MASTITIS IN HEIFERS: PREVALENCE, STRATEGY FOR CONTROL DURING THE PERIPARTURIENT PERIOD, AND ECONOMIC IMPLICATIONS

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SUMMARY

Intramammary infections in breeding age and pregnant heifers are much higher than previously thought. Many of these infections can persist for long periods of time, are associated with elevated somatic cell counts, and likely impair mammary development during gestation and affect milk production after calving. Prepartum intramammary antibiotic infusion of heifer mammary glands a few weeks before calving 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. Prepartum antibiotic-treated heifers produced significantly more milk and had significantly lower somatic cell count 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. Prepartum antibiotic treatment to reduce the rate of mastitis in heifers during early lactation was economically beneficial. Prepartum antibiotic-treated heifers produced 531 kg more milk than the untreated control group. Multiplying this increase by a milk price of $0.348/kg yielded a $184.54 (£122) per-heifer increase in gross revenue. Subtracting the cost of treatment from gross revenue (including the cost of testing for antibiotic residues), the net revenue from the actual production increase amounted to $174.92 (£115) per heifer.

INTRODUCTION

Intramammary infections (IMI) in breeding age and pregnant heifers are much higher than previously thought. Many of these infections can persist for long periods of time, are associated with elevated somatic cell counts (4, 21), and may impair mammary development (21) and affect milk production after calving. Until recently, little attention has focused on methods of controlling mastitis in heifers. Consequently, there are few management practices for controlling mastitis in heifers supported by research data. The purpose of this communication is to review literature published on the prevalence of mastitis in heifers and to share our findings on heifer mastitis, strategies for control, and economic implications based on research conducted at The University of Tennessee.

PREVALENCE OF INTRAMAMMARY INFECTIONS IN HEIFERS

Intramammary infections in unbred and pregnant heifers were once thought to be very low. However, it has been shown (9) that a high percentage of pregnant heifer mammary glands were infected during late gestation, at calving and during early lactation. During the last decade, 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. Marked herd variations in the rate of IMI and types of pathogens causing IMI have been reported (2, 7, 8, 10, 11, 12, 19, 20, 21).

The prevalence of IMI in unbred heifers and heifers during different stages of pregnancy may be high (21). 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 somatic cell counts than secretions from uninfected 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 (22). 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.

A study to determine the prevalence of mastitis and types of pathogens causing IMI in pregnant heifers prior to calving and during early lactation was conducted in a herd that was *Streptococcus agalactiae*-negative and had a low prevalence of *S. aureus* (14). 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 (9, 11), 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 *S. aureus*, 41.5% were infected with CNS and 1.9% were infected with streptococci other than *Str. agalactiae*. In another herd, 17.6% of quarters were uninfected, 23.1% were infected with *S. aureus*, 49.5% were infected with CNS and 9.9% were infected with *Streptococcus* species. In our studies (9, 10, 14, 15), CNS were isolated most frequently followed by environmental mastitis pathogens primarily *Streptococcus* species.

In one study 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 (19). Coagulase-negative *Staphylococcus* species were the most prevalent bacteria isolated and were found in 22.8% of heifers and 11.4% of quarters. Another study (7) indicated that 35.5% of colostrum samples were positive for 7 different *Staphylococcus* species. Species isolated most frequently were *S. chromogenes*, *S. aureus* and *Staphylococcus simulans*. *Staphylococcus* species were isolated from
about 18% of heifer mammary glands weekly for the first 5 wk of lactation. Some 19.7% of heifer mammary glands (59 of 300) were infected at calving. CNS caused 71.2% of these IMI in another study (11). 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 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 in multiparous cows (5, 12).

A large survey of 28 dairies in four states was conducted to determine the prevalence of IMI in unbred and pregnant dairy heifers and to determine potential factors that influenced herd variation (2). Most IMI were due to CNS and *S. 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. Thus, based on all studies reported thus far, 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.

In our studies, 8 to 10% of heifer mammary glands (10, 14 15) were infected with major mastitis pathogens near calving. Most major pathogen IMI were caused by environmental mastitis pathogens, primarily *Streptococcus* species, which was consistent with the pattern of IMI in lactating cows in these herds (13). Conversely, other studies (2, 21) indicated that *S. 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, it is reasonable to assume that heifers from herds with a high prevalence of contagious mastitis will likely be infected predominantly by contagious mastitis pathogens such as *Str. agalactiae* and *S. aureus*. Similarly, environmental mastitis pathogens such as *Streptococcus uberis* and Gram-negative bacteria such as *Escherichia coli* will likely be the predominant major pathogens isolated from heifer mammary glands from herds with an environmental mastitis problem.

**CONTROL OF MASTITIS IN PREGNANT HEIFERS**

Little research has focused on management strategies for controlling mastitis in heifers. 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. It has been demonstrated (24) 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. The minimum inhibitory concentrations of penicillin, cloxacillin, cepharin, ceftiofur, novobiocin, enrofloxacin, erythromycin and pirlimycin against 1494 microorganisms isolated from heifer mammary glands...
have been determined (25). 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

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 almost ten years ago (14). 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 200 mg sodium cloxacillin (Beecham Laboratories, Bristol, TN) 7 days before expected parturition, and group 3 (n=36) - intramammary infusion of all quarters with 200 mg cephalirin sodium (Bristol Myers, Evansville, IN) 7 days before expected parturition.

During the winter months, heifers were housed in loose housing and bedded on sawdust. In the spring, summer and fall, heifers were maintained on pasture. After calving, heifers were milked in a 12-stall trigon parlor. Milking machines were backflushed after removal and all teats of heifers were dipped with an effective postmilking teat disinfectant after milking machine removal. Lactating heifers were housed in free stalls bedded with dairy waste solids separated from a manure slurry.

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 at 3 (C+3), 10 (C+10), C+11-30, C+31-90, C+91-150, C+ 151-240, C+241-475 days after calving 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. Differences in the percentage of heifers and quarters infected in control and antibiotic treated groups during early lactation were determined by Student's t-test. Microbiological data were also expressed as percent of samples containing major pathogens, minor pathogens, and percent of samples bacteriologically negative at each of the above time periods.

Almost 90% of heifers were infected 7 days prior to expected calving (Fig. 1). During early lactation, 78% of control heifers and 44.5% of quarters were infected. In contrast, 17.6% of antibiotic-treated heifers and 5.4% of antibiotic-treated quarters were infected during early lactation (Fig. 1). 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 (Figs. 2 and 3). 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. *Strep. uberis, Streptococcus dysgalactiae* and coagulase-negative *Staphylococcus* species were isolated most frequently in both untreated controls and antibiotic-treated heifer mammary glands.

### Figure 3. Percent quarters infected and pathogens causing infections in heifers before and after antibiotic therapy.

![Figure 3. Percent quarters infected and pathogens causing infections in heifers before and after antibiotic therapy.](image)

#### 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 (18). Zones of inhibition > 16 mm in diameter were interpreted as positive for antibiotic residues. Sensitivity of the *B. stearothermophilus* disc assay for detection of cephapirin and cloxacillin has been reported to be 0.025 μg/ml and 0.031 μg/ml, respectively (1, 3).
About 17% of colostrum samples from heifer mammary glands infused with cloxacillin were positive for antibiotic residues by the *B. stearothermophilus* disc assay (Fig. 4). 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 antibiotic residues if intramammary infusion of cloxacillin occurred $\geq 7$ days before parturition. All samples obtained 3 days after parturition, the time when milk would likely be marketed for human consumption, were negative for antibiotic residues. 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, antibiotic residues were detected frequently during early lactation in samples from heifer mammary glands infused with cephapirin. Almost 85% of colostrum samples and 28.2% of samples obtained 3 days after parturition were positive for antibiotic residues (Fig. 4). 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 residues. Conversely, 4 heifers calved 10 days after cephapirin treatment and 6 of 16 samples were positive for antibiotic residues. All samples (n=24) from 6 heifers obtained 3 days after calving were negative for antibiotic residues if intramammary infusion of cephapirin occurred $\geq 11$ 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 residues in milk (15). In this study, 82 Jersey heifers were assigned randomly to two groups: 1) negative control (n=42) and 2) intramammary infusion of 200 mg cephapirin 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 analysed for residues by the *B. stearothermophilus* disc assay. Sixty of 150 samples (40%) from cephapirin treated quarters were positive at the first milking after calving (Fig. 5). 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 (14), the interval between prepartum antibiotic treatment and calving was related to persistence of residues during early lactation. Intramammary infusion of antibiotics earlier in the prepartum period reduced the occurrence of residues in milk during early lactation.

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 (Fig. 6). 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 (Fig. 6). Mammary secretions were also collected from antibiotic-treated and untreated control heifers throughout lactation and at the last milking of lactation immediately before drying off for microbiological evaluation (16). Throughout the
remainder of lactation, mastitis pathogens were isolated from about 45% of quarter samples from untreated control heifers (Fig. 7). Conversely, mastitis pathogens were isolated from an average of 12% of antibiotic-treated quarters throughout lactation (Fig. 7). 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 (14).
More recently, we conducted a study to determine if prepartum therapy of heifer mammary glands with penicillin-novobiocin (Pharmacia Upjohn, Kalamazoo, MI) or pirlimycin hydrochloride (Pharmacia Upjohn, Kalamazoo, MI) was effective for reducing the percentage of heifers and quarters infected with mastitis pathogens during early lactation (17). Almost 73% of Holstein heifers (40 of 55) and 34.3% of 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) became 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 coagulase-negative staphylococci (44%) and S. aureus (30%). 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 days before expected parturition, 75% (54 of 72) became 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 coagulase-negative staphylococci (61%), Streptococcus species, primarily Str. uberis (19%) and S. aureus (8%). 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. Studies are underway to determine the influence of penicillin-novobiocin or pirlimycin hydrochloride prepartum therapy on lactational performance and milk quality.

It has been demonstrated that intramammary infusion of an antibiotic formulation for nonlactating cows into breeding age and pregnant heifers during different trimesters of pregnancy was effective in reducing the prevalence of mastitis and somatic cell counts at parturition (23). However, efficacy of prepartum antibiotic therapy at 7 or 14 days prior to expected calving in our studies (14, 15) was considerably higher than that reported from Louisiana (23). 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, it has been indicated that the prevalence of heifer IMI was highest during the last trimester of pregnancy (2). 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.

INFLUENCE OF PREPARTUM INTRAMAMMARY ANTIBIOTIC TREATMENT OF HEIFERS ON LACTATIONAL PERFORMANCE: ECONOMIC IMPLICATIONS

We were also interested in determining the influence of prepartum antibiotic treatment on subsequent lactational performance of heifers. Milk production and somatic cell count score data from 82 control heifers and 111 heifers treated with antibiotics before calving were evaluated and data are presented in Table 1. Mean 305-day milk production was significantly higher in heifers treated with antibiotics. Heifers treated with antibiotics before calving had a significantly lower somatic cell count score than control heifers (2.63 vs. 2.04).
Table 1.   Lactational performance of antibiotic-treated and control heifers.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Milk production (kg)</th>
<th>Somatic cell count score</th>
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<tbody>
<tr>
<td></td>
<td>Actual</td>
<td>305-day</td>
</tr>
<tr>
<td>Control (n=82)</td>
<td>5195</td>
<td>5005</td>
</tr>
<tr>
<td>Treated (n=111)</td>
<td>5726*</td>
<td>5464*</td>
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* Significantly different ($P<0.05$).

Prepartum antibiotic treatment to reduce the rate of mastitis in heifers during early lactation was economically beneficial (6). Prepartum antibiotic-treated heifers produced 531 kg more milk than the untreated control group. Multiplying this increase by a milk price of $0.3476/kg yielded a $184.54 (£122) per heifer increase in gross revenue. Subtracting the cost of treatment from gross revenue, the net revenue from the actual production increase amounted to $174.92 (£115) per heifer. These net revenue figures included the cost of testing for antibiotic residues. The relationship between net revenue increases and the increase in milk produced due to treatment, given a wage rate of $6.25 (£4.13)/h and a milk price of $0.3476/kg was determined. Treatment would be profitable as long as the increase in milk production is greater than 27.7 kg (6).

CONCLUSIONS

Intramammary infections in breeding age and pregnant heifers are much higher than previously thought. Many of these infections can persist for long periods of time, are associated with elevated somatic cell counts, and likely impair mammary development during gestation and affect milk production after calving. Prepartum intramammary antibiotic infusion of heifer mammary glands a few weeks before calving 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. Prepartum antibiotic-treated heifers produced significantly more milk and had significantly lower somatic cell count 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. Prepartum antibiotic treatment to reduce the rate of mastitis in heifers during early lactation was economically beneficial. Prepartum antibiotic-treated heifers produced 531 kg more milk than the untreated control group. Multiplying this increase by a milk price of $0.348/kg yielded a $184.54 (£122) per-heifer increase in gross revenue. Subtracting the cost of treatment from gross revenue (including the cost of testing for antibiotic residues), the net revenue from the actual production increase amounted to $174.92 (£115) per heifer.
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