

FINAL REPORT

Efficacy Of An Experimental Teat Dip Against *Staphylococcus aureus* and *Streptococcus agalactiae* During Experimental Challenge

INTRODUCTION

The Hill Farm Research Station dairy herd of 144 cows was used in a controlled infection trial to determine efficacy of a teat dip for preventing intramammary infections (IMI) with *Staphylococcus aureus* and *Streptococcus agalactiae*. The products were provided by Monsanto Company, St. Louis, MO. The trial was carried out following the general procedures recommended by the National Mastitis Council (NMC). This report summarizes the results from Product One.

MATERIALS AND METHODS

Herd One was used to test Product One containing sodium dichloroisocyanurate (NaDCC).

Sampling Schedule. Bacteriologic status of mammary quarters was determined at initiation of the trial by collecting and culturing duplicate milk samples. A third sample was collected from specific quarters and cultured when results from the first two samples differed.

Milk samples were collected and analyzed weekly during the trial. In instances where either *S. aureus* or *S. agalactiae* was present for the first time in a previously uninfected quarter, a second sample was collected immediately and cultured. All quarters were eligible for new infections during the trial except (1) those infected with organisms of the same species as challenge organisms; and (2) those with deformed or abnormal teats. Fossomatic somatic cell counts were determined on one set of quarter milk samples collected at the initiation of the trial and biweekly thereafter.

Collection of Milk Samples. Prior to sampling, udders were predipped in sanitizing solution or washed using a hand-held hose and paper towels. After predipping or washing, udders were dried thoroughly with additional paper towels, and two or three streams of foremilk were discarded. Each teat apex was scrubbed for several seconds with a cotton pledget moistened with 70% alcohol. Teats on the opposite side of the udder from the technician were sanitized first, and milk samples were collected in reverse order in sterile, snap-cap plastic tubes and refrigerated at 5°C.

Laboratory Cultural Procedures. Samples were mixed by shaking, and a 0.01-ml aliquot was streaked on Trypticase Soy Agar (TSA) containing 5% bovine blood. Plates were incubated at 37°C for 48 h and examined to identify microorganisms present. An IMI was confirmed when (1) *S. aureus* or *S. agalactiae* was isolated from a clinical quarter; (2) two consecutive samples yielded 500 or more colony-forming units (cfu)/ml of the same pathogen; or (3) three consecutive samples contained 100 to 400 cfu/ml of the same pathogen.

Treatment Method. At the afternoon milking, Monday through Friday, the lower third of all four teats of each cow was experimentally exposed to a challenge suspension containing both *S. aureus* (Newbould 305) and *S. agalactiae* (McDonald 44) immediately after milking machines were removed. Within 5 to 10 seconds thereafter, two teats (right front, left rear) were dipped full length with teat dip; the remaining two teats served as undipped controls. Teats were exposed to challenge organisms to increase the number of pathogens impinging on the test apex, resulting in an increased rate of IMI.

Preparation of Challenge Suspensions. Stock suspensions of *S. aureus* (Newbould 305) were prepared weekly. The contents of one lyophilized vial of *S. aureus* were reconstituted in 6 ml of Tryptic Soy Broth (TSB) and incubated at 37°C for 5 to 7 h. This culture was used to inoculate 500 ml of TSB which was incubated on a gyratory shaker for 16 h. After incubation, bacterial cells were pelleted by centrifugation, washed twice with 0.1% Proteose-peptone No. 3, and resuspended to the original volume in proteose-peptone. Serial dilutions were made in proteose-peptone and 0.1 ml was plated on TSA. Plates were incubated for 24 h at 37°C and colonies counted to ascertain the microbial concentration of the stock suspension. This suspension was stored at 5°C and used daily for one week to prepare challenge of *S. aureus*.

Streptococcus agalactiae (McDonald 44) cultures were prepared by resuspending a lyophilized vial of *S. agalactiae* in 6 ml TSB, and a 0.01-ml aliquot was streak-plated onto each of five TSA plates. Plates were incubated at 37°C for 16 h and stored at 5°C to serve as stock cultures for a one-week period. Daily challenge suspensions of *S. agalactiae* were prepared by inoculating 6 ml of TSB with six colonies from a TSA stock plate. The 6-ml culture was incubated for 7 h at 37°C on a gyratory shaker. Specific aliquots of the culture were added to approximately 146 ml of pasteurized milk to adjust the concentration of *S. agalactiae* to approximately 5×10^7 cfu/ml.

An aliquot of the *S. aureus* stock suspension was added to the *S. agalactiae* suspension to obtain a concentration of approximately 5×10^7 cfu/ml of *S. aureus*. The bacterial suspension was taken immediately to the milking parlor to challenge teats during the afternoon milking. A plate count was conducted daily on challenge suspensions and recorded.

TABLE 1.
Mean teat skin condition scores¹ before and after the trial for teat dips containing NaDCC.

NaDCC (Herd One)			
<i>Before</i>		<i>After</i>	
Dipped	Control	Dipped	Control
1.02	0.98	1.0	0.99

¹ Teat Skin Condition Scoring:

Score	Characteristics
0	Teat skin has been subjected to physical injury (e.g. stepped on or frostbitten) not related to the treatment, or the quarter is nonlactating.
1	Teat skin is smooth, free from scales, cracks or chapping.
2	Teat skin shows some evidence of scaling.
3	Teat skin is chapped. Some small warts may be present.
4	Teat skin is chapped and cracked. Redness, indicating inflammation, is present. Numerous warts may be present.
5	Teat skin is severely damaged and ulcerative with scabs or open lesions. Large or numerous warts are present that interfere with teat end function.

TABLE 2.
Mean teat end condition scores² before and after the trial for teat dips containing NaDCC.

NaDCC (Herd One)			
<i>Before</i>		<i>After</i>	
Dipped	Control	Dipped	Control
1.03	1.04	1.05	1.06

² Teat Skin Condition Scoring:

Score	Characteristics
0	Teat end has been subjected to physical or chemical injury (e.g. stepped on or frostbitten) not related to the treatment, or the quarter is nonlactating.
1	Teat end sphincter is smooth with no evidence of irritation.
2	Teat end has a raised ring.
3	Teat end sphincter is roughened with slight cracks but no redness is present.
4	Teat end sphincter is inverted with many cracks, giving a "flowered" appearance. Teat end may have old but healing scabs.
5	Teat end is severely damaged and ulcerative with scabs or open lesions. Large or numerous warts are present that interfere with teat end function.

SCORING OF TEAT SKIN AND TEAT END CONDITION

Characteristics of teat skin surfaces and teat ends were scored immediately before the teat dip trial evaluating the NaDCC product was initiated and at the conclusion of the trial to determine effects of this germicide on teat skin condition. Teat skin was characterized according to the parameters established by Goldberg et al.¹; scoring characteristics are presented in Tables 1 through 4.

An analysis of teat skin condition scores demonstrated that mean score before and after the trial for dipped and control quarters throughout the herd was approximately 1 (Table 1), which is characterized as normal, smooth skin, free from scabs, cracks or chapping. Likewise, similar analyses of teat end condition scores (Table 2) showed that mean score across all variables was approximately 1, which is characterized as a normal, smooth teat end with no evidence of irritation.

At least 94.1% of teats were characterized as having normal (score = 1) teat skin condition before and after the trial for dipped and control quarters throughout the herd (Table 3). The percentages of teats with physical injuries (score = 0) were lower after the trial than before the trial for dipped and control quarters throughout the herd. For example, before the trial in Herd One, 2.7% of dipped teats exhibited skin scores of 0, and after the trial, 1.4% of dipped teats exhibited skin scores of 0.

In Herd One, the percentage of teats showing some evidence of scaling (score = 2) decreased in both dipped and control quarters from 0.7% before to 0% after the trial. The percentage of teats showing chapping (score = 3) among dipped quarters decreased from 2% before to 0.7% after the trial, while among control teats, there was no difference (0.7%). No teats in the herd exhibited scores of 4 or 5.

At least 88.2% of teats were characterized as having normal (score = 1) teat end condition before and after the trial for dipped and control quarters throughout the herd (Table 4). A very small percentage of teat ends exhibited physical injuries (score = 0) before or after the trial.

In Herd One, the percentage of teat ends with a raised ring (score = 2) increased from 1.3% before to 2.1% after the trial in dipped quarters, and in control quarters the increase was from 1.3% to 3.5%. The percentage of teat ends exhibiting a roughened sphincter with slight cracks (score = 3) changed little before and after the trial in dipped and control quarters.

¹ Goldberg, J.J., P.A. Murdough, A.B. Howard, P.A. Drechsler, J.W. Pankey, G.A. Ledbetter, L.L. Day, and J.D. Day. 1994. Winter evaluation of a postmilking powdered teat dip. *J. Dairy Sci.* 77:748-758.

TABLE 3.
Frequency of teat skin condition scores³ before and after the trial for teat dips containing NaDCC.

NaDCC (Herd One)				
Score	Before		After	
	Dipped	Control	Dipped	Control
0	2.7	4.0	1.4	2.8
1	94.7	94.7	97.9	96.5
2	0.7	0.7	0.0	0.0
3	2.0	0.7	0.7	0.7
4	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0

³ Teat Skin Condition Scoring:

Score	Characteristics
0	Teat end has been subjected to physical injury (e.g. stepped on or frostbitten) not related to the treatment, or the quarter is nonlactating.
1	Teat skin is smooth, free from scales, cracks or chapping.
2	Teat skin shows some evidence of scaling.
3	Teat skin is chapped. Some small warts may be present.
4	Teat skin is chapped and cracked. Redness, indicating inflammation, is present. Numerous warts may be present.
5	Teat skin is severely damaged and ulcerative with scabs or open lesions. Large or numerous warts are present that interfere with teat end function.

TABLE 4.
Frequency of teat skin condition scores⁴ before and after the trial for teat dips containing NaDCC.

NaDCC (Herd One)				
Score	Before		After	
	Dipped	Control	Dipped	Control
0	1.3	0.0	1.4	2.8
1	96.0	97.3	94.4	95.1
2	1.3	1.3	2.1	3.5
3	1.3	1.3	2.1	1.4
4	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0

⁴ Teat Skin Condition Scoring:

Score	Characteristics
0	Teat end has been subjected to physical or chemical injury (e.g. stepped on or frostbitten) not related to the treatment; or the quarter is nonlactating.
1	Teat end sphincter is smooth with no evidence of irritation.
2	Teat end has a raised ring.
3	Teat end sphincter is roughened with slight cracks, but no redness is present.
4	Teat end sphincter is inverted with many cracks, giving a "flowered" appearance. Teat end may have old but healing scabs.
5	Teat end is severely damaged and ulcerative with scabs or open lesions. Large or numerous warts are present that interfere with teat end function.

TABLE 1.
Summary of efficacy data for Product One against
Staphylococcus aureus and *Streptococcus agalactiae*.

Organism and Treatment	Quarters eligible for new IMI	New IMI										% Quarters	% Reduction
		Week									Total		
		1	2	3	4	5	6	7	8	9			
<u><i>Staph. aureus</i></u>													
Dip	137	1	1	1	3	2	0	1	0	1	10	7.3	
Control	140	11	0	2	5	5	2	3	0	2	30	21.4	69.0*
<u><i>Strep. agalactiae</i></u>													
Dip	147	2	0	1	0	0	1	3	0	1	8	5.4	
Control	148	4	1	3	3	3	0	3	3	1	21	14.2	63.5†

* Significant, $P < 0.001$. † Significant, $P < 0.01$.

RESULTS

Infection data collected during the trial are summarized in Table 1. In Herd One, in which the NaDCC dip was tested, a total of 40 new *S. aureus* IMI were confirmed: 30 in control quarters and 10 in dipped quarters (Table 1). Twenty-nine new *S. agalactiae* IMI were confirmed: 21 in control quarters and 8 in dipped quarters (Table 1). The teat dip reduced the infection rate for *S. aureus* 69.0% (significant, $P < 0.001$) and 63.5% for *S. agalactiae* (significant, $P < 0.01$).

In summary, the NaDCC product was highly effective in preventing new IMI, and was as efficacious as other products currently available on the market.