Influence of Prepartum Pirlimycin Hydrochloride or Penicillin-Novobiocin Therapy on Mastitis in Heifers During Early Lactation

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ABSTRACT

A study was conducted in 2 dairy research herds to determine whether prepartum therapy of heifer mammary glands with penicillin-novobiocin or pirlimycin hydrochloride was effective for reducing the percentage of heifers and mammary quarters infected with mastitis pathogens during early lactation. Almost 96% of Jersey heifers (67 of 70) and 71.3% of quarters (199 of 279) were infected 14 d 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 intramammary infections in Jersey heifers were due to coagulase-negative staphylococci (61%), *Streptococcus* species, primarily *Streptococcus uberis* (19%), and *Staphylococcus aureus* (8%). Almost 73% of Holstein heifers (40 of 55) and 34.3% of mammary quarters (73 of 213) were infected 14 d before expected calving. Of the quarters infected at 14 d 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 intramammary infections in Holstein heifers were due to coagulase-negative staphylococci (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 significantly reduced the percent-

age of heifers and quarters infected with mastitis pathogens during early lactation.

(Key words: heifer, mastitis, intramammary infection, prepartum and periparturient period)

INTRODUCTION

Intramammary infections in breeding age and pregnant heifers are much higher than previously thought. 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. Several additional studies on the prevalence of mastitis in heifers have been published (Oliver, 1987; Oliver and Sordillo, 1988; Trinidad et al., 1990a; Pankney et al., 1991; Matthews et al., 1992; Smith et al., 1994; Fox et al., 1995; Nickerson et al., 1995), and all of these studies suggest that IMI in heifers during the peripartum period occur frequently.

Many IMI in heifers can persist for long periods of time (Oliver et al., 2003b), are associated with elevated SCC (Trinidad et al., 1990b; Hallberg et al., 1995), impair mammary development during gestation (Trinidad et al., 1990b) and affect milk production after calving (Oliver et al., 2003b). Studies by Oliver et al. (1992, 1997, 2003b) demonstrated that prepartum intramammary infusion of heifer mammary glands with cephalirin sodium or sodium cloxacillin approximately 1 to 2 wk before expected calving was 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. Oliver et al. (2003b) also reported that prepartum antibiotic-treated heifers milked significantly longer, produced significantly more milk, 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 lacta-

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tion. The objective of the present study was to determine the efficacy of pirlimycin hydrochloride and penicillin-novobiocin intramammary antibiotic therapy of heifer mammary glands at 14 d before expected calving on mastitis during early lactation.

MATERIALS AND METHODS

This study was conducted with pregnant Holstein heifers ($n = 55$) at The University of Tennessee Middle Tennessee Experiment Station (MTES) dairy herd, Spring Hill, and pregnant Jersey heifers ($n = 70$) at The University of Tennessee Dairy Experiment Station (DES) dairy herd, Lewisburg. Heifers were blocked by expected date of calving and heifers on each dairy were assigned randomly to blocks with each block containing 3 heifers. Treatment groups were as follows: 1) intramammary infusion of all mammary quarters with a lactating cow antibiotic preparation containing 150 mg of novobiocin sodium plus 100,000 IU of penicillin G procaine (Albacillin, Pfizer Animal Health, Kalamazoo, MI) 14 d before expected calving, 2) intramammary infusion of all mammary quarters with a lactating cow antibiotic preparation containing 50 mg of pirlimycin hydrochloride (Pirsue, Pfizer Animal Health, Kalamazoo, MI) 14 d before expected calving, and 3) no intramammary infusion (negative control) but sampled following the protocol identified for heifers in treatments 1 and 2.

Duplicate samples of mammary secretion were collected aseptically 14 d prior to expected calving. Single foremilk samples were collected aseptically at 7, 14, and 30 d after calving. Samples from one mammary quarter of a Jersey heifer and 7 mammary quarters of Holstein heifers were not collected because these mammary glands were nonfunctional and therefore contained little to no secretion. All samples obtained during lactation were collected immediately before regular milking using standard procedures described by the National Mastitis Council (Hogan et al., 1999). Before sample collection, teats were dipped in a premilking teat disinfectant, cleaned thoroughly, and dried with individual disposable paper towels, and teat ends were sanitized with swabs containing 70% isopropyl alcohol.

Milk samples were examined following procedures recommended by the National Mastitis Council (Hogan et al., 1999). Samples of mammary secretion ($10 \mu\text{L}$) from each mammary gland were plated onto one quadrant of a trypticase soy agar plate supplemented with 5% defibrinated sheep blood (Becton Dickinson and Company, Franklin Lakes, NJ). Plates were incubated at 37°C , and bacterial growth was observed and recorded at 24-h intervals for 3 d. Bacteria on primary

culture medium were identified tentatively according to colony morphologic features, hemolytic characteristics, Gram stain reaction, and catalase test. Isolates identified presumptively as staphylococci were tested for coagulase production by the tube coagulase method. Isolates identified presumptively as streptococci were evaluated initially for growth in 6.5% NaCl, hydrolysis of esculin and sodium hippurate, and CAMP reaction. Streptococcal organisms were identified to the species level using the API 20 Strep system (bioMérieux Inc., Hazelwood, MO) and a streptococcal agglutination system (Streptex, Remel, Lenexa, KS). Gram-negative isolates were identified to the species level using the following biochemical tests: triple sugar iron, urea, oxidase, motility, indole, and ornithine decarboxylase and by the API 20 E identification system (bioMérieux Inc.).

A quarter was considered infected during the prepartum period if at least 1 cfu/ $10 \mu\text{L}$ of sample of the same pathogen was isolated from duplicate samples obtained 14 d before expected calving. Generally, there were several colony-forming units (10 to $>100/10 \mu\text{L}$ of sample) of the same mastitis pathogen in duplicate samples. If one of the duplicate samples contained a mastitis pathogen and the other duplicate sample did not, that mammary quarter was considered uninfected regardless of the number of colony-forming units observed in the one sample. A quarter was considered infected during early lactation if at least 1 cfu/ $10 \mu\text{L}$ of sample of the same organism isolated during the prepartum period was obtained at 7, 14, or 30 d after calving. A quarter was considered chronically infected if at least 1 cfu/ $10 \mu\text{L}$ of sample of the same mastitis pathogen(s) isolated during the prepartum period was isolated 7, 14, and 30 d after calving. A bacteriological cure was defined as an infection observed during the prepartum period that was negative for the presence of previously identified bacteria at 7, 14, and 30 d after calving. A quarter was considered newly infected during early lactation if at least 1 cfu/ $10 \mu\text{L}$ of sample of the same organism was isolated in 2 of 3 samples obtained at 7, 14, and 30 d after calving. Treatment comparisons within herd were done using Pearson's chi-square test (SAS release 8.02, SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Almost 96% of Jersey heifers (67 of 70) and 71.3% of mammary quarters (199 of 279) were infected 14 d 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 (Table 1). The bacteri-

Table 1. Influence of prepartum antibiotic treatment of heifer mammary glands with penicillin/novobiocin or pirlimycin hydrochloride on intramammary infections during early lactation.

Herd	Treatment ¹	No. heifers (quarters) enrolled by treatment	No. quarters infected before calving	No. quarters infected in early lactation	Cure rate (%)
DES ²	Penicillin/novobiocin ^a	24 (95)	72	18	75
	Pirlimycin ^a	23 (92)	70	9	87
	Untreated control ^b	23 (92)	57	25	56
MTES ³	Penicillin/novobiocin ^a	19 (74)	25	6	76
	Pirlimycin ^a	19 (73)	29	12	59
	Untreated control ^b	17 (66)	19	14	26

¹Cure rates of treatments within the same herd that share the same superscript are not significantly different at the 5% significance level.

²A total of 70 Jersey heifers and 279 mammary quarters was included in the study. DES = University of Tennessee Dairy Experiment Station.

³A total of 55 Holstein heifers and 213 mammary quarters was included in the study. MTES = The University of Tennessee Middle Tennessee Experiment Station.

ological cure rate was significantly higher in mammary glands of Jersey heifers treated with penicillin-novobiocin or pirlimycin during the prepartum period than in untreated controls. Five antibiotic-treated and 3 control mammary quarters developed new IMI during early lactation. The majority of IMI in Jersey heifers were due to CNS (61%), *Streptococcus* species, primarily *Strep. uberis* (19%) and *Staph. aureus* (8%) (Table 2).

Almost 73% of Holstein heifers (40 of 55) and 34.3% of mammary quarters (73 of 213) were infected 14 d before expected calving. Of the quarters infected at 14 d 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 (Table 1). The bacteriological cure rate was significantly higher in Holstein heifer mammary glands treated with penicillin-novobiocin or pirlimycin during the prepartum period than in untreated controls. Ten antibiotic-treated and 12 control mammary quarters developed new IMI during early lactation. The majority of IMI in Holstein heifers was due to CNS (44%) and *Staph. aureus* (30%) (Table 3).

To our knowledge, this is the first report describing use of pirlimycin and penicillin-novobiocin for controlling heifer mastitis during the periparturient period. Pirlimycin is a lincosaminide antibiotic that functions

Table 2. Pathogens causing intramammary infections in Jersey heifers before calving and during early lactation in the Dairy Experiment Station herd.

Mastitis pathogen	Treatment group	Before calving	Early lactation ¹	Chronic ²
CNS Species	Penicillin/novobiocin	49	12	3
	Pirlimycin	35	5	2
	Control	37	8	10
<i>Staphylococcus aureus</i>	Penicillin/novobiocin	6	0	2
	Pirlimycin	5	0	0
	Control	5	1	0
<i>Streptococcus</i> species ³	Penicillin/novobiocin	4	0	0
	Pirlimycin	12	1	0
	Control	5	2	0
Gram-negative ⁴	Penicillin/novobiocin	1	0	0
	Pirlimycin	3	0	0
	Control	2	0	0
Mixed ⁵	Penicillin/novobiocin	12	1	0
	Pirlimycin	15	1	0
	Control	8	2	2
Total		199	33	19

¹Same pathogen isolated 7, 14, or 30 d after calving.

²Same pathogen isolated 7, 14 and 30 d after calving.

³Primarily *Strep. uberis* (12 of 21) and *Strep. dysgalactiae* subsp. *dysgalactiae* (6 of 21).

⁴Primarily *Escherichia coli* (5 of 6).

⁵Primarily *Strep. uberis* and CNS (26 of 35).

Table 3. Pathogens causing intramammary infections in Holstein heifers before calving and during early lactation in the Middle Tennessee Experiment Station herd.

Mastitis pathogen	Treatment group	Before calving	Early lactation ¹	Chronic ²
CNS Species	Penicillin/novobiocin	7	3	0
	Pirlimycin	16	6	3
	Control	9	3	2
<i>Staphylococcus aureus</i>	Penicillin/novobiocin	5	1	1
	Pirlimycin	8	3	0
	Control	9	4	5
<i>Streptococcus dysgalactiae</i> subsp. <i>dysgalactiae</i>	Penicillin/novobiocin	5	0	0
	Pirlimycin	1	0	0
	Control	0	0	0
<i>Bacillus</i> species	Penicillin/novobiocin	6	1	0
	Pirlimycin	0	0	0
	Control	0	0	0
Mixed	Penicillin/novobiocin	2	0	0
	Pirlimycin	4	0	0
	Control	1	0	0
Total		73	21	11

¹Same pathogen isolated 7, 14, or 30 d after calving.²Same pathogen isolated 7, 14 and 30 d after calving.

by binding to the 50S ribosomal subunit of mRNA, inhibiting protein synthesis. Pirlimycin has been reported to be active against many gram-positive mastitis pathogens including *Staph. aureus*, *Strep. uberis*, *Streptococcus dysgalactiae* subsp. *dysgalactiae*, and *Streptococcus agalactiae* (Henke et al., 1988; Yancey et al., 1989). In the present study, pirlimycin was very effective in eliminating IMI due to CNS, *Staph. aureus*, and *Strep. uberis* in Jersey heifers from the DES herd and was significantly more effective than the untreated control group in the MTES herd. Results of the present study are consistent with recent studies by Gillespie et al. (2002) and Deluyker et al. (2001), who demonstrated that pirlimycin was effective in eliminating naturally occurring environmental *Strep. uberis* subclinical IMI, and more recently with Oliver et al. (2003a), who reported that pirlimycin was very effective in eliminating experimentally induced *Strep. uberis* IMI during early lactation.

The rate of prepartum infection and types of pathogens causing IMI in heifers from the 2 herds evaluated varied considerably. Almost 96% of Jersey heifers and 71% of quarters were infected 14 d before expected calving. Of the IMI observed in Jersey heifers during the prepartum period, 60.8% (121 of 199) were due to CNS, 10.6% (21 of 199) were due to environmental *Streptococcus* species, 8% (16 of 199) were due to *Staph. aureus*, and 3% (6 of 199) were due to gram-negative pathogens primarily *Escherichia coli* (Table 2). In addition, 26 of 35 mixed pathogen IMI were also due to CNS and *Streptococcus uberis*. Far fewer Holstein heifers and quarters were infected during the prepartum period. Almost 73% of Holstein heifers and 34.3% of mammary quarters were infected 14 d before expected calving. Of

the IMI observed in Holstein heifers during the prepartum period, 43.8% (32 of 73) were due to CNS (Table 3), and a much higher prevalence of *Staphylococcus aureus* was observed (30% of IMI) in Holstein heifers than in Jersey heifers. Since the 2 breeds of dairy heifers used in this study were from 2 different herds, it is impossible to distinguish between a breed and a herd effect.

An equivalent percentage of prepartum IMI in heifers from both herds resulted in chronic IMI. The majority of chronic IMI in heifer mammary glands from both herds were caused by CNS (67%) and *Staph. aureus* (27%), and most chronic IMI (19 of 30) were observed in untreated control heifer mammary glands. These data suggest that in the absence of antibiotic treatment many IMI in heifers can persist for long periods of time. This is consistent with a recent report by Oliver et al. (2003b) that indicated that prepartum antibiotic-treated heifers had a lower prevalence of mastitis pathogen isolation throughout lactation, and that prepartum antibiotic-treated heifers produced significantly more milk and had a significantly lower lactation average SCC score than untreated control heifers.

One common denominator observed in both herds evaluated in the present study was that CNS caused the majority of IMI in pregnant heifers during the prepartum period. This is consistent with other published reports on the prevalence of mastitis in heifers (Oliver, 1987; Oliver and Sordillo, 1988; Trinidad et al., 1990a; Pankey et al., 1991; Matthews et al., 1992; Smith et al., 1994; Fox et al., 1995; Nickerson et al., 1995). Collectively, these studies suggest that marked herd variation in the rate and types of pathogens causing IMI is common, that IMI in heifers during the prepartum period

occur frequently, that CNS will likely cause the majority of IMI in pregnant heifers, and that variation in the prevalence of IMI in heifers should be expected among herds.

In conclusion, prepartum therapy of heifer mammary glands with penicillin-novobiocin or pirlimycin hydrochloride was effective in eliminating many infections observed during the prepartum period and for reducing the percentage of heifers and mammary quarters infected with mastitis pathogens during early lactation. However, an equivalent number of new IMI during early lactation was observed in antibiotic-treated and control mammary quarters, suggesting that prepartum antibiotic therapy as done in the present study had little effect on prevention of new IMI during early lactation. The majority of prepartum IMI in heifers were due to CNS, *Streptococcus* species primarily *Strep. uberis*, and *Staph. aureus*. 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.

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