

## Germicidal Activities of Representatives of Five Different Teat Dip Classes Against Three Bovine *Mycoplasma* Species Using a Modified Excised Teat Model

R. L. Boddie, W. E. Owens, C. H. Ray, S. C. Nickerson, and N. T. Boddie

Mastitis Research Laboratory, Hill Farm Research Station,  
Louisiana Agricultural Experiment Station,  
Louisiana State University Agricultural Center,  
Homer 71040

### ABSTRACT

Six representative teat dips from five different teat dip classes were tested for germicidal activity against challenge exposure to *Mycoplasma bovis*, *Mycoplasma californicum*, and *Mycoplasma bovisgenitalium* using a modified excised teat model. All teat dip formulations tested were efficacious against all of the *Mycoplasma* species, providing bacterial logarithmic reductions above 4. The germicides performed best against *M. bovisgenitalium* with an average log reduction (LR) of 6.29. Average LR were 5.41 and 5.70 against *M. bovis* and *M. californicum*, respectively. The iodine and chlorhexidine products performed best against *M. bovis* and *M. californicum*, respectively, with complete kill of all organisms. The chlorhexidine and the barrier chlorine product also had complete kill of *M. bovisgenitalium* organisms.

(**Key words:** teat dip, *Mycoplasma* spp., excised teat model)

**Abbreviation key:** LR = log reduction.

### INTRODUCTION

Mastitis caused by *Mycoplasma* species is becoming recognized more frequently as veterinarians and laboratory personnel become familiar with the clinical symptoms of the disease and the culture technique needed to identify infections. *Mycoplasma* species are found as normal flora of cattle nasal mucous membranes, respiratory surfaces, and the urogenital tract, as well as on teat skin. Due to the severity of clinical mycoplasmal mastitis, the lack of approved antimicrobial therapy, and because the organism is found in an opportune site on teat skin for development of IMI;

proper udder hygiene is very important for reducing numbers of *Mycoplasma* species on teat skin (Laboratory Handbook, 1999). During mycoplasmal mastitis outbreaks it has been recommended that sanitary practices be upgraded to include segregation of infected cows, teat dipping, and dipping milking clusters in a sanitizer between cows (Jasper et al., 1976). In the past, the effectiveness of various teat dips against mycoplasmas was not considered relevant. Not until Jasper et al. (1976) tested seven teat dip and sanitizer products against *M. bovis* was there any research showing teat dip efficacy against *Mycoplasma* species. This study revealed that the chlorine, iodine, and chlorhexidine teat dips tested were very effective against *M. bovis* on teat skin. Since that initial research more than 25 yr ago, many new teat dips have been developed that have not been tested against *M. bovis*, the most common bovine mycoplasmal mastitis pathogen, or any of the other *Mycoplasma* species causing mastitis. The objective of this study was to evaluate teat dips representing different teat dip classes against the three most common bovine mycoplasmal mastitis pathogens using a modified excised teat model (Philpot et al., 1978).

### MATERIALS AND METHODS

Descriptions of the teat dips tested are found in Table 1. Teats that were used during each trial were collected from slaughtered dairy cows. Excess skin and tissue was trimmed, teats were washed in a mild detergent and warm water, rinsed in water, dried, and dipped in 70% ethyl alcohol. Teats were discarded that had rough skin, chaps, or abrasions. Teats were placed in plastic bags in a glycerin and water solution and frozen at  $-20^{\circ}\text{C}$  until further use. The mycoplasmal strains used for each trial were *Mycoplasma bovis* American Type Culture Collection 25523; *Mycoplasma californicum* Willet 9978 received from R. N. Gonzalez, QMPS, College of Veterinary Medicine, Cornell University, Ithaca, NY, and *Mycoplasma bovisgenitalium*, received from J. S. Cullor, University of California, Davis.

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Corresponding author: W. E. Owens; e-mail: wovens@agctr.lsu.edu.

**Table 1.** Description of teat dips tested during excised teat study.

Product class	Product name	Active ingredients	Mixing instructions	Manufacturer
Chlorine compound	Effercept Vet Sanitizing Teat Dip and Spray	Sodium dichloroisocyanurate Base: 0.64% Sodium Chlorite Activator: 3% Mandelic Acid 0.5% Chlorhexidine gluconate	4 tablets/1 gallon water Mix equal amounts of base and activator Use Undiluted	Activon Products, Fort Collins, CO Alcide Corp., Redmond, WA IBA, Inc., Millbury, MA
Chlorine compound Chlorhexidine	Uddergold Plus Blue Ribbon Sanitizing Teat Dip Theratec Pre and Post Sanitizing Teat Dip	0.5% Iodine 0.5% Hydrogen peroxide 1.7% Lactic acid Benzalkonium chloride	Use Undiluted Use Undiluted Use Undiluted	Westfalia-Surge, Inc., Naperville, IL Klenzade, Division of Ecolab, Inc., St. Paul, MN Baca Enterprises, LLC, Fresno, CA
Peroxygen compound Quaternary ammonium	Oxy-Gard Sanitizing Teat Dip Smart Dip			

For preparation of the challenge inoculum of each *Mycoplasma* species, six 10-ml tubes of mycoplasma broth (Becton Dickinson, Cockeysville, MD), to which enrichment supplements of 20% inactivated horse serum (Sigma Chemical Co., St. Louis, MO) and 2.5% yeast extract (Becton Dickinson) were added, were inoculated heavily with colonies actively growing on mycoplasma agar (Becton Dickinson). The mycoplasma agar was supplemented with 10% inactivated horse serum, 1% yeast extract, 0.05% thallium acetate (Sigma Chemical Co.), 0.002% DNA (Sigma Chemical Co.), and 1000 units/ml of penicillin (Sigma Chemical Co.). The inoculum was incubated at 37°C in 10% CO<sub>2</sub> atmosphere for 72 h. The optical density of the inoculum was determined using a 20 Genesys spectrophotometer (Spectronic Unicam, Rochester, NY) and the organism population was adjusted to approximately 1 × 10<sup>8</sup> cfu/ml of each organism by use of a standard curve made by data obtained from previous serial dilutions and standard plate counts. Serial dilutions of the challenge inoculum were made before each trial began, and a standard plate count conducted on mycoplasma agar. The teat dip quenching solution was letheen broth (Becton Dickinson) modified to contain 1% sodium thiosulfate (Sigma Chemical Co.).

Each trial used 10 teats for testing the products as well as the negative control. Frozen teats were thawed in warm water, dipped in 70% ethyl alcohol, dried with a paper towel, and suspended by metal clips from a glass rod. Teats were dipped in the challenge suspension once to a depth of approximately 15 mm and allowed to drain for 5 min, then dipped with the test product to a depth of approximately 30 mm and drained for an additional 10 min. For undipped negative control teats, the drainage time was 15 min.

Organisms were removed by rinsing each teat with approximately 5 ml of quencher expressed from a polyethylene wash bottle. The quencher was maintained at 5°C during each trial. The 5-ml rinse was collected in sterile blood cell-counter vials (VWR Scientific Products, Atlanta, GA). Plating of the rinse was in 0.1-ml amounts on mycoplasma agar. Rinses from negative control teats required plating at 10<sup>-4</sup> to achieve countable plates, while rinses from teats dipped with the test products were not diluted before plating. Plates were incubated for 7 d at 37°C in 10% CO<sub>2</sub> atmosphere, and colonies were counted using a stereo microscope at 3× magnification. The geometric mean of colonies counted was determined, multiplied by 50 to express the total volume of rinse, and then multiplied by the dilution factor to yield the total colony-forming units recovered from the rinse of each teat. The total colony-forming units recovered for each product tested was converted to log form, and this log value was subtracted from

**Table 2.** Germicidal activity of various teat dips against *Mycoplasma* species.

Product	Germicide	<i>M. bovis</i> Log reduction from control	<i>M. californicum</i> Log reduction from control	<i>M. bovis genitalium</i> Log reduction from control
Effercept <sup>1</sup>	Sodium dichloroisocyanurate	4.79	5.84	6.27
Uddergold <sup>2</sup> Plus	0.64% Sodium chlorite 3% Mandelic acid	5.96	5.73	—
Blue Ribbon <sup>3</sup>	0.5% Chlorhexidine gluconate	5.15	—	—
Theratec <sup>4</sup>	0.5% Iodine	— <sup>7</sup>	6.68	6.42
Oxy-Gard <sup>5</sup>	0.5% Hydrogen peroxide 1.7% Lactic acid	4.60	4.38	6.00
Smart Dip <sup>6</sup>	Benzalkonium chloride	6.56	5.88	6.49

<sup>1</sup>Action Products, Fort Collins, CO.

<sup>2</sup>Alcide Corp., Redmond, WA.

<sup>3</sup>IBA, Inc., Millbury, MA.

<sup>4</sup>Westfalia Surge, Inc., Naperville, IL.

<sup>5</sup>Klenzade, Division of Ecobb, Inc., St. Paul, MN.

<sup>6</sup>Boca Enterprises, LLC, Fresno, CA.

<sup>7</sup>Complete kill of all microorganisms by the teat dip.

the log value for the negative control to yield the log reduction (LR) for that product. In cases where organisms were not recovered from a teat, the value of 0.01 was substituted for zero in determining mean counts.

The same set of teats was used for each run within a trial. Between runs, teats were rinsed in warm water for 2 min with agitation, dried, rinsed in a 0.05% sodium thiosulfate solution (Sigma Chemical Co.) for 1 min, dried, rinsed in a solution containing 0.05% lecithin (Fisher Scientific, Fair Lawn, NJ) and 0.05% Tween 80 (Fisher Scientific) for 1 min, dried, rinsed in warm water for 1 min, dried, dipped in 70% ethyl alcohol, dried, and resuspended on the glass rod to air dry before commencement of the next run.

## RESULTS

An effective teat dip should achieve a 3 and preferably, a 4 or 5 LR of microorganisms using an excised teat model (Philpot et al., 1978). Data for the teat dips tested are presented in Table 2. All of the teat dips tested were efficacious against all of the *Mycoplasma* species, providing LR above 4. The germicides performed best against *M. bovis genitalium* with an average LR of 6.29. Average LR were 5.41 and 5.70 against *M. bovis* and *M. californicum*, respectively. It should be noted that the active ingredients in Effercept Vet Sanitizing Teat Dip (sodium dichloroisocyanurate) and Uddergold Plus (sodium chlorite and mandelic acid) are different, although both are classified as chlorine compounds. Both teat dips kill microorganisms by chlorination and oxidation of the cell and internal proteins, including enzymes (Boddie et al., 1994; Boddie and Nickerson, 1996).

Mycoplasmal microorganisms may seem to be fragile and easy to destroy under laboratory conditions; however, in the field they may be more resistant to teat dips

because of their protection in milk and other organic materials (Bushnell, 1984). The ability of the tested strains to survive on the negative control teats in this assay for 15 min indicates that these organisms can survive on teat surfaces long enough to be transmitted from cow to cow. *Mycoplasma bovis* was the most resistant to germicides of the common mastitis-causing species in this study. In a study of neutralization of germicidal activity of disinfectants by OM, a 2% iodophor and a chlorine product showed a low tolerance of OM, including whole milk powder. A 1.6% chlorhexidine acetate and an anionic acid were only moderately affected by the presence of OM (Gelinas and Goulet, 1983). *Mycoplasma* can be introduced into a herd in ways other than mycoplasmal mastitis. Cows or calves with *Mycoplasma pneumonia* spread the disease to other herd-mates and provide a reservoir of infection that could lead to mycoplasmal IMI (Bushnell, 1984). Teat dip use is only one tool to help eliminate *Mycoplasma* on teat skin. Cows and heifers purchased should be screened for mycoplasmal IMI and respiratory disease before entering the herd. Proper udder infusion procedures and sanitization of milk equipment will also reduce the spread of mycoplasmal IMI.

The spread of mycoplasmal mastitis does continue in herds that practice teat dipping, but teat dip use can still be a good procedure for reducing the numbers of *Mycoplasma* on teat skin, therefore reducing the rate of spread of mycoplasmal IMI.

## CONCLUSIONS

Mastitis caused by *Mycoplasma* species is being recognized more frequently than in the past. *Mycoplasma* species are commonly found on nasal mucous membranes, respiratory and urogenital surfaces, and teat skin. Udder infections are spread from cow to cow by

physical contact and are precipitated by breakdowns in sanitation, equipment maintenance, and udder infusion. Antibiotic therapy has proven to be ineffective in controlling the disease. Proper sanitation, including pre- and postmilking teat dipping, good udder infusion techniques, and culling of carrier animals is essential in controlling this microorganism in a dairy herd. Data obtained during this study proves that several generic classes of teat dips are efficacious against the most common mycoplasmal mastitis species on teat skin.

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