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THE USE OF TYLOSIN TO TREAT INTRAMAMMARY INFECTIONS DURING THE NON-LACTATING PERIOD OF HEIFERS AND DAIRY COWS

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THE USE OF TYLOSIN TO TREAT INTRAMAMMARY INFECTIONS DURING THE NON-LACTATING PERIOD OF HEIFERS AND DAIRY COWS

By

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ABSTRACT

THE USE OF TYLOSIN TO TREAT INTRAMAMMARY INFECTIONS DURING THE NON-LACTATING PERIOD OF HEIFERS AND DAIRY COWS

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There are two types of non-lactating periods during the lifespan of a dairy cow. The first type refers to heifers from infancy to puberty and gestation prior to the first calving. The second is referred to as the dry cow period, and is defined as the time when a mature cow is not milked between two lactations. During the non-lactation period there is a risk of acquiring an intramammary infections (IMI) for both heifers and dairy cows. This is the safest time to treat IMI, because antibiotic treatments can be administered at higher a dose to increase effectiveness while reducing the risk of milk residues. In the present thesis, systemic tylosin was used in mature dairy cows alone or in combination with intramammary cephapirin, to treat IMI at the end of lactation. While in dairy heifers, systemic tylosin was used 2 weeks prepartum to reduce IMI after calving.

Tylosin was effective in reducing IMI caused by Gram-positive bacteria in dairy cows when used alone or in combination with intramammary infused cephapirin. In dairy heifers, intramuscular tylosin reduced IMI caused by coagulase negative staphylococci. The use of tylosin in the non-lactating period of dairy animals offers a management option that may be useful on some farms, as a part of their udder health program. Deciding whether to use tylosin should be based on sound evaluations of environment, management and economic implication for the farm.

DEDICATION

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INTRODUCTION

Mastitis is the most common disease of dairy cows and the single greatest cause of economic loss to the dairy industry today. The economic impact on the dairy industry attributed to mastitis is estimated at $2 billion a year in the USA or approximately 6% of the value of production (DeGraves and Fetrow, 1993; Harmon, 1994; Wells and Ott, 1998). At the herd level, costs of mastitis are divided into four main losses including: reduced milk quality, less efficient milk production due to chronic subclinically infected cows, increased costs related to treatment, and increased replacement rate or culling of cows at suboptimal time in lactation (Østeras, 2000; Halasa et al., 2007). An additional consideration includes the cost of mastitis prevention that amounts to almost half of total costs of dairy herd health programs (Miller et al., 1993).

Mastitis is a multifactorial disease, in which both the environment and the animal play a role. This not only requires an understanding of animal physiology and defense against pathogens, but also how the environment affects the animal responses to pathogens when designing effective control programs.

Producers and dairy professionals have developed strategies to control mastitis and reduce economic losses based on an understanding of animal-environment interactions. One of these strategies is antibiotic treatment during the non-lactating period to prevent clinical and subclinical mastitis in the subsquent lactation. There are two types of non-lactating periods during the lifespan of a dairy cow. The first type refers to heifers from infancy to puberty and gestation prior to the first calving.

The second type is referred to as the dry cow period, and is defined as the time when a mature cow is not milked between two lactations.

Antibiotic treatment for prevention of mastitis in dry cows is called dry cow therapy (DCT). It was first proposed in the 1950's (Pearson, 1950), and is generally practiced by dairy farmers around the world (Dingwell et al., 2003). Similar to DCT, prepartum intramammary antibiotic treatment of heifers (Precalving Heifer Treatment, PCHT) has been used from early pregnancy until 2-6 wk before expected calving (Trinidad et al., 1990c). Antibiotics used in DCT and PCHT include beta-lactams, while other families of antibiotics such as macrolides are available but are less commonly used. The macrolide antimicrobials diffuse readily into the mammary gland. Commercial products including, tylosin, tylmicosin and tulathromycin are currently used in food animal practice but not in DCT or PCHT.

Tylosin has the advantage of being relatively inexpensive, safe and easy to use when compared to the other members of the macrolide family. As a macrolide antibiotic, it diffuses well into the udder compartment, because of its basic pK. Furthermore, clinical research has proven its efficacy and it is approved in other countries such as New Zealand to treat clinical mastitis during the lactating period (McDougall, 2007). The efficacy of tylosin as systemic DCT or PCHT, however, has not been well documented. We investigated the effectiveness of tylosin as a systemic antibiotic in DCT and PCHT, and found that administration of systemic tylosin for DCT and PCHT decreases the incidence of IMIs at parturition.

CHAPTER 1

Dry Period: The non-lactating period in dairy cows

The period of highest risk for new intramammary infections (IMI) in the lactation cycle of cows is during the early non-lactating period (dry period). In order to identify management programs that address this high-risk period, it is necessary to understand the biological events of the dry period, as well as management practices that address this risk. The following chapter will review the physiology and the management practices of the dry period.

The ideal dairy cow should attain peak milk production at 60 days in milk (DIM), become pregnant again by 100 DIM, dry off at 305 DIM and rest during the dry period for 60 days. The dry period is important for lactation development, since dairy cows without a dry period have at least a 20% decrease in total milk yield as compared to herd-mates with a 45 to 60 days dry period (Remond et al., 1997; Sorensen and Enevoldsen 1991). In a pioneer study, Smith et al. (1966) compared milk production by quarters in two cows. In each animal two quarters were milked throughout the dry period phase (60 days) and two were not milked. Quarters without a dry period produced 38% and 44% less milk in the subsequent lactation than their counterparts that had a 60-day dry period.

The length of the dry period is also important in terms of milk components. Fat and protein yield is maximized in a lactation that follows a 60-day dry period. In contrast, dry periods of 20 days or less result in substantial losses in fat and protein yield in the subsequent lactation (Kuhn et al., 2006). Udder health also is affected by dry period length, with long dry periods (45 to 70 days) improving somatic cell count

in the subsequent lactation. In general the ideal dry period length is 45 to 60 days (Kuhn et al., 2007), but as noted by Bachman and Schairer (2003), further research is needed to determine the ideal length of the dry period for the modern dairy cow that accounts for various management scenarios.

Involution of the mammary gland occurs in two stages during the dry period. The first is triggered by local stimuli, such as accumulation of milk within the alveoli, which initiates apoptosis in some milk producing cells, although re-initiating milk removal can reverse this process (Furth et al., 1997). This process is accompanied by a decrease in lactose and fatty acids secretion, an increase in lactoferrin and NAG secretion, and a more active intracellular transport of immunoglobulins (Hurley, 1989). Differences among species are found in the second stage. Involution is irreversible in mice and rats and is characterized by activation of proteases that destroy the lobular-alveolar structure of the gland by degrading the extra-cellular matrix and basement membrane, as well as a loss of alveolar cells (Walker et al., 1989). In contrast, alveolar structure is maintained in dairy cows. Cows are usually gestating at dry-off and pregnancy might explain the conservation of the mammary structure (Lund et al., 1996; Capuco et al., 2002).

There are morphological changes in the gland after the cessation of milking. Within 24 hours of the last milking, secretory vesicles and fat droplets accumulate within the cytoplasm of alveolar cells. After 2 or 3 days, the vesicles fuse and large vacuoles are formed. Accumulation of secretory products and vacuole formation is consistent with inhibition of milk secretion (Capuco and Akers, 1999). Within the first 48 hours after the last milking, many cells display a decrease in cytoplasmatic

organelles involved in milk synthesis. After two weeks, cells exhibit a reduction in apparent secretory capacity, but they are still viable (Capuco and Akers, 1999). During the dry period there is no net loss of mammary cells, but there is an enhanced turnover of mammary epithelial cells, suggesting that this time of rest permits replacement of damaged or senescent cells prior to the following lactation (Capuco et al., 1997).

Implications of the dry period

Endevolsen and Sorensen (1992) demonstrated that dry periods of 7 weeks were associated with the lowest risk of clinical health disorders when compared to periods of 4 and 10 weeks. However, the susceptibility to new IMI is higher during the early dry period and near calving (Oliver 1988). This increased incidence of new IMI results in an elevated number of infected quarters at calving, and is responsible for the high level of IMI during early lactation in many herds.

According to the National Mastitis Council guidelines (2001), the elevated rate of new infections during the early dry period in the absence of DCT may be due to one or several of the following: 1) flushing of colonized bacteria from the teat canal during milking is terminated; 2) udder sanitation and teat dipping are discontinued; 3) the teat canal becomes dilated and shortened due to udder fill at milk cessation, which allows organisms to enter the udder; 4) phagocytes are involved in removing milk components instead of bacteria; and 5) activity of lymphocytes is reduced. In contrast, the rate of new infections during the mid-dry period is very low. Mammary

gland resistance during this time may be attributed to: 1) a keratin plug forms in the teat canal which prevents mastitis pathogens from entering the udder; and 2) increased presence of antibacterial factors such as lactoferrin and immunoglobulins in the udder. Inhibition of growth due to antibacterial factors may be associated with the high rate of spontaneous elimination of some IMI (e.g., *Corynebacterium bovis*) during the dry period (Oliver and Juneja, 1990). Susceptibility to infection again increases near calving. This may be due to: 1) increased fluid volume and dilation of the teat canal; 2) decreased lactoferrin concentration; 3) reduced leukocyte numbers and phagocytic ability; and 4) utilization of milk components for bacterial growth.

Dingwell et al. (2003) outlined specific factors influencing susceptibility of cows to IMI infections during the dry period; such factors are at the level of the quarter, cow and herd. Quarter-level factors include, bacterial population at the teat end, teat end integrity, and formation of teat canal keratin plug. Cow-level factors include parity, milk production, and method of milk cessation. Herd-level factors are housing, bedding materials, udder health management practices and nutritional status.

Pathogens

Mastitis pathogens are divided into contagious and environmental groups. Contagious pathogens include *Staphylococcus aureus, Streptococcus agalagtiae, Mycoplasma bovis* and *Corynebacterium bovis,*. Environmental pathogens include *Streptococcus uberis, Streptococcus dysgalactiae, Escherichia coli, Klebsiella* spp, *Enterobacter* spp, *Prototheca* spp, Pastereulla spp, *Arcanobacterium pyogenes* and *Pseudomonas* spp. (Hogan et al., 1999).

Contagious organisms, as well as some environmental bacteria, are transmitted among cows and quarters during the milking process, and are the predominant infections at the time of dry-off. Effective long-acting antibiotic therapy offers the best opportunity to eliminate these existing infections. In contrast, exposure to environmental pathogens is likely to continue throughout the dry period. These organisms are primarily contracted from contamination of udders by fecal material and bedding. Among environmental pathogens, infections with *Klebsiella*, and *Enterobacter* occur more frequently early in the dry period. On the other hand, *E. coli* infections tend to occur immediately before and after calving (Leslie, 2001). Infections with *Streptococci* are frequent throughout the non-lactating period, and their incidence increases a few weeks before and around parturition (Eberhart, 1987).

Bradley and Green (2000) assessed the incidence of enterobacterial infection in mammary glands during the non-lactating period. A rise in the incidence of enterobacterial infections was detected around drying off and calving. Quarters that acquired an infection were more likely to develop mastitis in the subsequent lactation. The authors found that more than 70% of enterobacterial mastitis occurs following a period of subclinical infection in the dry period.

Intramammary infections in the dry period increase the risk of clinical mastitis in the following lactation, especially in the early stage. Furthermore, infections with one organism during the dry period may increase susceptibility to infection caused by another pathogen, resulting in clinical mastitis during lactation (Green et al., 2002).

Dry Cow Treatment

The DCT is defined as the use of antibiotics immediately after the last milking of lactation. It can decrease the number of existing infections and prevent new infection during the early weeks of the dry period (NMC, 2000). Dry cow therapy has advantages over lactation therapy including higher cure rate, a reduction in new IMI after calving, and a higher dose of antibiotic can be used with lower risk of milk residues (Ruegg, 2005). Two approaches can be used to administer DCT in dairy herds: 1) blanket DCT that involves the treatment of all cows at dry-off following the last milking of lactation and 2) selective DCT only treats cows with high SCC or IMI at dry-off. In general, DCT is a cost-effective measure compared with no treatment at dry-off (Berry et al., 2004; Robert et al., 2006; Huijps and Hogeveen, 2007). Intramammary dry treatment reduces IMI in herds with a high prevalence and high risk of new infections. The most common route of DCT is intramammary infusion into each productive quarter, however antibiotic therapy for DCT can be administered by intramammary, systemic or as a combination of both routes.

Intramammary dry cow therapy

Intramammary dry cow treatment alone is widely used and very effective in reducing IMI (Zwald et al., 2004; Dingwell et al., 2003; Berry et al., 2002; Dimmick, 2001; Sol et al., 1994). However, low efficacy against some coagulase negative staphylococci has been demonstrated (Rajala-Schultz et al., 2004). The use of the intramammary route started as early as 1950 when penicillin was used to prevent

bacterial infection in the dry period (Pearson, 1950). New formulations of antibiotics were developed in the 1960's, using cloxacillin and penicillin G to extend the duration of effective concentration for several weeks after infusion (Smith et al., 1967 a, b). Currently, different antibiotics are used for intramammary treatment and there are often differences within geographical regions regarding antimicrobials of choice. For example, a survey of dairy herds in Pennsylvania found that drugs commonly used in dry cow therapy were cephapirin (52%), novobiocin (27%), penicillin G procaine (24%), and cloxacillin (15%) (Sawant et al., 2004). In contrast, a similar survey was conducted in California where the most commonly used antibiotics were cloxacillin, penicillin/streptomycin, and cephalosporin. Most dairies chose a single type of antibiotic and used the same antibiotic over an extended period of time (Kirk, 2004). In general, DCT is used in 98% of conventional dairy farms in USA, 99% in United Kingdom and 76.5% in Canada (Zwald et al., 2004; Berry et al., 2002; Dimmick 2001).

Systemic therapy

Systemic dry cow therapy is less expensive and easier to administer than intramammary but has been reported to be less effective and therefore, rarely used. Nickerson (1999) administered systemic tilmicosin (5mg/kg b.w. subcutaneous) to cows with at least one quarter infected with *Staphylococcus aureus*. Cure rates for cows treated with systemic tilmicosin were 9.1%, which was much lower than the ones achieved by intramammary tilmicosin (74%) and intramammary cephapirin (78%).

In another study, Shpigel et al., (2006) used systemic cefquinome (1mg/kg b.w. intramuscular) in cows with IMI caused by *S aureus*, reporting 28% cure rates for the cefquinone group in contrast with 70% for the intramammary infused group (sodium nafcillin, procaine benzylpenicillin and dihydrostreptomycin). The authors also reported an increase of IMI due to *S. aureus* in the cefquinone treated group. Norfloxacin nicotinate and oxytetracycline-HCL have also been reported as therapies using this route (Soback et al., 1990).

Combined therapy

The use of systemic and intramammary antibiotics at the same time was initially applied in treating subclinical mastitis (Owens, 1988). In this study, cows with quarters subclinically infected with *S. aureus* were treated with intramammary amoxicillin plus systemic penicillin or intramammary amoxicillin only. Cows treated with the combination had a 51% cure rate compared to 25% of the intramammary group. Based on this study, it was inferred that a combined therapy could increase the effectiveness of intramammary treatment alone because it provided better diffusion of certain systemic antibiotics into the mammary gland.

Combined therapy in DCT has been reported by O'Boyle et al., (2006) who compared two combinations: 1) 12 g of oxytetracycline hydrochloride (OTC-HCl) administered intramuscularly plus intramammary cephapirin 300 mg and 2) 20 g of tylosin subcutaneously plus intramammary cephapirin 300 mg. A teat sealant was used in combination with both treatments. Tylosin plus cephapirin gave better results in reducing somatic cell counts (SCC) and intramammary infections after calving

than OTC-HCL plus cephapirin. Other combinations have been used. Erskine et al. (1994) compared efficacies of intramammary infusion of cephapirin benzathine alone against infusion of the same antibiotic combined with intramuscular oxytetracycline at 11 mg/kg once daily on days 7, 8, 9, and 10 after drying off for treatment of *Staphylococcus aureus* mastitis. Systemic oxytetracycline, in combination with intramammary dry cow treatment, did not improve the cure rate for *S. aureus* mastitis, however, many of these infections were chronic and the effect on other pathogens was not reported.

Combined therapy has advantages over systemic or intramammary administration of DCT. The use of two or more antibiotics at the same time expands the spectrum of the treatment and increases the possibility of synergism among the antimicrobials. A combined DCT could be advised in certain herds with higher rates of IMI or high SCC, but this recommendation must be based on a thorough analysis of the udder health of the dairy herd.

Internal teat sealants

Internal sealants infused into the teat canal can occlude the entry of bacteria into the gland. A commonly used sealant, bismuth subnitrate in paraffin base is infused after the last milking of lactation alone or combined with antibiotic therapy. Bismuth subnitrate was initially evaluated in the UK. Berry and Hillerton (2002) found fewer new infections at calving in quarters of cows treated with teat sealant than in quarters of untreated cows. The authors reported that the predominant pathogen causing new infections was *Streptococcus uberis*. The teat-sealed group had fewer IMI caused by

this agent (9/27 quarters) than the control group (44/93 quarters). Similarly, Huxley et al. (2002) observed that quarters treated with teat sealant had fewer IMI caused by *Escherichia coli* and all *Enterobacteriaceae* when compared to quarters treated with intramammary antibiotic. Both studies noted that the use of internal teat sealant was also useful in preventing new IMI around calving caused by *Enterobacteriaceae.*

In North America, Godden et al. (2003) demonstrated a significant effect of adding teat sealant to DCT, in which quarters sealed with bismuth subnitrate were 30% less likely to develop a new IMI between dry-off and calving. These quarters were also 31% less likely to have an IMI present at 1 to 3 DIM, and were 33% less likely to experience a clinical mastitis event between dry off and 60 DIM, with a significantly lower linear score at 1 to 3 DIM and 6 to 8 DIM, as compared with control quarters.

External teat sealants

Barrier teat dips with a germicide have been used in heifers and in cows to prevent new IMI during the dry period. However, no benefits of its use were demonstrated on new infection rate at parturition (Edinger et al., 2000; McArthur et al., 1984; Matthews et al., 1988). Duration of sealant adherence to the teat-end should be considered when evaluating the impact of teat sealant treatment at drying off on the level of infection after calving. Moreover, the use of new compounds with higher adherence and also repeated teat dipping reduced IMI suggesting that the use of dry-cow teat sealants have a beneficial impact on the level of infection at calving, if a durable seal is formed and remains on the teat-end (Lim et al., 2007).

The use of DCT does not have to be limited to the classical intramammary approach. There are other choices that include systemic antibiotics or a combination of both options. Teat sealant can be added, to ensure protection to the mammary gland when the risk of infection is higher at the beginning or the end of the dry period. But general management practices such as clean environment, comfortable hygienic bedding, and clean calving pens cannot be forgotten, since DCT will not replace a poorly managed transition and calving barn.

Macrolides

Macrolides were first isolated in 1950 from a strain of streptomyces, a type of bacteria found in soil. All of the antibiotics in this family share a macrocyclic lactone in their structure. The family is named for this feature and each member is classified according to the size of the macrocyclic ring with 12-, 14-, or 16- membered ring macrolides. The 12-membered drugs include methymicin, the 14-membered group includes Erythromycin, and the 16-membered group includes tylosin. A subgroup called triamilide includes new semisynthetic macrolides such as tulathromycin that contains 3 additional amino groups (Lewicki, 2006).

Macrolides are generally bacteriostatic. Bactericidal activity may occur under certain conditions such as increasing the dose or against specific microorganisms. Their macrocyclic ring reversibly binds to the 23S ribosomal RNA (rRNA) in the 50S subunit of susceptible organisms, and thus inhibits mRNA-directed protein synthesis. Moreover, they stimulate the dissociation of peptidyl-tRNA during translocation, suppressing RNA-dependent protein synthesis and inhibiting bacterial growth

(Blondeau et al., 2002). Macrolides diffuse very well into milk following systemic administration with mammary tissue concentrations higher than plasma concentrations. Their pK (pH at which concentrations of dissociated and undissociated antibiotic are equal) is basic which results in a milk to plasma concentration ratio of 5:1 (Riviere, 1999). Once the macrolides are in milk they are virtually trapped inside the mammary gland (ion trapping) because of their basic pK. Macrolides bear a unique cellular distribution. Scorneaux and Shryock (1999) described tilmicosin distribution in cellular organelles, finding that 70 to 80% of the drug was located in lysosomes. Furthermore, neutrophil antibacterial activity is based on phagocytosis, a process in which lysosomes play a key role. Thus, the propensity of macrolides for the lysosome may aid in bacterial killing (Paape and Capuco, 1997).

Macrolides are active against aerobic and anaerobic Gram-positive cocci such as coagulase negative staphylococci (CNS). They are not effective against Gram-negative anaerobes and some enterococci. The antimicrobial spectrum of macrolides generally includes Gram-positive cocci such as *Staphylococcus aureus*, CNS, β-hemolytic streptococci, other streptococci species and some enterococci. Additional activity has been documented, other agents including *Haemophilus influenzae*, some pathogenic *Neisseria* species, *Bordetella*, *Corynebacterium*, *Chlamydia*, *Mycoplasma*, *Rickettsia* and *Legionella* species. The modification of macrolide structure has been shown to affect antibacterial activity. These changes also cause differences in pharmacokinetics (Abu-Gharbieh et al., 2004).

Resistance to macrolides has been reported especially in the streptococci group A, by two distinct mechanisms: 1) ribosomal modification resulting from the presence

of an ermmethylase and 2) drug efflux conferred by a membrane protein encoded by the *mefA* gene. This resistance is specific for 14- and 15-member macrolides (erythromycin, azithromycin and clarithromycin); 16-member macrolides (e.g. josamycin, tylosin) are not affected (Weiss et al., 2001).

Tylosin

Tylosin is a mixture of four macrolide antibiotics produced by a strain of *Streptomyces fradiae*. The main component of the mixture (> 80%) is tylosin A, although tylosin B, tylosin C and tylosin D may also be present (Lewicki, 2006). All four components contribute to the potency of tylosin, which is not less than 900 IU/mg, calculated with reference to the dried substance (European Pharmacopoeia, 2004). A summary of tylosin physico-chemical properties is shown in Table 1.

Pharmacokinetics

Tylosin is administered to cattle either intramuscularly or subcutaneously. Following IM administration of tylosin to calves (10 mg/kg body weight) at 12 hours and 24 hours, the mean serum concentrations (± SE) at 25 and 26 hours were 1.47 ± 0.14 μg/mL and 1.25 ± 0.08, μg/mL (Burrows et al., 1986). Peak blood concentrations of tylosin were reached in 2-4 hours following intramuscular injection of tylosin base in 50% propylene glycol and the aqueous solution of the tartrate salt in cows, ponies and pigs (Sauter et al., 1962; Gingerich et al., 1977). In another study, peak serum concentrations in cattle following intramuscular injection of tylosin (12.5 mg/kg body weight) reached values of 2.5 μg/mL about 5-6 hours after injection with

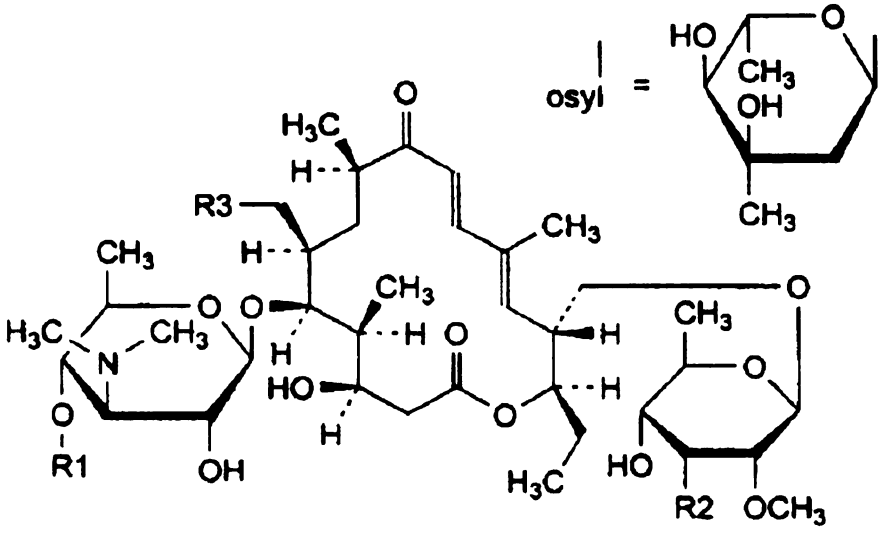
systemic bioavailability of 70-80% of the administered dose (Ziv and Sulman, 1973). Saurit et al. (2002) established peak concentration in cows for tylosin of 0.65 ± 0.07 μg/mL although the dose reported (10 mg/kg body weight) was lower than normally used.

With a pKa of 7.73 and featuring a high degree of lipid solubility, tylosin is well distributed in tissues. Calculated theoretical tissue: plasma ratios (k_{12}/k_{21}) of tylosin in cows were 2.05 (Baggot and Gingerich, 1976). Milk to plasma ratio calculated in milk samples was 5:1 (Riviere, 1999). Protein binding coefficients were 33.5-44% in cows (Ziv and Sulman, 1972; Ziv and Sulman, 1973). Reported half-life values for tylosin were 2.14, 1.62 and 2.24 hours (Ziv and Sulman, 1973; Baggot and Gingerich 1976; Saurit et al., 2002).

Primary metabolism occurs in the liver. Metabolites of tylosin include tylosin A, N-desmethyl-tylosin A, tylosin D, N-desmethyl-dihydro-tylosin A, O-desmethyl-tylosin, tylosin C and dihydrodesmycosin (JECFA, 1991). Tylosin is excreted mainly through the liver. Its concentration in bile at 24 hours of IM administration (7mg/ kg body weight) was 35.1μg/ml. Renal excretion is also important, urine concentration of tylosin was 12.9 μg/ml 24 hours after IM administration (7mg/ kg body weight), (Nouws and Ziv, 1977).

Table 1. Summary of physico-chemical properties of tylosin (Paesen et al., 1995abc; McFarland et al., 1997; The Merck Index, 2001).

Molecular formula:	$C_{46}H_{77}NO_{17}$
Molecular weight:	916.1
Appearance:	An almost white or slightly yellow crystalline powder
Melting point:	128-132 ^{o}C
Solubility:	5 mg/ml (water 25 ^{o}C), soluble in lower alcohols, esters, ketones, chlorinated hydrocarbons, benzene, ether, chloroform
Stability:	Solutions are stable at pH 4-9 (most stable at pH 7); Below pH 4 tylosin B (desmycosin) is formed as a result of acid hydrolysis; In neutral and alkaline pH – tylosin aldol (TAD) is formed together with polar degradation products of unknown identity; When tylosin solution is exposed to daylight, a photodegradation product - isotylosin A (isoTA) is formed
pKa:	7.73
Log P (octanol-water):	1.63
UV Absorption:	$UV_{max.}$ at 282 nm, extinction coefficient ($E_{1cm}^{1\%}$) is 245 at 282 nm



Name	Mol. Formula	R1	R2	R3
tylosin A	$C_{45}H_{77}NO_{17}$	osyl	OCH_3	CHO
tylosin B	$C_{39}H_{65}NO_{14}$	H	OCH_3	CHO
tylosin C	$C_{45}H_{75}NO_{17}$	osyl	OH	CHO
tylosin D	$C_{46}H_{79}NO_{17}$	osyl	OCH_3	CH_2 OH

Figure 1. Tylosin and associated metabolites. (Lewicki, 2006)

Cows receiving a single intravenous injection of tylosin tartrate (20 mg/kg b.w.) had peak concentrations of tylosin in milk of 10 μg/ml. In contrast, corresponding plasma concentrations of tylosin 4 hours after injection were only around 3.5 μg/ml. Lower peak values (approx. 6 μg/ml) were observed in cow's milk 6 hours after a single intramuscular injection of tylosin tartrate at the same dose. The ratio of peak normal milk (pH = 6.5-6.8) concentrations to peak serum concentrations of tylosin was approximately 2.5, while the ratio of peak mastitis milk (pH = 7.1-7.4) to peak serum concentrations was 1.6 (Ziv and Sulman, 1973). In cows receiving tylosin base intramuscularly (12.5 mg/kg b.w.) every 12 hours for 48 hours, the peak concentration of tylosin in milk (approx. 7 μg/ml) appeared after 60 hours and then rapidly decreased to 1.5 μg/ml at 72 hours. Milk:serum ratios corrected for differences in protein binding and calculated at various times ranged up to about 20:1 (Gingerich et al., 1977). Similar milk:serum ratios (up to approx. 17.5:1) were observed in cows after a single intramammary infusion of 200 mg of tylosin/quarter. When mastitic cows received repeated intramuscular injections of tylosin base at a dose of 10 mg/kg b.w. every 12 hours for 5 days, peak milk concentrations increased gradually up to 18 μg/ml at the fifth day after the onset of therapy (El-Sayed et al., 1986).

Table 2. Antimicrobial activity for tylosin. Reported minimum inhibitory concentrations (MIC 90) for different bacterial isolates (Catry et al., 2003. Odlan et al., 2002)

BACTERIA	MIC90 μg/ml
Staphylococcus chromogenes	1.00
Staphylococcus aureus B0329	0.5
Staphylococcus aureus ATCC29213	1-2
Streptococcus pneumoniae B0541	<0.125
Streptococcus pyogenes B0542	<0.125
Escherichia coli B0001	>64
Escherichia coli ATCC25922	>64
Haemophilus influenzae	16

Non-lactating heifers

The dairy heifer in lactogenesis faces several risk periods. During these periods, exposure to IMI can affect mammary development, and negatively influence milk production over her lifetime. Understanding lactogenesis and mammary development is essential in designing management protocols to reduce the risk of IMI at calving and optimize milk quality and production.

Mammogenesis

Mammary glands are exclusive of animals belonging to class *Mammalia*. These exocrine glands are modified sweat glands, which are located in anatomically different alignments among species. Each gland has a teat or nipple; this structure differs in formation depending on the infraclass either *Methateria* or *Eutheria*. *Methateria* animals (e.g. kangaroos) have nipples covered by a flap of skin forming the pouch. *Eutheria* animals have the nipples uncovered and with a wide variation of locations along the ventral line.

In the bovine embryo, mammogenesis follows six stages as described by Larson (1978). The first stage starts at 30 days of gestation with the formation of the mammary band, which is a thickening of the ventrolateral ectoderm. Then with further development, this group of epithelial cells becomes a mammary streak at 32 days. A mammary line is formed at 35 days, becoming a mammary crest by 37 days. At 40 days of gestation the embryo bears the mammary hillock that is transformed at 43 days to a localized area of epithelial development from which a mammary gland will arise regardless of species.

Larson (1978) also defined the pattern of development for the fetal mammary gland. After a mammary bud has been formed, further differentiation continues. Teats are formed at 65 days from a protruding structure of the mesenchyme surrounding the sprout. The primary sprout is recognized by 80 days of fetal age. This is a projection of the mammary bud into the surrounding mesenchyme. The number of projections determines the number of openings arising from each teat, in the cow there is only one, whereas humans have 15 to 25. By 90 days secondary sprouts develop; these are secondary structures that will become mammary ducts, due to a lack of a fatty pad, secondary sprout development is very limited in the male. At later stages secondary sprouts will branch and appear as the tertiary sprouts. These are the precursors of the secondary milk ducts. Canalization of the primary sprout is visible by 100 days. As the circumference of the sprout increases, a lumen is formed. This begins at the proximal end of the primary sprout (inner end) and proceeds in both directions, forming the gland cistern. The most distal region of the lumen will develop into the streak canal.

Larson (1978) also defined the complementary structures for the mammary gland. The fatty pad, which is originated in the mesoderm, is the adipose tissue structure into which the entire mammary complex of ducts and lobule alveoli penetrate. The pad provides space for growth and a source of energy for the epithelial cells. The structural tissues vary depending of the specie, e.g. in the bovine suspensory ligaments such as the central and lateral are developed by 200 days of fetal age. Myoepithelial cells are contractile epithelial cells that originate in the

epithelial layer of the gland and differentiate from the secretory epithelial cells. These cells are non-functional until lactation.

Prepubertal development

There are 2 stages of growth in the mammary gland of a female calf; isometric that occurs for the first 2-3 months of age, and allometric growth, which initiates at three months and reaches its plateau at 9 months of age (Hovey et al., 1999; Sinha and Tucker, 1969).

Isometric growth means that the gland grows at the same rate as the entire body. During this stage, there is no development of secretory lobular structures or secretory tissues. The increase in the size of the gland is the result of growth of fat pad and connective tissue (ansci.uiuc, 2006). In the female calf, a single primary duct extends from the teat. The base of the duct forms the gland cistern and the distal portion forms secondary and tertiary ducts to which mammary epithelium is related (Hovey et al., 1999).

At about 3 months after birth, allometric growth begins (growth rate faster than the rest of the body). In the calf, this includes extensive growth and development of the duct network, which invades the surrounding adipose tissue (fat pad). The allometric growth phase lasts until about 1 year of age, when the mammary growth rate returns to isometric growth (ansci.uiuc 2006). The allometric growth stage is highly dependent on hormonal changes. Prepubertal mammary epithelium proliferation is stimulated by progesterone and estrogen. The family of progesterone receptors (PR) and estrogen receptors (ER) mediate their influence. The ERs include

ERα, ERß, and three estrogen-related receptors (ERRs) (Meyer et al., 2007; Connor et al., 2005; Schams et al., 2003).

At 4 months of age, the mammary gland develops a limited ability to respond to lactogenic stimuli (insulin, prolactin and EGF) if primed with steroid hormones. By 7 months of age, priming with steroids is no longer necessary for histologic changes in response to lactogenic stimuli. By 10 months of age, changes in histologic appearance do not require in vivo steroid priming but functional differentiation and b-casein mRNA expression is only achieved when glands are cultured in the presence of the lactogenic hormones after steroid hormone priming. (Maple et al., 1998).

Other hormones and growth factors play a role in mammary gland development. Growth hormone (GH) directs allometric growth in heifers, but requires the presence of estrogen to promote growth. Local synthesis of various growth factors promotes the allometric growth stage. Among these factors is TGF ß1, which selectively acts on the stromal compartment of the bovine mammary gland by increasing cell proliferation and gene expression of extracellular matrix proteins. Fibroblast growth factors are also involved, including FGF-1, IGF-1, TGF-α and HGF (Hovey et al., 2002; Musters et al., 2004; Sinowatz et al., 2006).

Rearing heifers at an elevated level of nutrient intake especially a high energy diet impairs mammary development (Meyer et al., 2006, Capuco et al., 2005). Furthermore, leptin a hormone produced by adipocytes and elevated in serum of animals fed high-energy diets inhibits mammary development by reducing DNA synthesis in mammary gland associated epithelial cells (Silva et al., 2002). Its physiological pathway is still unclear since bovine mammary epithelial cells have

negligible leptin receptor expression, and do not respond to leptin in vitro (Thorn et al., 2006).

Puberty to Conception

At the onset of puberty, a 21-day estrous cycle begins in heifers, affecting growth of mammary gland. During estrus mammary DNA synthesis increases 115% and then decreases slowly through metestrus and diestrus until it reaches the lowest level just before estrus (Sinha and Tucker, 1969). The fundamental developmental unit in mammary tissue is the terminal ductule lobular unit (TDLU). After puberty it grows at an isometric rate, and only reaches full alveolar development after pregnancy (Hovey et al., 2002). Mammary development accelerates throughout pregnancy, and reaches its peak during the last trimester of pregnancy, coinciding with the most rapid period of fetal growth.

Intramammary infections

While mastitis in heifers has been recognized as a problem, most dairymen view heifers at parturition as being free of intramammary infections. Mastitis in heifers was first recognized in the 1940s by Palmer et al. (1941). Schalm (1942) traced IMI in heifers caused by *Streptococcus agalactiae* to suckling among calves. More recently the prevalence of IMI in prepartum heifers has been investigated more thoroughly. As many as 63% of heifers and 34% of their quarters may have intramammary infections (IMI) at first parturition and this causes an increase in SCCs and clinical mastitis cases after calving (Borm et al., 2006).

Intramammary infections affect dairy heifers from puberty throughout gestation and around calving. Trinidad et al., (1990a) reported IMI in 87% of the quarters of unbred heifers. Secretion samples from infected quarters had an increased SCC when compared to normal quarters. In another study, Trinidad et al. (1990b) collected mammary tissue samples from unbred heifers and found that secretory parenchyma from infected quarters had more connective tissue than samples from uninfected quarters. In addition, infected quarters exhibited greater leukocyte infiltration. Thus, IMI in puberty can impair mammary growth and development and influence future milk production.

The major problem associated with mastitis in heifers around calving is increased SCC (Waage et al., 1999). During the weeks of subclinical IMI, infected heifers would contribute elevated somatic cells to the bulk tank reducing the quality of milk for the whole farm. These infections can become clinical and cause further losses and premature culling. The heifer's IMI rate is reflective of the mastitis problem for the entire herd. A retrospective study (DeVliegher et al., 2004) using 117,496 four-weekly test-day records of 14,243 heifers revealed that heifers with a high test-day value SCC (200,000 or more) on days 7 to 14 after calving were likely to remain high during the whole first lactation. In the same study, the impact of early lactation SCC on milk yield was analyzed. Heifers with SCC 500,000 and 1,000,000 produced 119 and 155 kg less milk respectively as heifers with SCC of 50,000 (DeVliegher et al., 2004, 2005). In another study, prepartum antibiotic-treated heifers produced 531 kg more milk and also had lower SCC scores than the untreated control group (Oliver et al., 2003).

The main causes of IMI in unbred and primigravid heifers are CNS (Fox et al., 1995, Oliver et al., 2004, Trinidad et al., 1990). Some of the CNS are part of the normal skin flora and include the species *Staph simulans, Staph hyicus and Staph epidermis;* others are found in the environment such as novobiocin-resistant species. The CNS are opportunists and infect the teat canal and the gland from skin sources. Some may colonize the teat canal and persist for long periods (Aarestrup and Jensen 1997,Waage et al., 1999). Thus, while CNS are often grouped together, considerable variation in CNS species is reported and some are more problematic than others (Oliver et al., 2004). The duration of infection varies among *Staph* species, for example, *Staph chromogenes* can be found in infected quarters beginning 4 weeks before calving but its prevalence is reduced sharply after calving while other CNS such as *Staph simulans* are present 2 weeks before calving but its infection persists several weeks after calving (Aarestrup and Jensen, 1997).

Antibiotic treatment before calving

Prepartum antibiotic therapy has been evaluated in heifers. Trinidad et al. (1990c) evaluated intramammary antibiotics in breeding age and primigravid heifers during different trimesters of pregnancy and demonstrated that this practice was effective in reducing the prevalence of IMI at parturition. However, there were some issues related to antibiotic residues in milk due to use of antibiotics formulated for non-lactating cows.

Oliver et al. (1992) used either intramammary Cloxacillin (200 mg) or intramammary Cephapirin sodium (200 mg) 7 days before expected day of calving.

Both antibiotic formulations reduce IMI especially those caused by coagulase-negative staphylococci. However antibiotic residues in milk were found at days 3 and 5 after parturition. More recently, lactating cow formulations have been used 7 to 14 d before expected day of parturition. These treatments reduced IMI in 60% to 75% of quarters infected (Oliver et al., 2003; Borm et al., 2006).

Antibiotic infusion in mammary glands of pregnant heifers can reduce IMI prevalence and can increase milk production. In a study of one herd, prepartum antibiotic-treated heifers produced 531 kg more milk and also had lower SCC scores than the untreated control group (Oliver et al., 2003). Borm et al. (2006) analyzed intramammary antibiotic infusion in heifers from 9 herds in 7 different locations. The authors found that milk production and SCC from prepartum antibiotic-treated heifers did not differ significantly from the untreated control group. However, there were some differences depending on prevalence of IMI in each herd.

Antibiotic treatment immediately after calving

Some research groups have studied antibiotic treatment immediately after calving. Kreiger et al. (2007) used systemic (intramuscular) penethamate hydriodide, in heifers from herds with a high prevalence of *Staphylococcus aureus* infections. Heifers treated with antibiotics had fewer IMI and had higher milk production. However, this study consisted of a small number of animals and required a 5-day withholding for the milk produced by treated animals. This inconvenience did not provide an economically attractive protocol. Oliver et al. (2007) analyzed a different approach, using either pirlymicin or penicillin-novobiocin an intramammary

antibiotic after the first milking post-calving. No significant differences were observed when penicillin-novobiocin was compared with untreated control. Significantly, fewer IMI during early lactation were observed in heifers treated with pirlimycin compared to those in the untreated control group, although this treatment did not appear to be as effective as prepartum antibiotic-treatment of heifer mammary glands.

Internal teat sealants

Parker et al., (2007) tested a bismuth subnitrate teat-canal sealant 30 days before expected calving in heifers. They found that it improved udder health, reducing the risk of IMI caused by *Streptococci uberis* by 84% and clinical mastitis cases by 68%. However, the use of teat sealants based on bismuth subnitrate has been linked to the appearance of black spot defect (BSD) in Cheddar cheese. Lay et al. (2007) related this defect to residual levels of bismuth salt precursor unintentionally entrained within the cheese milk.

Tylosin use as systemic antibiotic in DCT and PCHT

Metaphylaxis to prevent new and treat chronic IMI using DCT or PCHT has been proven to be effective, however, effectiveness may vary depending on herd health status, bacteria type, and herd management issues. Systemic administration of antibiotics such as tylosin can be an alternative to DCT or PCHT alone or used in combination with intramammary treatment. In the following chapters, the use of tylosin alone or in combination with intramammary infusion of cephapirin and with

the addition of a teat sealant was tested in mature dairy cows at the time of dry off as DCT or in primigravid heifers 2 wk before calving as PCHT. We hypothesize that tylosin alone or in combination with intramammary antibiotic before calving as DCT or PCHT will lower the occurrence of IMI and reduce SCC after calving.

CHAPTER 2

Comparison of Systemic and Intramammary Dry Cow Treatments

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SYSTEMIC AND INTRAMAMMARY DRY COW TREATMENTS

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Summary

The objective of the study was to compare four different dry cow treatments and establish whether effectiveness at quarter level and by bacterial type increased when intramuscular tylosin therapy was added to intramammary infusion of cephapirin, and also evaluate tylosin alone intramuscularly as a systemic dry cow therapy (DCT). A total of 278 cows at the end of lactation were randomly assigned to one of 4 dry cow treatment groups: 1) Group CESE (n=89), cephapirin intramammary and teat sealant. 2) Group TYCESE (n=84), cephapirin intramammary, tylosin 12 g intramuscular and teat sealant. 3)Group TYSE (n=86), tylosin 12 grams intramuscular and teat sealant; 4) Group TY (n=76) tylosin 12 g intramuscular only. Quarter milk samples for culture were collected at dry-off and 1 and 2 wk after calving. Somatic cell counts (SCC) were taken from Dairy Herd Improvement Association (DHI) tests at dry-off, and the first two test days after calving. Milk production and health records were also monitored. Bacteria cure rate for Gram-positive intramammary infections (IMI) for group TYCESE was 93.6%, group CESE 78.9%, group TYSE 88.2%, and group TY, 78.1%. All four groups showed a decrease in the SCC at the first and second test after calving. The difference in means for log SCC between dry-off and fresh was highest for group TYCESE. Results demonstrated that use of systemic tylosin in combination with the intramammary antibiotics increased the effectiveness of DCT. The inclusion of this macrolide improves the cure rate of Gram-positive IMI.

Comparison of Systemic and Intramammary Dry Cow Treatments

Dry cow therapy (DCT) is defined as the use of antibiotics immediately after the last milking of lactation. There are three different approaches to DCT. First, intramammary dry treatment alone is widely used and very effective in reducing intramammary infections (IMI) (Berry & Hillerton, 2002; Dimmick, 2001; Sol et al., 1994; Zwald et al., 2004). However, low efficacy against some coagulase negative staphylococci IMI has been demonstrated (Rajala-Schultz et al., 2004). Another approach is systemic dry cow therapy (Soback et al., 1990; Nickerson et al., 1999), which is relatively inexpensive and easy to use but has been reported to be less effective and is therefore rarely used.

Finally a combination of systemic and intramammary antibiotics can also be administered; this method could increase the effectiveness over that of intramammary treatment alone because it provides advantages such as better diffusion of certain systemic antibiotics into the mammary gland. O'Boyle et al. (2006) compared two combinations, Oxytetracycline hydrochloride (OTC-hcl) 12 g intramuscular plus intramammary cephapirin 300 mg and tylosin subcutaneously 20 g plus intramammary cephapirin 300 mg. A teat sealant was used in combination with both treatments. Tylosin plus cephapirin gave better results in reducing somatic cell counts (SCC) and intramammary infections after calving than OTC-hcl plus cephapirin.

The use of tylosin alone as a systemic DCT has not been tested before. This antibiotic belongs to the macrolide family of antibiotics. One of the main advantages of this type of antimicrobial is an excellent diffusion into the mammary gland related to its basic pK (pH at which concentrations of dissociated and undissociated antibiotic

are equal), which results in a very high milk to plasma concentration ratio of 5:1 (Omura, 1984; Riviere, 1999). Once the macrolides are in milk they are virtually trapped inside the mammary gland. Because of these features tylosin was used as systemic DCT in this study.

Evaluation of combined DCT with tylosin as systemic antibiotic and cephapirin as an intramammary antimicrobial has not been performed at quarter level. O'Boyle's et al. (2006) results are based on composite samples; in this study, analysis of IMI was based on quarter cultures.

The objective of this study was to compare four different DCTs and establish whether effectiveness of DCT at quarter level increased when systemic (intramuscular) tylosin therapy was added to intramammary infusion of cephapirin, and also evaluate tylosin alone as a systemic DCT.

Materials and methods

A completely randomized design that included three treatments and a control was used. Cows on a commercial farm in Michigan were selected. Every week, starting from November 2005, and after confirming their gestation to be greater than 150 days, a group of mature Holstein cows due to go dry was assigned to one of four DCT groups (Table 1).

Treatment CESE (n=89 cows), reflecting standard practice on the farm, was used as control. After the last milking of lactation, animals were first infused in each productive quarter with a commercial preparation of 300 mg cephapirin (Tomorrow®, Fort Dodge, Ft Dodge, IA, USA) intramammary and then with 4 g of a commercially available internal teat sealant containing 65% bismuth subnitrate

(Orbeseal®, Pfizer. New York, NY, USA). Treatment TYCESE (n=84 cows) animals were first infused in each productive quarter with cephapirin intramammary 300 mg, then injected intramuscularly with tylosin (Tylosin, Agripharma. Westlake, TX, USA) 12 g and finally were infused with teat sealant. Group TYSE (n=86 cows) animals received tylosin 12 g intramuscular and then teat sealant was infused in each productive quarter. Group TY (n=76 cows) animals received 12 g tylosin intramuscularly. Intramammary infusions were administered by the partial insertion technique. Prior to instillation, all teat ends were prepared following the farm's milking preparation routine that included a predip containing 0.1% iodine, after which the dry teat end was disinfected with a cotton pad containing 70% isopropyl alcohol. Intramuscular injections were given in the neck, splitting the dose between two different sites. Tylosin dosage calculation was based in 18mg/kg dose for a 650 kg cow (average weight for mature cows on this commercial farm).

Quarter milk samples were taken following National Mastitis Council guidelines (Hogan et al., 1999) from all functional quarters at dry-off and at 1 and 2 wks after calving. Samples were immediately processed at the dairy's on-farm laboratory. A 10 μL aliquot from each sample was cultured on blood agar containing 5% esculin at 37°C for 24 h. Colonies were tentatively identified as staphylococci, streptococci, *Staphylococcus aureus*, coliforms or others; a presumptive diagnosis of staphylococci, streptococci, coliform, or other pathogens was made, based on colony growth, morphology and appearance, pattern of hemolysis, and catalase reaction. Staphylococcal isolates were tested for coagulase production with the tube coagulase test. Gram-negative bacteria were plated on MacConkey agar to facilitate

identification. Only staphylococci, streptococci and *S. aureus* were included in data analysis based on the assumption that cephapirin and tylosin are indicated to treat only Gram-positive infections. A cow was considered infected if at least one productive quarter had an IMI. A quarter was considered infected if five or more cfu of the same kind were identified, and was considered contaminated if three or more different colony types were present. Post calving samples were used to establish bacterial cure. A cow was considered cured if she had an IMI at dry-off and all productive quarters were negative at both samples post-calving. A cow was not cured if she had an IMI at dry-off and continued with the same quarter(s) infected with the same species at first and second test after calving. A newly infected cow had no IMI at dry-off and at least one quarter was positive at 1 and 2 wk after calving. A quarter was considered cured if it was positive at dry-off and negative in first and second post-calving samples. It was considered not cured if it was positive to the species isolated at dry-off and again 1 and/or 2 wk after calving. A quarter was considered newly infected if it was negative at dry-off and positive at 1 and 2 wk after calving.

Dairy Herd Improvement (DHI) tests were recorded to monitor SCC. The last test of lactation was considered the dry-test, and then linear SCC scores from subsequent monthly tests, were used to determine changes in SCC. Milk production records were collected by DHI personnel and total production was established as projected to 305 days mature equivalent (ME) by a herd management software (DairyComp®, Herd Management software, Valley Agricultural Software, Tulare, CA, USA) based on milk production at DHI test taken between 180 and 200 days in milk (DIM) after calving following the DCT assigned.

Cows with less than 30 d dry were excluded because of antimicrobial residue issues that required milk withdrawal. Cows with more than 100 d dry were also excluded. Of the 335 cows enrolled in the dry cow treatment trial, 278 had complete records and were included in the results. Culture results were analyzed using chi-square and SCC were analyzed by one-way ANOVA proc mixed procedure in SAS® (SAS Institute, 2003. Cary, NC, USA). Significance level was set at alpha=0.05.

Results

A total of 123 cows (44%) had IMI at dry-off. No differences in infection rate were found when comparing all four groups. Percentages of cows with IMI within each treatment group were as follows CESE 40%, TYCESE 39%, TYSE 45% and TY 53%. Cure rates at cow level were significantly higher for group TYCESE (75%) when compared to group TY (46%). Group CESE had a (66%) cure rate and TYSE (51%) and this difference was not significant. A summary of these results is shown in Table 1.

A total of 245 quarters (23% overall) were found infected with staphylococci, streptococci or *S. aureus*. IMI rates by quarters at dry-off are shown in Table 1. Group TY had 32.3% infected quarters and group TYSE had 24.6%, both were significantly higher ($p<0.05$) than TYCESE 17.3% and CESE 19.7%. Staphylococci accounted for 62.9% of the infections, streptococci for 32.6% and *S. aureus* for 4.5%. The staphylococcal infection rate was significantly higher in group TY when compared to TYCESE and TYSE ($p<0.05$) (Table 1). Infections caused by *S aureus* were found in 2 quarters of group CESE, 2 quarters of TYCESE, 6 quarters of TYSE and 1 quarter of TY. With such a small number of infections it was not possible to

establish differences among groups. Further studies are needed to evaluate tylosin efficacy alone or in combination against *S. aureus* IMI.

Bacterial cure rates for gram-positive bacteria by quarters are shown in Table 1. TYCESE had the highest cure rate (93.6%) and was significantly different ($p<0.05$) from TY (78.1%) and CESE (78.9%). No difference was found when TYCESE was compared to Group TYSE (88.2%). New infections rates were low, group CESE (1.8%), group TYCESE (2.6%), group TYSE (2.9%) and group TY (3.3%).

When comparing cure rates by bacteria type (Table 1) all four treatments had good efficacy against streptococcal infections, but there were differences in cure rates for staphylococcal infections. Group TYCESE had higher cure rates (92.5%) ($p<0.05$) than CESE (73.17%) and TY (72.4%).

All four treatments resulted in a decrease in the SCC at the first and second test after calving (Figure 2). When arithmetic means of logSCC (logarithm to base 2 of raw SCC count) at dry-off, first and second test after calving were compared (Fig 3), TYCESE had the greatest decrease in LogSCC between dry-off and first test (1.7) and group TY the lowest (0.5), although differences among groups were not significant.

Milk production at test date between 180 to 200 DIM was analyzed using 305 ME value. Milk production for each treatment group was as follows: TYCESE 10999 ± 2616 kg, CESE 10143 ± 2348 kg, TY 10074 ± 1968 kg and TYSE 10024 ± 1880 kg. When the difference between arithmetic mean of previous and current lactations was calculated for all groups, no significant differences were found.

Discussion

The randomization was effective in distributing IMI among groups at cow level, but was ineffective at quarter level generating a bias, observed in a higher number of quarter infections and a lower cure rate for the TY group. An ideal randomization would have included blocking after culturing quarters but this process would have represented major logistical problems on this commercial farm. However, all four DCTs were effective in reducing IMI.

Results for the combination of intramuscular tylosin plus intramammary cephapirin are similar to the results of O'Boyle et al. (2006). In his study cure rate for Gram-positive IMI was 87% when a combination DCT including tylosin was given subcutaneously. Furthermore, reduction of SCC was greater in group TYCESE, although not significant, similar to O'Boyle's results. There is a greater reduction in SCC when a DCT including systemic tylosin and intramammary cephapirin is used.

Bacterial cure rates for systemic tylosin (group TY 78.1%) are similar to those obtained by McDougall et al. (2007) in New Zealand, who administered tylosin intramuscularly to treat clinical mastitis during lactation and obtained 82% cure rates for Gram-positive infections. Systemic tylosin as DCT is as effective as intramammary cephapirin plus teat sealant (Group CESE 78.9%) to reduce IMI. Tylosin's effectiveness could be related to its distribution properties into the mammary gland and also to its unique intracellular distribution in neutrophils. It is known that udder immunological response is mainly mediated by neutrophils (Paape & Capuco, 1997). Neutrophil antibacterial activity is based on phagocytosis, a process in which lysosomes play a key role. The distribution in cellular organelles of

tilmicosin, another macrolide was described by Scorneaux & Shryock (1999), finding that 70 to 80% of the drug was located in lysosomes. This effect may be similar to that of tylosin by increasing the effectiveness of neutrophils in clearing IMI.

When analyzing cure rate by bacteria type, intramammary treatment alone could have been limited in its ability to eliminate infections during the dry period (CESE 73.2%). Because staphylococci are responsible for 49% of IMI in the dry period (Dingwell et al., 2003), an increase in the cure rate towards this bacteria type obtained by the addition of systemic tylosin to the intramammary DCT would be economically important.

In general DCT remains a cost-effective measure compared with no treatment at dry-off (Berry et al., 2004; Robert et al., 2006; Huijps & Hogeveen, 2007). Inclusion of a combined systemic and intramammary treatment has to be based on an economic analysis of the increased cost of such therapy versus its higher effectiveness. The use of systemic tylosin in combination with the intramammary cephapirin increased the effectiveness of intramammary DCT against Gram-positive IMI. There were no differences between the use of tylosin plus teat sealant and intramammary cephapirin plus teat sealant at dry-off. However, tylosin has the advantage of being cheaper and easy to use. Adding the teat sealant to the systemic treatment with tylosin at dry-off improved the response of the treatment. Further studies are needed to validate this effect in other dairy herds.

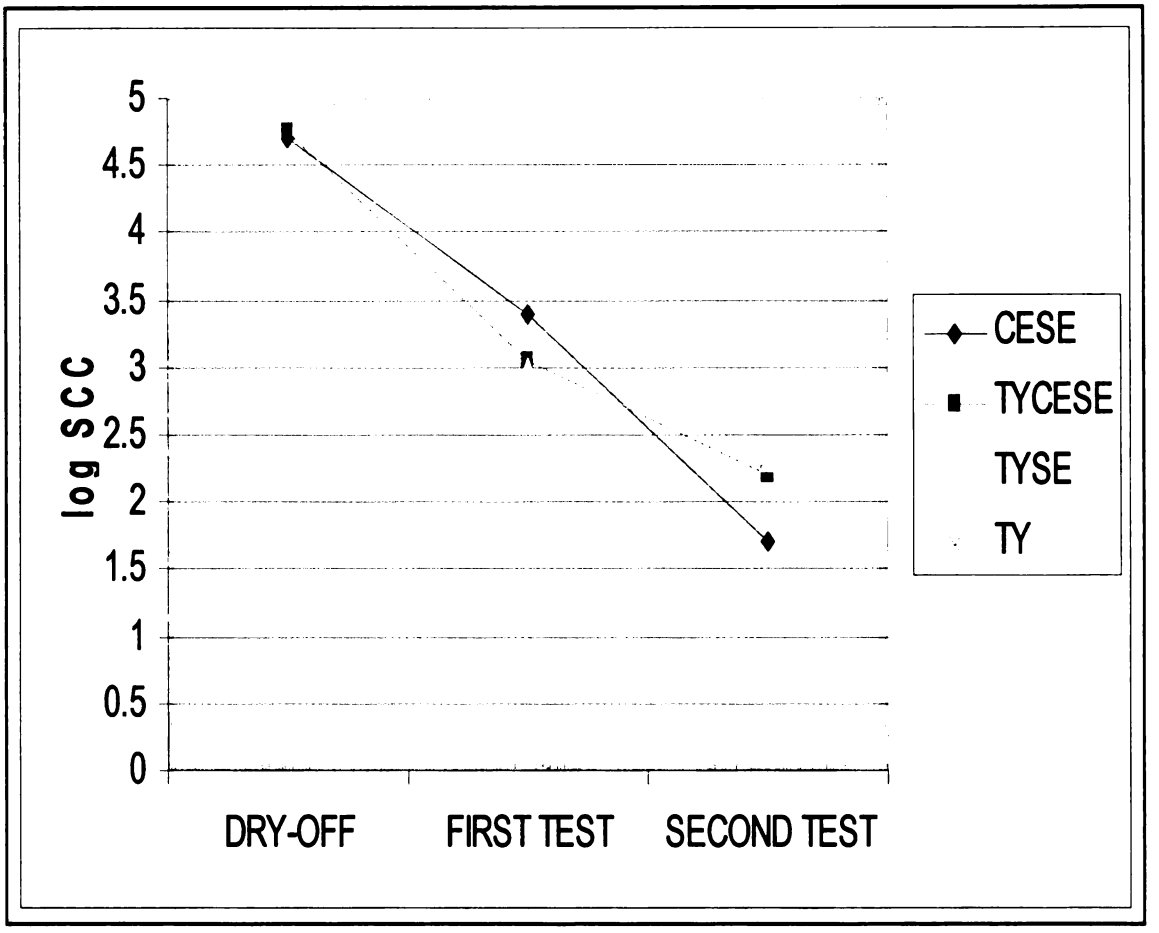

Figure 2. Mean values of logSCC at dry-off, first test and second test after calving for each treatment group.

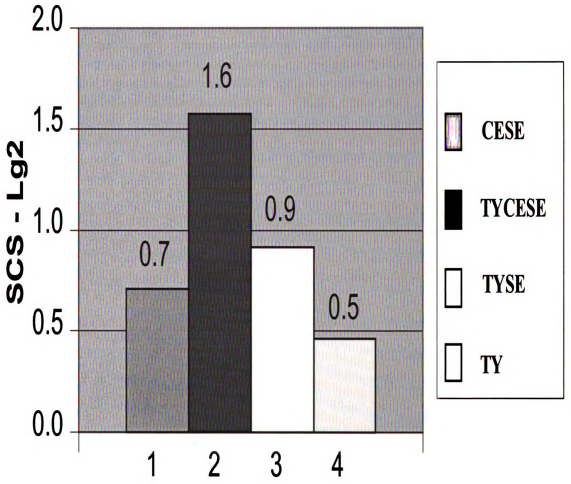

Figure 3. Somatic cell count reduction after calving explained by the difference between the log SCC at DHI dry-off test and log SCC at first DHI test after calving for each treatment

Table 3. Treatment designations, description and number of animals in each treatment group. Number of cows infected at dry-off, percentage of cows with IMI at dry-off. Number of cows cured after DCT and cure rate by treatment group. Number of cows with new IMI after calving. Number of quarters enrolled, non-functional quarters, number of quarters infected and percentage of Gram-positive bacterial infection of total number of quarters at dry-off. Number and percentage of quarters cured after calving. Number and percentage of bacterial infection by bacteria type at Dry-off in each treatment group, and number of quarters cured and cure rate after calving by bacteria type in each treatment. Values with the same letter are not different from each other ($p<0.05$).

	TREATMENT GROUP			
	CESE	TYCESE	TYSE	TY
Treatment description	Cephapirin 300 mg intramammary Teat sealant	Tylosin 12 g intramuscular Cephapirin 300 mg intramammary Teat sealant	Tylosin 12 g intramuscular Teat Sealant	Tylosin 12 g intramuscular
Cows Enrolled	75	71	72	60
Cows with IMI at dry-off	30	28	33	32
% of cows with IMI at dry-off	40%	39%	45%	53%
Cow cured after DCT at dry-off	20	21	17	15
% of cows cured after DCT at dry-off	66%ab	75%a	51%ab	46%b
Cows with new IMI after calving	4	4	6	5
Quarters Enrolled	289	271	276	226
Non-functional quarters	11	13	12	14
Quarters infected at dry-off	57	47	68	73
% of quarters with IMI at dry-off	19.7%b	17.3%b	24.6%a	32.3%a
Quarters cured after DCT at dry-off	45	44	60	57
% of quarters cured after DCT at dry-off	78.9%b	93.6%a	88.2%a	78.1%b
Staphylococci Number of quarters infected at dry-off	41	27	28	58
Staphylococci % quarters infected at dry-off	72%a	57%b	41%b	79%a
Staphylococci % quarters cured after DCT at dry-off	73%a	92%b	89%b	72%a
Streptococci Number of quarters infected at dry-off	14	18	34	14
Streptococci % quarters infected at dry-off	24%	38%	50%	19%
Streptococci % quarters cured after DCT at dry-off	100%	94%	91%	100%
S *aureus* Number of quarters infected at dry-off	2	2	6	1
S *aureus* % quarters infected at dry-off	3%	4%	8%	1%
S *aureus* % quarters cured after DCT at dry off	50%	100%	66%	100%

CHAPTER 3

Prepartum Systemic Antibiotic For Heifer Mastitis

A Practical Approach to Reducing the Incidence of Intramammary Infection in Heifers by Using Prepartum Systemic Tylosin Therapy

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Interpretative Summary

Reducing the incidence of intramammary infections in heifers by using prepartum systemic tylosin. By Contreras and Sears. The effectiveness for 20 g of tylosin administered intramuscularly to prepartum heifers 14 to 18 days before expected calving was analyzed. A control group of heifers with no antibiotic treatment was compared to a tylosin treated group for the prevention of intramammary infection at calving. The use of tylosin reduced the infection rate of intramammary infections due to coagulase negative staphylococci at quarter level. The treatment of heifers prepartum should not be advised without first evaluating udder health, management and economic implications on each individual dairy farm.

Abstract

Intramammary infusion of antibiotics has been studied as a method to prevent and treat intramammary infections (IMI) in heifers. Systemic administration of antibiotics could offer economic advantages related to drug cost and labor. In this study, the effectiveness of intramuscular administration of tylosin (20 g) was assessed. Heifers on a commercial farm in Michigan, due to calve within 14 to 18 d, were assigned randomly to one of two treatment groups. The control group (n=108 heifers) received no antibiotic treatment or teat sealants to prevent IMI. Group tylosin (n=112 heifers) animals were injected intramuscularly with 20 g of tylosin. Quarter milk samples were taken in duplicate from all functional quarters at 2 to 6 days (sample-1) and 7 to 15 days (sample-2) after calving for bacterial culture. Representative isolates from sample 1 were speciated. Somatic cell counts and milk production were recorded. At sample 1, 42% of the heifers, and 16.5% of the quarters were infected. Coagulase negative staphylococci (CNS) infected 10.8% of the quarters and streptococci 3.6%. No antibiotic residues were detected at either sample 1 or sample 2. At the heifer level, tylosin did not reduce IMI infection rate caused by Gram-positive bacteria. At the quarter level, tylosin reduced levels of IMI caused by CNS. No differences were observed in somatic cell count (SCC) and milk production between tylosin treated animals and controls, uninfected heifers had a lower somatic cell score (SCS). Tylosin administration to primigravid heifers 2 weeks before expected calving should not be advised without first evaluating udder health, management and economic implications on each individual dairy farm.

Introduction

While mastitis in heifers is recognized as a problem, most dairymen often view heifers at parturition as free of intramammary infections (IMI) For example, in a survey (Borm et al., 2006) that included farms in 7 states and 1 province, as many as 63% of heifers and 34% of their quarters had an IMI at calving. The predominant pathogen causing IMI in heifers from breeding age to first parturition are the coagulase negative staphylococci (CNS) (Trinidad et al., 1990a; Fox et al., 1995; Millys, 1995; Waage et al., 1999). IMI during lactogenesis can impair mammary development and cause a decrease in milk yield in the productive life at the heifer level. At the herd level IMI could cause an increase in somatic cell counts (SCC) and clinical mastitis cases (Trinidad et al., 1990b).

To prevent and treat IMI in heifers, infusion of antibiotics in the mammary gland before calving was proposed (Trinidad et al., 1990c; Oliver et al., 2003; Born et al., 2006). Intramammary antibiotics are usually effective in reducing IMI, but economic benefits can differ among farms depending on prevalence (Born et al., 2006). Furthermore, intramammary antibiotic infusions in heifers results in additional labor. Antibiotic administration immediately after calving, either intramammary (Oliver et al., 2007) or systemically (Kreiger at al. 2007) was previously attempted, but issues regarding antibiotic residues in milk were reported. Systemic administration to heifers before calving could offer advantages related to its relative lower cost and easier to administer when compared to intramammary infusion. Tylosin has been studied for use in systemic DCT (O'Boyle et al., 2006; Contreras et al., 2007). The major advantage of this antimicrobial is an excellent

diffusion into the mammary gland related to its basic pK (pH at which concentrations of dissociated and undissociated antibiotic are equal), which results in a very high milk to plasma concentration ratio of 5:1 (Omura, 1984; Riviere, 1999). In this study, we analyzed the effectiveness of tylosin injected intramuscularly at a dose of 20 g to prepartum heifers 14 to 18 d before expected calving in reducing IMI.

Materials and methods

Heifers on a commercial farm in Michigan, due to calve within 12 to 18 d, were selected every week between March and July 2007. A completely randomized design that included a systemic antibiotic treatment group and a negative control group was used. Before randomization, animals were properly identified and their gestational stage was confirmed. Heifers then were assigned randomly to one of two treatment groups. The control group (n=108 heifers), reflecting standard practice on the farm, received no antibiotic treatment or teat sealants to prevent IMI. Group tylosin (n=112 heifers) animals received 20 g of tylosin (Elanco, Greenfield, IN). Injections were administered intramuscularly in the neck and the 20 g dose was divided between three different sites.

Quarter milk samples were taken in duplicate following National Mastitis Council guidelines from all functional quarters at 2 to 6 d (sample-1) and 7 to 15 d (sample-2) after calving. Samples were stored in a container at 5°C and processed within 3h after collected. A 10 μL aliquot from each sample was cultured on blood agar containing 0.1% esculin at 37°C for 24h. Colonies were tentatively identified at 24h and 48h as CNS, streptococci, *Staphylococcus aureus*, coliform or others. A presumptive diagnosis was made based on colony growth, morphology and

appearance, pattern of hemolysis, and catalase reaction. Staphylococcal isolates were tested for coagulase production using the tube coagulase test. Gram-negative bacteria were plated on MacConkey agar to facilitate identification. For speciation, biochemical tests were performed on representative positive cultures from sample-1 of streptococci and staphylococci isolates by using the API20 Strep SYSTEM and the API-Staph SYSTEM, (BioMerieux, Hazelwood, MO). A heifer was considered infected if at least 1 quarter was infected at sample-1; considered newly infected if it was negative for all quarters at sample-1 but infected at sample-2; and was spontaneously cured if it was infected at sample-1 but negative at sample-2. Quarters were considered infected if at sample-1, five or more cfu of the same kind were identified, and were considered contaminated if three or more different colony types were present on both duplicate samples. A new infection was considered when a quarter was negative at sample 1 but was positive at sample-2. A quarter was considered spontaneously cured if it was infected at sample 1 but negative at sample-2.

Two composite milk (3ml) samples were collected during sampling-1 and 2. One of the samples was used to perform antibiotic residue test with the Delvotest® (DMS food specialties, Parsippany, NJ) and the second sample was used to determine SCC at the regional Dairy Herd Improvement (DHI) laboratory. Dairy Herd Improvement tests were used to monitor SCC and milk production during lactation. Milk production records were collected by DHI personnel and total production was calculated as projected to 305 d mature equivalent (ME) by a herd management

software based on milk production at DHI test taken between 180 and 200 d in milk (DIM) after calving.

Minimal inhibition concentration (MIC) values for the following representative isolates were determined using the Sensititre® system (Trek® diagnostics, Cleveland,OH): 6 *Streptococci uberis,* 6 *Enterococcus faecalis,* 10 *Staphylococcus chromogenes*, and 10 *Staphylococcus simulans.* The following antimicrobials were included in the Sensititre® plate format: ampicillin, ceftiofur, chlortetracycline, clindamycin, danofloxacin, enrofloxacin, gentamicin, neomycin, oxytetracycline, penicillin, spectinomycin, sulphadimethoxime, trimethropin/sulphamethoxazole, and tylosin.

Culture results were analyzed using chi-square and SCC scores were analyzed as repeated measures using a mixed model procedure (PROC MIXED; SAS Inst. Inc., Cary NC) following the equation:

$$Yijk = \mu + Gi + Ij + Gi * Ij + Sk + Sk * Gi + Sk * Ij + Sk * Gi * Ij + Eijk$$

Where Y_{ijk} is the dependent variable SCC score for a heifer in group *i,* with infection status *j,* at sample *k* as repeated measure, and E_{ijk} is the random error assumed to be correlated. The Satterthwaite's method for estimating degrees of freedom was used. Significance level was set at alpha=0.05.

Results and discussion

Infection levels for heifer and quarter are shown in Table 4. A total of 92 (42%) heifers were infected at calving. At sample-1, 50 (46%) heifers were infected at sample-1 in the control group and 42 (38%) in the tylosin group had an IMI. By the second sampling 76 (34.5%) heifers were infected, 43 (40%) in the control group and

33 (29%) in the tylosin group. This prevalence is similar to previous observations (Trinidad et al., 1990;Born et al., 2006). Spontaneous cures were observed in 19 heifers (38%) for control group and 21 animals (50%) in tylosin group. In both groups, 11% of the heifers acquired a new IMI in the first week after calving. At the heifer level, tylosin administration to prepartum heifers in this farm did not reduce IMI caused by gram-positive bacteria.

At the first sampling (Table 4), 145/879 quarters were infected. At sample 2, 97/879 (11.2%) quarters had an IMI. Quarter spontaneous cure rate for tylosin group was 61% and 50% for control group. New infections accounted for 5% of the quarters and no statistical difference was found between groups (Table 4).

Similar to previous studies, CNS were the principal agent causing IMI in heifers (Trinidad et al. 1990b; Fox et al., 1995; Oliver et al., 2004). The tylosin group had fewer CNS infections in sample-1, 36 quarters (8.1%) than the control group 59 quarters (13.5%) and this difference was significant ($P<0.01$), demonstrating that the high efficacy of tylosin against CNS, could have decreased infection rate due to these microbes (Table 4). At sample-2, CNS infected 25 quarters (5.6%) in tylosin group and 47 quarters (10.7%) in the controls, this difference was significant also ($P<0.01$), but could be related to the initial lower prevalence of CNS infection in tylosin group at sample-1.

Spontaneous cure for CNS was 61% for tylosin and 49% for controls. New infections accounted for 2.9% of quarters in tylosin group and 4.1% for the control group. Further studies are needed to evaluate economic and physiological

implications of transient infections of CNS in the first week of lactation in heifers, since at least half of the CNS infections will clear without antibiotic intervention.

Bacterial speciation results are shown in Table 5. Among CNS *Staphylococcus chromogens* -a natural inhabitant of teats skin flora, *S. simulans* and *S. epidermis* are highly prevalent around calving (Aarestrup and Jensen, 1997). Aarestrup and Jensen (1997) reported, a higher prevalence of *S. Chromogenes* (15% of the quarters) in the first two wk after calving, reflecting the normal presence of this species on the skin. Similar to their results, *S. chromogenes* was the most prevalent species among CNS in this study, representing 41.2% of CNS infections. In contrast, *S. simulans* affected 25% of the quarters in this trial, and only 3% in Aarestrup's trials (1997) but were persistent after several weeks after calving.

Streptococci infections made up 32/145 (22%) of all IMI after calving. Over all streptococci infected 3.6% of the quarters at sample-1 and 2% at sample-2. Streptococcal infection rate was equal for both groups (3.6%) at sample-1, but was slightly higher at sample-2 for tylosin (2.9%) than for control (1.1%). *Streptococcus uberis* was the most prevalent streptococcal infection, followed by *Strep. dysgalactiae.* In contrast, studies with pasture-grazed heifers in New Zealand (Compton at al., 2006) revealed a higher infection rate by *Strep. uberis* (10% of the quarters) in the first week after calving, reflecting changes in IMI prevalence due to housing systems. Spontaneous cures for streptococcal infection accounted for 43% of the quarters in the tylosin group and 56% in the control group. Four new infections were found in the tylosin group, and none in the control group. Contagious

pathogens, *Streptococcus agalactiae* and *Staphylococci aureus* were absent from the study animals.

Effectiveness of systemic treatment with tylosin varies depending dose and time interval between administration and calving. Parker et al. (2008) using pasture-grazed heifers in New Zealand, compared a commercial teat sealant, tylosin (5 g of tylosin base I.M. for 3 d at 24-h intervals) or a combination of both. Teat sealant reduced the risk of new IMI by 74% and reduced the risk on new IMI with *Strep uberis* by 70%. Tylosin did not have any effect in reducing risks. Differences in responses to tylosin treatment between this study and Parker's (2008) could have been related to the relative low dose used in New Zealand and timing of treatment (27 d before expected calving). A higher dose with a single injection was used in this trial, and the treatment was administered close to the programmed calving date (14 d). Tylosin's peak milk concentration was reported to be 10 μg/ml after a single injection at a dose of 20 mg/kg b.w.) (Ziv and Sulman, 1973), and 18 μg/ml after three repeated injections at a dose of 10 mg/kg b.w. (El-Sayed et al. 1986). In the present work, tylosin's dose was based on average calving weight for heifers (600kg) at 33mg/kg. Despite of the relative high dose used, antibiotic residues were not found in the composite samples taken from sample-1 and sample-2.

It is presumable that peak milk concentration in tylosin treated animals was higher than previously reported, therefore achieving bactericidal activity, which was observed for macrolides when used in high concentrations (Diarra et al, 1999). However, tylosin activity against enterococci was poor, and although had an MIC_{90}

0.5 μg/ml, for *Strep uberis*„ enterococci isolates were resistant (MIC_{90} 32 μg/ml) to this macrolide (Table 6).

It is unknown if the use of tylosin as a systemic treatment to eliminate or prevent IMI in heifers at calving is dose and time sensitive, thus further studies are needed to evaluate of extended therapy at different doses with this macrolide to improve its response. Because of the studies logistic limitations, multiple administrations and dosage based on individual weight was not possible.

Quarter infection rate by season is summarized in Table 8. No differences were observed for CNS infections in spring at any sampling for both groups. In contrast, tylosin treated heifers had fewer infections caused by CNS in summer when comparing to controls at sample-1 and sample-2 and this difference was significant ($P<0.05$). The use of systemic treatment might have a useful role in decreasing CNS infections during warmer months.

Log scores for SCC results are shown in Figure 4. Treatment effect was not a significant ($P=0.17$) source of SCS variation. At sample-1, tylosin group had a higher score (4.2±3) than control group (3.7±2.3), at sample-2, 1st and 2nd DHI tests both groups had similar means for the somatic cell scores. As expected, IMI status had a significant effect in SCS ($P<0.0001$). Infected heifers had a higher SCS than non-infected. When separating IMI by bacteria type for SCS results (Figure 5), CNS infected heifers had similar SCS values to animals infected with other pathogens. Despite of the high spontaneous cure rates for CNS, the infection status was still responsible of increased SCS.

Tylosin group had an average milk projection at 305 d of 9,515±1847 at the first test and 10,556±1790 at the second test. Similarly the control group had 9,495±1658 at the first and 10,346± by the second test. No difference in milk yield was found between the two groups.

Conclusion

Systemic administration of tylosin was effective in reducing levels of IMI in heifers caused by CNS at quarter level, but routine use on this farm may not be economically sound. Transient IMI occur early in lactations with at least half of the infections cleared without antibiotic intervention. Tylosin, administration to primigravid heifers 2 wk before parturition, should not be advised without a previous analysis of udder health, management, and economic implications in each individual farm. Furthermore, in this study 10% of the heifers acquire an IMI within the first week after calving, therefore special attention has to be given to environmental and housing issues. Antibiotic therapy will not replace poor management in a close-up, calving or fresh pens.

Table 4. Culture results at heifer level and quarter level at sample-1 and sample-2 including percent of spontaneous cure and percent of new infections, for control and tylosin groups. Staphylococci and streptococci infections at sample-1 and sample-2 for control and tylosin groups, including spontaneous cure and new infections. Percentages with different letters are considered to be different at P<0.05.

	PARAMETER	CONTROL	TYLOSIN	TOTAL	P value
	HEIFERS	108	112	220	
	IMI 2- 6 DAYS	50	42	92	0.18
	% INFECTED HEIFERS SAMPLE 1	46%	38%	42%	
HEIFER	IMI 7-14 DAYS	43	33	76	0.10
LEVEL	%INFECTED HEIFERS SAMPLE 2	40%	29%	34.5%	
	SPONTANEOUS CURE	19	21	40	0.24
	%SPONTANEOUS CURE	38%	50%	44%	
	NEW INFECTIONS	12	12	24	0.60
	% NEW INFECTIONS	11%	11%	11%	
	QUARTERS	436	443	879	
	IMI 2-6 DAYS	80	65	145	0.14
QUARTER	%INFECTED QUARTERS SAMPLE 1	18%	14.5%	16.5%	
LEVEL	IMI 7 - 14 DAYS	54	43	97	0.20
	%INFECTED QUARTERS SAMPLE 2	12.3%	9.7%	11%	
	SPONTANEOUS CURE	40	40	80	0.16
	%SPONTANEOUS CURE	50%	61%	55%	
	NEW INFECTIONS	18	18	36	0.85
	% NEW INFECTIONS	5%	5%	5%	
STAPH	QUARTERS	436	443	879	
INF	IMI 2-6 DAYS	59	36	95	0.009
	%INFECTED QUARTERS SAMPLE 1	13.5%a	8.1%b	10.8%	
	IMI 7 - 14 DAYS	47	25	72	0.005
	%INFECTED QUARTERS SAMPLE 2	10.7%a	5.6%b	8.2%	
	SPONTANEOUS CURE	29	22	51	0.9
	%SPONTANEOUS CURE	49%	61%	70%	
	NEW INFECTIONS	18	12	30	0.35
	% NEW INFECTIONS	4.1 %	2.9%		
STREP	QUARTERS	436	443	879	
INF	IMI 2-6 DAYS	16	16	32	0.96
	%INFECTED QUARTERS SAMPLE 1	3.6%	3.6%	3.6%	
	IMI 7 - 14 DAYS	5	13	18	0.06
	%INFECTED QUARTERS SAMPLE 2	1.1%	2.9%	2%	
	SPONTANEOUS CURE	9	7	16	0.47
	%SPONTANEOUS CURE	56%	43%	50%	
	NEW INFECTIONS	0	4	4	0.055

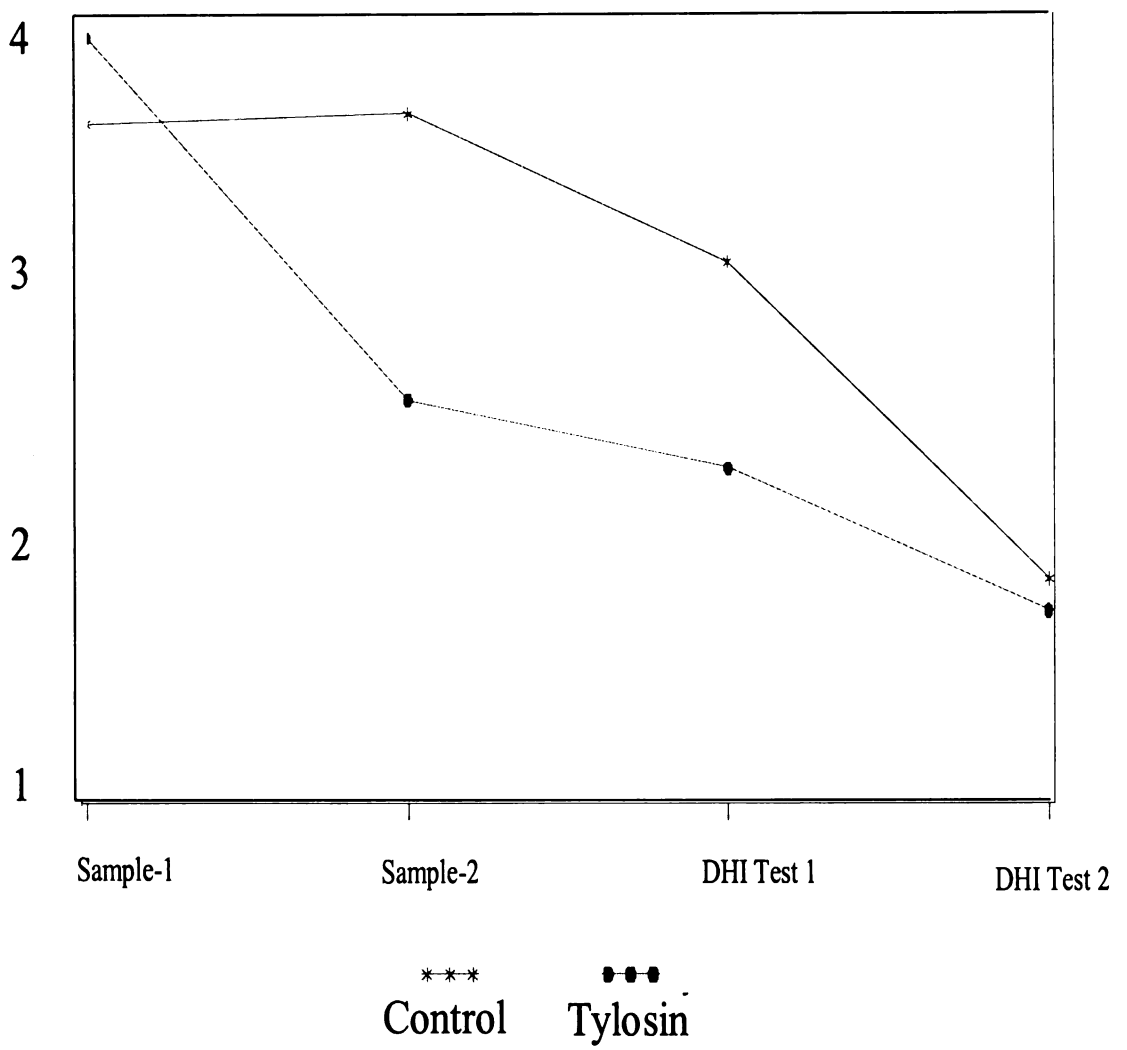

Figure 4. Means for somatic cell scores (SCS) at sample-1 and sample-2, SCS at 1st and 2nd DHI tests after calving, and projected 305d ME milk production at 1st and 2nd DHI tests for control and tylosin group.

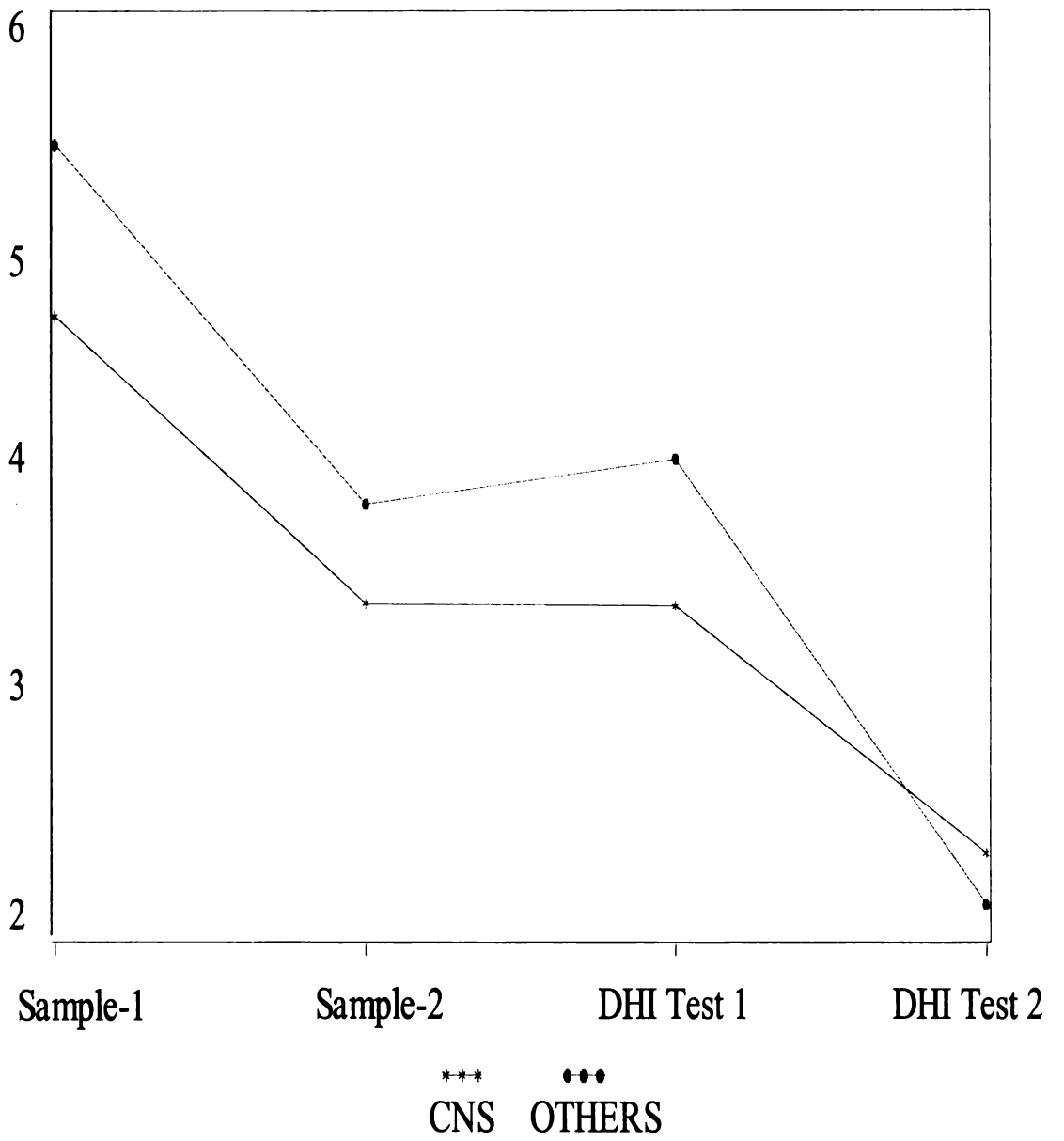

Figure 5. Means for somatic cell scores (SCS) at sample-1 and sample-2, SCS at 1st and 2nd DHI tests after calving, and projected 305d ME milk production at 1st and 2nd DHI tests for infected heifers. CNS and others

Table 5. Minimum inhibitory concentrations (MIC) of ampicillin (AMP), ceftiofur (CF), enrofloxacin (ENR), neomycin (NEO), penicillin (PEN) and tylosin (TY) for *S. chromogenes, S. simulans, Streptococci uberis, and Enterococcus faecalis.*

Organism	N		AMP	CF	ENR	NEO	PEN	TY
C_{max} Milk		μg/ml	3.4	214.7*	2.7	222*	0.385	10
S. chromogenes	10	MIC50	0.25	0.5	0.12	4	0.12	1
		MIC90	0.25	0.5	0.12	4	0.12	1
		MODE	0.25	0.5	0.12	4	0.12	1
		RANGE	<=0.2 5-16	0.25-0.5	0.12-0.5	4	0.12-8	0.5-2
S. simulans	10	MIC50	1	0.5	0.12	4	0.12	0.5
		MIC90	4	0.5	0.5	8	8	6
		MODE	0.25	0.5	0.12	4	0.12	0.5
		RANGE	<=0.2 5-16	0.25-1	0.12-2	4-8	0.12-8	0.5-32
Streptococci uberis	6	MIC50	16	0.25	0.5	16	0.5	0.5
		MIC90	16	0.25	0.5	16	8	0.5
		MODE	0.25	0.25	0.12	16	8	0.5
		RANGE	0.25-16	0.25	0.25-0.5	4-16	0.5-8	0.5-32
Enterococcus faecalis	6	MIC50	0.25	0.25	.5	8	.25	32
		MIC90	1	8	1	32	2	32
		MODE	0.25	0.25	1	8	0.25	32
		RANGE	<=0.2 5-1.0	<=0.25-8	0.25-1	5-32	0.25-4	32

*CF intramammary infusion
*NEO intramammary infusion

Table 6. Speciation of representative isolates at sample 1, from cultures at sample 1 for control and tylosin groups.

BACTERIA	TYLOSIN	CONTROL	TOTAL	%
S. Chromogenes	16	12	28	41.2%
S. Simulans	7	10	17	25%
S.Epidermis	0	1	1	1.5%
S. Xylosus	1	1	2	2.9%
Strep. Uberis	7	3	10	14.7%
Strep. Bovis	2	0	2	2.9%
Strep. dysgalactiae	3	1	4	5.9%
E. Faecalis	2	2	4	5.9%

Table 7. Culture results for tylosin and control groups at sample 1 and sample 2 in summer and spring seasons.

Sample	Season	Tylosin			Control			P-value CNS
	Culture result	Neg	Staph	Strep	Neg	Staph	Strep	
1	Spring	160	16	6	85	23	5	0.09
	Summer	221	20	10	174	36	11	0.04
2	Spring	162	15	4	269	22	3	0.08
	Summer	211	10	9	190	25	2	0.01

CHAPTER 4

Conclusion

Dairy cows have changed dramatically in the past 60 years with the average milk production increasing from 4622 pounds in 1950 to 17800 pounds per year in 2000 (Lake, 1971; US census, 2002). At the same time susceptibility to some infectious and metabolic diseases has increased proportionally (LeBlanc et al., 2006). There is a decline in number of dairy farms and animals are being concentrated in larger herds, where efficiency indexes have improved, but cows are put at a high risk for disease. Although major dairying developments like dry cow therapy, teat dips, and better milking systems have helped improve udder health, some things have not changed from the 1950's with mastitis still accounting for the single greatest economic loss (Lake, 1971; Wells and Ott, 1998).

To prevent and treat mastitis, antibiotic therapy during the non-lactating period was introduced in the 1950's for cows (Pearson, 1950) and for heifers in the 1990's (Trinidad et al., 1990c). Recently, issues regarding reduced effectiveness of DCT against certain pathogens like CNS were reported (Rajala-Schultz et al. 2004) revealing the need to improve efficacy by not only changing antibiotics but also the way they are administered. Although precalving heifer treatment (PCHT) using intramammary infusion is effective in reducing IMI, it is labor intensive and not practical in most dairy herds. This has stimulated discussion of alternative approaches to reduce IMI in heifers.

The use of tylosin in DCT offers the advantages of better diffusion into the mammary gland, increased effectiveness against CNS, a relatively low cost and a synergistic effect when used in combination with other antibiotics. If included in PCHT, tylosin administered systemically is less labor intensive than intramammary infusion. In this thesis, we evaluated the use of systemic tylosin (12 g) in DCT alone or in combination with intramammary cephapirin. We also evaluated the administration of systemic tylosin at a dose of 20 g in PCHT.

The use of systemic tylosin in combination with intramammary cephapirin increased the effectiveness of intramammary DCT against Gram-positive IMI, specifically CNS. Systemic tylosin plus teat sealant was as effective as cephapirin plus teat sealant in curing Gram-positive IMI. Similar to results observed in DCT, tylosin reduced the prevalence of IMI caused by CNS in the first week after calving when administered to heifers 14 to 18 days before expected calving.

Each farm has geographical and environmental conditions that when combined with different management styles make them unique. Systemic use of tylosin can help improve udder health in some herds, but should not be advised for all farms. Its use as a systemic antibiotic alone or in combination with other drugs in DCT or PCHT cannot be routinely advised. Tylosin is very effective against CNS, but antimicrobial activity against enterococci and some streptococci can vary. Since pasture grazed herds have a higher incidence of streptococcal infections (Parker et al, 2007), tylosin administration might not be as effective as in confined operations. Special attention has to be given to housing conditions for transition cows, fresh cows

and heifers. In the heifer experiment, a high rate of new infections was observed during the first week of lactation, demonstrating a higher IMI susceptibility during this early stage of lactation.

Further studies are required to test tylosin's effectiveness against contagious pathogens such as *S. aureus* and *Strep. agalactiae*. Although tylosin interactions with teat sealant were evaluated recently in heifers (Parker et al., 2008), an analysis of such interactions is required in dry cows. During the heifer trial a high rate of new IMI during the first week of lactation was observed. Furthermore, this high prevalence of IMI was followed by a similar high rate of spontaneous elimination by the second week of lactation. Additional studies are needed to evaluate the implications of this transient IMI in udder health and its economic impact.

The dairy industry must adapt to the changing requirements of the consumers. Quality dairy products are possible because of high quality milk produced on well-managed farms, where animal nutrition, preventive medicine and a good cow environment is employed. Tylosin is just another tool that may have its place on some farms as a part of their udder health program. The decision to use it has to be based on sound evaluations of environment, management and economics for the farm.

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