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Efficacy of a New Premilking Teat Disinfectant Containing a Phenolic Combination for the Prevention of Mastitis

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ABSTRACT

A teat disinfectant containing a phenolic combination was evaluated in a natural exposure study in two dairy research herds. Premilking teat disinfection was compared with a negative control using a split-udder experimental design. In both herds, premilking and postmilking teat disinfections with the phenolic combination were significantly more effective in preventing new intramammary infection (IMI) than was postmilking teat disinfection only. Clinical mastitis and new IMI by *Streptococcus uberis*, *Streptococcus dysgalactiae*, Gram-negative pathogens, and coagulase-negative *Staphylococcus* species were significantly lower in quarters of cows with teats predipped and postdipped than in quarters with teats postdipped only. No chapping or teat skin irritation was observed. Premilking teat disinfection with the phenolic combination in association with good udder preparation and postmilking teat disinfection can further reduce the occurrence of new IMI by numerous mastitis pathogens during lactation. (**Key words:** bovine mastitis, premilking teat disinfectant, phenolic combination, environmental mastitis pathogens)

INTRODUCTION

Postmilking teat disinfection is an effective procedure for the prevention of mastitis in dairy cows during lactation (Oliver et al., 1989, 1990; Pankey et al., 1984). Consequently, this procedure is widely recommended by dairy advisors throughout the world and adopted by dairy producers in increasing numbers because it is recognized as a simple, economical, and highly effective method for controlling mastitis.

Despite widespread acceptance of postmilking teat disinfection for mastitis control, there are limitations associated with most teat disinfectants currently available. The most significant limitation is that teat disinfectants do not afford equal protection against the numerous pathogens that cause mastitis. In particular, postmilking teat disinfection has not proven effective in controlling mastitis due to environmental pathogens such as coliforms and streptococci other than *Streptococcus agalactiae* (Oliver et al., 1991; Smith, 1983; Smith et al., 1985). Exposure of teats to environmental mastitis pathogens, which are ubiquitous in the dairy environment, continues between milkings when most teat disinfectants have lost effectiveness, which perhaps explains why postmilking teat disinfection is less effective against environmental pathogens.

Premilking teat disinfection was developed as a potential method to control environmental pathogens by reducing bacterial populations on teat skin before milking. Reports (Oliver et al., 1993a, 1993b, 1994) have documented the benefits of premilking teat disinfection with an effective germicide in combination with postmilking teat disinfection. The objective of the present study was to determine the efficacy of a new premilking teat disinfectant containing a phenolic combination. 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 (Block, 1991). The efficacy of this phenolic combination as a postmilking teat disinfectant was recently reported (Oliver et al., 1999).

MATERIALS AND METHODS

The premilking teat disinfectant containing a phenolic combination (MASTICIDE, Sporicidin International, Rockville, MD) was evaluated in a natural exposure study in two dairy research herds of The University of Tennessee; the Dairy Experiment Station dairy herd, Lewisburg (herd 1) and the Middle Tennessee Experiment Station dairy herd, Spring Hill (herd 2). Study duration in the Dairy Experiment Station herd

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was 18 mo. study duration in the Middle Tennessee Experiment Station dairy herd was 15 mo.

The teat disinfectant was evaluated following procedures recommended by the National Mastitis Council (Hogan et al., 1991). Premilking teat disinfection was compared with a negative control using a split-udder experimental design. Control teats (both right teats) were forestripped to check for abnormal milk; washed with a minimum amount of water, if necessary; and dried thoroughly with single-service paper towels in preparation for milking. Treatment teats (both left teats) were forestripped to check for abnormal milk; washed with a minimum amount of water, if necessary; and dried thoroughly with single-service paper towels. The premilking teat disinfectant was then applied with a contact time of at least 30 s, and the teats were dried thoroughly with single-service paper towel in preparation for milking. The phenolic combination was applied as a postmilking teat disinfectant to teats of all cows after milking machine removal.

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 on two occasions within 10 d 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 the National Mastitis Council (Hogan et al., 1999). Before sample collection, teats of cows were cleaned thoroughly and dried with individual disposable paper towels, and teat ends were sanitized with swabs containing 70% isopropyl alcohol.

Milk samples were examined following procedures described by the National Mastitis Council (Hogan et al., 1999). 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, TN). Plates were incubated at 37°C, and bacterial growth was observed and recorded at 24-h intervals for 2 d. Bacteria on primary culture medium were tentatively identified according to colony morphologic features, hemolytic characteristics, and catalase test. Isolates presumptively identified as staphylococci were tested for coagulase by the tube coagulase method. Isolates presumptively identified 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). 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, Inc.). *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, NJ).

A quarter was considered infected at the onset of the study when the same pathogen was isolated from duplicate samples. Diagnosis of IMI in mammary glands of cows after parturition was based on isolation of the same organism in samples obtained within the first 10 d 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 (e.g., only one *Streptococcus uberis* infection per quarter per lactation). Teats were observed visually for irritation (redness) and chapping throughout the study.

We determined the mean percentage reduction in the rate of new IMI among mammary glands with teats predipped and postdipped in the experimental teat disinfectant compared with the rate among quarters with teats postdipped only; we also determined the statistical reliability of the mean percentage reduction. Differences between treatment groups were assessed by Student's *t*-test as described previously (Oliver et al., 1993b).

RESULTS AND DISCUSSION

The percentage of quarters newly infected in herd 1 was 40.9% lower ($P < 0.0005$) in quarters with teats predipped and postdipped than in quarters with teats postdipped only (Table 1). New IMI by Gram-negative pathogens ($P < 0.05$), *Streptococcus dysgalactiae* ($P < 0.05$) and coagulase-negative staphylococci ($P < 0.0005$) were significantly lower in quarters with teats predipped and postdipped than in quarters with teats postdipped only (Table 1). Clinical mastitis was significantly lower ($P < 0.025$) in quarters with teats predipped and postdipped than in quarters with teats postdipped only (Table 2). The majority of clinical mastitis was due to *Escherichia coli*, *S. uberis*, and *S. dysgalactiae*.

The percentage of quarters newly infected in herd 2 was 38% lower ($P < 0.0001$) in quarters with teats predipped and postdipped in the phenolic combination than in quarters with teats postdipped only (Table 3).

Table 1. Efficacy of a premilking teat disinfectant containing a phenolic combination for the prevention of new intramammary infections in lactating dairy cows in herd 1.

Organism	Treatment group	New infections		Percentage reduction
		Number	% quarters	
Staphylococcus aureus	Control[1]	10	1.4	
	Treated[2]	6	0.8	
Streptococcus uberis	Control	7	0.9	
	Treated	9	1.2	
Streptococcus dysgalactiae	Control	13	1.8	61.1
	Treated	5	0.7	$P < 0.05$
Other *Streptococcus* species	Control	5	0.7	
	Treated	2	0.3	
Gram-negative bacteria	Control	19	2.6	47.5
	Treated	10	1.3	$P < 0.05$
Coagulase-negative *Staphylococcus* species	Control	100	13.5	40.7
	Treated	59	8.0	$P < 0.0005$
Corynebacterium bovis	Control	10	1.3	
	Treated	5	0.7	
Other pathogens	Control	12	1.6	
	Treated	8	1.1	
All pathogens	Control	176	23.8	40.9
	Treated	104	14.1	$P < 0.0005$

[1]n = 740 quarters available for infection.
[2]n = 740 quarters available for infection.

New IMI by *S. uberis* ($P < 0.005$) and coagulase-negative staphylococci ($P < 0.025$) were significantly lower in quarters with teats predipped and postdipped than in quarters with teats postdipped only (Table 3). Efficacy against *Staphylococcus aureus* approached significance ($P < 0.10$). Clinical mastitis in herd 2 was significantly ($P < 0.05$) lower in quarters with teats predipped and postdipped in the phenolic combination than in quarters with teats postdipped only (Table 4). The majority of clinical mastitis was due to *E. coli*, *Staph. aureus*, and *S. uberis*.

Table 2. Mastitis pathogens isolated from cows with clinical mastitis in herd one.

Organism	Experimental group	
	Control	Treated
Staphyloccocus aureus	3	2
Streptococcus uberis	6	6
Streptococcus dysgalactiae	8	2
Other *Streptococcus* species	1	1
Coagulase-negative *Staphylococcus* species	3	1
Escherichia coli	14	8
Other Gram-negative	4	1
Bacillus species	0	1
Bacteriologically negative	1	1
Total[1]	40	23

[1]Different from controls ($P < 0.025$).

One major difference between results of the present study and previous reports on efficacy evaluation of premillking teat disinfectants (Oliver et al., 1993a, 1993b, 1994) is that the teat disinfectant containing this phenolic combination was significantly effective against a wide variety of mastitis pathogens including *S. dysgalactiae, S. uberis*, Gram-negative pathogens, and coagulase-negative *Staphylococcus* species. In all of our previous studies on the efficacy evaluation of premilking teat disinfectants (Oliver et al., 1993a, 1993b, 1994), a split-udder experimental design was used and control teats and treatment teats were prepared similarly, except for the application of the premilking teat disinfectant to treatment teats. Thus, premilking udder hygiene was the same. In both herds, premilking and postmilking teat disinfection with the phenolic combination were significantly more effective in preventing new IMI than postmilking teat disinfection only. Therefore, it would appear that the disinfectant containing the phenol combination and not the method of premilking teat preparation was responsible for differences observed. An effective premilking teat disinfectant and good premilking udder preparation are two important factors to consider if premilking teat disinfection is used on dairy farms.

Phenol and phenolic compounds have been studied extensively as disinfectants and have been shown to have a wide spectrum of antibacterial activity against

Table 3. Efficacy of a premilking teat disinfectant containing a phenolic combination for the prevention of new intramammary infections in lactating dairy cows in herd 2.

Organism	Treatment group	New infections		Percentage reduction
		Number	% quarters	
Staphylococcus aureus	Control[1]	40	9.3	32.3
	Treated[2]	27	6.3	$P < 0.10$
Streptococcus uberis	Control	23	5.3	69.8
	Treated	7	1.6	$P < 0.005$
Streptococcus dysgalactiae	Control	6	1.4	
	Treated	2	0.5	
Other *Streptococcus* species	Control	12	2.8	
	Treated	8	1.9	
Gram-negative bacteria	Control	18	4.2	
	Treated	15	3.5	
Coagulase-negative *Staphylococcus* species	Control	39	9.0	43.3
	Treated	22	5.1	$P < 0.025$
Corynebacterium bovis	Control	39	9.0	
	Treated	28	6.5	
Other pathogens	Control	5	1.2	
	Treated	3	0.7	
All pathogens	Control	182	42.1	38.0
	Treated	112	26.1	$P < 0.0001$

[1]n = 432 quarters available for infection.

[2]n = 429 quarters available for infection.

both Gram-positive and Gram-negative pathogens (Block, 1991). 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 (Block, 1991). One theory for its effectiveness against environmental pathogens is the presence of residual antimicrobial activity.

A recent study (Oliver et al., 1999) reported the efficacy of the phenolic combination as a postmilking teat disinfectant. Results of that study demonstrated that the percentage of quarters newly infected by mastitis pathogens is 45% lower, and new IMI by *S. uberis*, *Staph. aureus*, coagulase-negative *Staphylococcus* species, and *C. bovis* were significantly lower in mammary glands with teats dipped in the phenolic combination teat disinfectant than in undipped controls. Results of the study by Oliver et al. (1999) coupled with results of the present study suggest that the phenolic combination has a broad spectrum of activity against both contagious and environmental mastitis pathogens and is efficacious as both a premilking and postmilking teat disinfectant.

Table 4. Mastitis pathogens isolated from cows with clinical mastitis in herd 2.

Organism	Experimental group	
	Control	Treated
Staphylococcus aureus	10	8
Streptococcus uberis	7	1
Streptococcus dysgalactiae	2	2
Other *Streptococcus* species	3	1
Coagulase-negative *Staphylococcus* species	1	1
Escherichia coli	12	8
Klebsiella pneumoniae	3	5
Klebsiella oxytoca	1	1
Yeast	1	1
Bacteriologically negative	2	0
Total[1]	42	28

[1]Different from controls ($P < 0.05$).

CONCLUSIONS

In both herds, premilking and postmilking teat disinfection with this phenolic combination were significantly more effective in preventing new IMI than postmilking teat disinfection only. Clinical mastitis and new IMI by *S. uberis*, *S. dysgalactiae*, Gram-negative pathogens, and coagulase-negative *Staphylococcus* species were significantly lower in quarters of cows with teats predipped and postdipped than in quarters with teats postdipped only. No chapping or irritation of teat skin was observed. Premilking teat disinfection with this phenolic combination in association with good udder preparation and postmilking teat disinfection will

significantly reduce the occurrence of new IMI by numerous mastitis pathogens during lactation.

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