Comparative Efficacy of Enrofloxacin and Oxytetracycline as Systemic Dry Period Therapy for the Control of Bubaline Mastitis

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ABSTRACT

This study was conducted to compare the efficacy of systemically administered Enrofloxacin and Oxytetracycline in buffalo during dry period as a measure for effective control of mastitis. A total of twenty seven dry pregnant buffaloes were selected and divided into three equal groups G1, G2 and G3. The animals of group G1 were treated with enrofloxacin 2.5 mg/kg (IM) at 14 days and 7 days prior to expected date of parturition, group G2 was treated with Oxytetracycline-HCl 11 mg/kg (IM) at 14 days and 7 days prior to the expected date of parturition and group G3 was kept as non-medicated control (G3). Mammary secretions from all group were collected aseptically 14 days prior to expected calving and milk samples from each quarter aseptically collected at day 7 and 14 post calving. The efficacy of treatments was evaluated through the prevalence of mastitic pathogens before and after calving and bacteriological cure rate. Post-calving prevalence of mastitic pathogens after systemic dry period therapy with enrofloxacin and oxytetracycline was lower than control group. The cure rate of infected quarters with enrofloxacin (91.67\%) and oxytetracycline (70\%) was significantly higher than that of control (21.43\%) (P<0.05); however, there was no significant difference between enrofloxacin and oxytetracycline treated groups (P>0.05). It was concluded that dry period therapy with antibiotics especially enrofloxacin helped in eliminating the existing intramammary infections and preventing new intramammary infections. It may be adopted as an integral part of management to bring this disease under control.

Keywords: Bubaline mastitis, dry period therapy, enrofloxacin, oxytetracycline

INTRODUCTION

Mastitis is one of the most economically important diseases of milk producing animals that cause the changes in glandular tissues affecting both the quantity and quality of milk (Ullah et al., 2005). Different strategies can be opted to avoid this problem and dry period therapy is considered as an essential part of mastitis control program (MCP). Dry cow therapy eliminates approximately 70\% to 98\% existing intra-mammary infections and prevents almost 50\% to 75\% new intra-mammary infections as a fundamental part of a successful MCP (Janosi and Huszenicaza, 2001; Petzer et al., 2009). The intra-mammary route is considered as the route of choice for delivery of dry period therapy for high absorption of drug into the udder region but at the same time there may be high risk of both physiological and anatomical damage to the streak canal and inoculation of organisms at the time of infusion (Bradley and Huxley, 2003). On the other hand, systemic dry period therapy has many advantages including better distribution of drug in the udder tissue which may lead to better cure of intra-mammary infections and avoidance of new infections which is possible risk at the time of administration of intramammary infusion (Ziv, 1980; Boddie and Nickerson, 1986). Systemic administration could simplify dry cow therapy routine. Systemic administration of antibiotics some weeks before parturition is very effective treatment for intra-
mammary infections, and is advisable for practice in the field conditions (Zecconi et al., 1999; Oliver et al., 2003). The study was conducted to evaluate the comparative efficacy of enrofloxacin and oxytetracycline as systemic dry period therapy in the control of bubaline mastitis with an objective to find out a better antibiotic for dry period therapy to control the bubaline mastitis, preventing new intramammary infections and eliminating the existing intramammary infections.

MATERIALS AND METHODS

Experimental Design

Dry pregnant buffaloes (n=27) were randomly selected from different livestock farms to be divided into three equal groups viz. G1, G2 and G3. The samples of mammary secretions were collected aseptically 14 days prior to the expected calving time for isolation and identification of prevalent mastitis pathogens. Methodology adopted was in accordance with the guidelines of National Mastitis Council (Anonymous, 1990). Each teat end was scrubbed vigorously with cotton gauze soaked with 70 percent ethyl alcohol. Immediately after samples collection from all groups, teat dipping was done using iodophores to seal the teat ends (Hovareshti, et al, 2007). Just after the collection of mammary secretions, antibiotic treatments were given to the animals as follows:

Group G1 = Pregnant buffaloes (n=9) were intramuscularly administered with enrofloxacin (Inj. Encure10™; Nawan Laboratories, Pakistan) 2.5 mg/kg on day 14th and 7th prior to the expected date of parturition

Group G2 = Pregnant buffaloes (n=9) were intramuscularly administered with oxytetracycline-HCl (Inj. Oxy-Kak™ LA; Kaksian, Pakistan) 11.0 mg/kg on day 14th and 7th prior to the expected date of parturition

Group G3 = Non-medicated pregnant buffaloes (n=9)

The milk samples (10 mL) from each quarter of all groups were collected aseptically in sterile glass vials at day 7th and 14th post calving and shifted to the Microbiology Laboratory, College of Veterinary and Animal Sciences, Jhang for isolation and identification of prevalent mastitis pathogens (Hogan et al., 1999).

Bacteriological Examination

Pre-and post-calving milk samples were processed for bacteriological examination. The procedure described by National Mastitis Council Inc., USA (Anonymous, 1990) was followed for culturing the samples and identification of mastitis pathogens. Briefly, milk samples were shaken gently to get a uniform dispersion of the pathogens. Using a platinum-rhodium loop, 0.01 ml of milk sample was streaked on blood agar and incubated at 37°C for 48 hours. A quarter was considered to be infected if 5 or more similar colonies were present on plate (Roberson et al., 1988). Absence of the bacterial colony in the cultured samples, collected at day 7th and 14th post-calving, was interpreted as a bacteriological cure.

The cultural and morphological characteristics of primary bacterial growth were studied by examination of colony characteristics and preparation of smears from different colonies. These smears were stained with Gram’s staining method and examined under the microscope. The primary growths were purified by frequent sub culturing on selective and differential media. Each of the isolate was identified on the basis of cultural and morphological characteristics, motility, hemolytic and biochemical properties as described earlier (Cruickshank et al., 1975). The genus of bacteria was determined on the basis of colony morphology, gram staining, hemolytic pattern and biochemical tests. (Anonymous, 1990).

Statistical analysis

Percent prevalence of mastitis was calculated in all groups. The cure rate of infected quarters among groups was calculated by using chi square test comparing treated groups and the control. All groups were compared with each other using two proportional Z-tests. All the values were considered significant at P<0.05.
RESULTS

Prevalence of mastitic pathogens in mammary secretions on day 14th pre-calving

Pre-calving analysis of mammary secretions revealed the presence of five different mastitic pathogens in the samples under study viz. Staphylococcus aureus, Streptococcus aglactiae, Escherechia coli, Coagulase negative staphylococci (CNS) and Corynebacterium spp. The percent prevalence of these bacteria in different quarters of animals in all groups is shown in Table 1.

Prevalence of mastitic pathogens in milk samples on day 7th post-calving

Bacteriological examination showed that in group G1, out of 36 milk samples collected from the individual quarters of 9 animals, only one sample was positive for Coagulase negative staphylococci with an overall prevalence rate of 2.78% of mastitic pathogens in group G1. Similarly only one sample was found positive in group G2 but the pathogen was Staphylococcus aureus. On the other hand, in non-medicated control group, all the mastitic pathogens identified in pre-calving secretions were found in milk secretions at day 7th post-calving except E. coli and Corynebacterium spp. The overall prevalence of mastitic pathogens in control group was 30.56% (Table 1).

Prevalence of mastitic pathogens in milk samples on day 14th post-calving

On day 14th post-calving, not a single sample was found positive for the presence of mastitic pathogens in group G1 administered with Enrofloxacin; whereas, in G2 administered with Oxytetracycline, 2 samples were positive for S. aureus, 1 sample for S. aglactiae and 1 sample for CNS with an overall prevalence of 11.11%. In group G3, a similar trend was observed as on day 7th post calving but the overall prevalence rate was much higher 41.67% (Table 1).

Postpartum cure rate of infected quarters

Post-calving cure rate of infected quarters at day 14th was 91.67% (cured/infected: 11/12) when treated with enrofloxacin (group G1); whereas, in animals administered with Oxytetracycline, 2 samples were positive for S. aureus, 1 sample for S. aglactiae and 1 sample for CNS with an overall prevalence of 11.11%. In group G3, this rate was 70% (cured/infected: 7/10). On the other hand, 21.43 % cure was also recorded in non-medicated control (group G3) that may be regarded as spontaneous cure. Statistical analysis revealed that difference in cure rates among medicated and control groups was significantly different (P< 0.05); whereas, among the treated groups, this difference was statistically non-significant (P> 0.05) (Table 2).

DISCUSSIONS

In the present study, comparative efficacy of enrofloxacin and oxytetracycline was studied in acquisition of better choice for dry period therapy in buffaloes for mastitis control. Five different mastitic pathogens viz. S. aureus, S. aglactiae, E. coli, CNS and Corynebacterium spp. were found in the mammary secretions of selected buffaloes. Earlier, different species of bacteria responsible for bubaline mastitis including S. aureus, S. aglactiae, CNS, A. pyogenese, corynebacterium and coliform spp. have been screened from buffaloes in different parts of the world (Trabla and Canavesio, 2003; Hovareshti et al., 2007).

On day 7th post-calving, a significantly lower prevalence rate of mastitic pathogens was detected in the animals of two groups (G1 and G2) medicated with antibiotics during dry period as compared to those of untreated group (G3); whereas, no significant difference was detected between the medicated groups. On day 14th prevalence rate of group G2 increased to 11.11%, in contrast, the 14th day prevalence rate of group G1 was dropped to zero; whereas, in untreated control group (G3), this prevalence rate increased to a much higher level (41.67%). On the whole, in this study enrofloxacin demonstrated better results as than oxytetracycline. This enhanced activity of enrofloxacin might be ascribed to with its large distribution volume, long half life and better activity against the pathogen involved (Soback et al., 1990). Hovareshi et al.( 2007) concluded that a higher efficacy of commercially available dry cow preparations as compared to intramuscular injections of tylosin and enrofloxacin but observed no difference in the tylosin or enrofloxacin antibiotic therapy for the control of mastitis. Although, dry cow preparations (intramammary tubes) give better results but in large dairy herds, it is almost impractical, tedious, dangerous and not expectable by the farmers (Shpigel et al., 2006). Additionally, this may introduce environmental bacteria and fungi into the quarters by unsanitary manipulation of udder. Moreover, a lower risk level of antibiotic
residue has also been demonstrated in systemic treatment as compared to intramammary infusions (Hovareshti et al., 2007). Systemic dry period therapy using different antibiotics had shown inconsistent results (Soback et al. 1990; Erskine et al. 1994; Smith and Hogan, 1998; Nickerson et al. 1999; Zecconi et al. 1999). In contrast to our findings, Shpigel et al. (2006) observed very low cure rate after systemic cefquinome treatment that was comparable to the spontaneous cure rate observed in untreated controls. The unfavorable results of the cefquinome systemic dry period therapy might reflect inadequate pharmacokinetic properties of the drug regarding poor udder penetration in subclinical mastitis and short antimicrobial effect.

Erskine et al. (1994) evaluated the efficacy of intramuscular oxytetracycline against S. aureus induced mastitis as a dry period treatment at drying off and found a low level of protection against S. aureus as compared to this study. This variation might be due to difference in the pre-calving medication time. Dry period length may be long and animal regain infections during dry period.

Post-calving maximum cure rate of infected quarters (91.67%) was recorded in the buffaloes of group G1 injected with enrofloxacin followed by those administered with oxytetracycline (G2) (70%). Control group (G3) also showed 21% cure rate that might be regarded as spontaneous cure. Cure rates against individual microbes were higher in enrofloxacin treated buffaloes (G1) as than those of treated (G2) and control groups. In G1, 100% cure was observed against S. aureus, S. agalactiae, E. coli and corynebacterium spp.; whereas, for coagulase negative staphylococci cure rate was 66.66%.

Similar findings have been reported by Petzer et al. (2009) who used intramammary preparation containing cephalaxin 250 mg and neomycin sulphate 250 mg. The cure rates in this study were 94.4 % for S. aureus, 100% for S. agalactiae and S. dysgalactiae, 78.1% for CNS and 100% for the other minor pathogens.

In the present study, four new infections (11.11%) were also occurred in the control group but no new infections appeared in the treated groups (G2 and G3). Natzke (1971) also reported the development of new infections during the dry period without dry period therapy. In some previous studies, new intramammary infections rates have been reported ranging from 13.10% to 34% during the dry period (Osteras et al., 1991; Schukken, et al., 1993; Williamson et al., 1995).

In conclusion, the systemic dry period therapy using enrofloxacin is probably very much effective to clear the existing intramammary infections and preventing new intramammary infections. It should be adopted as an integral part of mastitis management to bring this disease under control.


Table 1. Pre- and post-calving prevalence (%) of mastitic pathogens from individual quarters (n=9×4=36, each group) of pregnant buffaloes.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14 days before calving</td>
<td>14 days before calving</td>
<td>14 days before calving</td>
</tr>
<tr>
<td></td>
<td>First sample at day 7</td>
<td>Second sample at day 14</td>
<td>14 days before calving</td>
</tr>
<tr>
<td></td>
<td>N o.</td>
<td>%</td>
<td>N o.</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>5</td>
<td>13.89</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>2</td>
<td>5.56</td>
<td>0</td>
</tr>
<tr>
<td>E. coli</td>
<td>1</td>
<td>2.78</td>
<td>0</td>
</tr>
<tr>
<td>Coagulase Negative Staphylococcii</td>
<td>3</td>
<td>8.33</td>
<td>1</td>
</tr>
<tr>
<td>Corynebacterium spp.</td>
<td>1</td>
<td>2.78</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>33.33</td>
<td>1</td>
</tr>
</tbody>
</table>

G1=Enrofloxacin; G2=Oxytetracycline; G3=Non-medicated control
Table 2. Day 14\textsuperscript{th} post calving quarter based cure rate.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total number of quarter</th>
<th>Number of quarter infected before treatment</th>
<th>Percentage</th>
<th>Number of Quarter Cured</th>
<th>Cure Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>36</td>
<td>12</td>
<td>33.33</td>
<td>11</td>
<td>91.67</td>
</tr>
<tr>
<td>G2</td>
<td>36</td>
<td>10</td>
<td>27.78</td>
<td>7</td>
<td>70.00</td>
</tr>
<tr>
<td>G3</td>
<td>36</td>
<td>14</td>
<td>38.89</td>
<td>3</td>
<td>21.43*</td>
</tr>
</tbody>
</table>

\* = Significantly different (P>0.05).