 41.4 kg^{a}, SP = 40.3 kg^{b} and TM = 41.1 kg^{a,b}, $P = 0.0496$), FCM production (LSM: QT = 41.9 kg^{a}, SP = 40.7 kg^{b} and TM = 41.7 kg ^{a,b}, $P = 0.03$) and fat production (LSM: QT = 1.44 kg^{a}, SP = 1.39 kg^{b} and TM = 1.43 kg^{a,b}, $P = 0.03$).

Introduction

Dry cow mastitis refers to intramammary infections (**IMI**) that persist through the dry period from the previous lactation or that are acquired during the dry period. North American studies have estimated the proportion of quarters subclinically infected at dry off to be between 19 to 35% (Godden et al., 2003, Chapter 2 of this thesis) and the

proportion of quarters that develop a new subclinical IMI during the dry period to range between 8 and 25% (Godden et al., 2003, Cook et al., 2005, Chapter 2 of this thesis). Dry cow mastitis leads to presence of IMI at calving, which increases the risk for clinical flare ups in the next lactation and therefore affects long-term production and health parameters of dairy cows.

An important consequence of dry cow mastitis is future reduction of milk production. Subclinical mastitis has been shown to be one of the diseases that have the greatest detrimental effect in milk production, which has economic importance for the producer (Dohoo et al., 1984). Increased somatic cell count (**SC**C) is also an important consequence. Measures of SCC are commonly used to access milk quality and overall udder health status and it is known that high SCC are negatively associated with cheese yield and shelf life of pasteurized milk (APHIS, 2011). Producers that do not meet country specific SCC requirements suffer consequences that can vary from monetary penalties to suspension of selling permit (APHIS, 2008). Mastitis is also one of the main reasons for culling an animal, along with low milk production, poor reproductive performance and udder problems (Milian-Suazo et al., 1989). Green et al. (2005) identified that cows with a major pathogen isolated from one or more quarters during the late dry to calving period were more likely to be culled in the next lactation than those which were uninfected. Finally, Schrick et al. (2001) have reported that clinical and subclinical mastitis have detrimental effects on reproductive performance, and the impact can be even more severe when subclinical cases progress to clinical. All these

consequences of dry cow mastitis make it clear that control strategies are needed in order to prevent and control this disease.

Blanket dry cow period (**DCT**) is a procedure recommended by the National Mastitis Council for mastitis prevention and control. This practice has the potential to reduce the prevalence of IMI in early lactation, reducing the possibility of clinical and subclinical mastitis cases and therefore diminishing the detrimental consequences caused by those. However, studies comparing efficacy of different commercially available DCT products are extremely limited. One recent study conducted in two Florida dairy herds compared dry treatment with ceftiofur hydrochloride and penicillin/dihydrostreptomycin regarding the incidence of clinical mastitis from calving to 30 days in milk (**DIM**) and from calving to 60 DIM, and prevalence of high linear scores at 30 DIM and at 60 DIM (Pinedo et al., 2012). Results showed that treatment with ceftiofur resulted in a lower incidence of clinical mastitis and a lower prevalence of high linear scores for both interval periods (Pinedo et al., 2012). However, that study was limited to the study of two DCT products and geographically limited to two dairy herds located in the state of Florida. Furthermore, the authors did not report the actual clinical mastitis rates or proportions of cows with elevated SCC values by treatment group.

Three commonly used DCT products in the United States include QUARTERMASTER (**QT**, 100,000 IU procaine penicillin G and 1 g dihydrostreptomycin Pfizer Animal Health, New York, NY), SPECTRAMAST DC (**SP**, 500 mg ceftiofur hydrochloride, Pfizer Animal Health, New York, NY) and ToMORROW Dry Cow (**TM**, 300 mg

cephapirin benzathine, Boehringer Ingelheim Vetmedica, Inc., St Joseph, MO). Detailed descriptions of spectrum of activity, milk and meat withholding periods and dry period length can be found in Chapter 2. Previous analysis has shown there was no difference among these three DCT treatments when looking at quarter level outcomes measured shortly after calving, including prevalence of IMI post-calving, bacteriological cure during the dry period, development of new IMI during the dry period and clinical mastitis incidence (Chapter 2 of this thesis). However, producers are not necessarily interested in measuring bacteriological status at the quarter level, but instead are more likely to be interested in biologically and economically relevant outcomes including future lactation milk production, risk for clinical mastitis at the cow level, culling and death rates and reproductive efficiency.

The objective of the current study was to compare the efficacy of the three above mentioned commercial DCT products as measured by cow level outcomes evaluated between calving and 100 DIM. The outcomes evaluated included energy corrected milk (**ECM**) production, fat corrected milk (**FCM**) production, milk production per day, fat production per day, protein production per day, 305 mature equivalent (**ME**), linear score, risk for experiencing a clinical mastitis event, risk for leaving the herd and risk for pregnancy. The hypothesis was that there would be no difference among the three DCT products when evaluating the cow level outcomes milk production, linear score (**LS**), risk for clinical mastitis, risk for pregnancy, and risk for culling or death between calving and 100 DIM.

Materials and Methods

Herd Selection

A non-inferiority randomized clinical trial was conducted under IACUC approval between February 2011 and November 2011 in six commercial dairy herds located in California (n = 2), Iowa (n = 1), Minnesota (n = 1) and Wisconsin (n = 2). Inclusion criteria for study herds were to be within a reasonable driving distance from the respective collaborating institution, be on a regular Dairy Herd Information Association (**DHIA**) testing program, and comply with the study protocol. Herd descriptors such as herd size, housing, bedding, milk production annual rolling herd average and bulk tank milk SCC average are described elsewhere (Chapter 2 of this thesis).

Cow Enrollment, Follow-up and Data Collection

Cow enrollment was conducted weekly by trained university technicians. On the day of dry off, cows were identified in the parlor and visually assessed for body condition score, lameness and clinical mastitis. To be eligible for inclusion, cows had to be in good overall health, have four functioning quarters, have not received parenteral or intramammary treatment with an antibacterial or anti-inflammatory medication during a 30-day period prior to dry off and show no clinical signs of mastitis on the day of dry off. Eligible cows were randomly assigned to treatment with one of the three DCT products (QT, SP or TM). Randomization was blocked within farms. Immediately after the final milking, all four quarters were scrubbed with alcohol-soaked gauze and infused with the

respective DCT treatment and all four quarters massaged. An internal teat sealant product was then infused and cows were post-dipped and moved back to their facilities, where usual dry cow husbandry practices were undertaken. Besides treatment at dry off, milk samples were aseptically collected from all quarters of all study cows immediately prior to administration of intramammary treatment, and again at 0 to 6 DIM and at 7 to 13 DIM for microbiological culture. Udder hygiene was also scored at those events. Sampling details and a description of the prevalence and type of pathogens present at dry off and post-calving are reported in the previous Chapter of this thesis. Results of the effect of DCT treatment on quarter level outcomes such as bacteriological cure during the dry period, development of new IMI during the dry period and clinical mastitis incidence from calving to 100 DIM are also reported in Chapter 2.

All clinical mastitis, pregnancy, culling and death events occurring in the first 100 DIM were recorded by farm staff using an on-farm electronic record keeping system (DairyComp305, Valley Agricultural Software, Tulare, CA). DairyComp305 software was also utilized to capture electronic DHIA records for all study cows throughout the 100 DIM observation period. DHIA visit interval in participating dairy herds varied among and within herds, therefore the number of DHIA tests obtained per cow between calving and 100 DIM could possibly range from 1 to 3. Information collected from each DHIA test event included milk production at the test day (kg per day), percentage of fat produced at the test day (%), percentage of protein produced at the test day (%) and 305 ME milk production (kg) and LS at the test day.

Definitions of outcome variables

Clinical mastitis. A clinical mastitis event was defined as the identification of visibly abnormal milk accompanied or not by changes in the quarter. Cow level status of clinical mastitis was defined as at least one episode of clinical mastitis from calving to 100 DIM.

Linear Score. Linear score derives from a logarithm transformation of the somatic cell count (cells/ mL), ranging from 0 to 9 (DHI Glossary, 2012).

Energy Corrected Milk. This calculated outcome variable determines the amount of energy in the milk considering milk yield, fat and protein, and is adjusted for 3.5% fat and 3.2% protein (DHI Glossary, 2012). The ECM was calculated in kg using an Excel spreadsheet (2010 Microsoft Corporation, Santa Rosa, CA) and was based on milk, fat and protein yields obtained from each DHIA test, and using the following formula (Tyrrell and Reid, 1965):

ECM (kg) = (0.327 x milk yield in kg) + (12.95 x fat yield in kg) + (7.2 x protein yield in kg).

3.5% Fat Corrected Milk. This calculated variable adjusts the amount of milk produced to a standard fat of 3.5%: (milk yield in kg x 0.432) + (fat yield in kg x 16.216) (DHI Glossary, 2011).

305 Mature Equivalent. Corresponds to the 305 ME projected lactation milk production (kg) as calculated on a 305 day basis, using factors that adjust for days in milk on test day, milk frequency, season of calving, geographical location of the herd and age (DHI Glossary, 2012).

Statistical Analysis

The primary outcome for a priori sample size calculations for the current study was risk for a microbiological cure at the quarter level. This and other quarter level outcomes are reported in the previous Chapter. The maximum difference in cure rate to declare non-inferiority was pre-stated at 10%. To demonstrate non-inferiority, a total of 339 cows (1356 quarters) per group were estimated to be required, assuming $\alpha = 0.025$, $\beta = 0.2$, 10% losses to follow-up and that 30% of the quarters would be infected at dry off, and therefore at risk for a cure (non-inferiority tests for two proportions, Pass 2008; NCSS, Kaysville, UT, USA).

All statistical analyses for the current chapter were conducted at the cow level using the intent-to-treat approach using SAS version 9.2 (SAS Inst. Inc., Cary, NC). Initially descriptive statistics and plots were generated for exploratory data analysis and basic diagnostic techniques were used to evaluate normality, colinearity between independent variables and the presence of outliers. Characteristics of cows assigned to the three treatment groups including dry period length, parity, udder hygiene score at dry off, previous linear score and previous lactation milk production were initially compared at baseline (enrollment) using the chi-square test and ANOVA.

The effect of treatment on the dependent variables ECM, FCM, milk, protein and fat production per day, 305ME and LS (all computed as continuous variables) were analyzed by a Mixed Linear Model (PROC MIXED). In all models region was included as a fixed effect, herd was included as random effect and a repeated statement for DHIA test was

included in order to account for the clustering effects of herds within regions, cows within herds and multiple tests within cows, respectively. Explanatory variables offered to the model included DCT treatment group (forced), cow parity, previous lactation LS, previous lactation total milk production and dry period length. The variables previous lactation LS, previous lactation total milk production and dry period length were offered as continuous variables and cow parity was dichotomized in two categories: second parity and third or greater parities. The variable DHIA test number was categorized as tests 1, 2 or 3 according to the chronological order for each animal. The independent variable describing DIM on test day was highly correlated ($R^2 = 0.90$) to the variable DHIA test number, and therefore was not offered to the models. Univariate analysis was initially conducted to evaluate the relationship between each of the aforementioned explanatory variables and the dependent variable of interest. Variables significant at $P < 0.2$ in the univariate analyses were carried forward to offer into the full model. Correlation structures that did not stop due to infinite likelihood were selected. The correlation structure used for analysis of most outcome variables was the (UR) except for 305 ME, when AR (1) was used. Non-significant variables were removed from the final models one at a time in a backward stepwise approach with final significance declared at $P < 0.05$. A statistical trend was declared when $0.05 \leq P < 0.10$. First-order interactions between DCT treatment group and other remaining main effects were tested and included in the model if significant. Finally, quadratic and cubic terms for significant continuous terms were offered to the final models and kept if $P < 0.05$. Models were compared using

the -2 Residual Log Likelihood and model fit was evaluated by plotting marginal and conditional residuals. The Bonferroni correction method was applied in order to examine multiple comparisons (contrasts among the three DCT treatment groups) when the overall effect of DCT treatment was statistically significant. The Bonferroni method states that the P-value required for each comparison has to be less than or equal to α divided by the total number of study comparisons (Proschan and Waclawiw, 2000).

Cox proportional hazards regression (PROC PHREG) was used to describe the effect of DCT treatment on the survival distribution function of cows experiencing each of the following outcomes: a case of clinical mastitis, cows leaving the herd due to culling or death and cows getting pregnant. Cows were considered to be at risk for these events between calving and 100 DIM, with the failure date defined as the date when the cow was first reported to have the outcome. Cows not reported to have the outcome were classified as left censored either at the cow's culling or death date, if before 100 DIM, (for models predicting risk for clinical mastitis and risk for pregnancy) or right censored at 100 DIM (for all three models). Clustering at the herd level was controlled for with a Covsandwich statement. Explanatory variables offered to the model included DCT treatment group (forced), region (forced), cow parity, previous lactation LS, previous lactation total milk production and dry period length. Models were compared during the model building process using the -2 Log Likelihood statistics and the final model fit was assessed by plotting the deviance residuals.

Results

A total of 1,091 cows were enrolled into the study at dry off (QT = 373, SP = 349 and TM = 369). A total of 27 cows were either culled or died during the dry period (QT = 10, SP = 10 and TM = 7), therefore a total of 1,064 cows were at risk for the long-term outcomes evaluated from calving to 100 DIM. The three treatment groups did not differ at baseline regarding the following cow level parameters (overall mean ± standard deviation): parity (2.9 ± 1.2), previous LS (3.0 ± 1.6), previous total milk (12,551 ± 3,392 kg) and dry period length (54.2 ± 11.5 days) at dry off. Previous total milk was excluded for one cow due to an unrealistic reported value of 117 kg. A table with group-specific descriptive data regarding these parameters is reported in Chapter 2.

The total number of DHIA tests obtained from all study animals was 2,767. The average number of tests per cow between calving and 100 DIM was 2.6 (range 1 to 3). The mean DIM for test 1 was 22.2 days (median = 22, N = 1,019, range 1 to 89 days), the mean days in milk for test 2 was 53.5 days (median = 53, N = 962, range 31 to 91 days) and the mean days in milk for test 3 was 80.5 days (median = 81, N = 786, range 52 to 100 days).

Effect of treatment on energy corrected milk between calving and 100 days in milk

A total of 2,764 tests were used for the analysis of ECM, FCM and milk, fat and protein production (three records had missing information on previous milk production; SP = 3). The overall unadjusted average ECM from calving to 100 DIM was 37.8 kg (Table 3.1), with an effect of treatment on ECM production (least square means [**LSM**]: QT = 41.4

kg, SP = 40.3 kg and TM = 41.1 kg, $P = 0.0496$). The covariates test ($P < 0.01$), parity ($P = 0.03$), dry period length ($P < 0.01$) and previous milk production ($P < 0.01$), as well as a quadratic term for previous milk production ($P = 0.02$) were statistically significant and therefore included in the final model (Table 3.2).

Contrast analysis of the three DCT was conducted using the Bonferroni corrected *P*-value of 0.0167 (0.05/ 3 contrasts; $P < 0.0167$ to declare significance). Applying this method, there was a strong trend for cows treated with QT to produce more ECM (41.4 kg) than cows treated with SP (40.3 kg; $P = 0.0170$; Table 3.3). There was no difference on ECM production between TM and neither of the other two products.

Effect of treatment on fat corrected milk (kg per test day) between calving and 100 days in milk

Overall unadjusted average FCM from calving to 100 DIM was 38.3 kg (Table 3.1), and there was an effect of treatment on FCM production (LSM: QT = 41.9 kg, SP = 40.7 kg and TM = 41.7 kg, $P = 0.03$). The covariates test ($P < 0.01$), parity ($P = 0.02$), dry period length ($P < 0.01$) and previous milk production ($P < 0.01$) were statistically significant and therefore included in the final model (Table 3.4).

Contrast analysis using the Bonferroni corrected *P*-value of 0.0176 showed that cows treated with QT produced 1.22 kg more FCM than cows treated with SP ($P = 0.0109$; Table 3.5). There was no difference on FCM production when comparing cows treated with TM to either cows treated with QT or SP.

Effect of treatment on milk production (kg per test day) between calving and 100 days in milk

The unadjusted average milk production per test day from calving to 100 DIM was 39.4 kg (Table 3.1), and there was no effect of treatment on this outcome (LSM: QT = 42.9 kg, SP = 42.1 kg and TM = 42.8 kg, $P = 0.14$). The covariates test ($P < 0.01$), parity ($P < 0.01$), dry period length ($P < 0.01$) and previous milk production ($P < 0.01$) were statistically significant and therefore included in the final model (Table 3.6).

Effect of treatment on milk protein production (kg per test day) between calving and 100 days in milk

The unadjusted average milk protein production per test day from calving to 100 DIM was 1.10 kg (Table 3.1), and there no effect of treatment on this outcome (LSM: QT = 1.21 kg, SP = 1.18 kg and TM = 1.19 kg, $P = 0.18$). The covariates test ($P < 0.01$), parity ($P < 0.01$), previous milk production ($P < 0.01$) and a quadratic term for previous milk production ($P = 0.02$) were statistically significant and therefore included in the final model (Table 3.7).

Effect of treatment on milk fat production (kg per test day) between calving and 100 days in milk

Overall unadjusted average milk fat production from calving to 100 DIM was 1.31 kg (Table 3.1), and there was an effect of treatment on FCM production (LSM: QT = 1.44

kg, SP = 1.39 kg and TM = 1.43 kg, $P = 0.03$). The covariates test ($P < 0.01$), dry period length ($P < 0.01$) and previous milk production ($P < 0.01$) were statistically significant and therefore included in the final model (Table 3.8).

Contrast analysis using the Bonferroni corrected *P*-value of 0.0176 showed that cows treated with QT produced 0.05 kg more fat than cows treated with SP ($P = 0.0120$; Table 3.9). There was no difference on fat production among cows treated with TM and cows treated with either QT or SP.

Effect of treatment on 305 mature equivalent between calving and 100 days in milk

A total of 2,767 tests were used for analysis of 305 ME from calving to 100 DIM. The overall unadjusted average of 305 ME was 11,685 kg (Table 3.1), with no difference among treatments (LSM: QT = 11,587 kg, SP = 11,463 kg and TM = 11,540 kg, $P = 0.31$). Significant covariates in the model predicting 305 ME included test ($P < 0.01$) and parity group ($P < 0.01$; Table 3.10).

The effect of treatment on the different milk parameters is summarized on Table 3.11.

Effect of treatment on linear score between calving and 100 days in milk

A total of 2,767 tests were included in the analysis of LS. The overall unadjusted average LS was 1.9 (Table 3.1), with no effect of treatment on LS from calving to 100 DIM (LSM: QT = 1.9, SP = 2.0 and TM = 1.7, $P = 0.12$). Covariates included in the final model were test ($P < 0.01$), parity ($P < 0.01$) and previous LS ($P < 0.01$; Table 3.12).

Effect of treatment on risk for experiencing a clinical mastitis event between calving and 100 days in milk

A total of 1,058 cows were used to analyze the effect of treatment on risk or time for a clinical mastitis event. Six cows were omitted due to missing previous linear score information (QT = 3, SP = 1 and TM = 2). The overall crude incidence of clinical mastitis from calving to 100 DIM was 14.2% (Table 3.1), with no effect of treatment on days or time to a clinical mastitis event (crude incidence: QT = 14.8%, SP = 12.7% and TM = 15.0%, $P = 0.80$; Figure 3.1). Covariates that remained significant in the final multivariate model included parity ($P < 0.01$), region ($P < 0.01$) and previous LS ($P < 0.01$; Table 3.13).

Effect of treatment on risk for leaving the herd between calving and 100 days in milk

A total of 1,064 cows were used for the analysis of effect of treatment on risk for leaving the herd. The overall crude incidence for leaving the herd by either culling or death was 9.0% (Table 3.1), with no effect of treatment on risk for leaving the herd from calving to 100 DIM (crude incidence: QT = 7.5%, SP = 9.2% and TM = 10.3%, $P = 0.55$; Figure 3.2). Parity ($P > 0.01$) was significant and remained in the final model (Table 3.14).

Effect of treatment on risk for pregnancy by 100 days in milk

A total of 1,057 cows were used for the survival analysis on effect of treatment on risk or time for pregnancy between calving and 100 DIM. Seven cows were omitted due to missing previous total milk production (QT = 3, SP = 2 and TM = 2). The overall pregnancy rate from calving to 100 DIM was 28.2% (Table 3.1), with no effect of treatment on risk or time for pregnancy (QT = 31.5%, SP = 26.1% and TM = 26.9%, P = 0.26; Figure 3.3). The covariates previous total milk production (P = 0.01) and dry period length (P = 0.04) and region (P < 0.01) were significant in the final model (Table 3.15).

Discussion

To our knowledge, this is the first multi-herd multi-state non-inferiority randomized clinical trial conducted to compare efficacy of three commonly used DCT preparations regarding long-term outcomes related to dairy cow production and health in North American dairy herds. No differences in efficacy between the three DCT treatments were found when evaluated at the cow level for milk production, milk protein production, 305 ME, linear score, risk for a clinical mastitis event, risk for leaving the herd and risk for pregnancy between calving and 100 DIM. However, there was an effect of treatment on milk fat production and FCM. Cows treated with QT produced more milk fat and FCM compared to cows treated with SP between calving and 100 DIM. Finally, there was a strong trend for the effect of treatment on ECM production, cows treated with QT tended to produce more ECM compared to cows treated with SP from calving to 100 DIM.

There was no difference on fat production, FCM or ECM among cows treated with TM and cows treated with either QT or SP.

Other studies have reported quarter level outcomes such as microbiological cure and prevention of IMI during the dry period, but did not address long-term cow level outcomes (McDougall, 2011) and did not make direct comparisons between products (Hallberg et al., 2006). A recent study conducted on two Florida dairy herds compared the efficacy of the products QT and SP regarding risk for clinical mastitis from calving to 30 DIM and 60 DIM and risk for high linear scores at 30 DIM and 60 DIM (Pinedo et al., 2012). That study reported there was an effect of treatment on risk for clinical mastitis and on risk for high LS during the above mentioned periods. Possible explanations for differences in results between the aforementioned study and the current study will be addressed in the following discussion.

Strengths of the current study include the number of herds enrolled from different states and therefore the inclusion of different climates, different mastitis pathogen profiles, and different dry cow housing and management strategies (previously described in Chapter 2). Study herds did differ from average United States dairy herds in the following aspects: study herds were larger than average (2,230 lactating cows) when compared to the average number of lactating cows in US dairy herds (167 lactating cows; NAHMS, 2010) and when compared to herds that are enrolled on DHIA (129 lactating cows; DHIA Annual Summary, 2011), and had higher rolling herd average (**RHA**) and lower average

somatic cell count (RHA = 12,360 kg, SCC = 242,170 cells/mL) as compared to DHIA herds (RHA = 9,600 kg, SCC = 304,000 cells/mL; DHIA Annual Summary, 2011).

Effect of treatment on milk production parameters from calving to 100 days in milk

It is known that mastitis has a long lasting effect on milk yield (Rajala-Schultz et al., 2009). Given the fact that the current study found no quarter level effect of treatment on risk for presence of IMI after calving (Chapter 2 of this thesis), and no effect of treatment on clinical mastitis incidence between calving and 100 DIM, it is not surprising that this study found no effect of treatment on test day milk production and 305 projected total milk production in the first 100 DIM. There are currently no other studies available in the literature that directly compared commercial DCT products regarding milk production outcomes.

The analysis of effect of treatment on ECM showed a strong trend for cows treated with QT to produce more than cows treated with SP from calving to 100 DIM, but no difference among cows treated with TM and cows treated with either QT or SP. The authors decided to further investigate this finding and analysis of the effect of treatment on total milk, protein and fat produced at test day and FCM were also conducted. Final models indicated that the milk component responsible for the detected difference among QT and SP for ECM and FCM was milk fat production. Milk fat production was increased for cows treated with QT compared to cows treated with SP, and this was reflected on the outcomes FCM and ECM, which consider fat production in their

calculations. The authors have no ready explanation for this finding. There are no studies that investigated the effect of treatment with those or any other dry cow intramammary formulation on long term production outcomes throughout lactation. There is also a possibility that this is a spurious finding. Further investigation is needed for identification of possible explanations for this finding.

Effect of treatment on linear score between calving and 100 days in milk

The current study found no effect of treatment on LS between calving and 100 DIM. This finding also makes sense, given that previous analysis found no quarter level effect of treatment on risk for presence of IMI after calving (Chapter 2 of this thesis), and no effect of treatment on clinical mastitis incidence between calving and 100 DIM. This finding disagrees with a recent study (Pinedo et al., 2012) that reported that cows treated with SP had significantly lower odds (odds ratio = 0.51) of having a high somatic cell score (≥ 4.5) within 30 days after calving (first DHIA test) compared to cows treated with QT. In that study cows treated with SP also had significant reduced odds (odds ratio = 0.52) of having a high somatic cell score within 60 days after calving (second DHIA test) compared to cows treated with QT. Unfortunately those authors did not report group specific estimates (least square means) of SCC measures, or group-specific proportions of cows with elevated SCC. As such, the authors from the current study were unable to make direct comparisons of the SCC results between the aforementioned and the current study. There were notable differences in design between the two studies that may explain

some of the differences in findings. For example, the Pinedo et al. (2012) study was limited to two Florida dairy herds and had a shorter period of follow-up (60 days), while the current study included six herds from Minnesota, Wisconsin, Iowa and California and a 100 DIM follow-up period. These differences in study design could possibly contribute to between-study differences in pathogen profiles, risk for IMI during the dry period, risk for clinical mastitis in early lactation, and SCC in early lactation.

Effect of treatment on risk for experiencing a clinical mastitis event, risk for pregnancy and risk for leaving the herd between calving and 100 days in milk

The overall crude mastitis incidence in the first 100 DIM found in the current study (14.2%) is consistent with what has been previously reported in the literature. A study conducted in two dairy herds in Florida (Pinedo et al., 2012) reported a clinical mastitis incidence of 7.4% and 10.1% during the first 30 and 60 DIM, respectively. Another observational study conducted in two dairy herds in the state of New York reported a clinical mastitis incidence over the lactation of 19.6% and 28.8% for first and second-plus lactation cows, respectively, for one of the dairies, and 6.3% and 13.8% for first and second-plus lactation cows, respectively, for the other dairy (Wilson et al., 2004).

There was no effect of treatment on risk for experiencing a clinical mastitis event between calving and 100 DIM in the current study. This disagrees with results from a recent study (Pinedo et al., 2012) in which cows treated with SP had 0.38 and 0.27 lower odds of experiencing a clinical case during the first 30 DIM and during the first 60 DIM

respectively, when compared to cows treated with QT. As previously mentioned, the lack of information provided on treatment group specific estimates of clinical mastitis incidence for the Pinedo et al. (2012) study precludes the authors of the current manuscript to directly compare the magnitude of difference among the treatments with those from the current study. However, afore mentioned study differences might explain differences between the two studies.

The overall culling rate (9.0%) observed in the current study is in general agreement with rates reported in the literature. Hadley et al. (2006) calculated an average annual cull rate across 10 states in the United States over a 7-year period of 35.1%, including animals sold to other dairies. These estimates reflect annual herd culling rates. Our estimate corresponds to culling from calving to 100 DIM; therefore it is within the expected range. The current study found no effect of treatment on risk for leaving the herd between calving and 100 DIM or on risk for getting pregnant by 100 DIM. These findings are consistent with the previously reported finding that there was no effect of treatment on risk for presence of IMI at calving (Chapter 2 of this thesis) or on incidence of clinical mastitis between calving and 100 DIM.

Secondary Findings

Previous Linear Score. The current study found positive associations between LS before dry off and LS in the subsequent lactation (calving to 100 DIM), and also between LS before dry off and risk for a clinical mastitis episode between calving and 100 DIM. Our

findings agree with Pinedo et al. (2012), which reported that high average LS in the previous lactation was associated with increased odds for a clinical mastitis event up to 60 days after calving, and also had a positive association with LS at 30 and 60 DIM. Similar findings were reported in an observational study by Pantoja et al. (2009), wherein cows with SCC ≥ 200,000 cells/ mL across the dry period had a greater risk of being subclinically infected with a major pathogen at 30 DIM than cows with a SCC < 200,000 cells/mL, and also were at a greater risk of having a clinical mastitis flare up during the first 120 DIM. Another observational study conducted in Spain (Fouz et al., 2010) used more than 25,000 lactations and showed that animals that had a LS lower than 4 at the first test following calving had decreased odds of yielding high LS (> 4) over the entire lactation, and at subsequent dry off. High LS before dry off is an indirect indicator of presence of IMI. Due to the failure of cure all IMI during the dry period, these infections might persist in the gland after calving, thus increasing the risk for clinical cases (Green et al., 2007) and high LS at the start of the next lactation.

Parity. In the current study parity was positively associated with LS, risk for clinical mastitis and risk for leaving the herd between calving and 100 DIM. A positive association between parity and risk for acquiring new IMI during the dry period has been previously reported (Dingwell et al., 2004), as well as a positive association between parity and risk for clinical mastitis (Green et al., 2007; Pantoja et al., 2009) and between parity and culling (Hadley et al., 2006). The fact that older cows may have disrupted

natural defenses and might have been exposed to several IMI over time (Green et al., 2007) could possibly explain the associations found in the current study.

DHIA Test number. In the current study DHIA test number was positively associated with milk production. This is in agreement with what is expected from the lactation curve. Milk yield follows a predictable curvilinear function that peaks at 6 to 9 weeks of lactation (Nebel and McGilliard, 1993). Even though the formula for calculating 305ME is designed to adjust for DIM on test day, it is possible that the correction factors used are not perfect and that early test day projections underestimate future lactation milk projection. Furthermore, second and third test day projections may genuinely increase after cows are exposed to production-enhancing management strategies including the use of rBST (Posilac®. Elanco Animal Health Co. Indianapolis, IN) between 57 and 70 DIM. Test number was negatively associated with LS, since the first test of the lactation had higher LS when compared to the third test. The findings are in agreement with a study (Pinedo et al., 2012) that reported that 17.1% of cows had high LS at the first test day after calving compared to 16.1% of cows at the second test after calving. The rate of IMI during lactation is highest at calving and decreases as days in milk advances (Hogan and Smith, 1998). Therefore it is reasonable that higher LS are observed earlier in lactation, but tend to decrease as the cow progresses in her lactation.

Previous Milk Production. Previous lactation milk production was negatively associated with risk for pregnancy by 100 DIM. Data on the association between milk production and pregnancy rates are conflicting (Le Blanc, 2010), but a possible explanation would be

that high-producing cows could potentially spend more energy on milk production and less energy on preparation of the reproductive tract to initiate and support a new pregnancy.

Dry Period Length. Dry period length was positively associated with both milk production and risk for pregnancy by 100 DIM. It is essential that cows have enough time to go through physiological changes in order to prepare their mammary gland for the next lactation. The longer the dry period, the more time the udder has to reestablish its alveolar and lobar structures and more milk can potentially be produced throughout the next lactation. As previously mentioned, the lower the prevalence of IMI at calving, the lower risk for clinical mastitis cases early in lactation and therefore the better her reproductive efficiency is expected to be. A recent study, however, showed that there was no effect of dry period length on percentage of cured IMI or on prevention of new IMI when comparing dry period length of 30 days with 45 days or 60 days (Church et al., 2008).

Conclusions

The current study found no effect of treatment on milk or milk protein production per test day, 305 ME, linear score, risk for experiencing a clinical mastitis case, risk for leaving the herd or risk for pregnancy from calving to 100 DIM. The authors were not surprised with results from this study since a companion manuscript from the same study has already reported no difference among the three evaluated DCT treatments when

examining quarter level outcomes including risk for presence of IMI post-calving, risk for cure of IMI during the dry period, prevention of new IMI over the dry period and risk for a quarter to experience a clinical mastitis episode during the first 100 DIM (Chapter 2 of this thesis). Finally, cows treated with QT had a higher milk fat production, a higher FCM production and tended to have a higher ECM production from calving to 100 DIM compared to cows treated with SP. There was no difference on fat production, FCM production and ECM production among cows treated with TM and cows treated with either QT or SP. Producers should consider these study findings along with other product related characteristics such as dry period length, meat and milk withhold periods and cost, when selecting DCT products for use in their herds.

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Table 3.1. Unadjusted Average (standard deviation [**SD**]) 305 mature equivalent (305 ME), average (SD) energy corrected milk production (ECM), average (SD) linear score, culling or death incidence, pregnancy incidence and clinical mastitis incidence between calving and 100 days in milk, by treatment group and overall

Variable	QT[1]	SP[2]	TM[3]	Total
ECM (kg/day)	38.0 (15.0)	37.4 (14.7)	38.0 (15.0)	37.8 (15.0)
N[4]	953	883	928	2764
Fat Corrected Milk (kg/day)	38.5 (15.3)	37.8 (15.0)	38.5 (15.4)	38.3 (15.3)
N[4]	953	883	928	2764
Total Milk Production (kg/day)	39.4 (15.7)	39.2 (15.7)	39.5 (15.6)	39.4 (15.6)
N[4]	953	883	928	2764
Milk Protein Production (kg/day)	1.1 (0.4)	1.1 (0.4)	1.1 (0.4)	1.1 (0.4)
N[4]	953	883	928	2764
Milk Fat Production (kg/ day)	1.3 (0.6)	1.3 (0.5)	1.3 (0.6)	1.3 (0.6)
N[4]	953	883	928	2764
305 ME (kg)	11749 (1446)	11591 (1587)	11699 (1506)	11682 (1513)
N[4]	953	886	928	2767
Linear score	1.9 (2.1)	2.0 (2.1)	1.8 (2.0)	1.9 (2.1)
N[4]	953	886	928	2767
Clinical Mastitis (%)	14.8	12.7	15.0	14.2
N[4]	359	338	361	1058
Culling/death (%)	7.5	9.2	10.3	9.0
N[4]	359	338	361	1058
Pregnant by 100 days in milk (%)	31.5	26.1	26.9	28.2
N[4]	359	337	361	1057

[1]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin),
[2]SPECTRAMAST DC (500 mg ceftiofur hydrochloride),
[3]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[4]Total number of observations

Table 3.2. Final multivariate linear regression model for the analysis of effect of treatment on energy corrected milk production (kg per day) between calving and 100 days in milk

					95% CI[1]		Type III
Variable		LSM (SE)[2]	Coefficient	SE[3]	LCL	UCL	*P*-value
Intercept			43.82	5.40			
Treatment	QT[4]	41.4 (3.8)	0.31	0.45	-0.57	1.18	0.0496
	SP[5]	40.3 (3.8)	-0.78	0.46	-1.67	0.12	
	TM[6]	41.1 (3.8)	Referent				
Region	CA	31.4 (5.9)	-18.18	7.61	-33.10	-3.26	0.06
	IA	41.7 (8.3)	-7.90	9.62	-26.75	10.95	
	MN[7]	49.6 (4.8)	Referent				
Test number	1	35.8 (3.8)	-10.12	0.45	-11.00	-9.25	<0.01
	2	40.9 (3.8)	-5.04	0.31	-5.64	-4.45	
	3	46.0 (3.8)	Referent				
Dry period length			0.06	0.02	0.02	0.09	<0.01
Parity	2	40.5 (3.8)	-0.90	0.40	-1.69	-0.11	0.03
	>2	41.4 (3.8)	Referent				
Prev milk[8]			0.001	0.0003	0.0004	0.0015	<0.01
Prev milk*prev milk[9]			-2.1[-8]	0.00	-2.4[9]	-2.4[9]	0.02

[1]95% Confidence Interval (lower and upper confidence intervals)
[2]Least Square Means (Standard Error)
[3]Standard Error
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]The region MN corresponds to the herds from both states of MN and WI
[8]Total milk produced in the last lactation
[9]Quadratic term for total milk produced in the last lactation

Table 3.3. Results of contrasted analysis of the effect of treatment on energy corrected milk (kg per day) from calving to 100 days in milk

Contrast	Differences of LSM (SE)[1]	*P*-value[5]
QT[2] vs. SP[3]	1.08 (0.45)	0.0170
QT[2] vs. TM[4]	0.31 (0.45)	0.4937
SP[3] vs. TM[4]	-0.78 (0.46)	0.0880

[1]Differences of least square means (standard error)
[2]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[3]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[4]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[5]Bonferroni *P*-value used to declare significance ($P < 0.0167$)

Table 3.4. Final multivariate linear regression model for the analysis of effect of treatment on fat corrected milk production (kg per day) between calving and 100 days in milk

Variable		LSM (SE)[2]	Coefficient	SE[3]	95% CI[1] LCL	95% CI[1] UCL	Type III *P*-value
Intercept			48.68	5.06			
Treatment	QT[4]	41.9 (3.8)	0.24	0.47	-0.69	1.17	0.03
	SP[5]	40.7 (3.8)	-0.98	0.48	-1.92	-0.04	
	TM[6]	41.7 (3.8)	Referent				
Region	CA	31.8 (5.9)	-18.56	7.67	-33.60	-3.53	0.05
	IA	42.2 (8.4)	-8.11	9.69	-27.10	10.89	
	MN[7]	50.4 (4.8)	Referent				
Test number	1	36.3 (3.8)	-10.17	0.46	-11.08	-9.27	<0.01
	2	41.6 (3.8)	-4.91	0.32	-5.54	-4.27	
	3	46.5 (3.8)	Referent				
Dry period length			0.56	0.02	0.53	0.60	<0.01
Parity	2	41.0 (3.8)	-0.98	0.42	-1.80	-0.16	0.02
	>2	41.9 (3.8)	Referent				
Prev milk[8]			0.0003	0.02	-0.04	0.04	<0.01

[1]95% Confidence Interval (lower and upper confidence intervals)
[2]Least Square Means (Standard Error)
[3]Standard Error
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]The region MN corresponds to the herds from both states of MN and WI
[8]Total milk produced in the last lactation

Table 3.5. Results of contrasted analysis of the effect of treatment on fat corrected milk production (kg per day) from calving to 100 days in milk

Contrast	Differences of LSM (SE)[1]	*P*-value[5]
QT[2] vs. SP[3]	1.22 (0.47)	0.0109
QT[2] vs. TM[4]	0.24 (0.47)	0.6108
SP[3] vs. TM[4]	-0.98 (0.48)	0.0421

[1]Differences of least square means (standard error)
[2]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[3]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[4]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[5]Bonferroni *P*-value used to declare significance ($P < 0.0167$)

Table 3.6. Final multivariate linear regression model for the analysis of effect of treatment on milk production (kg per day) between calving and 100 days in milk

Variable		LSM (SE)[2]	Coefficient	SE[3]	95% CI[1] LCL	95% CI[1] UCL	Type III *P*-value
Intercept			48.06	4.77			
Treatment	QT[4]	42.9 (3.6)	0.09	0.47	-0.82	0.004	0.14
	SP[5]	42.1 (3.6)	-0.77	0.47	-1.70	0.17	
	TM[6]	42.8 (3.6)	Referent				
Region	CA	32.4 (5.6)	-18.68	7.20	-32.79	-4.56	0.03
	IA	44.2 (7.9)	-6.89	9.10	-24.72	10.94	
	MN[7]	51.2 (4.6)	Referent				
Test number	1	36.2 (3.6)	-11.89	0.47	-12.81	-10.98	<0.01
	2	43.6 (3.6)	-4.50	0.30	-5.10	-3.91	
	3	48.1 (3.6)	Referent				
Dry period length			0.06	0.02	0.02	0.09	<0.01
Parity	2	42.0 (3.6)	-1.17	0.41	-1.98	-0.36	<0.01
	>2	43.2 (3.6)	Referent				
Prev milk[8]			0.0005	0.00007	0.0004	0.0006	<0.01

[1]95% Confidence Interval (lower and upper confidence intervals)
[2]Least Square Means (Standard Error)
[3]Standard Error
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]The region MN corresponds to the herds from both states of MN and WI
[8]Total milk produced in the last lactation

Table 3.7. Final multivariate linear regression model for the analysis of effect of treatment on milk protein production (kg per day) between calving and 100 days in milk

Variable		LSM (SE)[2]	Coefficient	SE[3]	95% CI[1] LCL	95% CI[1] UCL	Type III *P*-value
Intercept			1.41	0.15			
Treatment	QT[4]	1.21 (0.11)	0.02	0.01	-0.01	0.04	0.18
	SP[5]	1.18 (0.11)	-0.003	0.01	-0.03	0.02	
	TM[6]	1.19 (0.11)	Referent				
Region	CA	0.91 (0.17)	-0.54	0.22	-0.96	-0.11	0.04
	IA	1.22 (0.24)	-0.22	0.27	-0.76	0.31	
	MN[7]	1.44 (0.14)	Referent				
Test number	1	1.04 (0.11)	-0.31	0.01	-0.34	-0.29	<0.01
	2	0.18 (0.11)	-0.17	0.01	-0.19	-0.15	
	3	1.35 (0.11)	Referent				
Parity	2	1.17 (0.11)	-0.04	0.01	-0.06	-0.01	<0.01
	>2	1.21 (0.11)	Referent				
Prev milk[8]			0.00002	7.59^{-6}	1.5^{-5}	3.5^{-5}	<0.01
Prev milk*prev milk[9]			-589^{-12}	0	5.7^{-34}	5.7^{-34}	0.02

[1]95% Confidence Interval (lower and upper confidence intervals)
[2]Least Square Means (Standard Error)
[3]Standard Error
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]The region MN corresponds to the herds from both states of MN and WI
[8]Total milk produced in the last lactation
[9]Quadratic term for total milk produced in the last lactation

Table 3.8. Final multivariate linear regression model for the analysis of effect of treatment on milk fat production (kg per day) between calving and 100 days in milk

Variable		LSM (SE)[2]	Coefficient	SE[3]	95% CI[1]		Type III *P*-value
					LCL	UCL	
Intercept			1.68	0.20			
Treatment	QT[4]	1.44 (0.14)	0.01	0.02	-0.03	0.05	0.03
	SP[5]	1.39 (0.14)	-0.04	0.02	-0.08	-0.001	
	TM[6]	1.43 (0.14)	Referent				
Region	CA	1.10 (0.23)	-0.65	0.30	-1.23	-0.07	0.09
	IA	1.43 (0.32)	-0.32	0.38	-1.05	0.42	
	MN[7]	1.74 (0.19)	Referent				
Test number	1	1.28 (0.15)	-0.31	0.02	-0.35	-0.28	<0.01
	2	1.40 (0.15)	-0.18	0.01	-0.21	-0.16	
	3	1.59 (0.15)	Referent				
Dry period length			0.002	0.0008	0.0007	0.004	<0.01
Prev milk[8]			9.96^{-6}	2.65^{-6}	-0.005	0.005	<0.01

[1]95% Confidence Interval (lower and upper confidence intervals)
[2]Least Square Means (Standard Error)
[3]Standard Error
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]The region MN corresponds to the herds from both states of MN and WI
[8]Total milk produced in the last lactation

Table 3.9. Results of contrasted analysis of the effect of treatment on milk fat production (kg per day) from calving to 100 days in milk

Contrast	Differences of LSM (SE)[1]	*P*-value[5]
QT[2] vs. SP[3]	0.05 (0.02)	0.0120
QT[2] vs. TM[4]	0.01 (0.02)	0.5879
SP[3] vs. TM[4]	-0.04 (0.02)	0.0494

[1]Differences of least square means (standard error)
[2]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[3]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[4]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[5]Bonferroni *P*-value used to declare significance ($P < 0.0167$)

Table 3.10. Final multivariate linear regression model for the analysis of effect of treatment on 305 mature equivalent milk production (305 ME, kg) between calving and 100 days in milk

					95% CI[1]		Type III
Variable		LSM (SE)[2]	Coefficient	SE[3]	LCL	UCL	*P*-value
Intercept			12279	445.51			
Treatment	QT[4]	11587 (345.6)	47.78	80.99	-110.96	206.52	0.31
	SP[5]	11463 (346.0)	-77.00	82.50	-238.70	84.71	
	TM[6]	11540 (345.7)	Referent				
Region	CA	11583 (536.7)	-713.86	694.25	-2074.59	646.87	0.17
	IA	10709 (757.6)	-1588.45	876.30	-3306.00	129.10	
	MN[7]	12297 (440.4)	Referent				
Test number	1	11438 (343.0)	-307.98	42.06	-390.41	-225.55	<0.01
	2	11405 (343.0)	-341.40	33.61	-407.27	-275.53	
	3	11746 (343.4)	Referent				
Parity	2	11774 (344.1)	488.06	67.38	356.00	620.12	<0.01
	>2	11286 (344.3)	Referent				

[1]95% Confidence Interval (lower and upper confidence intervals)
[2]Least Square Means (Standard Error)
[3]Standard Error
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]The region MN corresponds to the herds from both states of MN and WI

Table 3.11. Summary of mixed linear regression analysis of effect of treatment on different milk production parameters between calving and 100 days in milk

	Unadjusted mean (SD)	Least Square Mean (SE)[1]	Type III *P*-value
Milk production (kg/day)			
QT[2]	39.4 (15.7)	42.9 (3.6)[a]	0.14
SP[3]	39.2 (15.7)	42.1 (3.6)[a]	
TM[4]	39.5 (15.6)	42.8 (3.6)[a]	
Protein production (kg/day)			
QT[2]	1.09 (0.4)	1.21 (0.1)[a]	0.18
SP[3]	1.10 (0.4)	1.18 (0.1)[a]	
TM[4]	1.10 (0.4)	1.19 (0.1)[a]	
Fat production (kg/day)			
QT[2]	1.33 (0.6)	1.44 (0.1)[a]	0.03
SP[3]	1.29 (0.5)	1.39 (0.1)[b]	
TM[4]	1.33 (0.6)	1.43 (0.1)[a,b]	
Energy Corrected Milk (kg/day)			
QT[2]	38.0 (15.0)	41.4 (3.8)[a]	0.0496
SP[3]	37.4 (14.7)	40.3 (3.8)[b]	
TM[4]	38.0 (15.0)	41.1 (3.8)[a,b]	
Fat corrected milk (kg/day)			
QT[2]	38.5 (15.3)	41.9 (3.8)[a]	0.03
SP[3]	37.8 (15.0)	40.7 (3.8)[b]	
TM[4]	38.5 (15.5)	41.7 (3.8)[a,b]	
305 Mature Equivalent (kg)			
QT[2]	11,749 (1,446)	11,587 (345)[a]	0.31
SP[3]	11,601 (1,581)	11,463 (346)[a]	
TM[4]	11,699 (1,506)	11,540 (346)[a]	

[1]Different letters correspond to statistical difference on the contrast analysis using the Bonferroni correction factor ($P < 0.0167$)
[2]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[3]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[4]ToMORROW Dry Cow (300 mg cephapirin benzathine)

Table 3.12. Final multivariate linear regression model for the analysis of effect of treatment on linear score between calving and 100 days in milk

Variable		LSM (SE)[2]	Coefficient	SE[3]	95% CI[1] LCL	95% CI[1] UCL	Type III *P*-value
Intercept			1.16	0.19			
Treatment	QT[4]	1.9 (0.1)	0.17	0.12	-0.08	0.41	0.12
	SP[5]	2.0 (0.1)	0.25	0.13	0.005	0.50	
	TM[6]	1.7 (0.1)	Referent				
Region	CA	2.2 (0.1)	0.29	0.12	0.05	0.53	<0.01
	IA	1.4 (0.1)	-0.47	0.13	-0.73	-0.21	
	MN[7]	1.9 (0.1)	Referent				
Test number	1	2.0 (0.1)	0.26	0.07	0.12	0.40	<0.01
	2	1.8 (0.1)	-0.01	0.06	-0.14	0.11	
	3	1.8 (0.1)	Referent				
Previous LS[8]			0.25	0.03	0.18	0.31	<0.01
Parity	2	1.7 (0.1)	-0.40	0.11	-0.62	-0.18	<0.01
	>2	2.1 (0.1)	Referent				

[1]95% Confidence Interval (lower and upper confidence intervals)
[2]Least Square Means (Standard Error)
[3]Standard Error
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]The region MN corresponds to the herds from both states of MN and WI
[8]Last linear score before dry off

Table 3.13. Final Cox Proportional Hazards Regression model for the analysis of effect of treatment on risk for experiencing a clinical mastitis event between calving and 100 days in milk

Variable		Coefficient	SE[2]	Hazards Ratio[3]	95% CI[1] LCL	95% CI[1] UCL	*P*-value
Treatment	QT[4]	0.005	0.19	1.005	0.69	1.46	0.80
	SP[5]	-0.12	0.20	0.89	0.60	1.33	
	TM[6]	Referent		1.00			
Region	CA	-1.04	0.18	0.35	0.25	0.50	<0.01
	IA	-2.38	0.35	0.09	0.05	0.18	
	MN[7]	Referent		1.00			
Previous LS[8]		0.21	0.05	1.23	1.13	1.34	<0.01
Parity	2	-0.52	0.17	0.60	0.43	0.83	<0.01
	>2	Referent		1.00			

[1]Confidence Interval for the odds ratio, lower (LCL) and upper (UCL) confidence limits.
[2]Standard Error
[3]Hazard of experiencing a clinical mastitis event
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]The region MN corresponds to the herds from both states of MN and WI
[8]Last linear score before dry off

Table 3.14. Final Cox Proportional Hazards Regression model for the analysis of effect of treatment on risk for leaving the herd (culling or death) between calving and 100 days in milk

Variable		Coefficient	SE[2]	Hazards Ratio[3]	95% CI[1] LCL	95% CI[1] UCL	*P*-value
Treatment	QT[4]	-0.26	0.24	0.77	0.48	1.22	0.55
	SP[5]	-0.15	0.24	0.86	0.55	1.36	
	TM[6]	Referent		1.00			
Region	CA	0.28	0.24	1.33	0.82	2.14	0.48
	IA	0.24	0.26	1.28	0.60	1.76	
	MN[7]	Referent		1.00			
Parity	2	-1.09	0.24	0.34	0.23	0.59	<0.01
	>2	Referent		1.00			

[1]Confidence Interval for the odds ratio, lower (LCL) and upper (UCL) confidence limits.
[2]Standard Error
[3]Hazard of experiencing a clinical mastitis event
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]The region MN corresponds to the herds from both states of MN and WI

Table 3.15. Final Cox Proportional Hazards Regression model for the analysis of effect of treatment on risk for pregnancy between calving and 100 days in milk

Variable		Coefficient	SE[2]	Hazards Ratio[3]	95% CI[1] LCL	95% CI[1] UCL	*P*-value
Treatment	QT[4]	0.19	0.14	1.21	0.92	1.58	0.26
	SP[5]	-0.02	0.15	0.98	0.74	1.31	
	TM[6]	Referent		1.00			
Region	CA	-0.33	0.16	0.72	0.53	0.98	<0.01
	IA	0.35	0.14	1.42	1.08	1.88	
	MN[7]	Referent		1.00			
Previous milk production		-0.00005	0.00002	0.9999	0.9999	0.9999	0.01
Dry period length		0.01	0.005	1.01	1.001	1.02	0.04

[1]Confidence Interval for the odds ratio, lower (LCL) and upper (UCL) confidence limits.
[2]Standard Error
[3]Hazard of experiencing a clinical mastitis event
[4]QUARTERMASTER (100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin)
[5]SPECTRAMAST DC (500 mg ceftiofur hydrochloride)
[6]ToMORROW Dry Cow (300 mg cephapirin benzathine)
[7]The region MN corresponds to the herds from both states of MN and WI

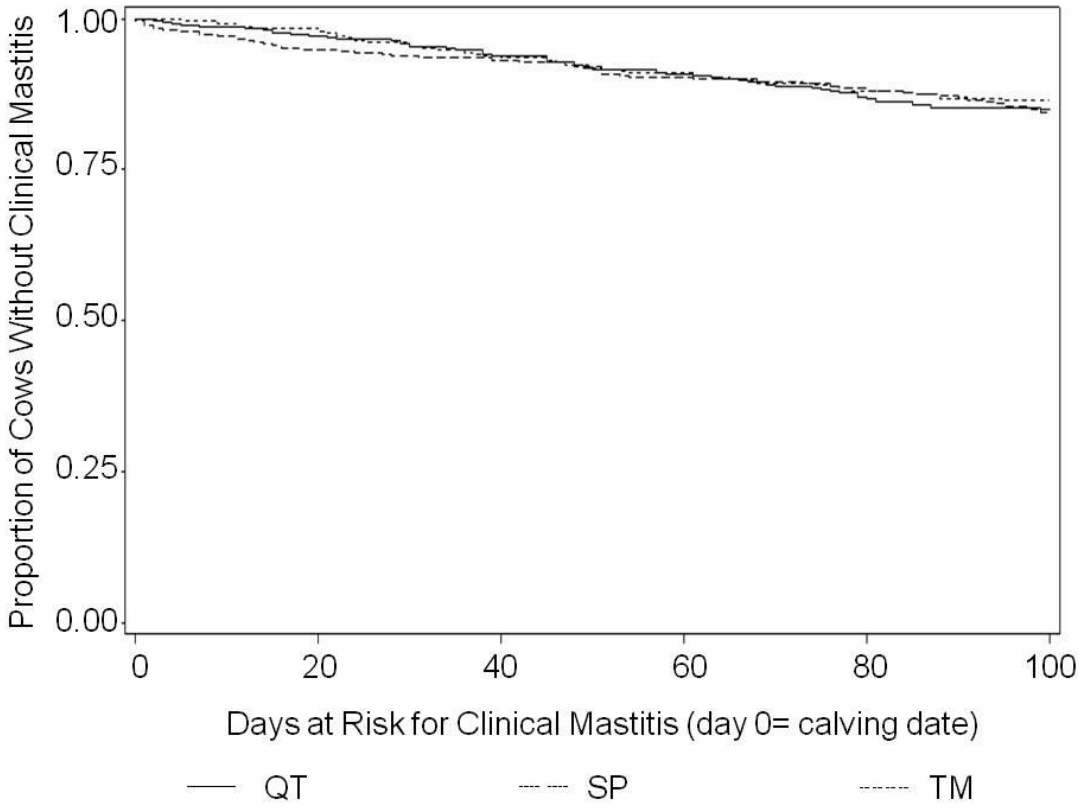

Figure 3.1. Survival distribution function for effect of dry cow therapy treatment on risk and days to a clinical mastitis event between calving (day 0) and 100 days in milk (unadjusted, QUARTERMASTER = 14.8%, SPECTRAMAST DC = 12.7% and ToMORROW Dry Cow = 15.0%)

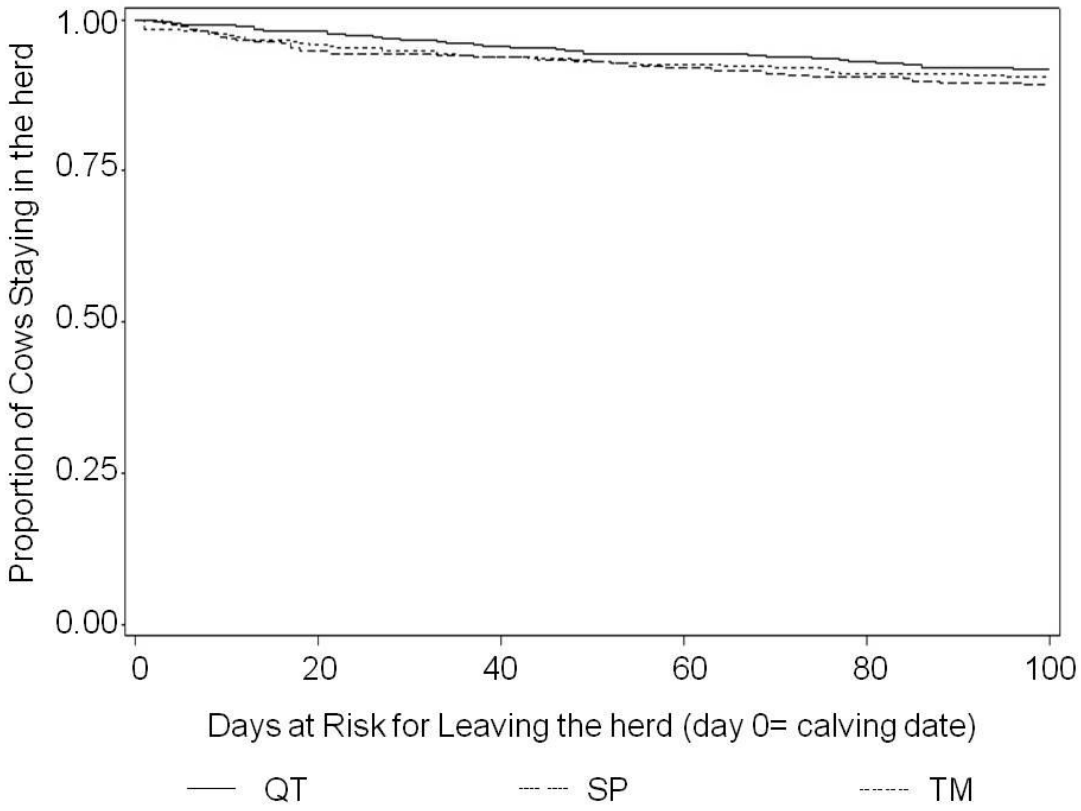

Figure 3.2. Survival distribution function for effect of dry cow therapy treatment on risk and days for leaving the herd (culling or death) between calving (day 0) and 100 days in milk (unadjusted, QUARTERMASTER = 7.5%, SPECTRAMAST DC = 9.2% and ToMORROW Dry Cow = 10.3%)

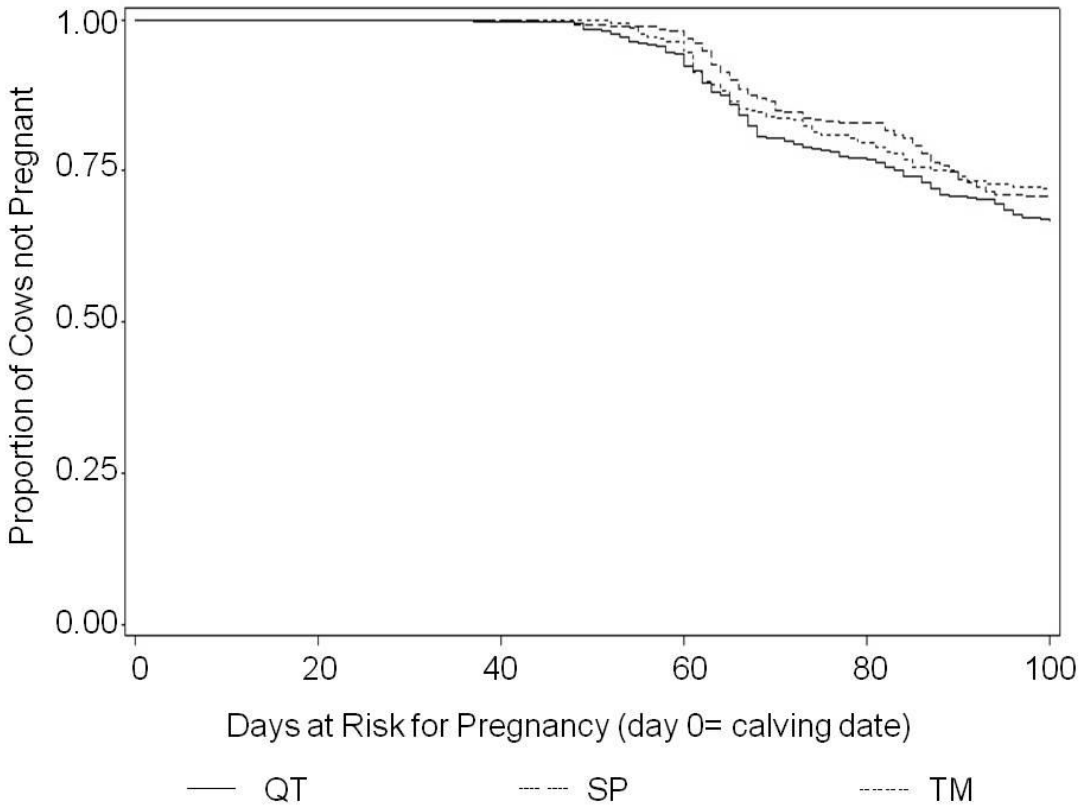

Figure 3.3. Survival distribution function for effect of dry cow therapy treatment on risk and days for pregnancy by 100 days in milk (unadjusted, QUARTERMASTER = 31.5%, SPECTRAMAST DC = 26.1% and ToMORROW Dry Cow = 26.9%)

CHAPTER 4

SUMMARY AND CONCLUSIONS

Chapter 4

Summary and Conclusions

Mastitis remains the most costly infectious disease affecting dairy herds despite the decades of research on its control and prevention. Persistence of preexisting intramammary infections (**IMI**) throughout the dry period and development of new IMI during the dry period are two important factors that influence the risk for manifestation of clinical mastitis in the next lactation. Therefore, many studies have been conducted in order to develop effective management to prevent and treat this costly disease (Chapter 1). The practice of blanket dry cow therapy (**DCT**) is one such strategy that has been highly successful. It is defined as the intramammary treatment of all quarters of all cows with a long-lasting antibiotic formulation at dry off, and it has the purpose of curing preexisting IMI and preventing new IMI that could be potentially acquired during the dry period.

Although dry cow antibiotic formulations are widely used in dairy herds across the United States, the efficacy of these products (as compared to a negative control) was typically established many years or decades ago, for the purpose of FDA approval. However, well-designed head-to-head studies comparing efficacy among DCT products have been largely lacking. The comparative efficacy of available DCT formulations deserves to be investigated so that producers can make informed science-based decisions when selecting DCT products for use in their herds. The main goal of this multi-herd,

multi-state study was to provide producers with information on the relative efficacy of three commercially available DCT products. This information could help guide in the selection of DCT products, thus promoting cow health and welfare, economic sustainability of the dairy farm and judicious drug use. The three products compared in this study were QUARTERMASTER (**QT**, 100,000 IU procaine penicillin G and 1 g dyhydrostreptomycin, Pfizer Animal Health, New York, NY), SPECTRAMAST DC (**SP**, 500 mg ceftiofur hydrochloride, Pfizer Animal Health, New York, NY) and ToMORROW Dry Cow (**TM**, 300 mg cephapirin benzathine, Boehringer Ingelheim Vetmedica, Inc., St Joseph, MO).

Efficacy of the products was assessed at both the quarter and cow level. The first objective of this study was to describe and compare the efficacy of the three aforementioned DCT formulations at the quarter level. The effects of treatment on risk for presence of an IMI after calving, risk for cure of an IMI during the dry period, risk for development of a new IMI during the dry period and risk of a clinical mastitis event between calving and 100 days in milk (**DIM**) were evaluated separately. The second objective was to describe and compare efficacy of the same three products regarding cow level health and production parameters for the first 100 DIM, including milk production, linear score (**LS**), risk of culling and death, risk for a clinical mastitis case and risk for pregnancy.

A total of 1,091 cows (4,364 quarters) from six commercial dairy herds in four different states (CA, IA, MN and WI) were enrolled and randomized to one of the three treatments

at dry off. Quarter milk samples were collected for bacterial culture prior to treatment at dry off, at 0 to 6 DIM and at 7 to 13 DIM. All clinical mastitis, pregnancy, culling and death events occurring in the first 100 DIM were recorded by farm staff using an on-farm electronic record keeping system. Dairy Herd Information Association electronic records were used to retrieve consecutive test data regarding milk production, milk composition and LS until 100 DIM, as well as previous lactation milk production and last LS before dry off.

The overall crude quarter level prevalence of infection at dry off was 19.2%. The most common pathogen isolated from milk samples at dry off was coagulase negative *Staphylococcus* (53.9%), followed by *Aerococcus* spp. (12.3%) and other *Streptococcus* spp. (7.4%). At the quarter level, there was no effect of treatment on risk for presence of an IMI at 0 to 6 DIM (least square means [**LSM**]: QT = 0.16 (95% CI: 0.14, 0.19), SP = 0.14 (95% CI: 0.12, 0.17) and TM = 0.16 (95% CI: 0.14, 0.19)), risk for a cure between dry off and calving (LSM: QT = 0.93 (95% CI: 0.87, 0.97), SP = 0.93 (95% CI: 0.86, 0.96) and TM = 0.94 (95% CI: 0.89, 0.97)), risk for development of a new IMI between dry off and 0 to 6 DIM (LSM: QT = 0.15 (95% CI: 0.12, 0.18), SP = 0.12 (95% CI: 0.10, 0.15) and TM = 0.14 (95% CI: 0.12, 0.17)) or risk to experience a clinical mastitis event between calving and 100 DIM (QT = 5.3%, SP = 3.8% and TM = 4.1%).

The cow level analysis showed there was no effect of treatment on milk production per day (LSM: QT = 42.9 kg, SP = 42.1 kg and SP = 42.8 kg, $P = 0.14$), milk protein production per day (LSM: QT = 1.21, SP = 1.18 and TM = 1.19, $P = 0.18$), 305 ME

(LSM: QT = 11,587 kg, SP = 11,463 kg and TM = 11,540 kg, $P = 0.31$), linear score (LSM: QT = 1.9, SP = 2.0 and TM = 1.7), risk for a clinical mastitis episode (QT = 14.8%, SP = 12.7% and TM = 15.0%), risk for leaving the herd (QT = 7.5%, SP = 9.2% and TM = 10.3%) or risk for pregnancy by 100 DIM (QT = 31.5%, SP = 26.1% and TM = 26.9%). There was an effect of treatment on ECM production (LSM: QT = 41.4 kg^{a}, SP = 40.3 kg^{b} and TM = 41.1 $kg^{a,b}$, $P = 0.0496$), FCM production (LSM: QT = 41.9 kg^{a}, SP = 40.7 kg^{b} and SP = 41.7 $kg^{a,b}$, $P = 0.03$) and fat production (LSM: QT = 1.44 kg^{a}, SP = 1.39 kg^{b} and TM = 1.43 $kg^{a,b}$, $P = 0.03$) by 100 DIM. Contrast analysis showed that cows treated with QT had a trend for higher ECM production by 100 DIM and produced more FCM and milk fat when compared to cows treated with SP.

All products evaluated in the current study are labeled to be effective against one or more Gram-positive organisms. Considering this, and considering that the majority of IMI in the current study were caused by Gram-positive organisms, it was not a surprise that the study found all three DCT products to have equivalent efficacy at the quarter level. Furthermore, the fact that no difference was reported for the quarter level outcomes was probably reflected in most of the cow level outcomes.

This was the first multi-herd multi-state non-inferiority clinical trial designed to compare three of the most commonly used dry cow mastitis formulations using a large sample size. The fact that six different herds with different management strategies in four different states in the United States were enrolled enhances the external validity of this study. However, characteristics of the study herds such as number of lactating cows,

rolling herd average and average annual somatic cell count differ from national average. Additionally, readers may want to consider herd management characteristics and mastitis pathogen profiles from the six study herds used when deciding on the generalizability of study findings to other commercial dairy herds. Future investigations are needed with respect to understanding the effect of treatment with QT and SP and the implication on milk fat production.

In conclusion, with the exception of ECM, FCM and milk fat production, this study found no difference in efficacy among the three commercial DCT antibiotic formulations evaluated. The findings regarding milk fat production need to be further investigated. Dairy farmers and herd managers should consider the findings of this study alongside with other product related characteristics such as dry period length, meat and milk withhold periods and cost, in order to help guide the selection of DCT products for use in their herds.

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