

Efficacy of Novel Antimicrobial Post-milking Teat Dip on the Rate of New Intramammary Infections with an Experimental Bacterial Challenge against Contagious Mastitis Organisms

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Summary and Implications

Teat dipping with effective products is a critical control point for mastitis prevention. Objective of this study was to determine efficacy of a novel antimicrobial solution containing citrate ion, methylene blue, parabens and emollients (ZuraLac^{SD}, Zurex PharmAgra) used as a post-milking teat dip against a positive control and negative control in reducing the incidence of new intramammary infections during a period of experimental exposure of teats to contagious mastitis organisms, *Streptococcus agalactiae* and *Staphylococcus aureus*. Novel germicide post milking teat dip significantly ($P < 0.01\%$) reduced the number of new infections of *Streptococcus agalactiae* and *Staphylococcus aureus* compared to both the negative control (95.3%) and positive control (80.9%) treatments. Incidence of clinical mastitis with a bacteriological positive identification of one of the challenge organisms was 40, 4 and 0 for negative control, positive control and novel germicide dips, respectively. Under the conditions of this study, no differences were visually observed for teat skin condition among the treatments.

The use of a novel germicide used in a post-milking teat dip significantly reduced the number of new intramammary infections (new IMI) caused by experimental challenge with contagious mastitis pathogens *Strep. agalactiae* and *Staphylococcus aureus* compared to no post-milking teat dipping (negative control) and to an industry standard, 0.5% iodine post-milking teat dip.

Introduction

Teat dipping with effective products is a critical control point for mastitis prevention. Objective of this study was to determine efficacy of a novel antimicrobial solution containing citrate ion, methylene blue, parabens and emollients (ZuraLac^{SD}, Zurex PharmAgra) used as a post-milking teat dip against a positive control and negative control in reducing the incidence of new intramammary infections during a period of experimental exposure of teats to contagious mastitis organisms, *Streptococcus agalactiae* and *Staphylococcus aureus*.

Materials and Methods

1. **Animal Selection:** 120 Holstein cows were used in an eight week trial during the fall / winter 2013. 40 cows were assigned to each of 3 treatments. Each treatment group consisted of 30% 1st lactation and 70% 2nd and 3rd lactation animals. All cows started the study within 60 - 122 days in milk and were milked 2X/day. Infection status of all quarters was determined 7 days prior to study initiation. Cows were not assigned to the study with *Streptococcus agalactiae*, *Staphylococcus aureus* and *Streptococci uberis* infected quarters and blemished teats.
2. **Treatments:** Three treatments were: experimental dip - ZuraLac^{SD} (Zurex PharmAgra, LLC) containing 10.0% trisodium citrate with 5.0% emollients used as a post-milking teat dip; positive control - Theratec^R (GEA Farm Technologies, Inc.) containing 0.5% iodine with a 3% triple emollient system used as a post-milking teat dip; and a negative control where no post-milking teat dipping occurred. For the teat dip treatments, teats were fore-stripped, pre-dipped with Theratec^R and dried 30 – 45 seconds later with dry cloth towels. For the negative control treatment, teats were fore-stripped and cleaned with moist cloth towels with subsequent drying with dry cloth towels. Milking machines were attached within two minutes of forestripping. All of the teats in each treatment were dipped immediately after machine removal at the morning milking with the experimental culture broth suspension of *Streptococcus agalactiae* (ATCC 55194) and *Staphylococcus aureus* (ATCC 12600). For the post-milking teat dipping treatments, the teats were manually dipped with the appropriate teat dip between two and five minutes after the teats being dipped with the culture broth suspension.
3. **Bacteriological Sampling:** Duplicate quarter samples were taken aseptically weekly (Monday AM milking) to determine the bacteriological status of each quarter. A third sample was taken at the Thursday AM milking when the results of the first two differed. Duplicate quarter milk samples were collected at the time of clinical mastitis.

4. **Criteria for Determining Infections:** Criteria for new IMI: Same bacterium was isolated from: 1) two consecutive samples during the trial (≥ 500 cfu/ml); 2) duplicate samples from a quarter with clinical mastitis (≥ 100 cfu/ml); and 3) three consecutive samples during the trial (≥ 100 cfu/ml). An individual quarter was eligible for only one infection of the challenge organism.
5. **Data Analysis:** Infection data were analyzed based on percent eligible quarters becoming infected with respective mastitis pathogens. The following statistic was applied:

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

where t = approximates Student's t statistic, x_1 = number of new IMI in control quarters, x_2 = number of 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 summation of quarter-days. The percent reduction in new infection rate in the treatment group compared with that in the control group was expressed as: $100 [(x_1/n_1) - (x_2/n_2)] / (x_1/n_1)$

Results and Discussion

Efficacy data for novel germicide product used as a post-milking teat dip against an experimental challenge of *Streptococcus agalactiae* and *Staphylococcus aureus* and compared against number of new IMI for a negative control and a positive control treatment are presented in Table 1.

Novel germicide post milking teat dip significantly ($P < 0.01\%$) reduced the number of new infections of *Streptococcus agalactiae* and *Staphylococcus aureus* compared to both the negative control (95.3%) and positive control (80.9%) treatments. Incidence of clinical mastitis with a bacteriological positive identification of one of the challenge organisms was 40, 4 and 0 for negative control, positive control and novel germicide dips, respectively. Under the conditions of this study, no differences were visually observed for teat skin condition among the treatments.

Overall Conclusion

The use of a novel germicide used in a post-milking teat dip significantly reduced the number of new intramammary infections (new IMI) caused by experimental challenge with contagious mastitis pathogens *Strep. agalactiae* and *Staphylococcus aureus* compared to no post-milking teat dipping (negative control) and to an industry standard, 0.5% iodine post-milking teat dip.

Table 1. Efficacy data of novel germicide when used as a post-milking teat dip against an experimental challenge of *Streptococcus agalactiae* and *Staphylococcus aureus* and compared with a negative control and a positive control treatment.

	New Infections (New IMI)				% reduction vs. control	
	Clin.**	Subclinical**	Total**	% Quarters*	Neg. ***	Pos. ****
Negative control	40	46	86	53.7%		
Positive control	4	17	21	13.1%	75.5%	
Novel germicide.	0	4	4	3.3%	95.3%	80.9%

* 160 eligible quarters per treatment group
 ** Number of confirmed clinical mastitis incidences; included in total of new infections.
 *** $P < 0.01$ Significant reduction in the number of new IMI compared to negative control.
 **** $P < 0.01$ Significant reduction in the number of new IMI compared to positive control.