Reduction of teat skin mastitis pathogen loads: Differences between strains, dips, and contact times

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ABSTRACT

The purpose of these experiments was to (1) assess differences in mastitis pathogen strain sensitivities to teat disinfectants (teat dips), and (2) determine the optimum time for premilking teat dips to remain in contact with teat skin to reduce pathogen loads on teat skin. Two experiments were conducted using the excised teat model. In experiment 1, the differences in mastitis pathogen strain sensitivities to 4 commercially available dips (dip A: 1% H₂O₂; dip B: 1% chlorine dioxide; dip C: 1% iodophor; and dip D: 0.5% iodophor) were evaluated. Four strains of 11 common mastitis pathogens (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma bovis*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Escherichia coli*, *Staphylococcus chromogenes*, *Staphylococcus epidermidis*, *Staphylococcus hyicus*, *Staphylococcus xylosus*, and *Staphylococcus haemolyticus*) were tested. In experiment 2, the percentage log reduction of mastitis pathogens (*Escherichia coli*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Klebsiella* species, *Staphylococcus chromogenes*, *Staphylococcus haemolyticus*, *Staphylococcus xylosus*, and *Staphylococcus epidermidis*) on teat skin with 3 commercially available teat dips: dip A; dip D; and dip E: 0.25% iodophor, using dip contact times of 15, 30, and 45 s, was evaluated. Experiment 1 results indicated significant differences in strain sensitivities to dips within pathogen species: *Staphylococcus aureus*, *Staphylococcus chromogenes*, and *Streptococcus uberis*. Species differences were also found where *Mycoplasma bovis* (97.9% log reduction) was the most sensitive to tested teat dips and *Staphylococcus haemolyticus* (71.4% log reduction) the most resistant. Experiment 2 results indicated that contact times of 30 and 45 s were equally effective in reducing recovered bacteria for dips D and E and were also significantly more effective than a 15-s contact

time. No differences were seen in recovered bacteria between tested contact times after treatment with dip A. It can be concluded that different mastitis pathogen species and strains within species may possess different sensitivities to teat dips, which may have implications in selection of teat dips on dairies. Furthermore, a 30-s premilking dip contact time for iodophors and 15 s for H₂O₂ dips may be optimal in reducing pathogen load in the shortest amount of time. A reduction in premilking teat dip contact time may improve milking parlor efficiency.

Key words: excised teat model, teat disinfectant, contact time, *Mycoplasma bovis*

INTRODUCTION

Teat dips have an important role in reducing the incidence of IMI. Postmilking teat dips have demonstrated efficacy in reducing the incidence of both contagious (Pankey et al., 1985) and opportunistic (Quirk et al., 2012) IMI. Premilking teat dips are effective in reducing the incidence of environmental IMI (Pankey et al., 1987). Contagious pathogens are transmitted from cow to cow, generally within the milking parlor. The opportunistic pathogens are part of the normal flora of the teat skin with the CNS being the primary pathogen group of interest, and the environmental pathogens are found ubiquitously within the environment. A teat dip's effectiveness is not simply dependent upon the concentration of the active ingredient. Therefore, uniform methods for evaluating teat dips have been discussed through the National Mastitis Council; these methods are 1) the natural exposure model, 2) the experimental challenge model, and 3) the excised teat model (Nickerson, 2001). The excised teat model is used to measure a germicide's ability to reduce viable bacteria on teat skin surfaces as a screening test (Nickerson, 2001). One study used the excised teat model to examine the germicidal activity of 2 postmilking teat dips against multiple strains of contagious and environmental pathogens (Schmidt et al., 1984), and the germicidal activity differed with different strains of pathogens within species. More recently, Azizoglu et al.

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(2013) found that some *Staphylococcus aureus* strains survived higher concentrations of iodine than others in vitro. The first experiment described herein was designed to determine if strains within species differed in their germicidal sensitivity to disinfectant formulations of 4 commercially available postmilking teat dips using the excised teat model.

Several have suggested that premilking teat dips be allowed to remain in contact with the teat skin for 30 s before removal and attachment of milking unit (Reneau, 2001; Bray and Shearer, 2012). In contrast, findings of controlled trials reported significant germicidal efficacy against mastitis pathogens with disinfectant contact times of 15 to 20 s (Galton et al., 1988) and 60 s (Peters et al., 2000). Longer premilking teat dip contact time with the teat skin potentially could result in reduced milking parlor efficiency as measured by cow throughput. No empirical evidence supports the recommendation that a 30-s contact time is optimum when considering both pathogen load reduction and milking parlor efficiency. The objective of the second experiment was to determine if the often advocated premilking dip contact time of 30 s reduced a greater or equal number of noncontagious (environmental and opportunistic) mastitis pathogens than dip contact times of 15 or 45 s.

MATERIALS AND METHODS

Strain Selection and Purification

For both experiments 1 and 2, 4 strains per pathogen species were randomly selected from the Washington State Mastitis Laboratory culture collection based on the following criteria. Strains were of bovine origin and from milk of cows with IMI. Contagious pathogen strains were from geographically isolated herds. Noncontagious pathogen strains were from geographically isolated herds or collected at least one year apart if from the same herd with the exception of the *Klebsiella* species. Two *Klebsiella* species strains were collected 9 mo apart from the same herd. Bacterial strains were purified by streaking for isolation on Colombia Blood agar (CBA; Hardy Diagnostics, Santa Maria, CA) and in the case of *Mycoplasma bovis*, purification was obtained using the filter cloning technique (Boonyayatra, 2010). Strains of the genus *Klebsiella* were not speciated but were identified at the genus level after isolation from MacConkey agar (Hardy Diagnostics) using Simmons Citrate agar and Motility agar (Hardy Diagnostics; Hogan et al., 1999). Purified strains were stored at -85°C and defrosted at ambient temperature when needed.

Bacterial Challenge Suspension

All bacterial species except *Mycoplasma bovis* were grown in brain-heart infusion broth (Becton, Dickinson and Company, Franklin Lakes, NJ) at 37°C . *Mycoplasma bovis* was grown in modified pleuropneumonia-like organism broth (Hardy Diagnostics) at 37°C with 10% atmospheric CO_2 . All bacterial strains grown in brain heart infusion broth were arrested in logarithmic growth at 4 h and *Mycoplasma bovis* strains at 72 h of incubation. Cultures were centrifuged at $3,000 \times g$ for 20 min at 5°C except for *Mycoplasma bovis*, which was centrifuged at $4,500 \times g$ for 30 min at 23°C . Pellets were resuspended in phosphate-buffered sterile saline (PBSS), washed 2 times, and resuspended with PBSS. Cultures were adjusted to an optical density corresponding to approximately 1×10^8 cfu/mL. Standardized samples were plated to confirm concentration and diluted at a factor of 1:10 into prepared sterile skim milk (Becton, Dickinson and Company), yielding a suspension of approximately 1×10^7 cfu/mL.

Experiment 1

The excised teat model was used to test strain variation response to 4 different teat dips as previously described by Philpot et al. (1978). Three contagious pathogens (*Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma bovis*), 3 environmental pathogens (*Streptococcus dysgalactiae*, *Streptococcus uberis*, and *Escherichia coli*), and 5 opportunistic pathogens (*Staphylococcus chromogenes*, *Staphylococcus epidermidis*, *Staphylococcus hyicus*, *Staphylococcus xylosus*, and *Staphylococcus haemolyticus*) were tested. Four strains per species were used as previous investigators observed differences utilizing 3 strains (Schmidt et al., 1984). All 4 strains of a bacterial species were tested on the same day. In brief, teats were collected from a commercial abattoir in June 2013 over the course of a week, frozen at -15°C , transported to the laboratory, and defrosted as needed at ambient temperature. Teats with a teat-end and skin score of ≤ 2 (Goldberg et al., 1994) were washed in mild detergent, rinsed with sterile water, dried, and marked with a felt-tip pen at depths 15 and 30 mm from the teat end. Teats were hung from a horizontal dowel rod, dipped with 70% isopropyl alcohol, and allowed to dry. In duplicate, teats were dipped to a depth of 15 mm with prepared bacterial challenge suspension and allowed to dry for 5 min. Teats were then dipped with 15 mL of fresh teat dip to a depth of 30 mm. Four commercially available teat dips (Thatcher Company, Salt Lake City, UT) were used: dip A: T-Prox a 1% H_2O_2 dip; dip B: Cloguard H1 a 1% chlorine

dioxide dip; dip C: Magnum a 1% iodophor; and dip D: T-505, a 0.5% iodophor. Teat dips remained on teat skin for 10 min and teats were rinsed with 10 mL of Letheen (Becton, Dickinson and Company) 1% thio-sulfate quench solution (Philpot et al., 1978) using a sterile 16-gauge needle fitted on a sterile syringe. A duplicate control was used for each strain, being dipped with the challenge solution but not with test teat dip. Rinse was collected in sterile 50-mL centrifuge tubes and refrigerated. Rinse from teats treated with dips B, C, and D was not serially diluted but plated using both 1 mL and 0.1 mL/plate for each collected rinse solution. Rinse from dip-A-treated teats was plated directly on agar using a 0.1-mL aliquot of rinse solution and a second aliquot was diluted with PBSS 1:10 and 0.1 mL of the dilution plated. Rinse from control teats were plated at dilution factors of 1:1,000 and 1:10,000 for each rinse solution. All rinse solutions were plated on CBA except rinse collected from *Mycoplasma bovis* challenged teats, which was instead plated on modified Hayflick's agar. Colombia blood agar plates were incubated at 37°C for 18 to 48 h and modified Hayflick's agar at 37°C with 10% atmospheric CO₂ for 10 d. Colonies on CBA were visually enumerated and colonies on modified Hayflick's agar were enumerated at 15× using a stereomicroscope (Biddle et al., 2003). Plates yielding between 20 and 200 cfu were selected from the duplicate plating and used as the representative number of organisms recovered from the respective teat. Counts were log₁₀ transformed.

Experiment 2

The excised teat model was used to evaluate dip contact times for 3 different teat dips as previously described by Philpot et al. (1978). Four environmental pathogens (*E. coli*, *Strep. uberis*, *Strep. dysgalactiae*, and *Klebsiella* species), and 4 opportunistic pathogens (*Staph. chromogenes*, *Staph. haemolyticus*, *Staph. xylo-sus*, and *Staph. epidermidis*) were tested. Four strains were used for each species. Selected teat dips were dip A: T-Prox a 1% H₂O₂ dip, dip D: T-505 a 0.5% iodophor (Thatcher Company); and dip E: DellaCare Enhanced, a 0.25% iodophor (DeLaval, Kansas City, MO). A sample size calculation was done to detect a 0.5 log₁₀ difference in concentration of bacteria as effected by treatment (Minitab 17, Minitab Inc., State College, PA) using variance data from experiment 1 (power = 0.80, α = 0.05). Disinfectant contact times of 15, 30, or 45 s were tested.

Excised teats were collected in November 2013 over 1 wk from a commercial abattoir, frozen at -15°C, and shipped overnight to the laboratory. Teats were defrosted, placed into small bags, and refrozen at -15°C

upon arrival. The preparation of teats was as described for experiment 1. Strain was used as the unit of replication for each treatment and each tested contact time utilized a control that was unique to each tested strain. The teat challenge protocol was the same as experiment 1 through dipping the teats with 15 mL of fresh teat dip. After dip remained on teat skin for prescribed test contact times, they were rinsed with 10 mL of Letheen (Becton, Dickinson and Company) 1% thio-sulfate quench (Philpot et al., 1978) that was collected into sterile 50-mL tubes and refrigerated as previously described. A 0.1-mL aliquot of the rinse solution from dips D and E was plated directly to agar. A second aliquot was diluted with PBSS 1:10 and 0.1 mL of the dilution was plated on agar. Rinse solutions from dip-A-treated teats were plated at dilutions of 1:100 and 1:1,000 per plate. Plating of rinse solution from control teats was done as described for experiment 1. Incubation and enumeration of colonies were as described for experiment 1. Counts were log₁₀ transformed.

Statistical Analysis

Teat dip efficacy was defined as the percentage log₁₀ reduction from the undipped negative control (Schmidt et al., 1984). Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). The Proc Mixed procedure was used for initial analysis and multiple comparisons were performed using Fisher's least significant difference test. Output was sorted using a Proc Mixed macro (Saxton, 1998). Six models were used.

Experiment 1. A first model was used to determine if use of dips A, B, C, or D, significantly reduced the log number of bacteria recovered from teat skin versus the undipped control. Recovered log cfu was the dependent variable. Treatment; dips A, B, C, D, and control ($n = 5$); and species ($n = 11$) were the independent variables and considered fixed effects. A second model was used to determine if the percentage log reduction of bacteria was different between species, bacterial strain nested within species, treatment, and their interactions. Dependent variable was percentage log reduction and the independent variables were species of mastitis pathogen (species; $n = 11$), strain of bacteria (strain; $n = 4$), and treatment with test disinfectant (dip; $n = 4$). All independent variables were fixed effects. A third model was developed to examine differences between mastitis pathogen groups (contagious, environmental, and opportunistic) and tested disinfectants. The dependent variable was percentage log reduction and the independent variables were pathogen groups ($n = 3$) and test disinfectants ($n = 4$), both fixed effects.

Experiment 2. A first model was used to determine if treatments, disinfecting inoculated teats with dips

A, D, and E, reduced a significant number of log cfu bacteria from teat skin compared with the untreated control. Log cfu was the dependent variable, and treatments ($n = 3$) and species ($n = 8$) were the independent variables, both fixed effects. A second model was used to determine if significant differences in the dependent variable, percentage log reduction of bacteria from undipped control, existed between the independent variables, tested contact times (contact time; $n = 3$), mastitis pathogen species (species; $n = 8$), test disinfectants (dip; $n = 3$), and their interactions. All were fixed effects. A third model was used to determine if the percentage log reduction of treatments differed significantly between the 2 mastitis pathogen groups, environmental and opportunistic, and test disinfectants ($n = 3$). Dip and pathogen group were fixed effects.

RESULTS

Experiment 1

A total of 436 samples were collected and used for data analysis across all treatment and control teats. Significant differences in recovered log cfu between treatments (control, dips A, B, C, and D; $P < 0.0001$) were detected. Dips A, B, C, and D each significantly reduced recovered log₁₀ cfu number when contrasted with undipped control ($P < 0.0001$) where treatments of all 4 dips and controls resulted in recovered log₁₀ cfu/mL of bacteria of 2.26, 0.35, 0.23, 0.44, and 4.82 (SE $\pm$ 0.06), respectively.

Bacterial species mean percentage log reductions achieved by dips were contrasted and summarized in Table 1. Significant differences in percentage log reductions were detected between bacterial species, between dips, and an interaction between dip and species ($P < 0.05$). *Mycoplasma bovis*, 97.9% log reduction, was the species most sensitive to tested dips and *Staph. haemolyticus* was the most resistant, with a 71.4% log reduction. Significant differences in the reduction of bacteria recovered between strains within species (*Staph. aureus*, *Staph. chromogenes*, and *Strep. uberis*) were also found (data not shown; $P < 0.05$). Mean percentage log reductions in bacteria recovered contrasted by dip and species are summarized in Table 2. Dip A was the least efficacious dip, effecting an overall mean of 52.9% log reduction, significantly different from dips B, C, and D ($P < 0.05$). Dip C achieved the largest percentage log reduction, 95.1%, which was significantly different from dip D, 90.9% log reduction ($P < 0.05$) but not from dip B, 92.9% ($P > 0.05$). Percentage log reductions of dip B and C were not significantly different ($P > 0.05$). Pathogens tended to be uniformly sensitive to dips B, C, and D. However, *Staph. aureus* was more resistant

Table 1. Mean percentage log reduction¹ across all strains and test disinfectants within mastitis pathogen species

Species	% log reduction ²
<i>Mycoplasma bovis</i>	97.9 ^A
<i>Streptococcus dysgalactiae</i>	87.8 ^B
<i>Staphylococcus hyicus</i>	85.3 ^{BC}
<i>Staphylococcus aureus</i>	83.7 ^{BCD}
<i>Streptococcus agalactiae</i>	83.7 ^{BCD}
<i>Staphylococcus chromogenes</i>	82.2 ^{CD}
<i>Staphylococcus epidermidis</i>	81.8 ^{CD}
<i>Staphylococcus xylosus</i>	80.8 ^{CD}
<i>Streptococcus uberis</i>	78.9 ^D
<i>Escherichia coli</i>	78.8 ^D
<i>Staphylococcus haemolyticus</i>	71.4 ^E

^{A-E}Percentage log₁₀ reductions not sharing the same superscript were significantly different ($P < 0.05$).

¹Percentage of log₁₀ cfu recovered from teats treated with test disinfectants relative to untreated control teats.

²The largest observed standard error of the means was ± 2.0 .

to dips B and D than dip C ($P < 0.05$) and *Staph. xylosus* was more resistant to dip D than dips B and C ($P < 0.05$). Dips were not significantly different in their efficacy against *M. bovis* ($P > 0.05$).

Across all dips, the percentage log reduction of contagious mastitis pathogens averaged 88.4%, which is significantly different from the reduction of environmental pathogens, 81.8%, and CNS, 80.0% ($P < 0.05$). The mean percentage log reduction of environmental pathogens as compared with CNS did not differ ($P > 0.05$).

Experiment 2

A total of 381 samples were collected and used for data analysis across all treatment and control teats. The first model indicated significant differences in recovered log cfu between treatments as dips A, D, and E, significantly reduced numbers of recovered log cfu as contrasted with undipped control ($P < 0.0001$). The number of recovered bacteria after treatment with dips A, D, E, and from controls, were 3.68, 3.00, 3.30, and 5.01 log₁₀ cfu/mL (SE $\pm$ 0.04). The range in reductions of recovered bacteria was 1.3 to 2.0 log₁₀ cfu/mL.

Significant differences were detected in percentage log reduction between bacterial species, which are contrasted and summarized in Table 3. Percentage log reductions of strains of the genus *Klebsiella* were greatest across all test dips and *Staph. chromogenes* strains were the most resistant to the germicidal activity of the test dips. Significant differences were detected between test dips, between contact times, and an interaction between species and dip in the dependent variable percentage log reduction ($P < 0.05$). The interactive term species by contact time was not significant ($P > 0.05$). Mean

Table 2. Log reductions¹ and percentage log reductions² within each mastitis pathogen species across test disinfectants³

Species	Dip A		Dip B		Dip C		Dip D	
	Log reduction	% log reduction ⁴	Log reduction	% log reduction ⁴	Log reduction	% log reduction ⁴	Log reduction	% log reduction ⁴
<i>Staphylococcus aureus</i>	2.76	56.9 ^C	4.34	89.4 ^B	4.86	100 ^A	4.28	88.5 ^B
<i>Streptococcus agalactiae</i>	2.22	46.9 ^B	4.55	96.2 ^A	4.52	95.6 ^A	4.53	96.1 ^A
<i>Mycoplasma bovis</i>	4.67	91.4 ^A	5.10	100 ^A	5.10	100 ^A	5.10	100 ^A
<i>Escherichia coli</i>	2.04	42.4 ^B	4.54	95.2 ^A	3.99	83.9 ^A	4.47	93.9 ^A
<i>Streptococcus dysgalactiae</i>	2.95	59.1 ^B	4.95	99.2 ^A	4.84	96.6 ^A	4.81	96.2 ^A
<i>Streptococcus uberis</i>	2.41	50.9 ^B	4.01	85.0 ^A	4.43	93.3 ^A	4.11	86.4 ^A
<i>Staphylococcus chromogenes</i>	2.60	53.9 ^B	4.34	90.6 ^A	4.57	95.1 ^A	4.29	88.6 ^A
<i>Staphylococcus epidermidis</i>	2.47	50.9 ^B	4.38	90.5 ^A	4.63	95.7 ^A	4.35	90.0 ^A
<i>Staphylococcus haemolyticus</i>	1.84	37.9 ^B	3.96	82.1 ^A	4.25	87.1 ^A	3.81	78.3 ^A
<i>Staphylococcus hyicus</i>	2.24	47.9 ^B	4.58	97.9 ^A	4.60	98.4 ^A	4.58	96.8 ^A
<i>Staphylococcus xylosus</i>	2.04	43.6 ^C	4.53	96.6 ^A	4.69	100 ^A	3.90	83.1 ^B
Overall mean ⁵		52.9 ^C		92.9 ^{AB}		95.1 ^A		90.8 ^B

^{A-C}Percentage log₁₀ reductions not sharing the same superscript in a row were significantly different ($P < 0.05$).

¹Reduction of log₁₀ cfu recovered from teats treated with test disinfectant relative to untreated control teats.

²Percentage of log₁₀ cfu recovered from teats treated with test disinfectants relative to untreated control teats.

³Dip A = 1% H₂O₂, dip B = 1% chlorine dioxide, dip C = 1% iodophor, and dip D = 0.5% iodophor.

⁴Largest observed standard error of the means was ± 4.1 .

⁵Largest observed standard error of mean dip effects ± 1.2 .

percentage log reductions stratified by dip and species are summarized in Table 4. Overall, dip D was the most efficacious dip, with a 40.3% log reduction, which was a significantly greater reduction than achieved by dip A and E ($P < 0.05$). Percentage log bacterial reductions with dip A and E were 26.5 and 34.3%, respectively; differences were significant ($P < 0.05$).

The interaction between dip and contact time in reducing recovered bacterial numbers approached significance ($P = 0.0561$) and is summarized in Table 5. No significant differences in percentage log reduction were seen between tested contact times when teats were treated with dip A ($P > 0.05$). Conversely, for dipo D and E, a contact time of 15 s resulted in a significantly lower bacterial percentage log reduction than contact

times of 30 or 45 s ($P < 0.05$), but no difference was seen between contact times of 30 or 45 s ($P > 0.05$). Overall, contact times of 15, 30, and 45 s resulted in mean log reductions of 30.0, 34.1, and 37.0%, all significantly different ($P < 0.05$).

A significant difference in the percentage log reductions in recovered bacteria between the environmental and CNS-grouped pathogens was detected ($P < 0.0001$). Environmental and CNS-grouped pathogens had overall means of 37.5 and 29.9% across all tested dipo.

DISCUSSION

Experiment 1

The results support our hypothesis that bacterial strains would differ in sensitivity within bacterial species when exposed to teat dipo. Significant differences in the percentage log reduction between bacterial strains after treatment with dipo occurred for 3 of the 11 tested pathogens: *Staph. aureus*, *Staph. chromogenes*, and *Strep. uberis*. These observed differences between bacterial strains are consistent with the findings of Schmidt et al. (1984), where investigators used the excised teat model and observed apparent differences in the number of recovered bacteria between strains of both *Staph. aureus* and *Strep. uberis* after exposure to teat dipo. Azizoglu et al. (2013) also described sensitivity differences between strains of *Staph. aureus* when exposed to diluted concentrations of iodine teat disinfectants in vitro. The results align with those of Piessens et al. (2012) where several, but not all, strains of *Staph.*

Table 3. Mean percentage log reduction¹ across all contact times and teat disinfectants within mastitis pathogen species

Species	% log reduction ²
<i>Klebsiella</i> spp.	46.9 ^A
<i>Escherichia coli</i>	38.1 ^B
<i>Streptococcus uberis</i>	36.0 ^B
<i>Staphylococcus epidermidis</i>	35.8 ^B
<i>Staphylococcus xylosus</i>	34.8 ^B
<i>Streptococcus dysgalactiae</i>	29.0 ^C
<i>Staphylococcus haemolyticus</i>	28.8 ^C
<i>Staphylococcus chromogenes</i>	20.3 ^D

^{A-D}Percentage log₁₀ reductions not sharing the same superscript were significantly different ($P < 0.05$).

¹Percentage of log₁₀ cfu recovered from teats treated with test disinfectants relative to untreated control teats.

²Largest observed standard error of the means was ± 1.6 .

Table 4. Log reductions¹ and percentage log reductions² within each mastitis pathogen species across test disinfectants³

Species	Dip A		Dip D		Dip E	
	Log reduction	% log reduction ⁴	Log reduction	% log reduction ⁴	Log reduction	% log reduction ⁴
<i>Escherichia coli</i>	1.11	22.8 ^B	2.32	47.5 ^A	2.15	44.0 ^A
<i>Klebsiella</i> spp.	1.63	34.5 ^B	2.44	51.5 ^A	2.58	54.8 ^A
<i>Streptococcus uberis</i>	1.12	22.0 ^C	2.35	47.0 ^A	1.96	39.0 ^B
<i>Streptococcus dysgalactiae</i>	1.55	30.8 ^A	1.52	30.3 ^A	1.30	25.9 ^A
<i>Staphylococcus chromogenes</i>	1.15	22.5 ^{AB}	1.18	23.0 ^A	0.78	15.4 ^B
<i>Staphylococcus epidermidis</i>	1.45	28.5 ^B	2.16	43.4 ^A	1.77	35.6 ^B
<i>Staphylococcus haemolyticus</i>	1.20	23.0 ^B	1.81	34.6 ^A	1.50	28.7 ^{AB}
<i>Staphylococcus xylosus</i>	1.45	28.1 ^B	2.35	45.3 ^A	1.61	31.0 ^B
Overall mean ⁵		26.5 ^C		40.3 ^A		34.3 ^B

^{A-C}Percentage log₁₀ reductions not sharing the same superscript in a row were significantly different ($P < 0.05$).

¹Reduction of log₁₀ cfu recovered from teats treated with test disinfectant relative to untreated control teats.

²Percentage of log₁₀ cfu recovered from teats treated with test disinfectants relative to untreated control teats.

³Dip A = 1% H₂O₂, dip D = 0.5% iodophor, and dip E = 0.25% iodophor.

⁴Largest observed standard error across the 4 tested strains of each species within test disinfectants ± 2.9 .

⁵Standard error of mean dip effects ± 1.0 .

chromogenes survived diluted concentrations of iodine and chlorhexidine teat disinfectants in vitro.

Dip A was the least efficacious dip tested. The hydrogen peroxide may have been reacting with the organic material of the teat skin or in the challenge suspension and thus had less germicidal activity. In vivo tests are needed to determine a dip's true efficacy because the excised teat model is an in vitro assay and cannot evaluate efficacy in terms of reducing IMI incidence.

The observed differences in teat dip sensitivities among mastitis pathogens were expected. Many authors have observed similar results both in vitro (Schmidt et al., 1984) and in vivo (Foret et al., 2005; Quirk et al., 2012). The contagious pathogen *M. bovis* was the most sensitive to tested dips with a 97.9% log reduction. This high sensitivity agrees with previous findings. Jas-

per et al. (1976) observed similar results in vivo when cow teats were challenged with *M. bovis* and researchers were unable to recover *M. bovis* from the teat skin after treatment with 3 of 6 tested dips. Boddie et al. (2002) used the excised teat model and was unable to recover single tested strains of *M. bovis* and *Mycoplasma californicum* after treatment with iodine and chlorhexidine teat dips. It could be concluded that *M. bovis* is extremely sensitive to postmilking teat dips. However, *M. bovis* is increasing in prevalence as an agent of bovine diseases (Kusiluka et al., 2000; Maunsell et al., 2011; Fox, 2012). Increased prevalence of mycoplasma mastitis is occurring despite the fact that disinfecting teats postmilking is widely practiced (USDA-APHIS, 2008). Although *M. bovis* is classified as a contagious mastitis pathogen, hygienic milking procedures that incorporate use of a postmilking teat dip have not been examined in their ability alone to control *M. bovis* on dairies. These procedures have proven effective against the other contagious pathogens *Staph. aureus* and *Strep. agalactiae* (Boddie et al., 2004). The fact that *M. bovis* prevalence is increasing despite the widespread use of postmilking teat dips suggest that other transmission pathways may exist for *M. bovis* mastitis.

Experiment 2

The objective of experiment 2 was to determine if the commonly recommended 30-s premilking teat dip contact time reduced a greater or equal number of bacteria than a contact time of 15 or 45 s. Overall, increased contact time increased percentage log reduction, which agrees with the findings of others (Schmidt et al., 1985; Gradle, 2009). A dip by contact time interaction ap-

Table 5. Percentage log reduction¹ within test disinfectants and test contact time

Dip ²	% log reduction ³		
	15 s	30 s	45 s
Dip A	26.1 ^A	25.5 ^A	28.1 ^A
Dip D	36.0 ^B	41.2 ^A	43.8 ^A
Dip E	28.0 ^B	35.7 ^A	39.2 ^A
Overall mean ⁴	30.0 ^C	34.1 ^B	37.0 ^A

^{A-C}Percentage log₁₀ reductions not sharing the same superscript in a row were significantly different ($P < 0.05$).

¹Percentage log₁₀ cfu recovered from teats treated with test disinfectants relative to untreated control teats.

²Dip A = 1% H₂O₂, dip D = 0.5% iodophor, and dip E = 0.25% iodophor.

³Standard error across all tested bacterial strains of each species within test contact time and test disinfectant ± 1.7 .

⁴Standard error of mean contact time effects ± 0.1 .

peared to exist. No differences were seen between the tested contact times with dip A in pathogen reduction on teat skin ($P > 0.05$). Results would suggest the active ingredient in dip A, hydrogen peroxide, is a fast-acting disinfectant as it is a reactive oxygen species (Remington et al., 2006). No difference in pathogen reduction on teat skin was seen between contact times of 30 and 45 s for dips D and E. A contact time of 30 and 45 s significantly reduced the number of bacteria recovered from the teat skin compared with the 15-s contact time for both dips D and E. A contact time of 30 s appears optimal when using an iodophor, but only 15 s is needed for hydrogen peroxide dips. Optimal is defined as reaching maximal reduction of teat skin pathogen load in the shortest amount of time.

The milking parlor is the most expensive facility on the dairy and is often the limiting factor in expansion and herd size (Smith et al., 1997). The average US dairy herd size has increased from 19 cows per herd in 1970 (USDA-ERS, 2006) to 196 cows per herd in 2013 (Halladay, 2014). Thus, it is not uncommon for farms to use the milking parlor 21 to 22 h a day (VanBaale et al., 2004). Increasing parlor efficiency can be achieved by maximizing use of the milking parlor by both increasing the number hours the parlor is used and by increasing the number of animals milked in a given time period. Data presented by Schuring (2007) suggest that premilking teat dip contact time is the most time consuming component of premilking preparation. Thus potential exists for improving milking parlor efficiency by reducing premilking cow preparation time. Decreasing premilking dip contact times could potentially increase the number of cows milked per hour and thus increase parlor efficiency. Results reported herein suggest optimum contact time for iodophor dips and hydrogen peroxide dips may differ and thus the optimum premilking teat dip contact time may be a function of the type of dip used.

The hydrogen peroxide dip, dip A, was least effective in reducing recovered bacteria the number of recovered bacteria from teat skin compared with dips D and E ($P < 0.05$). Despite the apparent reduced efficacy of dip A relative to dips D and E, dip A may possess an advantage. Dip A achieved its maximum efficacy within 15 s of contact time compared with the tested iodophors at 30 s. Milk iodine residues have been of concern to the dairy industry (Rasmussen et al., 1991) because elevated levels pose a risk to human health (National Institutes of Health, 2014). Hydrogen peroxide is presently not of significant concern to human health and has been used as a dairy preservative (Lück, 1962). Milk possesses catalase (Fox and McSweeney, 1998), an enzyme that degrades hydrogen peroxide to water and oxygen. Amin and Olson (1967) demonstrated that the

longer hydrogen peroxide is exposed to milk catalase and peroxidase, the greater its reduction in milk. Dip A significantly reduced teat skin pathogen load, which may or may not be biologically significant when contrasted with dips D and E in reducing the incidence of IMI.

CONCLUSIONS

Variation exists in sensitivity of mastitis pathogen strains to teat dips. Because variation exists, multiple strains of bacteria should be used when testing teat dips because using a single strain may yield biased results. It may be that dairy operations tailor their teat dipping program to counter the differences in strain sensitivities to dips. With respect to the 3 tested premilking teat dip contact times, it can be concluded that a 30-s contact time is optimal when using an iodophor and a 15-s contact time is optimal when utilizing a hydrogen peroxide dip. Reducing unnecessary premilking contact time may potentially increase parlor efficiency and therefore potentially increase the number of animals milked per hour. Improved milking parlor efficiency should have a positive financial effect on the dairy industry.

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