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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% H_2O_2; 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 H_2O_2 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) and 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 × *g* for 20 min at 5°C except for *Mycoplasma bovis*, which was centrifuged at 4,500 × *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