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Research Note

# Evaluation of a Postmilking Teat Disinfectant Containing a Phenolic Combination for the Prevention of Mastitis in Lactating Dairy Cows

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## ABSTRACT

A trial was conducted for 12 months in a herd of 120 Holstein cows in order to determine the efficacy of a teat disinfectant, which contained a phenolic combination, for the prevention of bovine intramammary infections during lactation. Postmilking teat disinfection was compared to a negative control using a split-udder experimental design. The percentage of quarters newly infected by mastitis pathogens was 45% lower in mammary glands with teats that had been dipped in the experimental teat disinfectant after milking than it was in undipped controls. New infections caused by *Streptococcus uberis*, *Staphylococcus aureus*, coagulase-negative *Staphylococcus* species, and *Corynebacterium bovis* were significantly lower in mammary glands with teats that had been dipped in the experimental teat disinfectant than in undipped controls. No statistical differences in the incidence of clinical mastitis between treatment groups were observed. No irritation or chapping of teats dipped in the experimental teat disinfectant were observed. The results of this study suggest that the experimental teat disinfectant containing a phenolic combination is an effective postmilking teat disinfectant for use in the prevention of new intramammary infections by both contagious and environmental mastitis pathogens.

Postmilking teat disinfection is one of the most effective procedures for reducing the rate of subclinical and clinical mastitis during lactation *(2, 5–11)*. Consequently, this procedure has been widely recommended by dairy advisors and has been adopted by dairy producers in increasing numbers because it is recognized as a simple, economical, and highly effective method for controlling mastitis.

Despite universal acceptance of postmilking teat disinfection as a method of mastitis control, there are limitations associated with most of the teat disinfectants that are currently available. The most significant limitation is that teat disinfectants do not afford equal protection against the vast array of bacteria that cause mastitis. While postmilking teat disinfection with an effective germicide reduces intramammary infections (IMIs) caused by contagious mastitis pathogens, such as *Streptococcus agalactiae* and *Staphylococcus aureus (2, 9, 10)*, it has not proven effective in controlling mastitis that is attributable to environmental pathogens, such as coliforms and streptococci other than *S. agalactiae (2, 8, 10, 11)*.

The lack of effectiveness of postmilking teat disinfectants, as related to the prevention of mastitis caused by environmental pathogens, is thought to be related to differences in the reservoirs of mastitis pathogens and to the time at which bacterial exposure takes place *(2, 10, 11)*. The primary reservoir of contagious mastitis pathogens in dairy herds is thought to consist of infected mammary glands *(10, 11)*. Exposure to contagious mastitis pathogens occurs during the milking process, as milk that contains bacteria and contaminated hands or milking-machine liners come into contact with the teats of uninfected mammary glands. Postmilking teat disinfection kills bacteria transferred to teats during milking, thus decreasing IMIs that are attributable to *S. agalactiae* and *S. aureus*. Reservoirs of environmental mastitis pathogens are not found only in infected mammary glands but are also found in the environment. Postmilking teat disinfection reduces the number of environmental pathogens on teat skin immediately after milking. However, exposure of teats to environmental pathogens continues between milkings, when most teat disinfectants have lost their effectiveness. Thus, reduced effectiveness of postmilking teat disinfectants (against environmental pathogens) is a significant limitation of this mastitis control procedure, since the prevalence of environmental mastitis appears to be increasing in herds that have eradicated *S. agalactiae* and reduced the prevalence of *S. aureus (2, 7, 10, 11)*.

The objective of the present study was to determine the efficacy of a postmilking teat disinfectant containing a phenolic combination for the prevention of bovine mastitis during lactation. Phenol and phenolic compounds have been studied extensively as disinfectants and have been shown to have a wide spectrum of antibacterial activity against both gram-positive and gram-negative pathogens,

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including *Mycobacterium bovis*, as well as viruses *(1)*. This study was conducted in a herd that was free of *S. agalactiae* but that had a high prevalence of *S. aureus* and *Streptococcus uberis* mastitis.

## MATERIALS AND METHODS

This study was conducted in The University of Tennessee Middle Tennessee Experiment Station research herd at Spring Hill, Tenn. An average of 120 Holstein cows, with a rolling herd average of approximately 9,545 kg of milk per cow, were lactating during each herd survey, and 194 cows were included in the 12-month study. Cows that were milking for at least 30 days were included in data analysis. The herd was *S. agalactiae*–negative, but the herd experienced mastitis caused by *S. aureus* and environmental pathogens, particularly *S. uberis*. Cows were milked twice daily in a double-five herringbone parlor equipped with a DeLaval milking-machine system (DeLaval, Kansas City, Mo.) with automatic milking-machine take-offs. Milking equipment was evaluated routinely and maintained per the manufacturer's recommendations. Lactating cows were housed in free stalls bedded with sawdust. Lactating cows were allowed on pasture for exercise for 4 h per day when weather permitted. All cows were dried off approximately 8 weeks before expected calving, and all quarters of cows were infused with antibiotic preparations approved for use in nonlactating cows following the last milking of lactation.

The experimental teat disinfectant was evaluated, following the procedures recommended by the National Mastitis Council *(4)*, in order to determine the efficacy of a postmilking teat disinfectant based on reduction of naturally occurring new IMIs. Postmilking teat disinfection was compared to a negative control using a split-udder experimental design. Milking procedures for cows in the postmilking teat disinfectant and negative control groups were identical, except for the application of the experimental teat disinfectant that contained a phenolic combination (MASTICIDE, Sporicidin International, Rockville, Md.) after milking-machine removal. Teats on the left side of udders were not dipped. Teats were forestripped to check for abnormal milk, washed (if necessary) with a minimum amount of water, and dried thoroughly (if washed) with single-service paper towels in preparation for milking.

Duplicate samples of foremilk were collected aseptically from all quarters of all lactating cows at the onset of the study and from cows with clinical mastitis. Single samples of foremilk were collected monthly thereafter for the duration of the study. In addition, quarter foremilk samples were collected aseptically from cows within 7 days after calving, from cows at drying off, and when animals left the herd. All samples were collected immediately before regular milking using standard procedures described by Harmon et al. *(3)*. Before sample collection, teats of cows were cleaned thoroughly, dried with individual disposable paper towels, and teat ends were sanitized with swabs containing 70% isopropyl alcohol.

Milk samples were examined following the procedures recommended by the National Mastitis Council and essentially as described by Oliver et al. *(5)*. Briefly, foremilk samples (10 μl) from each quarter were plated onto one quadrant of a trypticase soy agar plate supplemented with 5% defibrinated sheep blood (Laboratory Supply, Nashville, Tenn.). Plates were incubated at 37°C, and bacterial growth was observed and recorded at 24-h intervals for 2 days. Bacteria on primary culture media were identified tentatively according to colony morphologic features, hemolytic characteristics, and catalase test. Isolates identified presumptively as staphylococci were tested for coagulase by the tube coagulase method. Isolates identified presumptively as streptococci were evaluated initially for growth in 6.5% NaCl, hydrolysis of esculin, and CAMP reaction. Streptococcal organisms were identified to the species level using the API 20 Strep System (bioMerieux Vitek, Inc., Hazelwood, Mo.) upon first isolation of the organism from infected quarters. Gram-negative isolates were plated on MacConkey's agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.) and evaluated by the following biochemical tests: triple sugar iron, urea, oxidase, motility, indole, and ornithine decarboxylase. Gram-negative isolates were identified to the species level using the API 20E identification system (bioMerieux Vitek). *Corynebacterium bovis* was identified as catalase-positive, gram-positive rods that exhibited enhanced growth on brain heart infusion agar supplemented with 0.1% Tween 80 (Fisher Scientific Co., Fair Lawn, N.J.).

A quarter was considered infected at the onset of the study when the same pathogen was isolated from duplicate samples. Diagnosis of IMIs in mammary glands of cows after parturition was based on isolation of the same organism in samples obtained within the first 7 days of lactation. A quarter was considered newly infected when the same bacterial species was isolated from two consecutive monthly samples or in samples from mammary glands of cows with clinical mastitis. A quarter was eligible for only one infection per bacterial species during a lactation (i.e., only one *S. aureus* infection per quarter per lactation). Teats were regularly visually examined for irritation, chapping, or other abnormalities throughout the course of the study.

The mean percentage reduction in the rate of new IMIs achieved among mammary glands with teats that had been dipped in the experimental teat disinfectant after milking compared with the same rate in undipped controls, and the statistical reliability of the mean percentage reductions were determined. Differences between treatment groups were assessed using Student's *t* test.

## RESULTS AND DISCUSSION

A summary of the efficacy of the experimental postmilking teat disinfectant that contained a phenolic combination for the prevention of new IMIs is presented in Table 1. One hundred and four new *S. aureus* infections were observed—63 in undipped controls and 41 in mammary glands with teats that had been dipped in the experimental teat disinfectant after milking. The efficacy of the experimental teat disinfectant in the prevention of new *S. aureus* IMIs was 33.5% ($P < 0.05$). Seventy-five new *S. uberis* IMIs were detected—52 in undipped controls and 23 in mammary glands with teats that had been dipped in the experimental teat disinfectant after milking. The efficacy of the experimental teat disinfectant in the prevention of new *S. uberis* IMIs was 54.1% ($P < 0.001$). The efficacies of the experimental teat disinfectant in the prevention of new coagulase-negative *Staphylococcus* species IMIs and *C. bovis* were 62.8 ($P < 0.005$) and 53.3% ($P < 0.005$), respectively. The overall efficacy of the experimental postmilking teat disinfectant that contained a phenolic combination for the prevention of new IMIs against all mastitis pathogens was 45% ($P < 0.001$).

Sixty-two cases of clinical mastitis were observed, 38 in undipped controls and 24 in mammary glands with teats that had been dipped in the experimental teat disinfectant after milking; however, statistical differences between treat-

TABLE 1. *Efficacy of an experimental teat disinfectant containing a phenolic combination for the prevention of new intramammary infections in lactating dairy cows*

| Organism | Treatment group | New infections: Number | New infections: % of quarters | Percentage reduction |
|---|---|---|---|---|
| *Staphylococcus aureus* | Control[a] | 63 | 16.4 | 33.5[c] |
| | Treated[b] | 41 | 10.9 | |
| *Streptococcus uberis* | Control | 52 | 13.5 | 54.1[d] |
| | Treated | 23 | 6.2 | |
| *Streptococcus dysgalactiae* | Control | 11 | 2.9 | |
| | Treated | 9 | 2.4 | |
| Gram-negative bacteria | Control | 5 | 1.3 | |
| | Treated | 8 | 2.1 | |
| Coagulase-negative *Staphylococcus* species | Control | 30 | 7.8 | 62.8[e] |
| | Treated | 11 | 2.9 | |
| *Corynebacterium bovis* | Control | 46 | 12.0 | 53.3[e] |
| | Treated | 21 | 5.6 | |
| Other pathogens | Control | 6 | 1.6 | |
| | Treated | 1 | 0.3 | |
| All pathogens | Control | 213 | 55.5 | 45.0[d] |
| | Treated | 114 | 30.5 | |

[a] $n$ = 384 quarters available for infection.
[b] $n$ = 374 quarters available for infection.
[c] Different from controls ($P < 0.05$).
[d] Different from controls ($P < 0.001$).
[e] Different from controls ($P < 0.005$).

ment groups in terms of the incidence of clinical mastitis were not observed (Table 2). Most clinical mastitis was attributable to *S. aureus* (38.7%), *Streptococcus dysgalactiae* (24.2%), and *S. uberis* (19.4%). About 20% of *S. aureus*, 75% of *S. dysgalactiae*, and 16% of *S. uberis* IMIs detected during lactation resulted in clinical mastitis.

Phenol and phenolic compounds have been studied extensively as disinfectants, and they have been shown to have a wide spectrum of antibacterial activity against both gram-positive and gram-negative pathogens *(1)*. At higher concentrations, these compounds penetrate and disrupt the bacterial cell wall and precipitate proteins of the bacterial cell. At lower concentrations, phenol apparently inactivates important enzyme systems of the bacterial cell *(1)*.

One major difference between the results of the present study and previous reports on efficacy evaluation of postmilking teat disinfectants *(7–9)* is that the experimental postmilking teat disinfectant that contained a phenolic combination was significantly effective in preventing new *S. uberis* IMIs. One potential explanation for the effectiveness against this environmental pathogen could be the residual antimicrobial activity. Thus, on the basis of the present study, the phenolic combination–based teat disinfectant would likely be of considerable value as a postmilking teat disinfectant in herds that were experiencing *S. uberis* mastitis.

TABLE 2. *Mastitis pathogens isolated from cows with clinical mastitis*

| Organism | Experimental group: Control | Experimental group: Treated |
|---|---|---|
| *Staphylococcus aureus* | 13 | 8 |
| *Streptococcus uberis* | 6 | 6 |
| *Streptococcus dysgalactiae* | 10 | 5 |
| Coagulase-negative *Staphylococcus* species | 4 | 0 |
| *Escherichia coli* | 1 | 3 |
| *Klebsiella pneumoniae* | 3 | 1 |
| *Klebsiella oxytoca* | 0 | 1 |
| *Bacillus* species | 1 | 0 |
| Total[a] | 38 | 24 |

[a] Not different from controls ($P > 0.10$).

## CONCLUSIONS

The results of the present study indicate that the experimental teat disinfectant that contained a phenolic combination was effective in preventing new IMIs caused by *S. uberis*, *S. aureus*, coagulase-negative *Staphylococcus* species, and *C. bovis*. Under the conditions of this trial, no chapping or irritation of the teats was observed. The phenolic combination–based teat disinfectant used in the present study would be of considerable value as a postmilking teat disinfectant, especially in herds experiencing *S. uberis* mastitis.

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## REFERENCES

1. Block, S. S. 1991. Disinfection, sterilization, and preservation. Lea & Febiger, Philadelphia.
2. Bramley, A. J., and F. H. Dodd. 1984. Reviews of the progress of dairy science: mastitis control–progress and prospects. J. Dairy Res. 51:481–512.
3. Harmon, R. J., R. J. Eberhart, D. E. Jasper, B. B. Langlois, and R. A. Wilson. 1990. Microbiological procedures for the diagnosis of bovine udder infection. National Mastitis Council, Inc., Arlington, Va.
4. Hogan, J. S., D. M. Galton, R. J. Harmon, S. C. Nickerson, S. P. Oliver, and J. W. Pankey. 1990. Protocols for evaluating efficacy of postmilking teat dips. J. Dairy Sci. 73:2580–2585.
5. Oliver, S. P., B. E. Gillespie, M. J. Lewis, T. L. Ingle, and H. H. Dowlen. 1994. Evaluation of chlorhexidine as a premilking teat disinfectant for the prevention of intramammary infections during lactation. J. Food Prot. 57:614–618.
6. Oliver, S. P., S. H. King, M. J. Lewis, P. M. Torre, K. R. Matthews, and H. H. Dowlen. 1990. Efficacy of chlorhexidine as a postmilking teat disinfectant for the prevention of bovine mastitis during lactation. J. Dairy Sci. 73:2230–2235.
7. Oliver, S. P., S. H. King, P. M. Torre, E. P. Shull, H. H. Dowlen, M. J. Lewis, and L. M. Sordillo. 1989. Prevention of bovine mastitis by a postmilking teat disinfectant containing chlorous acid and chlorine dioxide in a soluble polymer gel. J. Dairy Sci. 72:3091–3097.
8. Oliver, S. P., M. J. Lewis, S. H. King, B. E. Gillespie, T. Ingle, K.

R. Matthews, H. H. Dowlen, P. A. Drechsler, E. E. Wildman, and J. W. Pankey. 1991. Efficacy of a low concentration iodine postmilking teat disinfectant against contagious and environmental mastitis pathogens in two dairy herds. J. Food Prot. 54:737–742.

9. Pankey, J. W., R. J. Eberhart, A. C. Cuming, R. D. Daggett, R. J. Farnsworth, and C. K. McDuff. 1984. Update on postmilking teat antisepsis. J. Dairy Sci. 67:1336–1353.

10. Smith, K. L. 1983. Mastitis control: a discussion. J. Dairy Sci. 66: 1790–1794.

11. Smith, K. L., D. A. Todhunter, and P. S. Schoenberger. 1985. Environmental mastitis: cause, prevalence, prevention. J. Dairy Sci. 68: 1531–1553.