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Effect of Prepartum Intramammary Treatment with Pirlimycin Hydrochloride on Prevalence of Early First-Lactation Mastitis

A.S. Leaflet R2101

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Summary and Implications
Holstein-Friesian heifers (n = 178) from a central Iowa dairy farm were enrolled in a study to determine whether prepartum intramammary treatment of dairy heifers with pirlimycin hydrochloride would reduce the prevalence of intramammary infection (IMI) and lower the somatic cell count (SCC) during early lactation or improve 305-day mature equivalent milk production. Heifers were assigned to treatment and control groups, and treated heifers received a single 50-mg dose of pirlimycin in each mammary quarter approximately 10 days prior to parturition.

Treated heifers had a higher overall cure rate and cure rate for IMI caused by coagulase negative staphylococci (CNS), but postpartum California mastitis test scores and prevalence of chronic IMI did not differ between groups. Mature equivalent 305-day milk production did not differ between treatment groups (trend for higher production when treated, p = 0.085). No pirlimycin residues were detected in postpartum milk samples.

Introduction
Research data accumulated over the past 20 years suggests that a high proportion of dairy heifers develop IMI prior to calving. Fox et al. reported that the prevalence of IMI in heifers was greatest during the last trimester of gestation. Heifers infected with staphylococci prior to calving tend to have higher numbers of leukocytes in mammary secretions and often develop scarring of udder tissue, which may have an impact on future milk production. Studies published to date agree that coagulase-negative staphylococci (CNS) is the most common cause of prepartum IMIs in dairy heifers, with prevalences of other pathogens varying from one study to the next. Given the high prevalence of mastitis pathogens in the mammary glands of prepartum heifers, the potentially detrimental effects of IMI on future productivity, and the possibility of introducing or perpetuating contagious mastitis pathogens in dairy herds, recent research has focused on prepartum intramammary antimicrobial treatment of heifers to reduce the prevalence of IMI during early lactation. Several studies have reported prepartum antimicrobial treatment of dairy heifers decreased the prevalence of IMI after calving, and 1 study reported a significant economic benefit through increased milk production. However, only 1 of these studies evaluated the effect of prepartum treatment with pirlimycin hydrochloride on the prevalence of mastitis pathogens during early lactation in dairy heifers, and although a significant reduction in the prevalence of postpartum IMI was found, the authors did not evaluate the effect of treatment on milk somatic cell count (SCC) or milk production. In addition, although the study included cattle from 2 herds, only 42 heifers treated with pirlimycin were included, and no data on pirlimycin residues in milk from treated heifers were provided. The potential for antimicrobial residues in milk from heifers treated prior to parturition has been studied previously, but to our knowledge, no study has evaluated residues in heifers treated with pirlimycin before parturition.

The purpose of this study was to determine whether prepartum intramammary treatment of dairy heifers with pirlimycin would reduce the prevalence of IMI, lower SCC during early lactation, and improve 305-day mature equivalent (ME) milk production. In addition, we wanted to determine whether prepartum intramammary treatment of dairy heifers with pirlimycin would result in detectable pirlimycin residues in milk collected after parturition.

Materials and Methods
One hundred seventy eight Holstein heifers on a 1200 cow commercial central Iowa dairy were used in the study. Heifers were enrolled longitudinally in the study on the basis of expected calving date over an 8 month period. Approximately 10 days prior to calving, cattle were systematically and equally assigned to 1 of 2 groups (treatment and untreated control), with every other heifer assigned to the treatment group.

Treatment and Sample Collection
Prepartum mammary gland secretions were aseptically collected from all functional mammary quarters of all cattle at the time of enrollment. Prior to collection of these samples, teats were treated with a germicidal dip, dried with individual towels, and vigorously scrubbed with cotton pads soaked in 70% isopropyl alcohol. Personnel collecting samples wore latex gloves. Following sample collection, animals in the treatment group received a single 50-mg dose of pirlimycin hydrochloride (Pirsue, Pfizer Animal Health) by aseptic intramammary infusion into each mammary quarter. Control cattle did not receive any intramammary injections prior to calving. No systemic antimicrobials were administered to cattle in either group prior to calving. After sample collection and treatment, teats were dipped in an external teat sealant (Stronghold, West Agro, Inc.).

For each heifer, the date when prepartum mammary gland secretions were collected and the calving date were recorded. Aseptic mammary quarter foremilk samples were
collected from all heifers approximately 1 and 4 weeks after calving and were submitted for bacterial culture using NMC protocols. California mastitis test (CMT) scores were obtained at calving. Actual 305-day ME milk production were recorded after each animal completed its first lactation.

A composite foremilk sample consisting of milk from all lactating mammary quarters was collected late on day one or day 2 of lactation and tested on farm for pirlimycin residues with a commercial test kit (Delvotest-P, DSM Food Specialties USA, Inc.)

Mammary quarters from which bacteria were isolated prior to calving were classified as cured if bacteria were not isolated from any milk sample obtained after calving and were classified as chronically infected if the same bacteria isolated prior to calving were isolated from both milk samples obtained after calving. Mammary quarters were classified as newly infected if the same new bacteria were isolated from at least 2 consecutive milk samples collected after calving.

**Data Analysis**

Proportions of mammary quarters infected prior to parturition, cured, chronically infected, and newly infected were compared between groups by means of the $\chi^2$ or Fisher exact test. Mammary quarters were treated as individuals because all quarters included in the data analysis had subclinical IMIs and communication between quarters was considered unlikely. A previous report on pre-partum treatment of heifers used a similar approach to analyze data. Milk production data were analyzed by means of two-way ANOVA on ranks. Days from treatment to calving and CMT data and days from calving to collection of the first and second postpartum milk samples were analyzed by means of Kruskal-Wallis one-way ANOVA on ranks. The Bonferroni multiple comparison test was used for pair-wise comparisons. For all analyses, values of $P \leq 0.05$ were considered significant.

**Results**

A total of 178 heifers were enrolled in the study (120 with complete data: 66 treated and 54 control). Twenty-seven quarters were excluded because intramammary antimicrobial treatment was given at the time of calving due to clinical mastitis (6 of 17 treated quarters and 2 of 10 control quarters that were excluded had gram-negative mastitis), 6 quarters were excluded because of missing data, and 4 quarters were excluded because the quarters were non-functional after calving. Thus, data were collected for 443 quarters (242 treated and 201 control).

Median time from collection of prepartum mammary gland secretions to calving was 9 days (range, 1 to 20 days) with no significant difference between treatment groups ($P = 0.796$). Median times between parturition and collection of the first and second postpartum milk samples were 6 days (range, 0 to 32 days) and 33 days (range, 15 to 92 days), with no significant difference between treatment groups ($P = 0.428$). 82% of heifers (98/120) were found to have prepartum IMIs.

There was no difference in prevalence of prepartum IMI between treatment groups ($P = 0.470$) (Table 1). Proportion of mammary quarters classified as cured was significantly higher for treated quarters ($P = 0.017$). CNS was most commonly isolated from prepartum mammary gland secretions, followed by environmental streptococci (Table 2). Proportion of mammary quarters with prepartum IMI caused by CNS that were classified as cured following parturition was significantly higher for the treatment group ($P = 0.013$). Mammary quarter CMT scores obtained at the time of calving were not significantly different between treatment groups ($P = 0.652$). For both groups, median CMT score was 1 (ranges, 0 to 4 and 1 to 3 for treatment and control groups, respectively).

Actual 305-day ME milk production data was available for 105 (59 treated and 46 control) heifers. Median 305-day ME milk production was 11,417 kg (range, 3,012 to 16,914 kg) for treated heifers and 10,476 kg (range, 3,801 to 16,252 kg) for control heifers. No significant ($P \geq 0.085$) differences in actual 305-day ME milk production were detected between groups. Also, no significant ($P \geq 0.167$) difference in actual 305-day ME milk production was detected between heifers that had a prepartum IMI and those that did not. Results of the commercial test kit for pirlimycin residues in all milk samples were negative.

Results of the present study suggest that prepartum treatment of heifers significantly reduced the prevalence of early lactation IMI, particularly IMI caused by CNS. There was no significant differences between groups in CMT scores at calving or in 305-day ME milk production (trend of higher production at $p = 0.085$, for treated animals). There were also no detectable pirlimycin residues (Delvotest-P) on day 2 of lactation for all animals.
Table 1—Outcome of prepartum intramammary treatment with pirlimycin hydrochloride among dairy heifers.

<table>
<thead>
<tr>
<th>Herd</th>
<th>Group</th>
<th>No. of heifers</th>
<th>No. of quarters</th>
<th>No. (%) of quarters with prepartum IMI</th>
<th>No. (%) of quarters cured</th>
<th>No. (%) of quarters chronically infected</th>
<th>No. (%) of quarters newly infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated</td>
<td>66</td>
<td>242</td>
<td>99 (41)</td>
<td>88 (89)</td>
<td>2 (1)</td>
<td>8 (4)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Control</td>
<td>54</td>
<td>201</td>
<td>90 (45)</td>
<td>67 (74)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* In each column, values with the same letter superscript were significantly ($P < 0.05$) different.

Mammary quarters from which bacteria were isolated prior to calving were classified as cured if bacteria were not isolated from any milk sample obtained after calving. Mammary quarters were classified as chronically infected if bacteria were isolated prior to calving and the same bacteria were isolated from both milk samples obtained after calving. Mammary quarters were classified as newly infected if the same new bacteria were isolated from at least 2 consecutive milk samples collected after calving. IMI = Intramammary infection.

Table 2—Pathogens isolated from prepartum mammary gland secretions and postpartum milk samples from heifers that underwent prepartum intramammary administration of pirlimycin and from untreated control heifers.

<table>
<thead>
<tr>
<th>Herd</th>
<th>Pathogen</th>
<th>Group</th>
<th>No. of quarters with prepartum IMI</th>
<th>No. (%) of quarters cured</th>
<th>No. (%) of quarters chronically infected</th>
<th>No. (%) of quarters newly infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS</td>
<td>Treated</td>
<td>91</td>
<td>80 (88)*</td>
<td></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>73</td>
<td>52 (71)*</td>
<td></td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Streptococcus spp</td>
<td>Treated</td>
<td>6</td>
<td>6 (100)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>11</td>
<td>10 (91)</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
<td>Treated</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>4</td>
<td>3 (75)</td>
<td></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Gram-negative bacteria</td>
<td>Treated</td>
<td>2</td>
<td>2 (100)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1</td>
<td>1 (100)</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mixed bacteria</td>
<td>Treated</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1</td>
<td>1 (100)</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* In each column, values with the same letter superscript were significantly ($P < 0.05$) different.

CNS = Coagulase-negative staphylococci. Streptococcus spp includes streptococci other than Streptococcus agalactiae. Mixed bacteria typically consisted of staphylococci and streptococci other than S agalactiae. See Table 1 for remainder of key.