Efficacy of Two Acidified Chlorite Postmilking Teat Disinfectants with Sodium Dodecylbenzene Sulfonic Acid on Prevention of Contagious Mastitis Using an Experimental Challenge Protocol

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ABSTRACT

Two acidified sodium chlorite postmilking teat disinfectants were evaluated for efficacy against Staphylococcus aureus and Streptococcus agalactiae by using National Mastitis Council experimental challenge procedures. The effect of these teat dips on teat skin and teat end condition was also determined. Both dips contained 0.32% sodium chlorite, 1.32% lactic, and 2.5% glycerin. Dips differed in the amount of sodium dodecylbenzene sulfonic acid (0.53 or 0.27%) added as a surfactant. Both dips significantly reduced new intramammary infection (IMI) rates compared with undipped controls. The dip containing 0.53% dodecylbenzene sulfonic acid reduced new IMI by Staph. aureus by 72% and Strep. agalactiae by 75%. The dip containing 0.27% dodecylbenzene sulfonic acid reduced new IMI by Staph. aureus by 100% and by Strep. agalactiae by 88%. Changes in teat skin and teat end condition for treatment and control groups varied in parallel over time. Teats treated with either teat dip had higher mean teat skin and teat end scores than control teats at some weeks. However, teat skin and teat end condition did not tend to change from the start to the completion of the trial. Application of the two new postmilking teat dips was effective in reducing new IMI from contagious mastitis pathogens.

(Key words: teat dip, contagious mastitis, chlorous acid)

Abbreviation key: DBSA = sodium dodecylbenzene sulfonic acid, TSB = trypticase soy broth.

INTRODUCTION

Postmilking teat antisepsis (teat dipping) remains the primary method for preventing new IMI caused by contagious pathogens. Yet the effectiveness of this procedure is not absolute, i.e., there are difficulties in balancing ingredients such that disinfection is maximized while skin irritation is minimized. To this end, factors such as the germicidal agent (active ingredient), surfactant, pH, and skin conditioner must be balanced to achieve an effective dip (Pankey et al., 1984a).

The acidified sodium chlorite disinfectant system results in the formation of the active ingredient, chlorous acid, a product of the reaction of sodium chlorite with an activating acid. Mandelic and lactic acids are typically used as the activating acids. It was noted that the use of an α-hydroxy organic acid as the activator to generate chlorous acid in a teat dip may afford continued germicidal activity on the teat surface after drying of the product (Boddie et al., 1994). In one of its commercial iterations, acidified sodium chlorite system has been incorporated into a teat dip as a polymer gel, and such incorporation may provide broad microbicidal activity when the teat dip remains moist on the teat skin surface (Oliver et al., 1989; Poutrel et al., 1990). Moreover, data indicate use of such a product can maintain teat skin condition (Boddie et al., 1994).

Surfactants improve the spreading characteristic of the disinfectant solution and its ability to penetrate crevices, cavities, and films of organic material (Huber, 1977). Sodium dodecylbenzene sulfonic acid (DBSA) teat dips have been found to significantly reduce IMI by contagious pathogens in experimental challenge (Pankey et al., 1984b) and natural exposure field studies (Pankey et al., 1985). Thus, the addition of a surfactant, with known efficacy as an active ingredient in teat dips, may increase the efficacy of acidified sodium chlorite teat dips.

The objective of this study was to evaluate the efficacy of two acidified sodium chlorite postmilking teat dips that contained DBSA on prevention of contagious IMI, and to determine their effects on teat skin and teat end condition.

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MATERIALS AND METHODS

Collection of Milk Samples and Determination of Intramammary Infection

The initial bacteriological status of all mammary quarters of cows eligible for the trial at the Washington State University Dairy Center was determined by aseptic collection and culture of milk samples (Hogan et al., 1999). A second collection and culture of milk from all mammary quarters was made 3 d after the first, and a third milk sample was collected from specific quarters and cultured when results from the first two samples were not in agreement. Quarter milk samples were collected and cultured weekly during the trial. Before sampling, udders were wiped by using reusable cloth towels to remove visible dirt and debris. After wiping, two or three streams of foremilk were discarded. Each teat apex was scrubbed for several seconds with a cotton pledget moistened with 70% ethanol aqueous solution. Milk samples were collected in sterile snap-cap plastic tubes and stored frozen at −20°C. Isolation of either Staphylococcus aureus or Streptococcus agalactiae for a first time from a previously uninfected quarter triggered a collection of another sample within 2 d after the first isolation. Results of this second sample were used to help confirm an IMI; the criteria used to determine IMI by the challenge pathogen follows. Sample collection from mammary quarters suspected of having new IMI was done at 2- to 3-d intervals. This collection sample string was continued until two consecutively collected samples were free of the challenge organism or as many as five consecutive samples yielded the pathogen in question. All mammary quarters were eligible for new IMI during the trial except those infected with organisms of the same species as the challenge pathogens and those mammary quarters that were not functional.

Mammary quarter milk samples were warmed to ambient conditions and vortex mixed. A 0.1-ml aliquot was spread on Columbia blood agar (Remel Inc.; Seattle, WA) containing 5% sheep blood. Plates were incubated at 37°C for 48 h and examined to identify microorganisms present. Staph. aureus were identified by colony morphology, hemolytic patterns, and by the tube coagulase test (Hogan et al., 1999). Strep. agalactiae were identified by hemolytic pattern, colony morphology, CAMP reaction (abbreviation of typical test for Streptococci, referred to in Hogan et al., 1999). An IMI was confirmed when 1) Staph. aureus or Strep. agalactiae were isolated from a mammary quarter with clinical mastitis, 2) two consecutive samples yielded ≥500 cfu (colony forming units)/ml of the same pathogen, 3) three consecutive samples contained 100 to 400 cfu/ml of the same pathogen, or 4) five consecutive samples contained >1 cfu/ml of the same pathogen.

Treatment Application

The efficacy of two test dips using the acidified sodium chlorite system, termed Red and Blue (Alcide Corporation, Redmond, WA), was evaluated for their ability to prevent IMI by contagious pathogens. Dips contained 0.32% sodium chlorite as base with 2.5% glycerin and DBSA, and 1.32% lactic acid as activator. Base and activator were combined in equal volumes and a fresh mixture was made daily. Red and Blue dips differed only by the concentration of DBSA, 0.27% and 0.53%, respectively. All lactating cows not enrolled on other trials at Washington State University’s Dairy Center (n = 165) were randomly assigned to two groups. At the morning milking, Monday through Friday, all four teats of each cow were immersed in a challenge suspension containing Staph. aureus (ATCC 29740) and Strep. agalactiae (ATCC 27956) immediately after the milking machines were removed. A mammary gland half, right or left, of each cow was randomly assigned to receive one of the postmilking teat dips, the other side remained as an undipped control. Teats receiving dip were immersed in the test germicide after both daily milkings, 7 d per week.

Preparation of Experimental Challenge Suspension

Challenge immersions were prepared following the procedure outlined by Boddie et al. (1994). Isolated colonies of Staph. aureus and Strep. agalactiae were used to inoculate 5 ml of trypticase soy broth (TSB). The TSB tubes were incubated at 37°C for 7 h. After incubation, TSB cultures were vortex mixed and used to inoculate 500-ml bottles of TSB and incubated for 15 h. Bacterial cells were then pelleted by centrifugation (3840 × g), washed twice with 0.1% proteose-peptone (Difco Laboratories, Detroit, MI), and resuspended with 500 ml of proteose peptone. Portions of the resuspended pathogens were serially diluted to 1 × 10⁻⁷, 1 × 10⁻⁸, 1 × 10⁻⁹, and a 0.1-ml portion was spread on Columbia blood agar to ascertain the microbial concentration of the stock suspension. A stock suspension was used daily for 1 wk to prepare challenge suspensions of Staph. aureus and Strep. agalactiae. Challenge suspensions were prepared by adding approximately 10 ml of stock culture to 146 ml of pasteurized skim milk in an attempt to achieve a concentration of 5 × 10⁷ cfu/ml of Staph. aureus and Strep. agalactiae. Serial dilutions of the challenge suspension were made daily, plated, and the concentration of Staph. aureus and Strep. agalactiae...
Table 1. The effects of 0.27 and 0.53% sodium dodecylbenzene sulfonic acid (DBSA) in a chlorous acid base teat dip with a lactic acid activator on new IMI (NIMI) with either Staphylococcus aureus or Streptococcus agalactiae over a 12- to 14-wk period.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Treatment</th>
<th>No. of quarters</th>
<th>NIMI</th>
<th>% 1</th>
<th>No. of quarters</th>
<th>NIMI</th>
<th>% 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph. aureus</td>
<td>Blue (0.53% DBSA)</td>
<td>198</td>
<td>3</td>
<td>1.5^a</td>
<td>195</td>
<td>12</td>
<td>6.15^b</td>
</tr>
<tr>
<td>Strep. agalactiae</td>
<td>Blue (0.53% DBSA)</td>
<td>198</td>
<td>12</td>
<td>6.1^a</td>
<td>195</td>
<td>47</td>
<td>24.1^b</td>
</tr>
<tr>
<td>Strep. agalactiae</td>
<td>Red (0.27% DBSA)</td>
<td>132</td>
<td>1</td>
<td>0.76^a</td>
<td>132</td>
<td>8</td>
<td>6.06^b</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>Red (0.27% DBSA)</td>
<td>132</td>
<td>0</td>
<td>0^a</td>
<td>132</td>
<td>6</td>
<td>4.55^b</td>
</tr>
</tbody>
</table>

^a,bPercentage of NIMI within a row not sharing a common superscript are significantly different (P < 0.05).

^1Percentage of quarters newly infected with applied contagious pathogen.

tiae determined. Based on these plate counts, the volume of stock suspension added to pasteurized milk was adjusted in an attempt to achieve a daily final concentration of 5 × 10^7 cfu/ml of both pathogens.

Statistical Methods

The effectiveness of both dips was determined by contrasting the differences between the percentages of quarters becoming infected in mammary gland halves that received postmilking disinfection compared with controls. The approximated t statistic test, as described by Hogan et al. (1990), was used as follows:

\[ t = \frac{(x_1/n_1 - x_2/n_2)}{(x_1 + x_2)/(n_1n_2)^{0.5}}, \]

where \( x_1 \) = number new IMI in control quarters, \( x_2 \) = number new IMI in treated quarters, \( n_1 \) = (number of control quarters) (time unit), and \( n_2 \) = (number of treated quarters) (time unit). The unit of time was quarter days at risk. After developing an IMI, a quarter was not considered to be at risk for another IMI by the same pathogen for the remainder of the study. The percent reduction in new infection rate in the treated group compared with that in the control group is expressed as follows: 100[(x1/n1) − (x2/n2)]/(x1/n1), where \( x_1, x_2, n_1, \) and \( n_2 \) were defined previously.

Scoring of Teat Skin and Teat End

Characteristics of teat skin surfaces and teat ends for both groups of cows were scored immediately before the trial, weekly during the trial, and at the conclusion of the trial, to determine possible effects of the germicidal solutions on teat skin condition. Condition scores for teat skin and teat ends were characterized according to the criteria that were established by Goldberg et al. (1994). Those scores for teat skin and the respective criteria are as follows: 1) teat skin is smooth and free from scales, cracks, or chapping; 2) teat skin shows some evidence of chapping and fewer than five warts; 3) teat skin is chapped and more than five warts are visible; 4) teat skin is chapped and cracked, redness indicating inflammation is present, and numerous warts are present; and 5) teat skin is severely damaged and ulcerative with scabs or open lesions, large number of warts are present that interfere with teat end function. Scores and description for teat end condition score were as follows: 1) teat end sphincter is smooth with no evidence of irritation; 2) teat end has a raised ring without stars (radial cracking); 3) teat end sphincter is roughened with slight cracks, and radial cracking apparent; 4) teat end sphincter is inverted with severe radial cracking; teat end may have old but healing scabs; and 5) teat end is severely damaged and ulcerative with scabs or open lesions, large or numerous warts present that interfere with teat end function.

RESULTS

Both dips significantly reduced new IMI rate from the challenge organisms compared with controls (Table 1). The Red teat dip (0.27% DBSA) significantly reduced the number of new Staph. aureus IMI by 100% (P < 0.05) and the number of new Strep. agalactiae IMI by 88% (P < 0.05). The Blue teat dip (0.53% DBSA) reduced the number of new Staph. aureus IMI by 72% (P < 0.05) and the number of new Strep. agalactiae IMI by 75% (P < 0.05).

Teat end scores for cows receiving Red dip are summarized in Figure 1. In general, the treatment group had higher scores for teat ends than the control group. There was a significant (P < 0.05) difference in the group teat end scores during certain weeks. However, teat end scores for treated and control teats had parallel changes over time. Teat end scores for cows receiving Blue dip are summarized in Figure 2. Overall, cows in the treatment group had higher scores than the control group, and statistical significance varied on a weekly basis. Yet, teat end scores were not significantly differ-
ent at the end of the trial. At least 85% of teat skin and teat ends were characterized as normal (score = 2) before and after the trial for both groups.

Teat skin scores for cows receiving Red dip are summarized in Figure 3. Teat skin scores for the treatment group were statistically higher ($P < 0.05$) than controls at 4 of 14 time points. Teat skin scores for cows receiving Blue dip are summarized in Figure 4. The treatment group had higher ($P < 0.05$) scores than controls during wk 3, 4, 8, and 10. Changes in teat skin scores by group and treatment were parallel; teat skin and end condition was not statistically different at the end of the trial for control versus treated teats.

**DISCUSSION**

The active ingredients in the teat dips tested in this trial were associated with a significant reduction in new IMI with contagious mastitis pathogens, consistent with other studies. Boddie and Nickerson (1998) found a chlorous acid teat dip that reduced new IMI from *Staph. aureus* by 92% and from *Strep. agalactiae* by 72%. Boddie et al. (1994) also studied two teat dips containing chlorous acid with either lactic acid or mandelic acid as the activator. The dip activated with mandelic acid reduced new IMI from *Staph. aureus* by 67% and from *Strep. agalactiae* by 56%. The dip activated with lactic acid reduced new IMI from *Staph. aureus* by 69% and from *Strep. agalactiae* by 35%, which was similar to a study conducted by Dreschler et al. (1990). Dreschler et al. (1990) reported that a chlorous acid barrier dip with a lactic acid activator reduced new IMI from *Staph. aureus* by 79% and from *Strep. agalactiae* by 53% under both experimental and natural exposure conditions.

Five commercial herds participated in a natural exposure study to compare a chlorous acid barrier teat dip containing lactic acid against a positive control product containing 0.5% iodophor (Poutrel et al., 1990). There was a 19% reduction in new IMI caused by all pathogens and a 14% reduction in major pathogen IMI for the chlorous acid group. Thus, the chlorous acid barrier dip was at least as effective as the positive control formulation.

In the present study, the experimental teat dips incorporated DBSA as a surfactant, which has been used as the active ingredient in other teat dip formulations. Barnum et al. (1982) evaluated a teat dip with 1.94% DBSA as the active ingredient and reported reduction rates of 80 and 71% against an experimental challenge of *Strep. agalactiae* and *Staph. aureus*. A potential of the experimental teat dips used in this present study is that the surfactant might also provide added protection.
against new IMI resulting from contagious mastitis pathogens. Data to suggest a synergistic effect between DBSA and the acidified chlorite system can be found in the study by Morelli et al. (2001). These researchers examined the reduction in Staph. aureus counts on artificial teats, where pathogen application followed 4 h after the application of the Red dip, contrasted with application of two other similar acidified chlorite system dips that contained a different surfactant, a combination of nonionic surfactants poloxamer and octylphenoxy polyethoxyethanol. The Red dip was associated with a severalfold greater reduction in Staph. aureus counts compared with the other dips (Morelli et al., 2001).

An effective teat dip must balance the active ingredient, the disinfectant, and inert ingredients such as wetting agents, skin conditioners, and vehicle to achieve efficacy in control of contagious mastitis. Chlorous acid, as an active ingredient in teat dips, is advantageous because it is presumed that residual lactic acid may remain on the teat skin to provide continued bacteriostatic and bactericidal activity (Boddie et al., 1994). This process is accomplished by the partial conversion of the chlorite ion (ClO$_2^-$) to chlorous acid (HClO$_2$) as activated by lactic acid, which is responsible for the germicidal activity of the teat dip (Gordon, 1972). Once dried, the organic acid exists as a lactic acid and lactate buffer. Active ingredients can have an adverse effect on teat skin. Teat skin conditioners such as emollients and humectants can be added to teat dips to counteract the irritating properties that the active ingredients may have on the teat skin (Pankey et al., 1984a). Both test dips in this study contained the humectant glycerin (2.5%) as the skin conditioner. Teat skin and teat end condition scores changed weekly in this study. Teat skin and end scores were significantly greater than controls at certain weeks, suggesting that the test dips may have been more irritating than the no-dip control. Poutrel et al. (1990) reported that healing was reduced when a similar chlorous acid-based dip was used in contrast to an iodophor dip. However, except for the teat end condition of teats treated with Red dip at wk 14, the condition scores of all teats were similar at the beginning and end of the trial, signifying that any irritating properties of the dips were not long-lived. It could be theorized that the teat epidermis went through a period of acclimation to the Red and Blue dips, accounting for higher skin and end scores during the weeks between the start and end of the trial. However, this theory would have to be tested in a trial of longer duration.

The only variable of difference between the dip formulation was the surfactant, and it could be argued that indeed one dip was tested with varying levels of surfactant. However, the thrust of this study was to compare the efficacy of two dips against a negative, no-dip, control. Thus, the study design precluded the comparison of the efficacy between the Red and the Blue dip. In conclusion, the two acidified sodium chlorite-based dips, Red and Blue, were effective in controlling contagious mastitis, demonstrated by using the experimental challenge protocol. The formulation of both dips included the surfactant DBSA, with known disinfectant properties. The addition of DBSA to the dips may afford added protection against contagious pathogens, although an in vivo efficacy study where formulations with and without DBSA in the dip were contrasted would more definitively establish if a DBSA advantage truly exists.

REFERENCES


