Clinical Study on the Treatment of Pirole against Bovine Mastitis

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Abstract

The study aims to investigate the efficacy of pirole and antibiotics in the treatment of bovine mastitis caused by Streptococcus uberis (S. uberis) and Escherichia coli (E. coli) during dry-milk period. 1880 cows in dry-milk period were divided into 4 groups and treated with penicillin G, ammonia benzyl penicillin, ceftiofur and pirole, respectively. The efficacy of each medicine in treating mastitis caused by E. coli intramammary infection (E. coli IMI) was followed: 31.2% for penicillin G, 36.9% for ammonia benzyl penicillin, 61.3% for ceftiofur, and 64.4% for Pirole. For those caused by S. uberis intramammary infection (S. uberis IMI), the efficacy of ceftiofur was 90% and pirole was 94.4%. The results indicated that pirole was more effective than the other three in treating the disease. The following analysis on milk samples demonstrated that there was no pirole residue in those treated cows’ milk. Based on these data, it can be predicted that pirole will have a bright future in treating cow intramammary mastitis.

Keywords: bovine mastitis, efficacy, pirole

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Original Article

บทคัดย่อ

การศึกษาทางคลินิกของ Piroline ในการรักษาโรคเต้านมอักเสบในโคนม

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จุดประสงค์ในการศึกษาครั้งนี้ เพื่อศึกษาประสิทธิภาพของ Piroline และยาปฏิชีวนะในการรักษาโรคเต้านมอักเสบในโคนมที่เกิดจากการติดเชื้อ Streptococcus uberis (S. uberis) และ Escherichia coli (E. coli) ในช่วงหยุดให้นม โคนมจํานวน 1880 ตัวถูกแบ่งเป็น 4 กลุ่มทดลอง และทำการรักษาด้วยยา Penicillin G, ammonium benzyl penicillin, ceftiofur และ Piroline ประสิทธิภาพของยา 4 ชนิดในการรักษาโรคเต้านมอักเสบที่ติดเชื้อ E. coli (E. coli IM) มีค่าร้อยละ 31.2 ของ Penicillin G, ร้อยละ 36.9 ของ ammonium benzyl penicillin, ร้อยละ 61.3 ของ ceftiofur และร้อยละ 64.4 ของ Piroline ประสิทธิภาพของยา 4 ชนิดในการรักษาโรคเต้านมอักเสบที่ติดเชื้อ S. uberis (S. uberis IM) มีค่าร้อยละ 90 ของ ceftiofur และร้อยละ 94.4 ของ Piroline จากผลการทดลองพบว่า Piroline มีประสิทธิภาพที่ดีกว่ามากที่สุดในการรักษา ผลการตรวจวิเคราะห์น้ํานม ไม่พบสารตกค้างของ Piroline ในน้ํานมของโคที่รักษา ซึ่งแสดงถึง Piroline ที่ใช้ในการรักษาโรคเต้านมอักเสบในโคนม

คำสำคัญ: โรคเต้านมอักเสบในโคนม ประสิทธิภาพ Piroline

Introduction

Bovine mastitis is not only a major disease affecting the dairy industry, but also one of the major influencing factors in milk production (Yuan et al., 1992). It causes great economic losses and decreases animal health. Although much progress has been made in control of cow mastitis, producers still cannot prevent and cure it effectively. As reported in a literature (Yuan et al., 1992), 3650 strains of 24 species of bacteria and fungi were isolated and identified from 3006 milk samples, in which 2060 strains of 12 species were closely related to cow mastitis. The isolation rate of pathogenic bacteria was 62.5%. Bacteria related to mastitis were mainly S. uberis (38.11%), E. coli (7.14%), etc. Buddle and Cooper reported (Buddle and Cooper, 1980) that about 32% of found cure spontaneously over the dry period.

Dry cow treatment (DCT) is an important step of a mastitis control program, the advantages of DCT include reducing incidence of intramammary infections (IMI) at parturition and increasing cure rate of IMI (Buddle and Cooper, 1980). The aims of DCT are to cure existing intramammary infection (IMI), and to prevent new infection during the dry period.

Materials and Methods

Herd: The DCT field trials were conducted in three factory-supply dairy herds under seasonal-calving
In conditions in Lanzhou, Gansu of China. Owners agreed to the following conditions: 1) provide calculated calving dates; 2) schedule dates for drying off, sampling, and treatment; and 3) permit some cows to serve as untreated controls. General information on several herds is presented in Table 1.

**Udder selection and milk sample collection:** Udders and milk samples were collected through recommended procedures. Composite milk samples were collected from 1880 lactating cows from three herds within 4 weeks prior to drying off. Duplicate quarter milk samples were collected at drying off. And within 21 days, single sample was collected from all quarters of cows in treatment 1 and 3 at the prepartum period prior to infection of the lactating cow product (LCP).

**Drugs:** Penicillin G (batch number, h0605071) and Ammonium benzyl penicillin (batch number h06050714) was purchased from North China Pharmaceutical Group Corporation, Shijiazhuang city, China. Ceftriaxone (batch number Y06030914) was from Hebei Yuan Zheng Pharmaceutical Co., Ltd. Shijiazhuang, China. And Piroline was made by Lanzhou Institute of Animal & Veterinary Pharmaceutics Sciences of Chinese Academy of Agricultural Sciences, Lanzhou city, China.

**Assignment of cows to treatment groups:** Cows with E. coli IMI, based on cultural results of composite milk samples, were assigned randomly to the four treatment groups, a minimum of 80 E. coli IMI was included initially in treatment 1 and 3 in anticipation of missed infusions due to early calving or incorrect calculated calving dates. Cow missed scheduled prepartum treatment were included in treatments 2 and 4, respectively. All four treatment groups were represented within each herd in table 2.

**Treatment regimens:** Each group of treatments was listed in table 2. Treatment 1 received an infusion at drying off with penicillin G 2,000,000 IU/50 ml (water), treatment 2 with ammonium benzyl penicillin 0.5 g/50 ml (water), treatment 3 with ceftriaxone 0.5 g/50 ml (water), and treatment 4 with piroline 0.4 g/50 ml(water) (Brander, 1969).

**Table 1** Information on cooperation dairy farms

<table>
<thead>
<tr>
<th>Herd</th>
<th>Number of cows</th>
<th>Wash udders</th>
<th>Spray treats</th>
<th>Treat dry-cows</th>
<th>Overall management</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>620</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Good</td>
</tr>
<tr>
<td>B</td>
<td>708</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Good</td>
</tr>
<tr>
<td>C</td>
<td>552</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Fail</td>
</tr>
</tbody>
</table>

**Table 2** Experimental design of dry-cow therapy field trial

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time of treatment</th>
<th>Drying off</th>
<th>Prepartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

*: Intramammary infections

**Microbiological procedures:** Method recommended by the National Mastitis Council, U.S.A., was followed. Presumptive identification of the following microorganisms was made; *Escherichia coli (E. coli)*; *Staphylococcus epidermidis* (SE); *Streptococcus galactiae* (SAg); *Streptococcus uberis* (S. uberis); other streptococci (OS); *Corynebacterium bovis* (CB) and Coliforms (CO).

**Definition of terms:**

**Infection:** the number of somatic cells in the milk samples exceeds regular range and determination of pathogenic bacteria in the milk shows positive.

**Cure:** clinical symptoms ease off or disappear, the number of somatic cells in the milk returns to regular range, and determination of pathogenic bacteria in the milk shows negative.

**Fail:** clinical symptoms do not ease off, the number of somatic cells in the milk does not return to regular range, and determination of pathogenic bacteria in the milk shows negative.

**Statistical analysis:** An analysis of variance was conducted only on the E. coli and S. uberis data. The number of IMI with other pathogens was insufficient to conduct valid analysis. Developed (%) = (developed quarters/treated quarters) x 100%, and Cured% = (cured quarters/treated quarters) x 100%.

**Results**

The efficacy was 61.3% against S. uberis IMI in treatment 3 and 64.4% in treatment 4 (Table 3), and the difference was not significant. The efficacy of treatment 4 was significantly greater (*p*<0.01) than the 36.9% in treatment 2, and treatment 4 was also significantly different from treatment 1 (31.2%, *p*<0.01). Treatment 4 and 3 were significantly different from treatment 1 and 2.

Approximately 10.5% of quarters developed from new IMI with E. coli or S. uberis during the dry period (Table 4). These resulting pathogens were accounted for 93% of new IMI. Other new IMI were caused by SAg and OS, about 3% for each one. Though differences were observed between the treatment groups, the incidence of the new IMI was similar to E. coli and S. uberis. In treatment 1 and 2, the incidence of IMI with S. uberis almost doubled than that in treatments 3 and 4 (Liang et al., 1993; Liang et al., 2000). Efficacy of prepartum treatment with the ceftriaxone and piroline against new SAg IMI was low, but it was 61.3% and 64.4% against the new IMI with S.uberis respectively (Table 3).

The rate of new dry period IMI with SU ranged from 3.6% to 4.9% of quarters for treatment 1, 2 and 3. 10.5% of quarters become infected with SU in treatment 4 (Table 4), prior prepartum samples were analyzed from cows in treatment 3 and 4. So efficacy of ceftriaxone and piroline were excellent, 90% and 94.4%, respectively for treatment 3 and 4. No prepartum samples were analyzed from cows in treatment 1 and 2 and spontaneous recoveries were determined for the entire dry period using samples collected postpartum.
### Table 3: Efficacy of dry-cow therapy against S. uberis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cows</th>
<th>Quarters</th>
<th>Quarter Number</th>
<th>Intramammary infections</th>
<th>Efficacy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Drying off</td>
<td>Postpartum</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>91</td>
<td>243</td>
<td>99</td>
<td>61</td>
<td>31.2</td>
</tr>
<tr>
<td>2</td>
<td>102</td>
<td>288</td>
<td>102</td>
<td>64</td>
<td>36.9</td>
</tr>
<tr>
<td>3</td>
<td>83</td>
<td>211</td>
<td>89</td>
<td>66</td>
<td>61.3</td>
</tr>
<tr>
<td>4</td>
<td>98</td>
<td>270</td>
<td>91</td>
<td>70</td>
<td>64.4</td>
</tr>
<tr>
<td>Totals</td>
<td>374</td>
<td>1012</td>
<td>381</td>
<td>261</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: New dry-period intramammary infections

<table>
<thead>
<tr>
<th>Group</th>
<th>Cows</th>
<th>Quarters</th>
<th>E. coli</th>
<th>S. uberis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Quarter Number</td>
<td>Developed(^a)</td>
<td>Cured(^b)</td>
</tr>
<tr>
<td>1</td>
<td>98</td>
<td>270</td>
<td>21</td>
<td>7.8</td>
</tr>
<tr>
<td>2</td>
<td>102</td>
<td>288</td>
<td>10</td>
<td>3.6</td>
</tr>
<tr>
<td>3</td>
<td>91</td>
<td>243</td>
<td>8</td>
<td>3.4</td>
</tr>
<tr>
<td>4</td>
<td>83</td>
<td>211</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>Totals</td>
<td>374</td>
<td>1012</td>
<td>54</td>
<td>5.3</td>
</tr>
</tbody>
</table>

\(^a\)Developed after prepartum treatment, \(^b\)Expressed as percentage cure of IMI that developed prepartum sampling.

### Discussion

Treatment 4, treated with piroline, did not significantly reduce the number of *E. coli* infections postpartum when compared with treatment 3. On the contrary, cefotiofur showed good effect in reducing the number of *E. coli* IMI significantly when compared with treatment 1 and 2. Treatment 4, treated with piroline, did not significantly reduce the level of *E. coli* IMI only with ammonium benzyl penicillin for prepartum treatment as compared with penicillin. The rate of spontaneous recovery for *E. coli* during dry period was consistent with the figures previously reported. Efficacy of piroline was 91.3% when compared with other reports on piroline against *E. coli.*

The rate of new *E. coli* dry period IMI could be reduced 50% by DCT and supported earlier work. The incidence of new IMI with *S. uberis* was similar to that observed with *E. coli* and was reduced about 50% by DCT (Brander, 1969; Brown et al, 1969; Christie et al, 1974; Buddlde and Cooper, 1980). However, the incidence of new *S. uberis* IMI was similar to that in treatment 3 and 4. Further studies are required to explain these results since no prepartum samples were collected and detected from cows in treatment 1 and 2. Additionally, the rate of spontaneous recovery was high for new *S. uberis* IMI during the late dry or early postpartum period. Philpot (Philpot, 1969; Philpot, 1979) reported that spontaneous recovery over the dry period was 70% for Streptococci, compared with 27% for *E. coli*.

Prepartum therapy with piroline was effective in eliminating over 90% of new *S. uberis* IMI, but it was not effective in eliminating new *S. uberis* IMI (Liang, 2000). The average efficacy against new *E. coli* IMI was less than 60%, but the number of new *E. coli* IMI was largely reduced. A wide variation in the efficacy was observed when compared with the earlier reports, but these data may not be conclusive.

The results validated many earlier reports on piroline reducing the incidence of new dry period IMI with *E. coli* and *S. uberis*. Prepartum therapy with piroline appeared to be of marginal benefit and probably would be of practical value in dairy herds experiencing significant clinical mastitis cows. Philpot reported a 68% reduction of clinical cases during the first week of lactation when cows received penicillin G at parturition and the end of lactation. Results from this field trial provided supportive evidence of the effectiveness of piroline, eliminating many new IMI by educating levels of infected quarters before and after parturition, especially for the IMI infected with *S. uberis*.

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### References


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