Evaluation of Two Iodophor Teat Germicides: Activity Against *Staphylococcus aureus* and *Streptococcus agalactiae*[1]

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ABSTRACT

Two germicides containing 0.5 and 1% titratable iodine were tested for efficacy against the development of new intramammary infections (IMI) caused by *Staphylococcus aureus* or *Streptococcus agalactiae*. The two trials for postmilking teat dip used a model for experimental challenge that was recommended by the National Mastitis Council. The 0.5% iodine formulation reduced new *Staph. aureus* IMI by 78.2% and reduced new *Strep. agalactiae* IMI by 73.2%. The 1% iodine product reduced new *Staph. aureus* IMI by 43.5% and reduced new *Strep. agalactiae* IMI by 46.4%. No adverse effects on the condition of teat skin or on teat ends were observed over the course of the trials. At the completion of each trial, the teat skin of dipped quarters was characterized as normal, smooth skin that was free from scales, cracks, or chapping; the teat orifice was characterized as smooth without evidence of irritation.
(**Key words**: iodophor, *Staphylococcus aureus*, *Streptococcus agalactiae*, teat dip)

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

INTRODUCTION

Iodine, a strong bactericide, was first used in human medical practice as a remedy for bronchocele, a dilation of the large air passages in the lungs (6). Other early uses were for wound disinfection and for killing anthrax spores. Presently, iodine is used as a topical antiseptic, germicidal handwash, surgical scrub, disinfectant of hard surfaces, and teat dip for dairy cows as an aid in the prevention of mastitis.

The first recorded use of a teat dip was in 1916 by Moak (8), who used a dilute pine oil solution to reduce the spread of streptococci. Hypochlorite dips predominated until 1958, when Newbould and Barnum (9) found that dipping teats in 0.1, 1, and 2.5% tinctures of iodine markedly reduced the numbers of staphylococci that were recovered from milking machine liners. Iodine has since been incorporated into many teat dip products as a pre- and postmilking germicide.

Elemental iodine is not very soluble in water; however, this problem is alleviated by adding complexing agents to form iodophors. An iodophor is a complex of elemental iodine and a complexing agent or carrier, which increases the solubility of the iodine and provides a sustained-release reservoir of iodine for germicidal activity. The development of new technological methods enables the concentration of free iodine to be increased without lowering germicidal activity, and an elevated concentration of free iodine has been found to increase the speed of germicidal action against microorganisms that colonize teat skin (3). The objective of this study was to evaluate two new iodophor teat germicides that contain a high concentration of free iodine against experimental challenge with *Staphylococcus aureus* and *Streptococcus agalactiae*.

MATERIALS AND METHODS

Cows

The dairy herd of 155 lactating Jersey cows at the Hill Farm Research Station (Homer, LA) was used in a 6-wk study to evaluate the two germicides. Cows were maintained on pasture and also utilized a free-stall barn as a loafing and feeding area. Hardwood shavings were used as the bedding material. Cows were milked in a double-two, side-opening, low line parlor. The herd was divided into two groups in order to test both products simultaneously. The 0.5% iodine teat dip was evaluated using a group of 65 cows, and the 1% iodine teat dip was evaluated using a group of 90 cows. A split-design was used: two teats of each cow in one group were dipped with the 0.5% iodine

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dip, and two teats of each cow in the other group were dipped with the 1% iodine dip. Undipped quarters in each group served as controls.

Sampling Schedule

The bacteriologic status of each quarter was determined by collecting and culturing duplicate milk samples 1 wk before the bacterial challenge was initiated. A third sample was collected if the results from the first two samples did not agree. Quarters that had an IMI before the start of the study with either of the challenge organisms (*Staph. aureus* or *Strep. agalactiae*) were not eligible for new IMI with that organism during the study. The herd was sampled weekly, and specific quarters were resampled within 48 h to confirm new IMI with the challenge bacteria. Abnormal or injured teats were not included as eligible quarters.

Treatment Method and Diagnostic Procedures

The herd was divided into two groups, and the germicides were assigned randomly by lottery to each group. To increase the incidence of IMI, teats were immersed daily (Monday through Friday) with the challenge organisms at the p.m. milking immediately after removal of the milking machine. Two contralateral teats (left front and right rear) of each cow were immersed in germicide to a depth of approximately 25 mm, and the remaining two teats served as undipped controls.

An IMI was confirmed when *Staph. aureus* or *Strep. agalactiae* were isolated from a quarter exhibiting clinical mastitis (obvious clots, flakes, or abnormal milk), when two consecutive samples yielded >500 cfu/ml of either challenge organism, or when three consecutive samples contained 100 to 400 cfu/ml of either challenge organism (7). After a new IMI with *Staph. aureus* or *Strep. agalactiae* was confirmed, the infected quarter was treated with a commercially available antibiotic product for lactating cows and was considered to be ineligible for a new IMI with either microorganism for the remainder of the study.

Udder Preparation and Sample Collection

Prior to the collection of milk samples, the foremilk was discarded, and the teat apex was sanitized using cotton pledgets moistened with 70% ethyl alcohol. Approximately 10 ml of milk were collected in sterile, plastic tubes prior to the plating of 0.01 ml on trypticase soy agar (**TSA**) that contained 5% bovine calf blood and 0.1% esculin. Plates were incubated at 37°C for 48 h. The *Staph. aureus* were identified presumptively by hemolytic pattern and confirmed by the tube coagulase test. The *Strep. agalactiae* were identified to the serogroup level by the Phadebact Streptococcus Test® (Boule Diagnostics AB, Huddinge, Sweden).

Preparation of the Bacterial Challenge

Experimental challenge suspensions of bacteria were prepared weekly. A frozen stock of *Staph. aureus* ATCC 29740 stored in trypticase soy broth (**TSB**) and glycerin was thawed, plated on TSA, and incubated at 37°C for 24 h. This culture was used to inoculate six 6-ml tubes of TSB that were incubated at 37°C for 8 h. This 8-h culture was used to inoculate 500 ml of TSB, which was incubated on a gyratory shaker for 16 h. Bacterial cells were then 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 a 0.1-ml aliquot was plated on TSA. Plates were incubated for 24 h at 37°C, and colonies were counted to determine the microbial concentration of the stock suspension. This suspension was stored at 5°C and used daily for 1 wk.

Cultures of *Strep. agalactiae* ATCC 27956 were prepared; a frozen stock vial of the organism in TSB was thawed, and 0.01 ml was plated on each of five TSA plates. Plates were incubated at 37°C for 24 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 five 6-ml tubes of TSB with six colonies from a TSA stock plate. This culture was incubated for about 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. Specific aliquots of the culture were added to approximately 146 ml of 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 5×10^7 cfu/ml of *Staph. aureus*. This bacterial suspension was taken immediately to the milking parlor to challenge teats during the p.m. milking. A plate count was conducted daily on challenge suspensions.

Description of Teat Germicides

The teat germicides were provided ready to use. Both products contained a nonylphenoxypolyethoxyethanol iodine complex. The 0.5% iodine product con-

tained 2.5% of the complexed iodine, provided a minimum of 0.5% titratable iodine and 10 ppm of free iodine, and contained the teat skin conditioners glycerin (1%), lanolin (0.5%), and aloe vera (0.5%) (Bac-Stop; IBA, Inc., Millbury, MA). The 1% iodine product contained 5% of the bonded iodine, provided 1% minimum titratable iodine and 10 ppm of free iodine, and contained 2% glycerin as a humectant and skin conditioner (FS-103 II; IBA, Inc.).

Statistical Analysis of Data

The number of quarter milkings per new IMI was determined using the following equation (10): $Q = N \times M \times D/I$ where Q = number of quarter milkings per new IMI, N = number of quarters eligible for new IMI, M = number of milkings per day, D = number of days, and I = number of new IMI.

Differences between the percentage of quarters becoming infected in treatment groups were tested using an approximated *t* statistic defined by Hogan et al. (7):

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

where x_1 = number new IMI in control quarters, x_2 = number 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. After developing a new IMI, a quarter was not considered to be at risk for another IMI for the remainder of the study. The percentage of reduction in the rate of new IMI for dipped quarters compared with that for the undipped control quarters was expressed as $100[(x_1/n_1) - (x_2/n_2)]/(x_1/n_1)$. Teat germicides generally are considered to be efficacious in preventing new IMI when the mean percentage reduction in new IMI is ≥40%, and the lower confidence limit of the mean is >25% (7).

Condition Scoring of Teat Skin and Teat End

Characteristics of teat skin surfaces and teat ends for both groups of cows were scored immediately before and at the conclusion of the trials to determine any effects of the two germicides on teat condition. Condition scores for teat skin and teat ends were characterized according to the parameters that were established by Goldberg et al. (5) and outlined in Table 2.

RESULTS AND DISCUSSION

The data that were collected during the two trials are summarized in Table 1. The 0.5% iodine germicide significantly reduced the new IMI rate from *Staph. aureus* or *Strep. agalactiae*. Thirty-four new *Staph. aureus* IMI were confirmed in the 0.5% iodine group: 27 were in control quarters, and 7 were in dipped quarters. New IMI were thus reduced 78.2% ($P < 0.001$). Twenty-seven new *Strep. agalactiae* IMI were confirmed in the 0.5% iodine group: 21 in control

TABLE 1. Summary of efficacy data of 0.5 and 1% iodine teat germicides against *Staphylococcus aureus* and *Streptococcus agalactiae*.

Challenge organism	Quarters eligible for new IMI	New IMI	Quarter days at risk for new IMI	New IMI per 100 quarter days at risk	Reduction
	(no.)				(%)
Staph. aureus					
0.5% Iodine	123	7	3608	0.194	78.2****
Control	120	27	3039	0.888	
Strep. agalactiae					
0.5% Iodine	130	6	4708	0.127	73.2***
Control	128	21	4412	0.476	
Staph. aureus					
1% Iodine	154	13	4686	0.277	43.5*
Control	161	23	4687	0.491	
Strep. agalactiae					
1% Iodine	177	16	6396	0.250	46.4**
Control	174	28	5996	0.467	

****$P < 0.001$.

***$P < 0.005$.

**$P < 0.05$.

*$P < 0.1$.

quarters and 6 in dipped quarters; the reduction in new IMI was 73.2% ($P < 0.005$). The IMI rates for undipped control quarters were 22.5% for *Staph. aureus* and 16.4% for *Strep. agalactiae*.

Thirty-six new *Staph. aureus* IMI were confirmed in the 1% iodine group: 23 in control quarters and 13 in dipped quarters. New IMI were reduced 43.5% ($P < 0.1$). Forty-four new *Strep. agalactiae* IMI were confirmed in the 1% iodine group: 28 in control quarters and 16 in dipped quarters; the reduction in new IMI was 46.4% ($P < 0.05$). The IMI rates in undipped control quarters for *Staph. aureus* and *Strep. agalactiae* were 14.3 and 16.1%, respectively.

The incidence of clinical mastitis caused by *Strep. agalactiae* in the 0.5% iodine group was 1.6% in control quarters and 0.8% in dipped quarters. The 0.5% iodine group had no occurrence of clinical *Staph. aureus* IMI. The incidence of clinical mastitis caused by *Strep. agalactiae* in the 1% iodine group was 3.4% in control quarters and 0.6% in dipped quarters, and the incidence of clinical *Staph. aureus* IMI was 1.9% in control quarters and 0.6% in dipped quarters.

Conventional, complexed teat dips containing 1% iodine (10,000 ppm) commonly contain only 1 to 2 ppm of free iodine, which is known to kill bacteria faster than complexed iodine; however, recent technological innovations allow iodophors to be formulated that have 0.1 to 0.5% total available iodine while generating 4 to 6 ppm of free iodine (3). Bray et al. (4) compared low iodine concentration teat dips containing increased free iodine with a 1% iodophor and revealed that new IMI were fewer in quarters dipped with iodine concentrations of 0.1% (3.5 ppm of free iodine) and 0.25% (4 ppm of free iodine) than in those quarters dipped with an iodine concentration of 1% (1 ppm of free iodine). Both products in the present study provided 10 ppm of free iodine, and the ability to prevent new IMI compared favorably with other iodophors that released 4 to 8 ppm of free iodine (1, 2, 4).

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

An analysis of condition scores for teat skin demonstrated that the mean score (on a scale of 0 to 5) before and after the trial for dipped and control quarters was approximately 1 (Table 2), which is characterized as normal, smooth skin that is free from scabs, cracks, or chapping. A similar analysis of condition scores for teat ends (Table 2) showed that the mean score across all variables was approximately 1, which is characterized as a normal, smooth teat end with no evidence of irritation.

TABLE 2. Mean teat skin[1] and teat end[2] condition scores before and after the trial for teat dips containing 0.5 and 1% iodine.

	Teat skin	Teat end
0.5% Iodine		
Before		
Dipped	1.05	1.07
Control	1.04	1.04
After		
Dipped	1.00	1.12
Control	1.04	1.10
1% Iodine		
Before		
Dipped	1.02	1.02
Control	1.00	0.99
After		
Dipped	1.01	1.00
Control	1.01	0.99

[1]Teat skin condition scoring (5): 0 = teat skin has been subjected to physical injury (e.g., stepped on or frostbitten) unrelated 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, and large or numerous warts are present that interfere with teat end function.

[2]Teat end condition scoring (5): 0 = teat end has been subjected to physical or chemical injury (e.g., stepped on or frostbitten) unrelated 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, and large or numerous warts are present that interfere with teat end function.

For the 0.5% iodine product, the percentage of dipped teats exhibiting normal skin scores increased from 97.3% before the trial to 100% after the trial. This increase was due to a decrease in the percentage of teats showing evidence of skin scaling (score 2) and chapping (score 3); the percentage of control teats exhibiting normal skin scores before and after the trial remained the same (95.4 and 95.6, respectively; data not shown). For the 1.0% iodine product, the percentage of dipped teats exhibiting a normal skin score remained the same before and after the trial (97.9 and 97.1, respectively), but, for control teats, this percentage decreased from 100 to 97.1% because of increases in scores of 0 and 3 (data not shown).

For the 0.5% product, the percentage of dipped and control teats exhibiting normal teat end condition decreased from 93.6% before the trial to 88.9% after

the trial because of increases in teats with condition scores of 2 and 3; for the 1% product, no appreciable changes in teat end condition scores were observed before and after the trial (data not shown).

CONCLUSIONS

The two iodophor germicide formulations reduced new *Staph. aureus* and *Strep. agalactiae* IMI under conditions of experimental exposure to these mastitis pathogens. The 0.5% iodophor product significantly reduced new *Staph. aureus* IMI 78.2% and *Strep. agalactiae* IMI 73.2%; the 1% iodophor product reduced new *Staph. aureus* 43.5% and new *Strep. agalactiae* IMI 46.4% and was efficacious at lower significance levels than the 0.5% iodophor. Teat end and teat skin condition scores for both iodophor groups were characterized as normal and without irritation at the completion of the teat dip trial.

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