

HEIFER MASTITIS: PREVALENCE, RISK FACTORS AND CONTROL STRATEGIES

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Introduction

Intramammary infections (IMI) in unbred and pregnant heifers were once thought to be very low. However, during the last two decades, several studies have clearly shown that IMI in heifers occur frequently during the prepartum and peripartum periods. Many of these infections can persist for long periods of time, are associated with elevated somatic cell counts (SCC), and may impair mammary development and affect milk production after calving. The purpose of this communication is to review literature published on the prevalence of mastitis in heifers, potential risk factors associated with heifer mastitis, and to describe results of different approaches that have been taken to control mastitis in heifers.

Prevalence of IMI in Heifers

Mastitis in heifers was first recognized over 60 years ago (Palmer et al., 1941; Schalm, 1942). However, IMI in unbred and pregnant heifers were thought to be very low. Over 20 years ago, Oliver and Mitchell (1983) showed that a high percentage of pregnant heifer mammary glands were infected during late gestation, at calving and during early lactation. During the last two decades, several additional studies on the prevalence of mastitis in heifers have been published. All of these studies suggest that IMI in heifers during the prepartum period occur frequently. However, marked herd variation in the rate of IMI and types of pathogens causing IMI have been reported (Aerstrup and Jensen, 1997; Fox et al., 1995; Jonsson et al., 1991; Matthews et al., 1992; Myllys, 1995; Nickerson et al., 1995; Oliver, 1987; 1988; Oliver and Sordillo, 1988; Oliver et al., 1992; Pankey et al., 1991; Smith et al., 1994; Trinidad et al., 1990a; Waage et al. 1999).

Trinidad et al. (1990a) demonstrated that the prevalence of IMI in unbred heifers and heifers during different stages of pregnancy was very high. Unbred heifers had a higher percentage (86.7%) of infected quarters compared with the overall mean for pregnant heifers (70%). *Staphylococcus* species were observed most frequently and 8 different species were isolated. The three most common species isolated from unbred and pregnant heifer mammary glands were *Staphylococcus chromogenes*, *Staphylococcus hyicus* and *Staphylococcus aureus*. Coagulase-negative *Staphylococcus* species (CNS) accounted for 67.4% of bacteria isolated. Mammary secretions from infected mammary glands had significantly higher SCC than secretions from uninfected mammary quarters. In addition, tissue from mammary glands of unbred heifers infected with CNS exhibited greater leukocyte infiltration and increased connective tissue compared with tissue from uninfected mammary glands (Trinidad et al., 1990b). Thus, infection of heifer mammary glands by mastitis pathogens can occur at a very early age and some of these infections may impair mammary growth and development and influence future milk production.

Pankey et al. (1991) reported that approximately 46% of heifers and 19% of quarters were infected during early lactation based on duplicate samples obtained from 382 heifers within 3 days after calving. CNS were the most prevalent bacteria isolated and were found in 22.8% of heifers and 11.4% of quarters. Matthews et al. (1992) indicated that 35.5% of colostrum samples were positive for 7 different *Staphylococcus* species. Species isolated most frequently were *Staph. chromogenes*, *Staph. aureus* and *Staph. simulans*. *Staphylococcus* species were isolated from about 18% of heifer mammary glands weekly for the first 5 weeks of lactation. Oliver and Sordillo (1988) showed that 19.7% of heifer mammary glands (59 of 300) were infected at calving and CNS caused 71.2% of these IMI. During early lactation, 15.7% of heifer mammary glands (47 of 300) were infected and 48.9% were due to CNS. Thus, the number of mammary quarters infected with CNS decreased significantly from calving to early lactation suggesting that some CNS isolated from heifer mammary glands were either colonizing the teat duct and subsequently eliminated as a result of the milking procedure or that a high rate of spontaneous elimination occurred. Similar findings were reported by Harmon et al. (1986) and Oliver (1988) in multiparous cows.

Oliver et al. (1992) conducted a study to determine the prevalence of mastitis and types of pathogens causing IMI in pregnant Jersey heifers prior to calving and during early lactation. This study was conducted in a herd that was *Streptococcus agalactiae*-negative and had a low prevalence of *Staph. aureus*. This pattern of infection would be typical of many dairy herds that practice postmilking teat disinfection and antibiotic dry cow therapy. Heifers (n=115) were sampled 7 days before expected calving, and 3 (C+3) and 10 (C+10) days after calving. About 90% of heifers and 61% of quarters were infected during the prepartum period. The majority of IMI (243 of 279) were due to CNS. This is higher than what we observed previously in a study conducted in another herd (Oliver and Mitchell, 1983; Oliver and Sordillo, 1988), but types of mastitis pathogens isolated were similar. Trinidad et al. (1990a) also observed considerable herd-to-herd variation both in prevalence of IMI and mastitis pathogens causing IMI in unbred and pregnant heifers. For example, in one herd, 44.3% of quarters were uninfected, 12.3% were infected with *Staph. aureus*, 41.5% were infected with CNS and 1.9% were infected with streptococci other than *Strep. agalactiae*. In another herd, 17.6% of quarters were uninfected, 23.1% were infected with *Staph. aureus*, 49.5% were infected with CNS and 9.9% were infected with *Streptococcus* species. In our studies (Oliver and Mitchell, 1983; Oliver, 1987; Oliver et al., 1992; Oliver et al., 1997a; Oliver et al. 2003a), CNS were isolated most frequently followed by environmental mastitis pathogens primarily *Streptococcus* species.

Fox et al. (1995) reported on a large survey of 28 dairies in four states to determine the prevalence of IMI in unbred and pregnant dairy heifers and to determine potential factors that influenced herd variation. Most IMI were due to CNS and *Staph. aureus*. Location, herd, season, and trimester of pregnancy significantly influenced prevalence of IMI in heifers. Heifers in the third trimester of pregnancy had the highest prevalence of IMI.

Myllys (1995) indicated that CNS were the most frequently (57.8%) isolated bacteria from mammary secretions obtained from 200 heifers with mastitis and from 65 non-mastitic control heifers followed by *Staph. aureus* (20.1%), and streptococci (11.3%). *Staphylococcus simulans*, *Staph. hyicus*, *Staph. xylosus*, and *Staph. chromogenes* were frequently found in milk from heifers with clinical mastitis after calving, whereas other CNS were equally or more often found in non-mastitic control heifers.

Aaerstrup and Jensen (1997) reported that *Staph. chromogenes* was isolated from 15% of all mammary quarters and was the most commonly found bacterial species in heifer mammary secretions obtained before parturition. However, *Staph. chromogenes* IMI decreased shortly after parturition to around 1% of mammary quarters. Infections caused by *Staph. simulans* and *Staph. epidermidis* occurred in 1 to 3% of mammary quarters both before and after parturition. *Staphylococcus simulans* IMI persisted for several weeks while *Staph. epidermidis* IMI tended to be more transient. *Streptococcus dysgalactiae* subsp. *dysgalactiae* (*Strep. dysgalactiae*) was isolated from 4 to 6% of mammary quarters before and immediately after calving, and the prevalence of *Strep. dysgalactiae* decreased during early lactation. Infections due to *Staph. aureus* were rarely observed before calving, but the rate of *Staph. aureus* IMI increased greatly the first week after calving. The presence of an IMI in a mammary quarter before parturition increased the risk of IMI for the lactating cow (Aaerstrup and Jensen, 1997).

One common denominator of all studies on heifer mastitis is the high prevalence of CNS IMI. Thus, CNS will likely cause the majority of IMI in unbred and pregnant heifers and variation in the prevalence of CNS IMI in heifers should be expected among herds. The designation coagulase-negative staphylococci or CNS is loosely used to include all the staphylococci and micrococci isolated from milk samples that are not *Staph. aureus*. As a rule they are coagulase-negative, however, there are exceptions. The commonly isolated CNS are part of the normal skin flora and include the species *Staph. simulans*, *Staph. hyicus*, and *Staph. epidermidis*. In contrast, novobiocin-resistant species (*Staph. xylosus*, *Staph. saprophyticus*, *Staph. sciuri*, and *Staph. cohnii*) are found free-living in the environment. The CNS appear to be opportunists and infect the teat canal and gland from skin sources. Infections by novobiocin-resistant species may originate from the environment. *Staphylococcus chromogenes* and *Staph. hyicus* appear to readily colonize the teat canal and may persist for longer periods of time than the other CNS. Many CNS infections are transient and cow to cow spread is thought to be a low risk for infection (Oliver et al., 2004a). *Staphylococcus chromogenes* was isolated most frequently in three separate studies (Aaerstrup and Jensen, 1997; Matthews et al., 1992; Trinidad et al., 1990a). However, isolation of other CNS varied considerably. For example, *Staph. simulans* was isolated frequently in studies by Aaerstrup and Jensen (1997) and Matthews et al. (1992) while *Staph. hyicus* was isolated frequently by Trinidad et al. (1990a). Thus, while CNS are often grouped together, considerable variation in the frequency of CNS isolation between herds has been reported and it is possible that some CNS may be more problematic than others.

Similarly, the prevalence of mastitis pathogens other than CNS also varies considerably. In our studies (Oliver, 1987; Oliver et al., 1992; Oliver et al., 1997a; Oliver et al., 2003a), 8 to 10% of heifer mammary glands were infected by environmental mastitis pathogens, primarily *Streptococcus* species, which was consistent with the pattern of IMI in lactating cows in these herds. Conversely, other studies (Fox et al., 1995; Trinidad et al., 1990a) indicated that *Staph. aureus* was the most prevalent major mastitis pathogen isolated from unbred and pregnant heifer mammary glands. Differences in the incidence of IMI and types of bacteria causing IMI in pregnant heifers is likely due to the prevalence of mastitis pathogens in the herds evaluated. Thus, a reasonable hypothesis is that heifers from herds with a high prevalence of contagious mastitis will likely be infected predominantly by contagious mastitis pathogens. Similarly, environmental mastitis pathogens will likely be the predominant major pathogens isolated from heifer mammary glands from herds with an environmental mastitis problem.

Some IMI in heifers result in clinical mastitis during the prepartum period and during early lactation. Nickerson et al. (1995) indicated that 29% of heifers and 15% of mammary quarters exhibited clinical mastitis at breeding age as evidenced by clots or flakes in mammary secretions. *Streptococcus dysgalactiae* and *Strep. uberis* were isolated from 34.4% and 19.5%, respectively, of heifers with clinical mastitis occurring from puberty up to 14 days after calving in a large study involving bacterial analyses of 2069 udder secretions isolated from 1481 heifers with clinical mastitis in Sweden (Jonsson et al., 1991). Bacterial species generally regarded as important pathogens in the summer mastitis complex including *Actinomyces (Arcanobacterium) pyogenes*, Stuart-Schwan coccus and strictly anaerobic bacteria such as *Peptostreptococcus indolicus*, *Fusobacterium necrophorum* and *Bacteroides melaninogenicus*, were isolated at low frequencies (13.2, 6.3, 9.4, 3.8, and 1.3%, respectively). When cases of clinical mastitis were restricted to those appearing in heifers prepartum during the summer mastitis season (May 15 to October 14), these bacterial species were isolated at higher percentages (27.1, 14.4, 21.4, 13.5, and 5.2%, respectively). There were no significant differences in the frequency of *A. pyogenes* isolated during different seasons of the year. There were geographical differences in bacterial incidence, e.g. *Staph. aureus* was isolated significantly more often in northern regions whereas *Strep. dysgalactiae* was more common in the south. These data support the theory that *A. pyogenes* and strictly anaerobic bacteria are 'secondary invaders' that depend on *Strep. dysgalactiae* to cause a primary infection. Authors stressed that udders of all heifers should be examined daily so that cases of mastitis can be treated immediately (Jonsson et al., 1991).

More recently, Waage et al. (1999) reported results of a one-year field investigation of clinical mastitis in heifers in Norway. The study included 1,361 cases of clinical mastitis in 1,040 heifers that occurred prepartum or within 14 days after calving. Mastitis pathogens isolated most frequently from mammary quarters with clinical mastitis were *Staph. aureus* (44.3%), *Strep. dysgalactiae* (18.2%), *Staph. aureus* together with *Strep. dysgalactiae* (1.2%), CNS (12.8%), *Arcanobacterium pyogenes* (3.5%), *A. pyogenes* together with *Strep. dysgalactiae* (0.5%) or *Staph. aureus* (0.4%), and *Escherichia coli* (6.4%). Of the CNS, *Staph. simulans* (53.7%), *Staph. hyicus* (14.8%), and *Staph. chromogenes* (14.8%) were the most prevalent species. Except for a higher relative percentage of *A. pyogenes* in cases that occurred before parturition (8.2%) than in cases that occurred after parturition (2.7%), no significant differences were observed in the distribution of the various organisms among prepartum and postpartum cases. Regional variations were observed in the distribution of organisms. *Staphylococcus aureus* and *A. pyogenes* clinical mastitis were highest in late autumn and early winter, CNS clinical mastitis was lowest in late autumn and early winter, and *E. coli* clinical mastitis was highest in the summer.

Heifer Mastitis Risk Factors

Several potential heifer mastitis risk factors have been identified. In an epidemiological survey on 171 dairy farms from five regions of Spain, Martin-Richard et al. (2001) found that risk factors for heifer mastitis were calving in summer, high herd SCC, presence of *Staph. aureus* and *Mycoplasma spp.*, absence of fly control, feeding calves mastitic milk, contact among calves, absence of antibiotic therapy to heifers, contact with adult cows, inadequate milking practices, and poor housing conditions. Other heifer mastitis risk factors identified include an increase in the incidence of clinical mastitis in a herd, a decrease in the bulk tank SCC, an increase in herd

mean milk yield, calving in late spring or early summer, increased age at first calving, and milk leakage (Waage et al., 1998); blood in the milk, udder and teat edema (Waage et al., 2001); and presence of pathogens on heifer body sites (Roberson et al., 1998). Presence of IMI before calving increased risk of infection during lactation (Aarestrup and Jensen, 1997); IMI at calving increased the risk of clinical mastitis within the first week after calving, and mastitis prior to parturition and mastitis within the first week after calving increased the risk of further cases of mastitis and culling during the first 45 days of lactation (Edinger et al., 1999).

Studies have provided convincing evidence that the horn fly (*Hameotobia irritans*) is an important vector in the transmission of *Staph. aureus* mastitis in heifers. *Hameotobia irritans* can be colonized with *Staph. aureus* during feeding activities and can remain colonized for several days with substantial numbers of organisms present. When *Staph. aureus* colonized horn flies were allowed to feed on teats of uninfected dairy heifers, IMI with the same *Staph. aureus* DNA fingerprint subtype resulted (Gillespie et al., 1999). This indicates that the horn fly can transmit *Staph. aureus* to heifer teats if a sufficient source of organisms is present. That source was shown to be present in existing scabs on teat ends of heifers (Owens et al., 1998). High concentrations of *Staph. aureus* ($>10^7$ colony forming units/mg) were found in scab material present on heifer's teats. When uncolonized flies were allowed to feed on this material they became colonized with *Staph. aureus* just as readily as flies that had fed on experimentally infected blood. Thus, a vector shown capable of transmitting infection is readily present. When a source of *Staph. aureus* exists such as scabs on heifer teats, the potential for passage of IMI from heifer to heifer via horn flies exists. The threshold number of flies needed to transmit IMI is unknown. However, since fly populations can rapidly increase to several thousand per animal under favorable conditions, the need for early fly control on dairy heifers is apparent. Once scabs are obvious and fly populations are high, spread of new infections is likely. Prevention of initial high populations of flies on heifers is important to help reduce new infections.

Susceptibility of Pathogens Causing Mastitis in Heifers to Antibiotics

Considerable evidence suggests that IMI in pregnant heifers occurs frequently and that some infections may be detrimental to mammary gland development and influence subsequent lactational performance. Methods of controlling mastitis in heifers may eliminate or markedly reduce the deleterious effects of prepartum infections. One common denominator of all studies on heifer mastitis is the high prevalence of CNS IMI. Trinidad et al. (1990d) demonstrated that 90% of 311 staphylococcal isolates (primarily CNS) from heifer mammary glands were susceptible to antibiotics in vitro. Some variability to antimicrobial susceptibility of bacteria obtained within and among herds was noted; however, in general, bacteria were highly susceptible to all antibiotics evaluated. Watts et al. (1995) determined minimum inhibitory concentrations of penicillin, cloxacillin, cephalosporin, ceftiofur, novobiocin, enrofloxacin, erythromycin and pirlimycin against 1494 microorganisms isolated from heifer mammary glands. The majority of *Staphylococcus* species were susceptible to the antimicrobial agents evaluated. However, antimicrobial susceptibility was variable for *Streptococcus* species and poor against Gram-negative enteric organisms. These data suggest that antibiotic therapy may be an effective means of eliminating *Staphylococcus* species IMI that have been shown to cause the majority of IMI of heifer mammary glands.

A Simple and Effective Method for Controlling Mastitis in Heifers

The following is a discussion of research conducted at The University of Tennessee describing the development of a simple and effective method for controlling mastitis in heifers. Our initial study to determine if prepartum infusion of lactating cow antibiotic preparations into heifer mammary glands influenced rates of IMI during early lactation was published over 10 years ago (Oliver et al., 1992). Pregnant Jersey heifers (n = 115) from The University of Tennessee Dairy Experiment Station research herd at Lewisburg were assigned alternately to three treatment groups as follows: group 1 (n = 41) - no intramammary antibiotic infusion (negative control), group 2 (n = 38) - intramammary infusion of all quarters with sodium cloxacillin 7 days before expected parturition, and group 3 (n = 36) - intramammary infusion of all quarters with cephapirin sodium 7 days before expected parturition. Samples of mammary secretion for microbiologic evaluation were collected from all quarters of heifers in duplicate at 7 days before expected calving (C-7), and single quarter samples were obtained during early lactation (C+3 and C+10), at intervals throughout lactation and at the last milking of lactation immediately before drying off. A quarter was considered infected during the prepartum period if the same mastitis pathogen was isolated from duplicate samples obtained 7 days before expected calving. A quarter was considered infected during early lactation if the same mastitis pathogen isolated before treatment was present in samples obtained at 3 or 10 days after parturition.

Almost 90% of heifers were infected 7 days prior to expected calving. During early lactation, 78% of control heifers and 44.5% of mammary quarters were infected. In contrast, 17.6% of antibiotic-treated heifers and 5.4% of antibiotic-treated quarters were infected during early lactation. Fewer ($P < 0.001$) antibiotic treated heifers and quarters were infected during early lactation than in controls. Intramammary antibiotic therapy before calving was highly effective ($P < 0.001$) against CNS. It should be noted, however, that 24 of 88 (27.4%) CNS IMI in control heifers were not detected during early lactation suggesting a high rate of spontaneous elimination. Nine of 14 major pathogen IMI in control heifers and 3 of 22 major pathogen IMI in antibiotic treated mammary glands of heifers persisted into early lactation. Differences in major pathogen IMI between antibiotic treated and controls during early lactation were significant ($P < 0.025$).

Mastitis pathogens were isolated from 76% of samples obtained from untreated control quarters 7 days before expected calving, 47% of samples obtained 3 days after calving, and 29% of samples obtained 10 days postpartum. Throughout the remainder of lactation, mastitis pathogens were isolated in about 30% of control quarters. A similar percentage of samples (70%) was positive for mastitis pathogens at C-7 prior to antibiotic treatment. However, only 8% of samples obtained at 3 days after calving and 4% of samples obtained 10 days postpartum from quarters of antibiotic-treated heifers contained mastitis pathogens. Throughout the remainder of lactation, mastitis pathogens were isolated from an average of about 11% of quarters. Percent of samples with mastitis pathogens was higher in untreated controls than in antibiotic-treated quarters at most sampling intervals during lactation. *Streptococcus uberis*, *Strep. dysgalactiae* and CNS were isolated most frequently in both untreated controls and antibiotic-treated heifer mammary glands.

Antibiotic Residues in Milk Following Prepartum Treatment

One disadvantage of prepartum antibiotic administration for controlling mastitis in heifers is the potential for antibiotic residues in milk. This is especially important if heifers calve sooner than expected. To address this concern, samples of mammary secretion from all quarters of 98 heifers were collected at the first and sixth milking after calving and at 10 days after calving for antibiotic residue analysis. Samples were analyzed qualitatively for antibiotic residues by the *Bacillus stearothermophilus* disc assay. Zones of inhibition >16 mm in diameter were interpreted as positive for antibiotic residues. Sensitivity of the *B. stearothermophilus* disc assay for cephalosporin and cloxacillin has been reported to be 0.025 µg/ml and 0.031 µg/ml, respectively (Bishop and White, 1984; Ginn et al., 1982).

About 17% of colostrum samples from heifer mammary glands infused with cloxacillin were positive for inhibitors by the *B. stearothermophilus* disc assay. The majority of positive samples were from heifers that calved within 5 days of treatment. Only 4 of 88 samples obtained at the first milking after parturition were positive for inhibitors if intramammary infusion of cloxacillin occurred ≥ 7 days before parturition. All samples obtained 3 days after parturition, the time when milk would likely be marketed for human consumption, were negative for inhibitors. Thus, the cloxacillin formulation used in the present study should not result in antibiotic residue problems in marketable milk even if heifers calve earlier than expected.

In contrast, inhibitors were detected frequently during early lactation in samples from heifer mammary glands infused with cephalosporin. Almost 85% of colostrum samples and 28.2% of samples obtained 3 days after parturition were positive for inhibitors. Marked variability between time of antibiotic treatment and parturition with persistence of antibiotic residues was observed. For example, two heifers calved 8 days after treatment and all samples obtained 3 days after parturition were negative for inhibitors. Conversely, 4 heifers calved 10 days after cephalosporin treatment and 6 of 16 samples were positive for inhibitors. All samples (n = 24) from 6 heifers obtained 3 days after calving were negative for inhibitors if intramammary infusion of cephalosporin occurred ≥ 11 days before calving. Thus, it would appear that antibiotic treatment of heifer mammary glands earlier in gestation may be advantageous from an antibiotic residue standpoint. However, the timing of antibiotic treatment and subsequent persistence of antibiotics in mammary secretions following treatment could impact efficacy.

We conducted another study to determine if antibiotic treatment of heifer mammary glands earlier in the prepartum period reduced the occurrence of inhibitors in milk without influencing efficacy (Oliver et al., 1997a). In this study, 82 Jersey heifers were assigned randomly to two groups: 1) negative control (n = 42) and 2) intramammary infusion of cephalosporin sodium (n = 40) 14 days prior to expected calving. Mammary secretions were collected 14 days before calving, and at the first and sixth milking after calving and were analyzed for inhibitors by the *B. stearothermophilus* disc assay. Sixty of 150 samples (40%) from cephalosporin treated quarters were positive at the first milking after calving. However, only 4 of 127 samples (3.1%) obtained from antibiotic treated quarters at the sixth milking after calving were positive and 3 of the 4 positive samples were from a heifer that calved within 3 days of treatment. Thus, as observed in our earlier experiment (Oliver et al., 1992), the interval between prepartum antibiotic treatment and calving was related to the presence of

inhibitors in milk during early lactation. Intramammary infusion of antibiotics earlier in the prepartum period reduced the occurrence of inhibitors in milk during early lactation.

Mammary secretions were also collected 14 days before expected calving, at 3 days after calving, at intervals throughout lactation and at the last milking of lactation for microbiological evaluation. Mastitis pathogens were isolated from 67% of samples obtained from control mammary glands 14 days prior to expected calving, 56% of samples obtained 3 days after calving and 36% of samples obtained 30 days postpartum. Throughout the remainder of lactation, mastitis pathogens were isolated from about 45% of quarter samples (Oliver et al., 1997b). A similar percentage of samples (64%) were positive for mastitis pathogens prior to antibiotic treatment. However, only 16% of samples obtained at 3 days after calving and 8% of samples obtained 30 days postpartum from quarters of antibiotic-treated heifers contained mastitis pathogens. Throughout the remainder of lactation, mastitis pathogens were isolated from an average of 12% of antibiotic-treated quarters. Percent of samples with mastitis pathogens was higher in untreated control quarters than in antibiotic-treated quarters at every sampling interval during lactation. Coagulase-negative staphylococci were isolated most frequently followed by environmental mastitis pathogens. Bacteriological results during early lactation were similar to what we observed in our earlier work (Oliver et al., 1992).

More recently, we conducted a study to determine if prepartum therapy of heifer mammary glands with penicillin-novobiocin or pirlimycin hydrochloride was effective for reducing the percentage of heifers and quarters infected with mastitis pathogens during early lactation (Oliver et al., 2000, Oliver et al., 2003b). Almost 96% of Jersey heifers (67 of 70) and 71.3% of quarters (199 of 279) were infected 14 days before expected calving. Of the quarters infected at 14 d before expected parturition, 75% (54 of 72) were uninfected following treatment with penicillin-novobiocin; 87% (61 of 70) were uninfected following treatment with pirlimycin, and 56% (32 of 57) were uninfected in the untreated negative control group. The majority of IMI in Jersey heifers were due to CNS (61%), *Streptococcus* species, primarily *Strep. uberis* (19%) and *Staph. aureus* (8%). Almost 73% of Holstein heifers (40 of 55) and 34.3% of mammary quarters (73 of 213) were infected 14 days before expected calving. Of the quarters infected at 14 days before expected parturition, 76% (19 of 25) were uninfected following treatment with penicillin-novobiocin; 59% (17 of 29) were uninfected following treatment with pirlimycin, and 26% (5 of 19) were uninfected in the untreated negative control group. The majority of IMI in Holstein heifers were due to CNS (44%) and *Staph. aureus* (30%). In both herds, the bacteriological cure rate was significantly higher in heifer mammary glands treated with penicillin-novobiocin or pirlimycin hydrochloride than in untreated controls. Prepartum therapy of heifer mammary glands with penicillin-novobiocin or pirlimycin hydrochloride was an effective procedure for significantly reducing the percentage of heifers and quarters infected with mastitis pathogens during early lactation.

Influence of Heifer Prepartum Intramammary Antibiotic Treatment on Lactational Performance

Oliver et al. (2003a) determined the influence of prepartum antibiotic treatment on subsequent lactational performance of Jersey heifers. Milk production and SCC score data from 82 control heifers and 111 heifers treated with antibiotics before calving were evaluated (Table 1). Milk production (actual and 305-day) was significantly higher in heifers treated with antibiotics.

Heifers treated with antibiotics before calving had a significantly lower SCC score than control heifers (2.63 vs. 2.04).

Table 1. Lactational performance of antibiotic-treated and control heifers.

Treatment group	Actual milk production pounds (kg)	305-day milk production pounds (kg)	SCC score
Control (n=82)	11,429 (5,195)	11,011 (5,005)	2.63*
Treated (n=111)	12,597* (5,726)	12,021* (5,464)	2.04

* Significantly different from untreated controls ($P < 0.05$).

Economic Implications

Prepartum antibiotic treatment to reduce the rate of mastitis in Jersey heifers during early lactation was economically beneficial (Oliver et al., 2003a). Actual milk production averaged 11,429 pounds (5,195 kg) for untreated heifers and 12,597 pounds (5,726 kg) for antibiotic-treated heifers. Multiplying the increase in actual milk production (1168 pounds, 531 kg) in prepartum antibiotic-treated heifers by a milk price of \$18.50/cwt (\$0.407/kg) yielded a \$216.24 per-heifer increase in gross revenue. Treatment costs of \$15.60 were as follows: teat hygiene (\$0.10) which included the cost of a premilking teat disinfectant, barrier postmilking teat disinfectant and disposable paper towel; antibiotics (\$10.00); and labor (\$2.50). Another cost that may arise is the cost of testing for antibiotic residues in milk of heifers that calve too soon after treatment that we estimated to be \$3.00. Subtracting the cost of treatment (\$15.60/heifer) from gross revenue resulted in a net revenue increase of \$200.64 per heifer. These net revenue figures included the cost of testing for antibiotic residues for all antibiotic-treated heifers.

Break-even analysis indicated that it would be profitable to treat heifers before calving as long as the milk price was above \$0.013 per pound or \$1.30/cwt (\$0.029/kg). Milk price would not likely fall low enough to make treatment of prepartum heifers unprofitable. A similar relationship between the increase in net revenue and the hourly wage rate of labor was determined. Given a milk price of \$0.185/pound (\$0.407/kg), net revenue is equal to zero where the hourly wage rate of labor equals \$812.56 per hour. This suggests that treating heifers with antibiotics before calving would be profitable for wage rates below \$812.56/hour. The relationship between net revenue increases and the increase in pounds (kg) of milk produced due to treatment, given a wage rate of \$10.00/hr and a milk price of \$0.185/pound (\$0.407/kg) was determined also. Treatment would be profitable as long as the increase in milk production is greater than 84 pounds (38.2 kg; Oliver et al., 2003a).

Multistate Research Project on Prepartum Treatment of Heifers

Investigators in Washington, Louisiana, Ohio, Tennessee, New York, Connecticut and in Ontario, Canada are currently conducting a large field study to further evaluate the influence of prepartum antibiotic treatment of heifer mammary glands on IMI, SCC, milk production, and reproductive status of heifers similar to the approach described by Oliver et al. (2003a). Results of this study will be presented at the 2004 Annual Meeting of the National Mastitis Council.

Other Strategies for Controlling Mastitis in Heifers

Prepartum treatment of heifer mammary glands with antibiotics involves treatment of heifers at a time when animals are not generally worked with, would be an additional step in the management of heifers, and also might require restraining facilities for safe treatment that may not be readily available. A more “user friendly” approach would likely benefit the majority of producers that are interested in controlling mastitis in heifers, and this might enhance adoption of techniques shown to be effective for controlling mastitis in heifers.

Evaluation of a more “user friendly” approach was conducted to determine efficacy of intramammary antibiotic therapy of heifer mammary glands following the first milking after parturition on mastitis during early lactation and to determine if antibiotic treatment influenced lactational performance of heifers (Oliver et al., 2004b). Jersey (n = 43) and Holstein heifers (n = 36) from two dairy research herds were assigned to one of three treatment groups: 1) no intramammary infusion following the first milking after parturition, 2) intramammary infusion of all mammary glands with pirlimycin hydrochloride following the first milking after parturition, and 3) intramammary infusion of all mammary glands with novobiocin sodium plus penicillin G procaine following the first milking after parturition. Almost 93% of Jersey heifers (40 of 43) and 73.1% of quarters (125 of 171) were infected at the first milking after parturition. Of the mammary quarters infected at parturition, 76.7% (33 of 43) were cured following treatment with pirlimycin, 61.8% (21 of 34) were cured following treatment with penicillin-novobiocin, and 39.6% (19 of 48) were uninfected in the untreated negative control group. Significantly fewer infections were observed in pirlimycin or penicillin-novobiocin treated mammary glands of Jersey heifers during early lactation than in untreated control mammary glands. Almost 89% of Holstein heifers (32 of 36) and 52.8% of quarters (76 of 144) were infected at the first milking after parturition. Of the mammary quarters infected at parturition, 57.1% (12 of 21) were cured following treatment with pirlimycin, 41.4% (12 of 29) were cured following treatment with penicillin-novobiocin, and 23.1% (6 of 26) were uninfected in the untreated negative control group. Significantly fewer infections were observed in pirlimycin treated mammary glands of Holstein heifers during early lactation than in untreated control mammary glands. However, no significant differences in infections during early lactation were observed following penicillin-novobiocin treatment of Holstein heifers after the first milking of lactation compared to untreated control quarters. Coagulase-negative staphylococci and *Streptococcus* species, primarily *Strep. uberis* and *Strep. dysgalactiae*, were isolated most frequently in heifers from both herds. The SCC score of milk from Jersey heifers during the first 90 days of lactation was significantly lower in pirlimycin treated heifers than in untreated controls. However, no significant difference was observed in the SCC score of milk from penicillin-novobiocin treated heifers when compared to untreated controls. In addition, there was no evidence of an antibiotic treatment effect on milk production in Jersey heifers during the first 90 days of lactation. Thus, a more “user friendly” approach for controlling mastitis in heifers based on intramammary treatment following the first milking after parturition did not appear to be as effective as prepartum antibiotic treatment of heifer mammary glands. Differences are likely due to the timing of antibiotic treatment, persistence of antibiotics in mammary tissue and secretions, and the time when new IMI occur in heifers during the periparturient period.

Another strategy that has received considerable research attention is based on intramammary treatment of heifer mammary glands with a dry cow antibiotic formulation during different trimesters of pregnancy (Owens et al., 1991; 1994; 2001; Owens and Ray, 1996; Trinidad et al., 1990c). Mammary quarters of 35 breeding-age and primigravid Jersey heifers were infused with a nonlactating cow antibiotic formulation containing penicillin/streptomycin. Thirty-eight breeding-age and primigravid Jersey heifers served as untreated controls (Trinidad et al., 1990c). Of the 35 treated heifers, 34 (97.1%) were infected at time of treatment. In the untreated control group, all 38 heifers (100%) were infected at treatment time. At parturition, prevalence of IMI in treated heifers decreased to 40%, whereas prevalence in the control group remained about the same (97.4% of heifers). Prevalence of *Staph. aureus* mastitis in treated heifers was reduced from 17.1% to 2.9% after treatment. In the control group, prevalence of *Staph. aureus* mastitis decreased from 26.3% to 15.8%. Heifers treated during the second trimester of pregnancy had the greatest reduction in prevalence of mastitis. Results of this study suggest that intramammary treatment of primigravid heifers during pregnancy was effective in reducing prevalence of mastitis and SCC at parturition.

However, efficacy of prepartum antibiotic therapy at 7 or 14 days prior to expected calving (Oliver et al., 1992; Oliver et al., 1997a; Oliver et al., 2003a) was considerably higher than that reported by Trinidad et al. (1990c). This could be due, in part, to the time when heifers were treated with antibiotics, differences in the pathogens causing IMI, and the time when IMI occur. In support of this hypothesis, Fox et al. (1995) indicated that the prevalence of heifer IMI was highest during the last trimester of pregnancy. Thus, methods of controlling mastitis in heifers would likely be more effective if administered during the last trimester of pregnancy as opposed to early gestation.

In another study, a nonlactating cow antibiotic formulation containing cephalixin benzathine was evaluated in pregnant and nonpregnant Jersey heifers for its effect on experimentally induced *Staph. aureus* mastitis (Owens et al., 1991). Cephalixin was detectable in mammary secretion of nonpregnant heifers for up to 5 weeks and in tissue for 1 week after intramammary infusion. *Staphylococcus aureus* was not detectable in tissue and secretion of treated quarters at 1 and 3 weeks but was not eliminated from two quarters of one heifer tested at 6 weeks after treatment. Histologic evaluation of mammary tissue from nonpregnant heifers revealed significant differences in leukocytosis between uninfected and *Staph. aureus* infected mammary quarters but no differences in epithelium, lumen, and stroma, indicating no difference in secretion potential or glandular development. Pregnant Jersey heifers (n=25) were experimentally infected in two mammary quarters with *Staph. aureus* 12 to 14 weeks prepartum. After 1 to 3 weeks, 13 heifers were infused in 21 *Staph. aureus*-infected mammary quarters with a commercial cephalixin formulation approved for use in nonlactating cows. Nine infected mammary quarters were left untreated. All treated mammary quarters were bacteriologically negative both at calving and through 2 months after calving. Of the 9 infected mammary quarters not treated prepartum, 1 spontaneously cured and 2 became non-functional. The remaining quarters were treated at calving with a commercial cephalixin formulation approved for use in lactating cows. Of these, 3 cured and 3 failed to resolve. Heifers with cured *Staph. aureus* IMI produced 16.4 kg of milk per day while heifers that remained infected with *Staph. aureus* produced 14.5 kg of milk per day, or 11% less (Owens et al., 1991).

In a subsequent study, Owens et al. (1994) showed that intramammary infusion of a nonlactating cow formulation containing cephalixin into mammary quarters of 18 Jersey heifers 10 to 12 weeks

prepartum resulted in cure rates of existing IMI of 96% (24/25), 100% (4/4), and 90% (28/31) for *Staph. aureus*, *Streptococcus* species, and *Staphylococcus* species, respectively. Cure rates of IMI that had been treated with a commercial cephalosporin formulation approved for use in lactating cows at parturition were 62.5% (15/24), 100% (22/22), and 100% (3/3) for *Staph. aureus*, *Streptococcus* species, and *Staphylococcus* species, respectively. Initial SCC of secretions from infected mammary quarters were greater than from uninfected mammary quarters. At 2 months postpartum, the SCC of milk from treated and cured mammary quarters were reduced in comparison with mammary quarters that remained infected. Cephalosporin was present at detectable concentrations in 94, 80, 68, and 61% of treated mammary quarters at 1, 2, 3, and 4 weeks, respectively after infusion of the commercial cephalosporin formulation approved for use in nonlactating cows. At parturition, 24% of treated mammary quarters were positive for inhibitors, however, no mammary quarters remained positive for inhibitors at 5 days postpartum. An additional 40 heifers from a commercial herd were sampled and infused in all mammary quarters with the commercial cephalosporin formulation approved for use in lactating cows at 16 to 20 weeks prepartum. Cure rates for the commercial herd were 94% (29/31), 94% (16/17), 100% (44/44), and 100% (3/3), respectively, for mammary quarters infected by *Staph. aureus*, *Streptococcus* species, *Staphylococcus* species, and coliforms.

A follow-up study in 42 dairy heifers was conducted (Owens and Ray, 1996). Prepartum bacteriologic examination of mammary secretions from 42 dairy heifers 12 to 14 weeks prepartum revealed a total of 24 *Staph. aureus* infected mammary quarters, 53 *Staphylococcus* species infected mammary quarters, and 20 *Streptococcus* species infected mammary quarters. Prepartum intramammary therapy of primigravid dairy heifers with two commercially available antibiotic formulations approved for use in nonlactating cows (penicillin-novobiocin or cephalosporin) resulted in cure rates of 94%, 97%, and 100% for *Staph. aureus*, *Staphylococcus* species, and *Streptococcus* species, respectively. No protective effect was observed for dry cow treatment of uninfected mammary quarters of heifers for either of the commercially available antibiotic formulations approved for use in nonlactating cows. No antibiotic was detectable in heifer secretions collected at parturition indicating that antibiotic concentrations may have fallen below protective levels prior to parturition.

In a much larger study, 233 dairy heifers were treated 0 to 90, 90 to 180, or 180 to 270 days prepartum with one of five different antibiotic formulations for use in nonlactating cows to determine the best time to treat and the most effective product to use (Owens et al., 2001). At the initial sampling, 56.5% of mammary quarters were infected and 15.4% of mammary quarters were infected with *Staph. aureus*. Treatments included a commercially available cephalosporin dry cow product, a commercially available penicillin-novobiocin dry cow product, a commercially available penicillin-streptomycin dry cow product, an experimental dry cow product containing tilmicosin, and a cephalonium dry cow product not available in the United States. Cure rates for the five antibiotic products were equally effective against *Staph. aureus* and all were significantly more effective than the spontaneous cure rate observed in untreated control mammary quarters. Furthermore, no differences in efficacy were observed due to the different treatment times prepartum. However, fewer new *Staph. aureus* infections occurred after treatment in the third trimester of pregnancy. Fox et al. (1995) indicated that the prevalence of heifer IMI was highest during the last trimester of pregnancy. Thus, methods of controlling mastitis in heifers would likely be more effective if administered during the last trimester of pregnancy as opposed to early gestation.

Vaccination as a method to control mastitis in heifers also been conducted, however, results of those studies are equivocal. Nordhaug et al. (1994) used 108 heifers in a placebo-controlled multicenter study to evaluate an experimental *Staph. aureus* mastitis vaccine containing whole, inactivated bacteria with pseudocapsule, alpha and beta toxoids, and a mineral oil as adjuvant. Heifers were injected in the area of the supramammary lymph nodes twice before calving. None of the vaccinated animals developed clinical *Staph. aureus* mastitis, and 8.6% developed subclinical *Staph. aureus* mastitis. Conversely, 16.0% of control heifers developed clinical or subclinical *Staph. aureus* mastitis. Mean SCC in vaccinated and control heifers were the same throughout lactation. In the statistical analyses, when cow was used as the unit of concern, no significant differences occurred between groups. However, when all parameters on udder health were considered together, results indicated a potential protective effect of this vaccine during the entire lactation. More recently, Nickerson et al. (2000) suggested a positive effect of vaccination with a polyvalent *Staph. aureus* vaccine by increasing antistaphylococcal antibody titers and in preventing new *Staph. aureus* infections when the program was initiated at an early age in heifers from a herd with a high exposure to *Staph. aureus*.

A placebo-controlled field study was performed to evaluate the effect of a herd-specific vaccine against *Staph. aureus* on IMI, SCC, and clinical mastitis (Tenhagen et al., 2001). Heifers in the vaccination group (n = 164) were vaccinated twice at 5 and 2 weeks before expected calving. Heifers in the control group (n = 157) received the same treatment with a placebo containing no bacterial antigen. The prevalence of *Staph. aureus* in quarter milk samples taken at calving and 3 to 4 weeks after calving did not differ significantly between the vaccine and control group. Incidence of clinical mastitis during the first 3 months after calving and the prevalence of *Staph. aureus* in quarter milk samples taken before the onset of treatment did not differ significantly between groups. The SCC was lower in vaccinated than in control heifers. However, the difference was only significant on the third milk test day. Use of a herd-specific vaccine against *Staph. aureus* did not prove to be effective on this farm.

Thus, based on the few studies that have been reported, data are equivocal regarding efficacy of vaccination for the prevention of mastitis in heifers. One significant advantage of strategies based on vaccination is that this is a non-antibiotic approach for controlling mastitis and potential problems associated with antibiotic residues and antibiotic resistance are avoided. One important disadvantage, however, is that vaccination is pathogen specific. Since mastitis in heifers is caused by many different pathogens, vaccination against a single pathogen will not eliminate IMI caused by pathogens not targeted in the vaccine.

Another approach for controlling mastitis in heifers was based on prepartum teat disinfection with a germicide barrier teat disinfectant (Edinger et al., 2000). The effect of teat dipping with a barrier teat dip prior to parturition on IMI and clinical mastitis during the first 5 days postpartum was investigated in a split udder trial in 149 Holstein-Frisian heifers. Two mammary quarters were dipped three times weekly with a barrier teat disinfectant containing 0.1% polyvidon iodine from day 260 of gestation until parturition, and the remaining mammary quarters served as untreated controls. Bacteria were isolated from 52.2% of quarter milk samples collected immediately after parturition prior to first machine milking. *Staphylococcus aureus* and CNS were isolated most frequently (29.2 and 35.6% of the positive samples, respectively). At parturition, 6.7% of heifers showed signs of clinical mastitis and another 27.5% developed signs

of clinical mastitis during the first five days of lactation. No significant differences in IMI and clinical mastitis were found between mammary quarters dipped in the barrier teat dip prior to parturition and undipped control quarters. Authors concluded that teat disinfection prior to parturition in primigravid dairy heifers did not improve udder health in this trial (Edinger et al., 2000).

Conclusions

Intramammary infections in breeding age and pregnant heifers is much higher than previously thought. Many of these infections can persist for long periods of time, are associated with elevated SCC, and may impair mammary development during gestation and affect milk production after calving. Several potential heifer mastitis risk factors have been identified including calving in summer, high herd SCC, presence of *Staph. aureus* and *Mycoplasma spp.*, absence of fly control, feeding calves mastitic milk, contact among calves, absence of antibiotic therapy to heifers, contact with adult cows, inadequate milking practices, poor housing conditions, increased incidence of clinical mastitis in a herd, an increase in herd mean milk yield, calving in late spring or early summer, increased age at first calving, milk leakage, blood in the milk, udder and teat edema, and presence of pathogens on heifer body sites. Presence of IMI before calving increased risk of infection during lactation, IMI at calving increased the risk of clinical mastitis within the first week after calving, and mastitis prior to parturition and mastitis within the first week after calving increased the risk of further cases of mastitis and culling during the first 45 days of lactation. Prepartum intramammary antibiotic infusion of heifer mammary glands is an effective procedure for eliminating many infections in heifers during late gestation and for reducing the prevalence of mastitis in heifers both during early lactation and throughout lactation. Two studies reported that prepartum antibiotic-treated heifers produced significantly more milk than control heifers and had significantly lower SCC scores than untreated control heifers. These observations are likely associated with or due to the lower prevalence of mastitis pathogen isolation in prepartum antibiotic-treated heifers throughout lactation. One disadvantage of this strategy for controlling heifer mastitis is the potential for antibiotic residues in marketable milk. If heifers are treated with antibiotics before calving, dairy producers must make sure that milk is free of inhibitors since milk contaminated with antibiotics is unfit for human consumption.

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