ABSTRACT

We tested two postmilking teat dips for efficacy against *Staphylococcus aureus* and *Streptococcus agalactiae* using experimental challenge procedures recommended by the National Mastitis Council. The chlorine dioxide teat dip that contained 0.7% sodium chlorite reduced the number of new intramammary infections (IMI) caused by *Staph. aureus* by 86.6% and reduced new IMI caused by *Strep. agalactiae* by 88.4%. The 0.5% iodophor teat dip reduced the number of new IMI caused by *Staph. aureus* by 92.9% and reduced the number of new IMI caused by *Strep. agalactiae* by 43.4%. Teat skin and teat end conditions were evaluated before and after the study, and no deleterious effects were noted among dipped quarters compared with undipped control quarters for either teat dip.

(Key words: chlorine dioxide, *Staphylococcus aureus*, *Streptococcus agalactiae*, teat dip)

Abbreviation key: TSA = trypticase soy agar, TSB = trypticase soy broth.

INTRODUCTION

Chlorine was first used in a practical application as a gas and water solution that was used to bleach textiles. The development of sodium and calcium hypochlorite as applications followed shortly thereafter. Chlorinated lime was used to treat sewage in London as early as 1854. In 1881, Robert Koch, the German bacteriologist, demonstrated that bacteria in a laboratory environment could be destroyed by hypochlorite. Later, hypochlorite and chloride of lime were used in the United States to purify water. Widespread use of chlorine as a disinfectant began during World War I when, in 1915, Dakin (1915) used a 0.5% sodium hypochlorite solution to disinfect wounds.

Chlorine dioxide is one of the five major groups of chlorine sanitizing compounds. It is an extremely reactive compound and, consequently, cannot be made and shipped in bulk. Therefore, the compound is prepared at the place of consumption. One of the methods by which chlorine dioxide is produced is by reacting an acid with a chlorite (Dychdala, 1991). In 1978, Alliger (1978) developed a germicide at the site of application by combining solutions of sodium chlorite and acid, resulting in production of chlorine dioxide. Chlorine dioxide is reported to have 2.5 times the oxidizing or killing power of chlorine (Benarde et al., 1967).

In 1958, Newbould and Barnum (1958) found that dipping teats in 0.1, 1, and 2.5% tinctures of iodine greatly reduced the numbers of staphylococci that were recovered from milking machine liners. This research prompted teat dip manufacturers to incorporate iodine into many teat dip products that are commercially available today. Iodine has a low reactivity with proteins (milk, manure, and blood); therefore, it can more efficiently disinfect because the halogen concentration available for the actual degemming reaction is much greater than chlorine or bromine (Gottardi, 1991).

The objective of this study was to evaluate new chlorine dioxide and iodophor teat dips using experimental challenge procedures to determine their suitability for on-farm use.

MATERIALS AND METHODS

Cows

The Jersey dairy herd of 117 cows at the Hill Farm Research Station (Homer, LA) was used in a 9-wk controlled infection trial to evaluate the two teat dips. Cows utilized a free-stall barn as a loafing and feeding area and also had access to pasture. Hardwood shavings were used as the bedding material in a free-stall barn.
Cows were milked in a double-two, side-opening, low line parlor.

**Sampling Schedule**

The bacteriological status of mammary quarters was determined at the initiation of each study by the collection and culture of duplicate milk samples 1 wk before bacterial challenge was initiated. A third sample was collected from specific quarters and cultured when re-infection was initiated. All quarters were eligible for new IMI caused by Staph. aureus or Strep. agalactiae except those quarters infected with organisms of the same species as challenge organisms and those with deformed or abnormal teats.

**Collection of Milk Samples**

Before quarter milk sampling, the ventral surfaces of udders and teats that were excessively dirty were washed with a hand-held hose and paper towels. After washing, udders and teats were dried thoroughly with additional paper towels, and two or three streams of foremilk were discarded. Each teat apex was scrubbed for several seconds with a cotton pledget moistened with 70% alcohol until the teat was thoroughly clean. Teats on the side of the udder opposite from the technician were sanitized first, and milk samples were collected in reverse order into sterile snap-cap plastic tubes and refrigerated at 5°C. For teats that were visibly clean before collection, washing was omitted, and only cotton pledges moistened with 70% alcohol were used to sanitize teat ends.

**Culture and Diagnostic Procedures**

Samples were mixed by shaking, and a 0.01-ml aliquot was streaked on trypticase soy agar (TSA; Becton Dickinson, Cockeysville, MD) containing 5% bovine calf blood. Plates were incubated at 37°C for 48 h and examined to identify the microorganisms that were present. Contaminated quarters were resampled to confirm the presence or absence of challenge organisms. Colonies of Staph. aureus were identified presumptively by the hemolytic pattern and confirmed by the tube coagulase test. Colonies of Strep. agalactiae were identified to serogroup by the Phadebact Streptococcus Test (Boule Diagnostics AB, Huddinge, Sweden). A new IMI was confirmed when 1) Staph. aureus or Strep. agalactiae were isolated from a clinical quarter, 2) two consecutive samples yielded ≥500 cfu/ml of the same pathogen, or 3) three consecutive samples contained 100 to 400 cfu/ml of the same pathogen (Hogan et al., 1990).

**Description of Experimental Teat Dips**

The chlorine dioxide teat dip (Bi-Sept, Westfalia-Surge, Naperville, IL) was provided as two separate parts, and each part was mixed in equal quantities before use. Part 1 was an activator containing 2.9% lactic acid and 8% glycerin, and it was mixed in equal quantity with part 2, which was a base containing 0.7% sodium chlorite and 2% glycerin. The iodophor teat dip (Derma Kote, Westfalia-Surge) contained 0.5% iodine and was used without dilution.

**Treatment Method**

The milking herd of the Hill Farm Research Station was divided into two groups. A group of 59 cows was used to test the chlorine dioxide dip, and a group of 58 cows was used to test the 0.5% iodophor dip. During the afternoon milking, Monday through Friday, all teats of each cow were immersed to a depth of approximately 25 mm in a challenge suspension containing Staph. aureus (ATCC 29740) and Strep. agalactiae (ATCC 27956) immediately after milking machines were removed. Immediately following challenge, the distal 25 mm of two contralateral teats were dipped with the experimental teat dip; the remaining two teats served as undipped controls.

**Preparation of the Challenge Suspension**

Suspensions of Staph. aureus and Strep. agalactiae were prepared as described by Boddie et al. (1994). Stock suspensions of Staph. aureus were prepared weekly. The contents of one lyophilized vial of Staph. aureus were reconstituted in 6 ml of trypticase soy broth (TSB; Becton Dickinson) and incubated at 37°C for 5 to 7 h. This culture was used to inoculate a 500-ml volume of TSB, which was incubated on a gyratory shaker for 16 h. After incubation, bacterial cells were pelleted by centrifugation, washed twice with 0.1% proteose-peptone (Difco Laboratories, Detroit, MI), and resuspended to the original volume in proteose-peptone. Serial dilutions were prepared in proteose-peptone, and 0.1 ml was plated on TSA with 5% bovine calf blood. Plates were incubated for 24 h at 37°C, and colonies were counted to ascertain the microbial concentration of the stock suspension. This suspension was stored at 5°C and was used daily for 5 d to prepare challenge suspensions of Staph. aureus.
Cultures of *Strep. agalactiae* were prepared by suspension of a lyophilized vial of *Strep. agalactiae* in 6 ml of TSB, and a 0.01-ml aliquot was streak plated on each of five TSA plates. Plates were incubated at 37°C for 16 h and stored at 5°C to serve as stock cultures for 5 d. Daily challenge suspensions of *Strep. agalactiae* were prepared by the inoculation of 6 ml of TSB with six colonies from a TSA stock plate. The 6-ml culture was incubated for approximately 15 h at 37°C and was used to inoculate 500 ml of TSB. The 500-ml culture was incubated for 7 h at 37°C on a gyratory shaker. Aliquots of the culture were added to approximately 150 ml of nonsterile pasteurized milk to adjust the concentration of *Strep. agalactiae* to approximately $5 \times 10^7$ cfu/ml.

An aliquot of the Staph. aureus stock suspension was added to the *Strep. agalactiae* suspension to obtain a concentration of approximately $5 \times 10^7$ cfu/ml of Staph. aureus. This bacterial suspension was taken immediately to the milking parlor to challenge teats after the afternoon milking. A plate count was conducted daily on challenge suspensions.

**Statistical Methods**

Differences between the percentages of quarters that became infected in treatment groups were tested as described by Hogan et al. (1990) using an approximated statistic defined as follows: $t = [(x_1/n_1) - (x_2/n_2)]/[(x_1 + x_2)/n_1/n_2]^{0.5}$ where $x_1$ = number of new IMI in control quarters, $x_2$ = number of new IMI in treated quarters, $n_1$ = (number of control quarters)/(time unit), and $n_2$ = (number of treated quarters)/(time unit). The denominators $n_1$ and $n_2$ were expressed as the sum of quarter-days. A quarter was eligible for only one IMI per organism during each study. The percentage reduction in the rate of new IMI in the treated groups compared with that in the control groups was expressed as $100[(x_1/n_1) - (x_2/n_2)]/x_1$. Teat dips generally are considered to be efficacious when the mean percentage reduction of new IMI is ≥40% and the lower confidence limit of the mean is ≥25% reduction (Hogan et al., 1990).

**Scoring of Teat Skin and Teat End Condition**

Characteristics of teat skin and teat ends were scored immediately before and at the conclusion of the study to determine the effects of these germicides on teat condition. Teat skin and teat end conditions were delineated according to the parameters established by Goldberg et al. (1994). Condition scores for teat skin and teat ends before and after the trial were analyzed mixed model, in which cow within herd was considered a random effect (Gill and Hafs, 1971) as follows: model: $Y_{ijklm} = \mu + H_i + C(H)_{ij} + D_k + T_l + HD_{ik} + HT_{il} + DT_{kl} + HDT_{ikl} + \varepsilon_{ijklm}$, where: $Y_{ijklm}$ = dependent observation on teat skin or end score, $\mu$ = overall mean, $H_i$ = herd i, $C(H)_{ij}$ = cow j nested within herd i, $D_k$ = dip treatment k (control k = 0; dipped k = 1), $T_l$ = time l (before trial l = 0; after trial l = 1), $HD_{ik}$ = interaction between herd i and treatment k, $HT_{il}$ = interaction between herd i and time l, $DT_{kl}$ = interaction between treatment k and time l, and $HDT_{ikl}$ = interaction between herd i, treatment k, and time l. Mean squares for cow within herd were used to test herd main effect. All other sources were tested against the residual mean square.

**RESULTS AND DISCUSSION**

The chlorine dioxide teat dip reduced the number of new IMI caused by *Staph. aureus* by 86.6% ($P < 0.001$) and reduced the number of new IMI caused by *Strep. agalactiae* by 88.4% ($P < 0.001$) (Table 1). The infection rates for *Staph. aureus* in control and dipped quarters were 19.6 and 2.7%, respectively. Infection rates for *Strep. agalactiae* were 20.0 and 2.7% for control and dipped quarters, respectively.

The 0.5% iodine teat dip reduced the number of new IMI caused by *Staph. aureus* by 92.9% ($P < 0.001$) and reduced the number of new IMI caused by *Strep. agalac-

---

**Table 1. Summary of efficacy data on a chlorine dioxide teat dip against Staphylococcus aureus and Streptococcus agalactiae.**

<table>
<thead>
<tr>
<th>Challenge organism</th>
<th>Quarters eligible for new IMI</th>
<th>New IMI</th>
<th>Quarters at risk for new IMI</th>
<th>New IMI per 100 quarter-days at risk</th>
<th>Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staph. aureus</em></td>
<td>106</td>
<td>3</td>
<td>5051</td>
<td>0.0594</td>
<td>86.6*</td>
</tr>
<tr>
<td>Dip</td>
<td>112</td>
<td>22</td>
<td>4946</td>
<td>0.4448</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>113</td>
<td>3</td>
<td>5464</td>
<td>0.0549</td>
<td>88.4*</td>
</tr>
<tr>
<td><em>Strep. agalactiae</em></td>
<td>115</td>
<td>23</td>
<td>4855</td>
<td>0.4737</td>
<td></td>
</tr>
</tbody>
</table>

* $P < 0.001$.
Table 2. Summary of efficacy data on a 0.5% iodine teat dip against Staphylococcus aureus and Streptococcus agalactiae.

<table>
<thead>
<tr>
<th>Challenge organism</th>
<th>Quarters eligible for new IMI</th>
<th>New IMI</th>
<th>Quarters-days at risk for new IMI</th>
<th>New IMI per 100 quarters-days at risk</th>
<th>Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(no.)</td>
<td>(%)</td>
<td></td>
<td>(%)</td>
<td></td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>Dip</td>
<td>109</td>
<td>2</td>
<td>6045</td>
<td>0.0331</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>108</td>
<td>25</td>
<td>5366</td>
<td>0.4659</td>
</tr>
<tr>
<td>Strept. agalactiae</td>
<td>Dip</td>
<td>115</td>
<td>9</td>
<td>5723</td>
<td>0.1573</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>115</td>
<td>17</td>
<td>6122</td>
<td>0.2777</td>
</tr>
</tbody>
</table>

*P < 0.20.
**P < 0.001.

by 43.4% (P < 0.20) (Table 2). The infection rates for Staph. aureus in control and dipped quarters were 23.1 and 1.8%, respectively. Infection rates for Strept. agalactiae were 14.8 and 7.8% for control and dipped quarters, respectively.

Characteristics of lateral teat skin surfaces and those of teat ends were scored immediately before the initiation of the teat dip trials and at the conclusion of the trials to determine any effects of these germicides on teat condition.

An analysis of condition scores for the lateral teat skin (six-point scale where 0 = teat skin has been subjected to physical injury not related to treatment to 5 = teat skin is severely damaged with scabs or lesions) demonstrated that the mean score before and after the trial for dipped and control quarters was approximately 1 for both products (Table 3). A similar analysis of condition scores for teat ends (six-point scale where 0 = teat subject to physical injury not related to treatment to 5 = teat end is severely damaged and ulcerative with scabs or warts) showed that the mean score across all variables was approximately 1, ranging from 0.98 to 1.17 (Table 3). Thus, differences in the condition scores of teat skin or teat ends before or after the study for the treated or control quarters were not detected for either product using α < 0.05 probability of a type 1 error as the criterion for rejecting the null hypothesis of no difference.

The chlorine dioxide teat dip tested during this trial compared favorably with other chlorous acid-chlorine dioxide teat dips tested using experimental challenge procedures. Boddie et al. (Boddie et al., 1998) evaluated a chlorous acid-chlorine dioxide teat dip containing 0.98% phosphoric acid and 0.65% sodium chlorite. This experimental dip reduced new Staph. aureus IMI by 91.5% and new Strept. agalactiae IMI by 71.7%. Drechsler et al. (1990) tested a chlorous acid-chlorine dioxide teat dip containing 2.64% lactic acid and 0.64% sodium chlorite using experimental exposure procedures. Efficiencies against Staph. aureus and Strept. agalactiae were 78.9 and 52.5%, respectively. A teat dip containing 0.64% sodium chlorite and 3% mandelic acid reduced new IMI by Staph. aureus 68.7 and 56.4% for Strept.

Table 3. Mean condition score of teat skin and teat ends before and after the evaluation of a teat dip containing 0.7% sodium chlorite and a teat dip containing 0.5% iodine.1

<table>
<thead>
<tr>
<th></th>
<th>Teat skin2</th>
<th>Teat end3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chlorite</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before trial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dipped</td>
<td>1.01</td>
<td>1.17</td>
</tr>
<tr>
<td>Control</td>
<td>1.02</td>
<td>1.13</td>
</tr>
<tr>
<td>After trial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dipped</td>
<td>1.00</td>
<td>1.05</td>
</tr>
<tr>
<td>Control</td>
<td>0.99</td>
<td>1.07</td>
</tr>
<tr>
<td>Iodine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before trial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dipped</td>
<td>0.98</td>
<td>1.08</td>
</tr>
<tr>
<td>Control</td>
<td>1.00</td>
<td>1.05</td>
</tr>
<tr>
<td>After trial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dipped</td>
<td>1.03</td>
<td>1.05</td>
</tr>
<tr>
<td>Control</td>
<td>1.13</td>
<td>1.06</td>
</tr>
</tbody>
</table>

1Treatment means were not significantly different at α = 0.05.
2Teat skin condition scoring (Goldberg et al., 1994): 0 = teat skin has been subjected to physical injury (e.g., stepped on or frostbitten) that is not related to the treatment, or the quarter is nonlactating; 1 = teat skin is smooth and free from scales, cracks, or chapping; 2 = teat skin shows some evidence of scaling; 3 = teat skin is chapped, and some small warts may be present; 4 = teat skin is chapped and cracked; redness, indicating inflammation, is present; and numerous warts may be present; and 5 = teat skin is severely damaged and ulcerative with scabs or open lesions; large or numerous warts are present that interfere with teat end function.
3Teat end condition scoring according to (Goldberg et al., 1994): 0 = teat end has been subjected to physical or chemical injury (e.g., stepped on or frostbitten) that is not related to the treatment, or the quarter is nonlactating; 1 = teat end sphincter is smooth with no evidence of irritation; 2 = teat end has a raised ring; 3 = teat end sphincter is roughened with slight cracks, but no redness is present; 4 = teat end sphincter is inverted with many cracks, giving a flowered appearance; and 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 are present that interfere with teat end function.
agalactiae during an experimental challenge study (Boddie et al., 1994).

The 0.5% iodophor teat dip tested during the present study prevented new Staph. aureus IMI at a higher percentage (92.9%) efficacy, than a 0.5% iodophor dip in a previous study at the same location in which efficacy was 78.2% (Boddie and Nickerson, 1997). Efficacy in the previous study against Strep. agalactiae was 73.2% in contrast to 43.4% in the present study (Boddie and Nickerson, 1997).

CONCLUSIONS

The chlorine dioxide teat dip significantly reduced new IMI caused by Staph. aureus and Strep. agalactiae under experimental exposure to these pathogens. The 0.5% iodine teat dip significantly reduced new IMI caused by Staph. aureus (92.9%) but did not show a significant reduction against new Strep. agalactiae IMI (43.4%). The condition of teat ends and teat skin for both teat dips was characterized as normal and without irritation at the completion of the efficacy studies.

ACKNOWLEDGMENTS

The partial financial support of this study by Westfalia-Surge is gratefully acknowledged. The technical assistance of Nancy Boddie, Corinne Ray, and the Hill Farm Research Station dairy personnel and the secretarial support of Sondra Blackwell also are appreciated.

REFERENCES