Efficacy of a 0.1% Iodine Teat Dip Against *Staphylococcus aureus* and *Streptococcus agalactiae* During Experimental Challenge

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ABSTRACT

An experimental challenge trial was performed according to the guidelines recommended by the National Mastitis Council (NMC). A 0.1% iodine teat dip (Quartermate with I-Tech) was examined. This product gave an 87.9% reduction of new intramammary infections with *Staphylococcus aureus* and a 66.5% reduction for *Streptococcus agalactiae* compared with a negative control. Teat end and teat skin characteristics remained excellent throughout the trial.

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

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

INTRODUCTION

Intramammary infection of the bovine udder is the culmination of events beginning with contamination of the teat skin with a pathogen, entry through the teat canal, and establishment of bacterial growth within milk secretory tissue. Decreasing the microbial population on teat skin decreases the probability that the udder will become infected with a mastitis pathogen. Dipping teats with an effective germicide after milking will reduce new IMI by 50% or more for most gram-positive organisms (Pankey et al., 1984).

The experimental challenge model (NMC protocol B) is the industry standard for manufacturers of agricultural disinfectants for determining efficacy of new teat dip products. Testing of products following these guidelines provides dairy farmers with a “pool” or list of tested products of proven efficacy, and ensures that such products adhere to minimum standards of performance (National Mastitis Council, 2003).

The Hill Farm Research Station dairy herd of 53 Jersey cows was used in a experimental infection trial

to determine the efficacy of an iodine teat dip for preventing new IMI with *Staphylococcus aureus* and *Streptococcus agalactiae*. The product was provided by West Agro, Inc., Kansas City, MO. The trial was carried out following the general procedures recommended by the National Mastitis Council (NMC) with a published efficacy protocol (Hogan et al., 1990). The trial was performed to provide the manufacturer with a published efficacy protocol that appears in the NMC bibliography of protocol tested teat dips.

MATERIALS AND METHODS

Fifty-three Jersey cows from the Hill Farm Research Station (Homer, LA) dairy herd were used in an 8-wk experimental controlled infection trial to evaluate the teat dip. The trial was performed during the months of September to November 2002. 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. During the trial cows were not predipped.

Sampling schedule. The bacteriologic status of mammary quarters was determined at the initiation of the trial by collecting and culturing duplicate milk samples. A third sample was collected from specific quarters and cultured when results from the first 2 samples differed. Premilking preparation consisted of washing of teats with water, drying with individual paper towels, and forestripping prior to attachment of teat cups.

The herd is also sampled monthly throughout the year so that all cows have lifetime culture records for all lactations. When needed, this information is used to resolve discrepancies. Milk samples were collected and analyzed weekly during the trial. In instances in which either *Staph. aureus* or *Strep. agalactiae* was present for the first time in a previously uninfected quarter, a second sample was collected immediately and cultured. All quarters were eligible for new infections during the trial except 1) those infected with organisms of the same species as challenge organisms,

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and 2) those with deformed or abnormal teats. Quarters infected during the trial were treated by intramammary infusion of a cephalosporin lactating cow product after confirmation of infection. Quarters were treated at each milking for 6 consecutive milkings. Once infected with a challenge organism that quarter was no longer eligible for infection with that organism, regardless of status after therapy.

Collection of milk samples. Prior to sampling, 2 or 3 streams of foremilk were discarded. Each teat apex was scrubbed for several seconds with a cotton pledget moistened with 70% alcohol. Teats on the opposite side of the udder from the technician were sanitized first, and milk samples were collected in reverse order in sterile, snap-cap plastic tubes and refrigerated at 5°C.

Laboratory culture procedures. Samples were mixed by shaking, and a 0.01-mL aliquot was streaked on trypticase soy agar (TSA) containing 5% bovine blood. Plates were incubated at 37°C for 48 h and examined to identify microorganisms present. An IMI was confirmed when 1) *Staph. aureus* or *Strep. agalactiae* was isolated from a clinical quarter; 2) 2 consecutive samples yielded 500 or more cfu/mL of the same pathogen; or 3) 3 consecutive samples contained 100 to 400 cfu/mL of the same pathogen.

Method of challenge exposure. At the afternoon milking, Monday through Friday, the lower third of all 4 teats of each cow was experimentally exposed to a challenge suspension containing both *Staph. aureus* (Newbould 305) and *Strep. agalactiae* (McDonald 44) immediately after milking machines were removed. Within 5 to 10 s thereafter, 2 teats (right front, left rear) were dipped full length with teat dip; the remaining 2 teats served as undipped controls. Teats were exposed to challenge organisms to increase the number of pathogens impinging on the teat apex, resulting in an increased rate of IMI.

Preparation of challenge suspensions. Stock suspensions of *Staph. aureus* (Newbould 305) were prepared weekly. The contents of one lyophilized vial of *Staph. aureus* were reconstituted in 6 mL of tryptic soy broth (TSB) and incubated at 37°C for 5 to 7 h. This culture was used to inoculate 500 mL 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, and resuspended to the original volume in proteose-peptone. Serial dilutions were made in proteose-peptone, and 0.1 mL was plated on TSA. 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 used daily for 1 wk to prepare challenge suspensions of *Staph. aureus* (Hogan et al., 1990).

Table 1. Effect of the product on average teat skin condition and teat end condition scores before and at the end of the trial.

	Start of trial	End of trial
Skin ¹		
Dipped	1.00	1.00
Control	1.00	1.00
End ²		
Dipped	1.01	1.00
Control	1.01	1.00

¹For skin condition scores, 1 = teat skin is smooth, free from scales, cracks, or chapping; 2 = teat skin shows some evidence of scaling; 3 = Teat skin is chapped. Some small warts may be present; 4 = teat skin is chapped and cracked. Redness, indicating inflammation is present. Numerous warts may be present; and 5 = teat skin is severely damaged and ulcerative with scabs or open lesions. Large and/or numerous warts present, which interfere with teat end function.

²For teat end scores, 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. Teat end may have old but healing scabs; and 5 = teat end is severely damaged and ulcerative with scabs or open lesions. Large and/or numerous warts present, which interfere with teat end functions.

Streptococcus agalactiae (McDonald 44) cultures were prepared by thawing a frozen vial of *Strep. agalactiae*, and a 0.01-mL aliquot was streak plated onto each of 5 TSA plates. Plates were incubated at 37°C for 16 h and stored at 5°C to serve as stock cultures for a 1-wk period. Daily challenge suspensions of *Strep. agalactiae* were prepared by inoculating 6 mL of TSB with 6 colonies from a TSA stock plate. The 6-mL culture was incubated for 7 h at 37°C on a gyratory shaker. Specific aliquots of the culture were added to pasteurized milk as needed 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 5×10^7 cfu/mL of *Staph. aureus*. This bacterial suspension was taken immediately to the milking parlor to challenge teats during the afternoon milking. A plate count was conducted daily on challenge suspensions and recorded.

Statistical methods. The Student *t* test was used to evaluate the statistical difference between the dipped and control quarter data. This procedure was described by Hogan et al. (1990). The statistical probability of difference in IMI between the control and dipped quarters is measured from the value of *t*.

$$t = [(x_1/n_1) - (x_2/n_2)] / [(x_1 + x_2)/(n_1 n_2)]^{-5}$$

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)(days of exposure), n_2 = (number of

Table 2. Summary of efficacy data for the product against *Staphylococcus aureus* and *Streptococcus agalactiae*.

Organism and treatment	Quarters eligible for new IMI	New IMI										Quarter days at risk for new IMI	%	
		Week											Quarters infected	Reduction
		1	2	3	4	5	6	7	8	Total				
<i>Staph. aureus</i>														
Dip	96	0	0	0	0	0	2	0	0	2	5075	2.1		
Control	95	1	1	3	2	2	2	4	0	15	4594	15.8	87.9*	
<i>Strep. agalactiae</i>														
Dip	100	0	1	2	1	0	0	1	0	5	5102	5.0		
Control	98	3	0	1	3	2	1	3	1	14	4788	14.3	66.5**	

* $P < 0.001$.** $P < 0.05$.

treated quarters)(days of exposure). Percentage reduction = $100[(x_1/n_1) - (x_2/n_2)]/(x_1/n_1)$. A teat dip is considered efficacious if the reduction is at least 40% (Foret et al., 2003).

Scoring of teat condition. Characteristics of teat end and teat skin condition in dipped and control teats were scored immediately before the trial was initiated and at the conclusion of the trial to determine any effects of the teat dip products on the condition of teat ends and lateral teat skin. The teats were given a score of 1 to 5 using visual and tactile observation (Goldberg et al., 1994; Neijenhuis et al., 2004). See Table 1 for a description of the ordinal scale and results of the scoring.

Product description. The teat dip was provided ready to use from West Agro Inc., Kansas City, MO. The teat dip (Quartermate with I-Tech) evaluated contains 0.1% available iodine and 2% glycerin. The I-Tech iodine technology is described in US Patent 4,271,149 (Winicov et al., 1981) and US Patent 5,368,868 (Winicov, 1994).

RESULTS AND DISCUSSION

Infection data collected during the trial are summarized in Table 2. A total of 17 new *Staph. aureus* IMI were confirmed; 15 in control quarters and 2 in dipped quarters. Nineteen new *Strep. agalactiae* IMI were confirmed; 14 in control quarters and 5 in dipped quarters. The teat dip reduced the infection rate for *Staph. aureus* 87.9% ($P < 0.001$) and 66.5% for *Strep. agalactiae* ($P < 0.05$). Teats were scored for chapping, cracks, and other forms of irritation, both prior to and at the end of the trial. There were no changes in teat end or teat skin characteristics during the trial (Table 1).

The 0.1% iodine teat dip significantly reduced the IMI caused by *Staph. aureus* by 87.9% and *Strep. aga-*

lactiae by 66.5%. Skin characteristics and teat end condition remained excellent throughout the trial.

Results indicate that this 0.1% product has sufficient germicidal activity to be efficacious in preventing new IMI. Iodine teat dips have been a mainstay of post dip teat antiseptics for many years. High concentrations of iodine in dips have raised concern of potential residues in milk, especially with the advent of premilking application of dips. New formulations and technologies have allowed a decrease in total iodine concentration while maintaining or enhancing bactericidal activity. The I-tech iodine technology has proven to be effective in maintaining iodine bactericidal activity at lowered iodine concentrations, thereby reducing the risk of residues.

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