Reduction of Mastitis Caused by Experimental Challenge with *Staphylococcus aureus* and *Streptococcus agalactiae* by Use of a Quaternary Ammonium and Halogen-Mixture Teat Dip

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ABSTRACT

A teat-dip formulation containing sodium dichloroisocyanuric acid, bronopol, and quaternary ammonium was tested for efficacy against *Staphylococcus aureus* and *Streptococcus agalactiae* intramammary infections (IMI) using an experimental challenge model. Sixty-two Jersey cows from the Hill Farm Research Station (Homer, LA) were used in an 8-wk controlled infection trial to evaluate the teat dip. During the afternoon milking, Monday through Friday for 8 wk, all teats of each cow were immersed to a depth of approximately 25 mm in a challenge suspension containing approximately $5 \times 10^7$ cfu of *Staphylococcus aureus* and approximately $5 \times 10^7$ cfu of *Streptococcus agalactiae* immediately after milking machines were removed. Immediately after challenge, the distal 25 mm of two contralateral teats were dipped with the experimental teat dip; the remaining two teats served as undipped controls. The experimental teat dip reduced the number of new *Staph. aureus* IMI by 70.9% and reduced the number of new *Strep. agalactiae* IMI by 60.0%. Teat end and teat skin condition were characterized as normal and without irritation at the completion of the study. The combination of the three germicides in this experimental teat dip is unique and an effective formulation without adverse effects on condition of teat ends or teat skin. (Key words: sodium dichloroisocyanuric acid, bronopol, quaternary ammonium, teat dip)

INTRODUCTION

Chloroisocyanurates are organic chloramines that are used as household bleaches, dishwashing compounds, and sanitizing compounds for the food and dairy industries. Similarly, bronopol has widespread application as a preservative in pharmaceuticals and cosmetics. In addition, quaternary ammonium products are used as surgical hand scrubs and surgical instrument sterilization and for disinfection of hospital floors, walls, and equipment surfaces. Quaternary ammonium compounds are also used safely in mouthwashes, contact lens solutions, and topical skin preparations (Block, 1991; Pankey et al., 1984). The objective of this study was to evaluate a teat dip product containing chloroisocyanurate, bronopol, and quaternary ammonium for its ability to prevent new IMI after experimental challenge with *Staphylococcus aureus* and *Streptococcus agalactiae*.

MATERIALS AND METHODS

Cows

Sixty-two Jersey cows from the Hill Farm Research Station (Homer, LA) dairy herd were used in an 8-wk controlled infection trial to evaluate the teat dip. Cows used 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 free stalls. 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 the study by the collection and culture of duplicate milk samples from each quarter of 62 cows 1 wk before bacterial challenge was initiated. A third sample was collected from specific quarters and cultured when results from the first two samples differed. Milk samples from each quarter were collected and analyzed bacteriologically during each week of the study. Whenever *Staph. aureus* or *Strep.*
agalactiae were isolated for the first time in a previously uninfected quarter, a second sample was collected and cultured within 48 h after the first isolation to confirm the establishment of a new IMI. All quarters were eligible for new IMI caused by Staph. aureus or Strep. agalactiae, except those quarters previously 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 using 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 it 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 pledgets 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 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 the Experimental Teat Dip

The teat dip (Actisept Pre Post, Activon Products, Fort Collins, CO) was provided as a powder concentrate. One gallon (3.8 L) of warm tap water and shaking well. This solution was used no longer than 14 d after mixing.

Treatment Method

A group of 62 cows was used to test the teat dip. During the afternoon milking, Monday through Friday for 8 wk, all teats of each cow were immersed to a depth of approximately 25 mm in a challenge suspension containing approximately 5 × 10^7 cfu of Staph. aureus [American Type Culture Collection (ATCC) 29740, Rockville, MD] and approximately 5 × 10^7 cfu of Strep. agalactiae (ATCC 27956) immediately after milking machines were removed. Immediately after 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 × 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.

### Scoring of Teat Skin and Teat End Condition

Characteristics of lateral teat skin surfaces and those of teat ends were scored immediately before the initiation of the teat dip trial and at the conclusion of the trial to determine any effects of the germicide on teat condition. Condition scores for the lateral teat skin were on a six-point scale where 0 = teat skin had been subjected to physical injury not related to treatment to 5 = teat skin had been severely damaged with scabs or lesions (Goldberg et al., 1994). Condition scores for teat ends were also recorded on a six-point scale where 0 = teat had been subjected to physical injury not related to treatment to 5 = teat end was severely damaged and ulcerative with scabs or warts (Goldberg et al., 1994).

### 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 $t$ statistic defined as follows: 

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

where $x_1 = n$ number of new IMI in control quarters, $x_2 = n$ number of new IMI in treated quarters, $n_1 = (n$ number of control quarters $)$ $($time unit$)$, and $n_2 = (n$ 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 the study. The percentage reduction in rate of new IMI in the treated group compared with that in the control group was expressed as $100[(x_1/n_1) - (x_2/n_2)]/(x_1/n_1)$. Teat dips generally are considered to be efficacious when the mean percentage reduction of new IMI is $\geq 40\%$ and the lower confidence limit of the mean is $\geq 25\%$ reduction (Hogan et al., 1990).

Condition scores for teat skin and teat ends before and after the study were analyzed by repeated measures ANOVA. The design was a split block in time adapted from Gill and Hafs (1971) using the following model: 

$$ Y_{ijk} = \mu + A_i + B(A)_j + C_k + AC_{ik} + e_{ijk} $$

where $Y_{ijk}$ = dependent observation, $\mu$ = overall mean, $A_i$ = teat dip $I$, $B(A)_j$ = cow $j$ nested within teat dip, $C_k$ = time $k$ (before or after study), $AC_{ik}$ = interaction of teat dip and time, and $e_{ijk}$ = residual error. The effect of teat dip was tested using $B(A)_j$ as the error term using $\alpha < 0.05$ probability of a Type 1 error as the criterion for rejecting the null hypothesis of no difference. Time and the interaction of teat dip and time were tested using the residual as the error term. If a significant interaction was detected, the effects of the teat dip were examined within time by general linear models ANOVA.

### RESULTS AND DISCUSSION

The teat dip reduced the number of new IMI caused by *Staph. aureus* by 70.9% ($P < 0.001$) and reduced the number of new IMI caused by *Strep. agalactiae* by 60.0% ($P < 0.05$) (Table 1). The infection rates for *Staph. aureus* in control and dipped quarters were 25.9 and 8.6%, respectively. Infection rates for *Strep. agalactiae* were 13.6 and 5.7% for control and dipped quarters, respectively.

The teat dip tested during this trial compared favorably with other teat dips containing either sodium dichloroisocyanuric acid (NaDCC), bronopol, or quaternary ammonium, which were tested in separate trials using experimental challenge procedures. A 0.2% bromine teat dip was tested by Philpot and Pankey (1975), and efficacies of 91.8 and 78.7% were obtained against *Staph. aureus* and *Strep. agalactiae*, respectively. However, in a subsequent study, lower efficacies were obtained with a 0.2% bronopol teat dip (Philpot et al.; 1978b); reduction in new *Staph. aureus* IMI was 54.4% and that for *Strep. agalactiae* was 41.7%. Philpot and Pankey (1978a) tested 0.18 and 0.5% quaternary ammonium teat dips and obtained efficacies of 84.8% against *Staph. aureus* for the 0.18% formulation and 62.0 and 50.5% against *Staph. aureus* and *Strep. agalactiae* for the 0.5% formulation. Boddie et al. (1983) tested a 0.2% quaternary ammonium complex dip and obtained efficacies of 89.3 and 60.8% against *Staph. aureus* and *Strep. agalactiae*, respectively. A 0.6% sodium dichloro-triazenetrione (SDT) teat dip, which is homologous to NaDCC, yielded a 79.0% efficacy against *Staph. aureus* (Philpot and Pankey, 1978a). In another trial, a 1% SDT teat dip yielded 75.9 and 63.2% efficacies against *Staph. aureus* and *Strep. agalactiae*, and a 1.7% SDT dip tested against *Strep. agalactiae* reduced new IMI by 48.1% (Pankey et al., 1983). More recently, two NaDCC formulations were tested by Boddie and Nickerson (1996); the formulation that contained 2800 mg/kg of available chlorine reduced new *Staph. aureus* IMI by 73.6% and new *Strep. agalactiae* by 65.1%, and the formulation that contained 3000 mg/kg of available chlorine reduced new *Staph. aureus* and *Strep. agalactiae* IMI by 69.0 and 63.5%, respectively.

Mean scores of teat skin condition before and after the trial for dipped and control quarters were approximately 1 for the product and ranged from 0.99 to 1.08 (Table 2). A similar analysis of condition scores for teat
ends showed that the mean score across all variables was also approximately 1, ranging from 0.96 to 1.05 (Table 2). Thus, differences in the condition scores of teat skin or teat ends before or after the study between the treated and control quarters were not detected.

**CONCLUSIONS**

A teat dip composed of a novel mixture of halogens and quaternary ammonium significantly reduced new IMI caused by *Staph. aureus* and *Strep. agalactiae* under experimental exposure. This product reduced new *Staph. aureus* IMI by 70.9% and new *Strep. agalactiae* IMI by 60.0%. Teat end and teat skin condition were characterized as normal and without irritation at the completion of the study. The combination of the three germicides in this experimental teat dip is a unique formulation. In the authors’ experience, this formulation has not been tested using an experimental challenge model before. Evidence indicates that small additions of bromine to chlorine solutions greatly enhance the bactericidal activity of chlorine and that the killing time of chlorine is increased considerably if ammonium is added to the solution at a concentration less than one-eighth of the total available chlorine (Dychdala, 1991).

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**REFERENCES**


