Evaluation of 1.6% Phenol as a Premilking and Postmilking Teat Dip in Preventing New Bovine Intramammary Infections[1]

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ABSTRACT

We evaluated the effectiveness of a 1.6% phenol-based teat dip using both a teat skin assay and natural exposure field trial. A teat skin assay was conducted to ascertain the concentration of phenol + phenate to be used in the field study. One percent and 0.5% iodine, and 0.5, 1.1, and 1.6% phenol + phenate were compared using *Escherichia coli* and *Staphylococcus aureus*. Logarithmic reductions for *S. aureus* were 2.2 and 2.8 for 0.5 and 1% iodine, and 1.3, 2.1, and 2.8 for 0.5, 1.1, and 1.6% phenol + phenate, respectively. Logarithmic reductions for *E. coli* were 3.3 and 3.8 for 0.5 and 1% iodine, and 1.2, 1.9, and 2.6 for 0.5, 1.1, and 1.6% phenol + phenate, respectively. A concentration of 1.6% phenol + phenate was chosen as experimental teat dip, and 0.5% iodine served as control. The field study was conducted at Beltsville (n = 185) and Clarksville (n = 100) dairy herds using a split herd design. Teat dips were used premilking and postmilking for 12 mo. The number of new intramammary infections (IMI) for the Beltsville herd in iodine and phenol + phenate teat dipped cows were: 29 and 35 for major pathogens, and 81 and 72, for minor pathogens. For the Clarksville herd, number of new intramammary infections in iodine and phenol + phenate teat dipped cows was 9 and 10 for major pathogens, and 50 and 60 for minor pathogens. Rates of IMI per quarter day per lactation were not different for either herd or when herd data were combined. The number of clinical mastitis cases per 100 cows per month were similar in both treatments. The incidences of new IMI and clinical mastitis were similar using both dips.

(**Key words:** phenol, teat dip, mastitis)

Abbreviation key: CNS = coagulase-negative staphylococci, **NMC** = National Mastitis Council.

INTRODUCTION

The National Mastitis Council (**NMC**) recommends teat dipping because it prevents new IMI and improves udder health and milk quality (15). In the marketplace, many different products with various active ingredients are available for teat dipping (1). But none has phenol as the active ingredient, even though a 1% solution is an effective germicide and fungicide (8, 19). A 1% solution is also used extensively to inactivate rabies virus in vaccine preparations. In reviews of literature, Streiker (23) and Bonda et al. (3) reported that a 1% solution of phenol increased the permeability of the bacterial cell wall and caused leakage of essential metabolites and glutamic acid. Furthermore, phenol had a deleterious effect on bacterial enzymes.

An FDA monograph regarding use of over-the-counter active ingredients on skin lists several products that may include 1.5% phenol or less aqueous and alcoholic preparation (6). Such uses are antimicrobial soaps, patient preoperative skin preparations, skin antiseptic, skin wound cleanser, skin wound protectant, and surgical hand scrubs. The FDA has also published a monograph concluding that phenol is safe and effective as an "over-the-counter" anesthetic and analgesic active ingredient for topical use on mucous membranes of the mouth and throat when used within dosage limits set forth in the monograph (5).

A mouthwash, Chloraseptic, with phenol as the main active ingredient has been widely available to consumers since 1962. The chemical constituents of Chloraseptic solution are phenol (<1.4%), sodium phenolate, menthol, thymol, sodium tetraborate, glycerin, and chlorophyll. Chloraseptic is an alkaline solution with a pH of 8.5. Studies evaluating Chloraseptic have demonstrated that it is a safe and efficacious anesthetic mouthwash (18). A new product with chemistry similar to Chloraseptic, Masticide, is being evaluated in this research for potential use as a premilking and postmilking teat dip.

Objectives of these experiments were: 1) to determine the concentration of phenol + phenate to be used in a field study by comparing the germicidal activity among

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0.5, 1.1, and 1.6% total phenol based teat dips (Sporicidin) to 0.5 and 1% iodophor teat dips on the teat skin of live cows; 2) to evaluate the efficacy of the 1.6% phenol + phenate formulation in preventing new IMI when used as a premilking and postmilking teat dip as compared with 0.5% iodine; 3) to compare the number of clinical cases of mastitis in cows teat dipped with 1.6% phenol + phenate product with those dipped in 0.5% iodine.

MATERIALS AND METHODS

Experiment I. Testing Efficacy of Phenol + Phenate in Reducing Bacterial Flora on the Teat Skin of Live Cows

We adopted the procedures of Sears et al. (22) for this experiment. Ten cows were used to evaluate the germicidal activity of formulations against *Staphylococcus aureus* and *Escherichia coli*. The germicidal activity of phenol + phenate (0.5, 1.1, and 1.6%) and 0.5 and 1% iodophor were evaluated separately on all 10 cows. Hair on udders was clipped 1 d before challenge. On the day of challenge, debris on teat skin was removed by applying a warm aqueous solution of iodine (0.25%) with disposable paper towels. The teats were dried with single-use paper towels. To ensure that the teat surface was free of iodine residues, they were wiped with gauze pads moistened in 70% alcohol until the pledget remained white. Each teat was submerged in 70% isopropanol and allowed to air dry for 5 min.

All teats were dipped with a challenge suspension of either *S. aureus* or *E. coli* (10^8 colony forming units (cfu)/ml) approximately 15 mm above the teat orifice and allowed to air dry for 10 min. Following this exposure to the mastitis pathogen the two front teats were dipped up to 30 mm from teat orifice with either the experimental phenol + phenate teat dip or the iodine teat dip. The left front teat was dipped first with the germicidal solution. After a 15-s lag period, the right front teat was dipped. The two rear teats of each cow were undipped, negative controls.

After 1 min of exposure to the germicide, the treated teats were rinsed with the appropriate quencher. Bactoletheen broth (Difco Laboratories, Detroit, MI) containing 1% sodium thiosulfate was used to inactivate iodine, and bactoletheen broth (Difco Laboratories) containing 0.5% polyoxyethylenesorbitan monooleate (Tween80) was used to inactivate phenol + phenate. Quencher solution (10 ml) was delivered to rinse the teats starting 15 to 20 mm above the teat orifice. The syringe was angled so the quencher thoroughly covered the teat surfaces. The rinse was collected in sterile polypropylene cups held directly under the teat and covered immediately. The same quencher and procedure were used on the untreated rear teats.

Table 1. Evaluation of the germicidal activity of three concentrations of phenol and phenate (PP) and two concentrations of iodine after 1 min of contact with *Staphylococcus aureus* when used as a premilking teat dip on live teat skin.

Teat dip	Treated teats (n)	Logarithm of cfu for negative control[1]	Logarithmic reduction in cfu[2]	SEM
0.5% PP	20	5.1	1.3[c]	0.17
1.1% PP	20	5.4	2.1[b]	0.17
1.6% PP	20	5.3	2.8[a]	0.17
0.5% Iodine	12	5.2	2.2[b]	0.21
1% Iodine	20	5.4	2.8[a]	0.17

[a,b,c]Means within a column followed by any identical superscript are not different ($P < 0.05$).

[1]Logarithm for negative control = mean logarithm of cfu/ml recovered from negative control teats averaged over cows.

[2]Logarithmic reduction = logarithm of cfu/ml for negative control minus logarithm of cfu/ml for treated teats averaged over cows.

The recovered rinse was placed on ice and returned to the laboratory. Four dilutions were made from each recovered rinse (undiluted, 10^1, 10^2, and 10^3 dilutions). One hundred microliters of each dilution was streaked on duplicate plates of trypticase soy blood agar containing 5% blood and 0.1% esculin (Remel Regional Media Laboratories, Lenexa, KY). Plates were incubated aerobically at 37°C for 48 h before determining the number of colony-forming units. *Escherichia coli* cultures were identified by colony morphology on MacConkey agar. This medium was prepared in the laboratory with components obtained from Difco Laboratories. *Staphylococcus aureus* was identified by colony morphology and hemolytic patterns, and the tube was tested for free coagulase.

The numbers of colony-forming units from control and experimental groups were compared. The raw data were the mean of duplicate plate counts (cfu/ml) for each collection of the rinse recovered from the teats. Raw data were logarithm transformed (base 10) before analysis. Logarithms of colony-forming units for positive and negative control quarters were averaged within each cow. The difference between these two values (logarithms reduction resulting after application of germicide) was calculated for each cow.

The data are summarized in Tables 1 and 2. Based on the logarithm reductions in colony-forming units, 1.6% phenol + phenate was chosen for use in the field trial following procedures outlined by the NMC (11, 12).

Experiment 2. Testing Efficacy of 1.6% Phenol + Phenate in Reducing New Intramammary Infections

This study was designed to measure the combined effect of premilking and postmilking teat dipping on

rate of new intramammary infection and, thus, deviates from the NMC protocols that are designed to measure rate of new IMI from only premilking (11) or postmilking (12) teat dipping. The experiment lasted 12 mo and involved two dairy herds. The total number of cows used in both herds (USDA, Beltsville, MD, and University of Maryland dairy farm at Clarksville) was 385. All animals were in lactations 1 to 9 (mean = 2.5).

A phenol + phenate concentration of 1.6% was chosen for the field study because of its higher efficacy among the concentrations tested. One-half percent iodine was chosen as the teat dip for a positive control. We chose 0.5% iodine because no 1% iodine teat dips have been evaluated as premilking teat dip with the NMC protocols (1) and, thus, no 1% iodine products in the NMC teat dip bibliography are labeled for premilking teat dipping. In addition, 1% iodine has been shown to increase milk iodine residues compared with lower iodine concentrations (4, 9). Perhaps this explains why commercial companies have not invested resources to evaluate 1% iodine teat dips for predipping.

Premilking and postmilking teat sanitizers were evaluated in a split herd study. Efficacy was based on comparing new IMI and the number of clinical mastitis cases in control and experimental teat dip groups. Cows in the control group were teat dipped with a 0.5% iodine product (Surge Theratec, Babson Bros. Co., Naperville, IL) of proven efficacy (19). Cows that were to be dipped with 1.6% phenol + phenate were identified by colored bands on the rear leg. Initially, cows were alternatively assigned to treatments based on stage of lactation, parity, and bacteriological status of quarters. As new cows freshened and entered the study, they were assigned to treatments, balanced for number of animals and the previous criteria described.

Table 2. Evaluation of the germicidal activity of three concentrations of phenol and phenate (PP) and two concentrations of iodine after 1 min of contact with *Escherichia coli* when used as a premilking teat dip on live teat skin.

Teat dip	Treated teats (n)	Logarithm of cfu for negative control[1]	Logarithmic reduction in cfu[2]	SEM
0.5% PP	20	5.4	1.2[e]	0.15
1.1% PP	20	5.3	1.9[d]	0.15
1.6% PP	20	5.5	2.6[c]	0.15
0.5% Iodine	12	5.4	3.3[b]	0.18
1% Iodine	20	5.3	3.8[a]	0.15

[a,b,c]Means within a column followed by any identical superscript are not different ($P < 0.05$).

[1]Logarithm for negative control = mean logarithm of cfu/ml per ml recovered from negative control teats averaged over cows.

[2]Logarithmic reduction = logarithm of cfu/ml for negative control minus logarithm of cfu/ml for treated teats averaged over cows.

Cows were milked twice daily, and the same milking procedures were used for control and treated cows within each herd. At Beltsville, excess dirt from udder and teats was manually removed, milk was forestripped into a strip cup, premilking teat dip was applied with a dip cup for a minimum contact time of 20 s, and teats were dried thoroughly with a single-service towel. Teats and udders of cows that were excessively dirty were washed first with water and dried, and then premilking teat dip was applied. Cows' udders and teats at the Clarksville herd were first washed with water, dried, and forestripped on the floor, and the premilking teat dip was applied with a cup and left on teats for a minimal contact interval of 20 s. Teat dip was removed by wiping teats with individual paper towels before attaching milking machine. Cows were milked and, after machine removal, the full lengths of all teats were immediately dipped with a cup applicator.

Cows were classified as having clinical mastitis based on the appearance of abnormal milk (15) in the strip cup at Beltsville or on the floor at Clarksville.

The microbiological status of each quarter was determined immediately before the study by collecting and culturing single quarter milk samples once each week for 2 consecutive wk. A third sample was collected and cultured when the results from the first two samples differed.

Quarter milk samples were collected every month from all lactating cows for 12 mo. The first monthly sampling was started in October 1995 and ended in September 1996 at Beltsville, and November 1995 to October 1996 at Clarksville. Duplicate samples were collected at drying off, when the cow left the herd, and at freshening (no earlier than 3 d and no later than 7 d after calving). Clinical cases of mastitis in one or more quarters were also recorded, and duplicate milk samples were taken from all quarters.

After 20 s of exposure to the teat dip, teats were dried with individual paper towels and teat ends were thoroughly scrubbed with 70% alcohol saturated swabs until the teat dip was completely removed from the teats. Sterile tubes were used to collect the milk samples. Tubes were held at a 45° angle at the bottom of the teat and approximately 5 ml of milk was collected aseptically. Caps were replaced immediately after sample collections.

Ten microliters of each milk sample was streaked onto the surface of trypticase soy blood agar plates containing 5% blood and 0.1% esculin. Plates were observed at 24 and 48 h. One colony-forming units was considered as an infection for any bacterial species. Colonies were identified according to procedures of NMC (16). Occasionally, milk samples were stored at –4°C immediately after collection and cultured within 4

wk. It has been shown that freezing milk samples for up to 6 wk does not affect the viability of mastitis pathogens (14).

Only quarters free of IMI at the beginning of the study were eligible for new IMI. The criterion for new IMI of a quarter was isolation of the same bacterial species for 2 consecutive mo. An individual quarter was eligible for one infection by each pathogen during a lactation. Quarters infected in one lactation were eligible for new infection in the subsequent lactation if the infection cleared spontaneously or as a result of dry cow antibiotic therapy.

In experiment I, mean logarithmic reduction of the number of bacterial colonies for different concentrations of phenol + phenate and iodine was tested using the GLM procedure of SAS®, version 6.11. The model was as follows:

$$Y_{ij} = \mu + COW_i + TRT_j + \varepsilon_{ij},$$

where:

Y_{ij} is the observation for the ith cow and jth treatment,
μ is the overall mean,
COW_i is the fixed effect of the ith animal (i = 1 to 10),
TRT_j is the fixed effect of the jth treatment (j = 0.5%, 1.1%, 1.6% phenol + phenate or 0.5% , 1% iodophor), and
ε_{ij} is the random effect associated with the Y_{ij} observation, assumed NID $(0,\sigma_\varepsilon^2)$.

Means were compared using least significant difference.

In experiment II, the MIXED procedure of SAS, version 6.11 (21) was used to analyze new IMI (per quarter day) combined for both herds. The model was as follows:

$$Y_{ijklmn} = \mu + HERD_i + TRT_j + PARITY_k + STAGE_l + TRT_j * PARITY_k + TRT_j * STAGE_l + HERD_i * PARITY_k + HERD_i * STAGE_l + \delta_{m(ijkl)} + \varepsilon_{n(ijklm)},$$

where:

Y_{ijklmn} is the observation for the ith herd, jth treatment, kth parity, lth stage of lactation, mth cow and nth quarter,
μ is the overall mean,
$HERD_i$ is the fixed effect if the ith herd (i = 1 or 2),
TRT_j is the fixed effect of the jth treatment (j = 1.6% pheno/phenate or 0.5% iodophor),
$PARTY_k$ is the fixed effect of the kth parity (k = 1 or 2 or greater),
$STAGE_l$ is the fixed effect of the lth stage of lactaiton (l = early, mid, or late),
$TRT_j * HERD_i$ is the fixed interaction effect of the jth treatment with the ith herd,
$TRT_j * PARITY_k$ is the fixed interaction effect of the jth treatment with the kth parity,
$TRT_j * STAGE_l$ is the fixed interaction effect of the jth treatment with the lth stage,
$HERD_i * PARITY_k$ is the fixed interaction effect of the ith herd with the kth parity,
$HERD_i * STAGE_l$ is the fixed interaction effect of the ith herd with the lth stage,
$\delta_{m(ijkl)}$ is the random effect of the mth cow nested within the ith herd, jth treatment, kth parity, and lth stage of lactation, assumed NID $(0,\sigma_\delta^2)$, and
$\varepsilon_{n(ijklm)}$ is the random effect of the nth quarter nested within the ith herd, jth treatment, kth parity, lth stage of lactation and mth cow, assumed NID $(0,\sigma_\varepsilon^2)$.

Because observed differences were not significant, the required difference between treatment means to be significant at the 5% level with 90% power (sensitivity) given the observed variance was computed for each dependent variable. A second model was used to analyze the rate of clinical mastitis (per 100 cow mo) combined for both herds. The model was as follows:

$$Y_{ijklmn} = \mu + HERD_i + TRT_j + PARITY_k + STAGE_l + TRT_j * HERD_i + TRT_j * PARITY_k + TRT_j * STAGE_l + HERD_i * PARITY_k + HERD_i * STAGE_l + \varepsilon_{m(ijkl)},$$

where:

Y_{ijklmn} is the observation for the ith herd, jth treatment, kth parity, lth stage of lactation, and mth cow,
μ is the overall mean,
$HERD_i$ is the fixed effect of the ith herd (i = 1 or 2),
TRT_j is the fixed effect of the jth treatment (j = 1.6% pheno/phenate or 0.5% iodophor),
$PARTY_k$ is the fixed effect of the kth parity (k = 1 or 2 or greater),
$STAGE_l$ is the fixed effect of the lth stage of lactation (l = early, mid, or late),

$TRT_j * HERD_i$ is the fixed interaction effect of the jth treatment with the ith herd,
$TRT_j * PARITY_k$ is the fixed interaction effect of the jth treatment with the kth parity,
$TRT_j * STAGE_l$ is the fixed interaction effect of the jth treatment with the lth stage,
$HERD_i * PARITY_k$ is the fixed interaction effect of the ith herd with the kth parity,
$HERD_i * STAGE_l$ is the fixed interaction effect of the ith herd with the lth stage, and
$\varepsilon_{m(ijkl)}$ is the random effect of the mth cow nested within the ith herd, jth treatment, kth parity and lth stage of lactation, assumed NID $(0,\sigma_\varepsilon^2)$.

RESULTS AND DISCUSSION

Experiment I: Teat Skin Assay

The procedure used to prepare bacterial solutions was consistent across treatments. This correspondence was reflected in the small range (mean = 5.3, range = 5.5 to 5.1) observed in the logarithms of cfu/ml for the negative controls across treatments (Tables 1, 2). The concentration of bacterial solution used to dip the teats was 10^8 cfu/ml, and an average of 10^5 cfu/ml was recovered in the rinse solution (negative control).

Increasing the concentration of phenol + phenate in the teat dip formulation increased the logarithmic reduction in cfu/ml for both *S. aureus* and *E. coli* (Tables 1 and 2). The logarithm reduction of *S. aureus* was identical for 1% iodine and 1.6% phenol + phenate (Table 1), but 0.5 and 1% iodine were more effective than 1.6% phenol + phenate against *E. coli* (Table 2). The logarithm reduction of cfu for 1% iodine was comparable to the results of Sears et al. (22) in which 1% iodine reduced colony-forming units of *E. coli* and *S. aureus* by 3.7 and 3.3 logarithms, respectively. Similar results were obtained by Boddie et al. (2), who found that 1% iodine reduced colony-forming units by 3.8 logarithms for *S. aureus.*

Experiment II

The prevalence of IMI was determined in both herds at the start of the study. In the Beltsville herd, 125 cows were evaluated and the number of quarters with IMI for each mastitis pathogen were as follows: *S. aureus* (2), *Streptococcus* spp. (nonagalactiae) (7), *E. coli* (5), *Corynebacterium* spp. (14), and coagulase-negative staphylococci (**CNS**) (102). The number of quarters with

Table 3. Rate of new IMI per quarter day and total number of new IMI for major and minor mastitis pathogens in 1.6% phenol and phenate (PP) and 0.5% iodine teat disinfectant treatments in Beltsville herd.[1]

Organism	Trt[2] Group	Rate of new IMI per quarter day[1]	Total number of new quarter IMI	SEM (IMI per quarter day)	*P*[4]	Sensitivity[5]
Major pathogens						
Staphylococcus aureus	Iodine	0.00007	3	0.00007	0.90	0.0007
	PP	0.00009	5	0.00009		
Streptococcus spp. (nonagalactiae)	Iodine	0.00037	11	0.00002	0.26	0.0004
	PP	0.00022	8	0.00004		
Coliforms	Iodine	0.00017	11	0.00009	0.43	0.0004
	PP	0.00027	14	0.00011		
Pseudomonas spp.	Iodine	0.00009	4	0.00007	0.17	0.0003
	PP	0.00024	8	0.00010		
Total	Iodine	0.00077	29	0.00012	0.62	0.0007
	PP	0.00089	35	0.00019		
Minor pathogens						
Coagulase-negative staphylococci	Iodine	0.00147	80	0.00024	0.15	0.0009
	PP	0.00102	65	0.00022		
Corynebacterium spp.	Iodine	0.00002	1	0.00002	0.40	0.0003
	PP	0.00010	7	0.00009		
Total	Iodine	0.00150	81	0.00025	0.24	0.0010
	PP	0.00111	72	0.00022		

[1]Number of eligible quarters for infection in iodine group, 367; phenol group, 363.

[2]TRT = Treatment.

[3]Least square means.

[4]*P* = Probability of a type I error if the null hypothesis is rejected.

[5]Difference required between treatment means to be significant at the 5% level with 90% power given the observed variance.

Table 4. Rate of new IMI per quarter day and total number of new IMI for major and minor mastitis pathogens in 1.6% phenol and phenate (PP) and 0.5% iodine teat disinfectant treatments in Clarksville herd.[1]

Organism	Trt[2] Group	Rate of new IMI per quarter day[1]	Total number of new quarter IMI	SEM (IMI per quarter day)	P[4]	Sensitivity[5]
Major pathogens						
Staphylococcus aureus	Iodine	0.00035	8	0.00009	0.08	0.0003
	PP	0.00016	5	0.00008		
Streptococcus spp. (nonagalactiae)	Iodine	0.00000	0	0.00001	0.45	0.0002
	PP	0.00004	2	0.00005		
Coliforms	Iodine	0.00000	1	0.00004	0.35	0.0002
	PP	0.00008	3	0.00007		
Pseudomonas spp.	Iodine	0.00000	0	0.00000	1.00	0.0002
	PP	0.00000	0	0.00000		
Total	Iodine	0.00032	9	0.00012	0.83	0.0005
	PP	0.00028	10	0.00013		
Minor pathogens						
Coagulase-negative staphylococci	Iodine	0.00147	43	0.00034	0.65	0.0012
	PP	0.00128	54	0.00029		
Corynebacterium spp.	Iodine	0.00028	7	0.00008	0.59	0.0004
	PP	0.00022	6	0.00011		
Total	Iodine	0.00186	50	0.00036	0.53	0.0013
	PP	0.00159	60	0.00031		

[1]Number of eligible quarters for infection in iodine group, 171; phenol group, 197.

[2]Trt = Treatment.

[3]Least square means.

[4]P = Probability of a type I error if the null hypothesis is rejected.

[5]Difference required between treatment means to be significant at the 5% level with 90% power given the observed variance.

IMI from 74 cows in the Clarksville herd was: *S. aureus* (11), *Streptococcus* spp. (nonagalactiae) (7), *E. coli* (2), *Corynebacterium* spp. (58), and CNS (103).

Differences in the number of new IMI between iodine and phenol + phenate treatments were not significant for any major or minor pathogens or for totals of major or minor pathogens (Tables 3 and 4) when herds were analyzed individually or when herds were combined (Table 5). Stage of lactation, parity, and herd × treatment interaction also were not significant sources of variation ($P > 0.05$). The only difference in new IMI that approached statistical significance ($P = 0.08$) was for *S. aureus* in the Clarksville herd (Table 4). The total number of new quarter *S. aureus* IMI in cows dipped with phenol + phenate in the Clarksville herd tended to be lower than for quarters dipped with iodine (Table 4). A similar decrease was not observed in the Beltsville herd (Table 3). A decrease was probably not observed because cows chronically infected with *S. aureus* were routinely culled before and during the study, but this was not done in the Clarksville herd. Because *S. aureus* is a highly contagious pathogen (17) retention of *S. aureus* infected cows in the Clarksville herd may have spread the infection to herdmates, which may have been a contributing factor to the higher infection rate of *S. aureus* in that herd.

The total number of new IMI caused by minor pathogens in each herd was greater than IMI caused by major pathogens (Table 5). Prevalence of CNS was higher than other pathogens in both herds. Coagulase-negative staphylococci accounted for the majority of the new infections in each herd (Tables 3 and 4). Similar results were observed by Hogan et al. (13), who found that percent quarters infected with *Staphylococcus* spp. other than *S. aureus* was higher (6.5%) than those infected with *S. aureus* (1.6%). *Staphylococcus* spp. other than *S. aureus* is the bacterial group most frequently isolated from teat skin (7, 10).

The total number of new IMI due to minor pathogens was 81 in iodine treatment compared with 72 in phenol + phenate treatment (p = 0.24) in the Beltsville herd (Table 3), and 61 were infected in iodine treatment compared with 49 for the cows teat dipped with phenol + phenate ($P = 0.53$) in the Clarksville herd (Table 4).

To compare the number of new IMI with those reported in other studies on an equal number of quarter-days bases, we performed the following weighting. Because the occurrence of an infection follows the binomial distribution, probability of a quarter being infected in Y = number of days was calculated by using the following formula: $[1 - (1–X)^Y]$, where X = number of new IMI and Y = number of days animals were in the study.

Table 5. Rate of new intramammary infection IMI per quarter day and total number of IMI for major and minor mastitis pathogens in 1.6% phenol and phenate (PP) and 0.5% iodine teat disinfectant treatments in both herds combined.[1]

Organism	Trt[2] Group	Rate of new IMI per quarter day[1]	Total number of new quarter IMI	SEM (IMI per quarter day)	P[4]	Sensitivity[5]
Major pathogens						
Staphylococcus aureus	Iodine	0.00021	11	0.00006	0.30	0.0003
	PP	0.00012	10	0.00007		
Streptococcus spp. (nonagalactiae)	Iodine	0.00018	11	0.00006	0.50	0.0002
	PP	0.00013	10	0.00005		
Coliforms	Iodine	0.00008	12	0.00005	0.29	0.0002
	PP	0.00017	17	0.00007		
Pseudomonas spp.	Iodine	0.00005	4	0.00004	0.17	0.0001
	PP	0.00012	8	0.00005		
Total	Iodine	0.00054	38	0.00011	0.81	0.0005
	PP	0.00058	45	0.00012		
Minor pathogens						
Coagulase-negative staphylococci	Iodine	0.00147	123	0.00022	0.27	0.0009
	PP	0.00115	119	0.00019		
Corynebacterium spp.	Iodine	0.00015	8	0.00004	0.96	0.0002
	PP	0.00016	13	0.00003		
Total	Iodine	0.00167	131	0.00023	0.28	0.0009
	PP	0.00135	132	0.00020		

[1]Number of eligible quarters for infection in iodine group, 538; phenol group, 560.

[2]Trt = Treatment.

[3]Least square means.

[4]P = Probability of a type I error if the null hypothesis is rejected.

[5]Difference required between treatment means to be significant at the 5% level with 90% power given the observed variance.

Poutrel et al. (20) tested the efficacy of a postmilking barrier teat dip and a 0.5% iodine teat dip as positive control. This field study lasted for 12 mo in five herds (number of cows = 269). The difference between that study and this study was the frequency of milk sampling and criteria for defining new IMI. In their study, milk samples were collected at freshening, during the first 5 d after calving, during the 4th mo of lactation, at dry off, and again at the end of the study. In the current study, milk samples were collected at freshening, at monthly intervals, at drying off, and for clinical cases. One colony-forming unit per 10 μl of milk plated was considered as new IMI. In contrast, Poutrel et al. (20) required 3 cfu per 25 μl of milk plated. In their study, the number of new IMI (per 305 quarter d) due to *S. aureus*, coliforms, and *Streptococcus* spp. (nonagalactiae) in 0.5% iodine treatment were 0.014, 0.02, and

Table 6. Rate of clinical cases of mastitis per 100 cows per month in Beltsville herd[1].

Organism	Iodine	SEM	Phenol/ Phenate	SEM	P[2]
Staphylococcus aureus	0.05	0.07	0.32	0.14	0.85
Streptococcus spp. (nonagalactiae)	0.16	0.10	0.29	0.23	0.60
Coliforms	0.61	0.18	0.33	0.23	0.33
Pseudomonas spp.	0.09	0.04	0.36	0.02	0.07
Coagulase-negative staphylococci	0.52	0.18	0.21	0.09	0.12
No growth[3]	0.20	0.09	0.15	0.12	0.79
Total	1.54	0.36	1.72	0.48	0.76

[1]Total number of lactating cows: iodine group, 111; phenol group, 111.

[2]P = Probability of a type I error if the null hypothesis is rejected.

[3]Clinical mastitis but bacteriologically negative.

Table 7. Rate of clinical cases of mastitis per 100 cows per month in Clarksville herd[1].

	Treatment				
Organism	Iodine	SEM	Phenol/ Phenate	SEM	P[2]
Staphylococcus aureus	0.38	0.09	0.33	0.16	0.78
Streptococcus spp. (nonagalactiae)	0.14	0.10	0.00	0.00	0.14
Coliforms	0.25	0.18	0.38	0.35	0.74
Pseudomonas spp.	0.00	0.00	0.05	0.04	0.23
Coagulase-negative staphylococci	0.14	0.007	0.03	0.05	0.18
No growth[3]	0.12	0.07	0.01	0.04	0.19
Total	0.97	0.28	0.90	0.42	0.89

[1]Total number of lactating cows: iodine group, 59; phenol group, 79.

[2]P = Probability of a type I error if the null hypothesis is rejected.

[3]Clinical mastitis but bacteriologically negative.

Table 8. Rate of clinical cases of mastitis per 100 cows per month in both herds combined[1].

	Treatment				
Organism	Iodine	SEM	Phenol/ Phenate	SEM	P^2
Staphylococcus aureus	0.21	0.06	0.33	0.12	0.39
Streptococcus spp. (nonagalactiae)	0.15	0.08	0.14	0.11	0.96
Coliforms	0.45	0.14	0.36	0.22	0.78
Pseudomonas spp.	0.04	0.19	0.21	0.08	0.05
Coagulase-negative staphylococci	0.32	0.10	0.11	0.05	0.06
No growth[3]	0.17	0.06	0.12	0.08	0.66
Total	1.25	0.25	1.31	0.35	0.89

[1]Total number of lactating cows: iodine group, 170; phenol group, 190.

[2]P = Probability of a type I error if the null hypothesis is rejected.

[3]Clinical mastitis but bacteriologically negative.

0.035, respectively, compared with 0.062, 0.024, and 0.0534 (per 305 quarter d) for the respective pathogens in the current study. The differences in the frequency of milk sampling and the criteria for defining new IMI might explain the higher number of new IMI in the present study.

The number of clinical cases of mastitis per 100 cows per month in iodine and phenol + phenate teat dipped cows was not different ($P > 0.05$) between treatments in either herd or when data from both herds were combined (Tables 6, 7, and 8). When data from both herds were combined, the number of clinical cases of mastitis due to coliforms accounted for the majority of infections in both phenol + phenate and iodine treatments (Table 8), and the number of clinical cases of mastitis due to *Pseudomonas* spp. was higher in phenol + phenate treatment than iodine treatment ($P = 0.05$). Clinical mastitis due to *Staphylococcus* spp. was higher in iodine treatment compared with phenol + phenate treatment ($P = 0.06$). The average rate of clinical cases of mastitis per 100 cows per month for cows teat dipped with iodine and phenol + phenate were 1.54 and 1.72, respectively, in the Beltsville herd ($P = 0.76$) (Table 6), and 0.97 and 0.90, respectively, in the Clarksville herd ($P = 0.89$) (Table 7). Coliforms were the predominant cause of clinical cases in the Beltsville herd while *S. aureus* was the predominant causative organism in the Clarksville herd.

CONCLUSIONS

In the present study, 1.6% phenol + phenate efficacy was not different from 0.5% iodine for reducing new IMI and clinical cases of mastitis.

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