Bacteriological Cures and New Intramammary Infection of Mild to Moderate Clinical Mastitis Caused by Gram Positive after Treatment with Cephapirin Sodium

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Abstract

The objectives of the present study were to determine bacteriological cure rates and subsequent new infection rates of mild and moderate clinical mastitis cases caused by gram positive pathogens after treatment with either oxytocin or antibiotic intramammary infusion with cephapirin sodium (ToDay™, Zoetis, Thailand). A randomized controlled field trial was conducted between January and August 2014 using small-holder dairy farms in northern Thailand. Cows with clinical mastitis included in the study were randomly assigned into 1) treatment with oxytocin (OXY); 2) treatment with intramammary cephapirin sodium after milking for 2 (C2X) and 4 (C4X) consecutive times. The main isolated pathogens were Streptococcus uberis (26.4%) and other streptococci (15.6%). Clinical cure rates were 36.4%, 68.8% and 68.8% and bacteriological cure rates were 36.4%, 81.3% and 75.0% for OXY, C2X and C4X, respectively. The new intramammary infections were found at day 10 in both C2X (38.5%) and C4X (33.3%) groups, but not in OXY. In conclusion, treatment of mild and moderate clinical mastitis with cephapirin sodium has more bacteriological cure rates than treatment without antibiotics.

Keywords: cephapirin sodium, mastitis treatment, bacteriological cure, new intramammary infection

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Introduction

Mastitis is the most costly disease in the dairy industry worldwide (Piepers et al., 2007; Pinzón-Sánchez et al., 2011). Mastitis can be separated into clinical and subclinical mastitis. Cows with clinical mastitis give rise to economic loss from discarded milk, transient reductions in milk yield and premature culling (Fetrow, 2000). Discarded milk accounts for more than half the cost. Successful treatment of clinical mastitis minimizes this loss.

Treatment is compulsory when a cow is obviously sick, but in many instances treatment of mastitis is voluntary (Ruegg, 2011; Wilson et al., 1999). When cows present with mild cases of mastitis, clinical signs will normally abate within 4-6 days, regardless of antibiotics treatment (Guterbock et al., 1993), but disappearance of clinical signs does not always indicate that the infection has been successfully treated (Ruegg, 2012). In general, antibiotic treatments are necessary for mastitis caused by gram positive pathogens but may not be needed for cases caused by gram negative pathogens or cases where the cow’s immune system has successfully killed the bacteria (Ruegg et al., 2009). Antimicrobial susceptibility determined \textit{in vitro} has been considered as a prerequisite for treatment (Hess et al., 2003; Pyörälä, 2009). In northern Thailand, cephalosporin group were reported in many previous studies as the most susceptible antibiotics for mastitis pathogens (Suriyasathaporn, 2010; Suriyasathaporn, 2011; Suriyasathaporn et al., 2012).

Several factors related to successful treatment of clinical mastitis include mastitis pathogens and duration of treatment. For example, \textit{Staphylococcus aureus} yielded bacteriological cure at 49%, while \textit{Streptococcus agalactiae} yielded the cure rate at 77% (Wilson, 1999). Cure rates of mastitis from \textit{Staphylococcus aureus} at day 10 were higher in 5-day extended therapy with cephapirin but needed more milk withdrawal times (Panyamongkol et al., 2015; Roy et al., 2009). Cows with first mastitis had a cure rate 7 times higher than recurrent cases (Pinzón-Sánchez et al., 2011). The low bacteriological cure rate might be caused by antimicrobial resistant pathogens or by a new intramammary infection (IMI); the latter cases were both defined as unsuccessful treatment.

Therefore, the objectives of the present study were to determine bacteriological cure rates and subsequent new infection rates of mild and moderate clinical mastitis cases caused by gram positive pathogens after treatment by either intramammary infusion with 2 protocols with cephapirin sodium (ToDay™, Zoetis, Thailand) and intramuscular administration with oxytocin.

Materials and Methods

A randomized controlled field trial was conducted between January and August 2014 using small-holder dairy farms in Chiang Mai Province, Thailand. All participating farmers were informed about study designs and their responsibilities in the study. Most farms were members of local dairy cooperatives and owned on average 10-20 milking cows. The cooperatives’ mastitis control program included monthly somatic cell count (SCC) testing at farm levels, price penalty on high bulk SCC, and a fine of 30 times the cost of milk revenue when the milk sold was contaminated with antibiotics (Suriyasathaporn et al., 2012). Most cows were Holstein Friesian crossbred. They were tied in stall barns and milked twice daily using bucket-type milking machines with an average of 6 cows/milking unit.

The farmers were immediately informed by the researcher (T. Punyamongkol) when cows...
had mild and moderate signs of clinical mastitis diagnosed by visible abnormal milk (color, viscosity or consistency) or abnormal udders (heat, pain, redness or swelling of the quarter). None of the cows had previous dosage of antibiotics for at least two weeks. Within 2 hours after receiving information, the researcher visited the farms, checked for the selection criteria, and decided which cows to include in the study. Data on cows including parity, mastitis treatment history, and signs of clinical mastitis were collected. Milk samples were aseptically collected, kept on ice, transported to the milk quality laboratory, and immediately tested for bacterial identification. After 24 hr, colonies were tested for bacterial gram strains, and only milk samples with positive gram strain were included in the study.

The selected cows were randomly assigned into 1 of 3 treatment groups namely 1) oxytocin treatment (OXY) by intramuscular administration of 20 unit of oxytocin before milking for consecutive 6 milking times; 2) treatment with 2 times of cephapirin sodium, 200 mg cephapirin sodium (ToDay™), (C2X) by intramammary infusion every 12 hours after milking for 2 consecutive times; and 3) treatment with 4 times of cephapirin sodium (C4X). All quarters in each cow received the same treatment. Intramammary infusion was practiced by udder cleaning, milking out from the infected quarter, using 70% alcohol for soaking infected teat end, 5-mm insertion of intramammary infusion tips into the teat of infected quarter, administration of antibiotic into quarters and using antiseptic teat dipping. After 3 days (D3) of treatment, the treated quarters were observed for their clinical signs and the healthy quarters were determined as clinical cures. At D10, D17 and D33, milk samples were aseptically collected from treated quarters for bacterial identification.

Bacterial identification was performed in accordance to guidelines of the National Mastitis Council (NMC, 1999). Briefly, 0.01 ml of milk sample was streaked on a blood agar plate, and incubated at 36°C for 24 h. Samples from which 3 or more isolated pathogens were classified as contaminated. Pathogens were further differentiated based on Gram stain characteristics, colony morphology, catalase test, coagulase tests, CAMP test, and selected biochemical tests.

**Statistical Analysis**

Percentages were used to describe data on pathogens, clinical cure, bacteriological cure, and new infection after bacteriological cure. The treated quarters were evaluated for their clinical cure at D3, and for bacteriological cure at D10. Bacteriological cures were determined when results of bacterial identification at D10 differed from that before treatment. For bacteriological cured quarters, the new IMI were defined at D10, D17 and D33 as their isolates were different from that at D0. Rates of clinical cure, bacteriological cure, and new infections at D10, D17, and D33 among treatment groups were evaluated using Fisher’s Exact Chi-square Tests.

**Results**

From a total of 83 clinical mastitis cases, 18 (21.6%), 3 (3.6%) and 2 (2.4%) cases were excluded due to no growth, gram negative pathogens, and yeast and other microorganisms, respectively (Table 1). Numbers of cows with 1 and 2 treated quarters were 40 and 10, respectively. From 60 cases of mastitis with gram positive pathogens, 11 (18.3%) and 6 (10.0%) cases were excluded due to farmer’s decision on study termination and mistakes in intramammary infusion procedures, respectively. The final data included 43 cases in OXY (n=11), C2X (n=16) and C4X (n=16),
respectively. Pathogens causing clinical mastitis are shown in Table 1. The most common mastitis pathogens were *Streptococcus uberis* (26.4%), other streptococci (15.6%), *Streptococcus dysgalactiae* (12%), and coagulase negative staphylococci (CNS) (10.8%).

**Table 1** Pathogens causing mild and moderate clinical mastitis\(^1\) of dairy cows in small holder dairy farms in Chiang Mai province, Thailand (n=83)

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>n</th>
<th>%</th>
<th>OXY(^2)</th>
<th>C2X(^2)</th>
<th>C4X(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus agalactiae</em></td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><em>Streptococcus uberis</em></td>
<td>22</td>
<td>26.4</td>
<td>3</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td><em>Streptococcus dysgalactiae</em></td>
<td>10</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Other streptococci</td>
<td>13</td>
<td>15.6</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Other staphylococci</td>
<td>9</td>
<td>10.8</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><em>Corynebacterium bovis</em></td>
<td>1</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Coliform bacteria</td>
<td>2</td>
<td>2.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yeast and other microorganisms</td>
<td>3</td>
<td>3.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NG</strong></td>
<td>18</td>
<td>21.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>83</td>
<td>100</td>
<td>11</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

\(^1\)“Mild” clinical mastitis was identified by the changes of color or characteristics of normal milk, and “moderate” was determined when udder showed inflammatory signs.

\(^2\)All clinical mastitis quarter causing by gram positive bacteria were randomly assigned into OXY (treatment with 20 IU of oxytocin), C2X (treatment with 2 times of cephapirin intramammary infusion, Today™), or C4X (treatment with 4 times of Today™).

Percentages of clinical cure, bacteriological cure, new IMI at D10, D17 and D33 separating among treatment groups are shown in Figure 1. Clinical cure rates of OXY, C2X and C4X were 36.4%, 68.8% and 68.8% and bacteriological cure rates were 36.4%, 81.3% and 75.0%, respectively. Clinical cure rates were not associated with treatment groups (P=0.17). An association between treatment groups and bacteriological cure rates was found (P<0.05), indicating that bacteriological cure rates of both antibiotic groups were significantly higher than OXY.

For evaluation of new IMI rate, 29 bacteriological cure cases (67.4%), including 4, 13, and 12 cases for OXY, C2X and C4X, respectively, were used. The new IMI were found at day 10 after starting treatment in both C2X (38.5%) and C4X (33.3%) groups, but not in OXY. Numbers of new IMI were simultaneously increased on D10, D17, and D33 after treatment (Figure 1). At D33, associations between treatment groups and new IMI rates were found (P=0.05), indicating that the new IMI was significantly highest in C2X (84.6%) compared to C4X (50.0%) and OXY (25.0%).
Figure 1 Percentages of clinical cure and bacteriological cure of mild and moderate clinical mastitis (n=43) after treatment with oxytocin (OXY), C2X (2 times of cephapirin intramammary infusion) and C4X (4 times of cephapirin intramammary infusion), and number of new intramammary infection at D10, D17, and D33 of bacteriological cured quarters (n=29). **indicating significant different among treatment groups at P<0.05.

Discussion

This study was designed to resemble the real situation for mastitis treatment in small-holding dairy farms. The successful treatment of mild and moderate severities of clinical mastitis depends on types of intramammary pathogens (Barkema et al., 2006; Wilson et al., 1999). In addition, 1-day waiting period for bacterial identification is not suitable for more severe mastitis, such as peracute mastitis or systemic illness of cows, that need an immediately treatment (Erskine et al., 2002). In this study, in almost 20% of the mild and moderate clinical mastitis cases, the owners refused the use of intramammary antibiotic infusion. In general: treatment of mastitis is voluntary for mild and moderate cases (Ruegg, 2011; Wilson et al., 1999), and owners had to weigh up the economic loss of continuous infected quarters from unsuccessful spontaneous cure against milk discard from the use of antibiotic treatment.

Pathogens causing mastitis are shown in Table 1. In confirmation of previous studies in this area, most mastitis pathogens, for both clinical and subclinical cases, were environmental streptococci and other staphylococci (Paitoonchaiporn et al., 2012; Suriyasathaporn, 2010; Suriyasathaporn, 2011; Suriyasathaporn et al., 2012). In contrast to studies in western countries, the main pathogens causing clinical mastitis were coliform (Lago et al., 2011a; Lago et al., 2011b; Pinzón-Sánchez et al 2011) and other staphylococci (Pol and Ruegg, 2007). Differences in mastitis causing pathogens in this study might be the limiting clinical mastitis criteria to mild and moderate cases. In addition, the optimal temperature and
humidity in Thailand, as a tropical country, exacerbates the numbers of environmental bacteria on the floor and also various cow surface areas, especially the udder (Suriyasathaporn et al., 2012).

From Figure 1, bacteriological cure rates from the use of antibiotic intramammary infusion, regardless of whether 2 or 4 times, were significantly higher than treatment with oxytocin, but the clinical cure rates of C2X and C4X were only slightly higher (P=0.17) in comparison to OXY. These conclusions indicated the advantages of antibiotic intramammary infusion on mastitis treatment for either decreasing mastitis symptoms or killing pathogens as supported by previous studies (Hillerton and Semmens, 1999). In general, treatment of mild clinical mastitis with oxytocin was not different from treatment with antibiotic infusion (Guterbock et al., 1993). However, in that study, most cases were caused by minor pathogens. In this study, no difference between C2X and C4X was observed (Figure 1). In general, the extended therapy for treatment of mastitis, for example 5-d and 8-d treated with ceftiofur, were more effective than the standard 2-d treatment (Oliver et al., 2004a; Oliver et al., 2004b). The bacteriological cure rates of extended treatment depended on the pathogens causing mastitis (Truchetti et al., 2014). In addition, overall bacteriological cure rates were not different after 8-d treatment with cequinome compared with 1.5-d treatment (Swinkels et al., 2014). A single dose of intramammary infusion using 250 mg cefoperazone for clinical mastitis resulted in an overall high clinical cure (Wilson et al., 1986).

Many previous studies defined bacteriological cure when the infected quarters had not shown any positive bacterial identification at approximately d-20 (Guterbock et al., 1993; Truchetti et al., 2014). This might lead to new IMI, for example the use of molecular technique could indicate that Streptococcus uberis could re-infect into the same quarter after its spontaneous cure (Leelahapongsathon and Suriyasathaporn, 2015). Some bacteria may require a longer period of adaptation to the mammary environment, after which they are able to colonize and infect the mammary gland (Rambeaud et al., 2003). In an experimental IMI, viable Streptococcus uberis were detected in milk within 24 h after infusion (Pryor et al., 2009), indicating that a new IMI can occur within 24 h after antibiotic depletion from treated quarters. Quarters with spontaneous cures by their udder immunity had lower new IMI rates than quarters with antibiotic treatments. For quarters with spontaneous cure, development of innate immunity for killing invading pathogens, for example increases of complement and immunoglobulin (Suriyasathaporn et al., 2000) might remain in the quarter and help to prevent new infection.

**Conclusion**

Treatments of mild and moderate clinical mastitis by gram positive pathogens with cepahpirin sodium have higher bacteriological cure rates than treatment without antibiotic. After treatment, a new infection can be observed within 10 days after starting treatment and this may lead to confusion between unsuccessful treatment and a new infection. Spontaneous cure or cure without antibiotic treatment have lower new IMI rate than treatment with antibiotic. Higher cure rates in cepahpirin intramammary infusion are more advantageous in reducing economic losses due to clinical mastitis than spontaneous cure. For the best practice, farmers need to follow an optimal mastitis control program to prevent new IMI after treatment.
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References


Hillerton, J.E. and J. E. Semmens. 1999. Comparison of treatment of mastitis by oxytocin or antibiotics following detection according to changes in milk electrical conductivity prior to visible signs. Journal of dairy science. 82(1), 93-98.


Paitoonchaiporn, K. and W. Suriyasathaporn. 2012. Factors related to clinically cure rates of clinical mastitis after treatment


