Germicidal efficacy testing of Forticept Udder Wash (pre-dip) and Forticept Udder Forte (post-dip) in reducing the new intramammary infection rate and SCC under natural exposure to mastitis pathogens, with additional in vitro testing of germicidal activity

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Abstract

Teat disinfection, both before and after milking, is the most important mastitis management tool for reducing the incidence of new intramammary infections in dairy cows. Between milkings, cows are exposed to pathogenic mastitis-causing microorganisms in the environment in which they are managed that are present in soil, manure, bedding materials, water, and mud. These pathogens contaminate the teat skin of the udder, and include environmental bacteria such as *Streptococcus uberis* and *Escherichia coli*. The practice of pre-dipping reduces the bacterial load with these environmental pathogens, which subsequently reduces the new infection rate. During the milking process, teats are exposed to the contagious organisms such as *Streptococcus agalactiae*, *Staphylococcus, aureus*, and *Mycoplasma* species. Post-dipping by immersion of teats in a disinfectant immediately after milking cluster removal kills the majority of contagious bacteria, thereby preventing the establishment of new infections during the post-milking period. Whether pre- and/or post-dipping are used, dairy producers must ensure that they are using products that have been proven effective against mastitis-causing bacteria through valid scientific testing. In this study, the germicidal efficacies of Forticept Udder Wash (pre-dip) and Forticept Udder Forte (post-dip) (Lidan, Inc., NY, NY) in reducing the new intramammary infection rate under natural exposure to mastitis pathogens were evaluated and compared with a proven iodine pre- and post-dip product as a positive control. Results of the 6-month trial demonstrated a new infection rate of 10.5% among mammary quarters pre-dipped and post-dipped in Forticept products, and a rate of 5.8% among quarters pre-dipped and post-dipped in the positive control iodine product; the difference was not significant ($P < 0.06$). In addition, average somatic cell count (SCC) was 304,000/ml among mammary quarters pre-dipped and post-dipped in Forticept products, and 239,000/ml among quarters pre-dipped and post-dipped in the positive control product; the difference was not significant ($P < 0.06$). Average teat condition scores were very similar ($P < 0.96$) among quarters pre-dipped and post-dipped in Forticept products (Score = 1.40), and quarters pre-dipped and post-dipped in the positive control (Score = 1.46). Findings suggest that under the conditions of this study, the new mammary quarter infection rate, SCC, and teat condition scores were similar among quarters dipped in Forticept products and quarters dipped in the positive control product.

Introduction

The underlying principle on which the control of mastitis rests involves prevention of the disease. This is best accomplished by minimizing the number of mastitis pathogens to which teats are exposed during the: (1) pre-milking; (2) milking; (3) post-milking; and (4) inter-milking periods. A high level of exposure to mastitis pathogens will invariably lead to an increased rate of intramammary infection, while lowering the level of exposure to these organisms will reduce the

rate of infection.

Hygiene can be described as preventive medicine. When used in the broadest sense, hygiene is the sum of all attempts to manage the total environment of the cow to minimize the number of mastitis organisms to which teats and udders are exposed during both lactation and the dry period. Thus, the ultimate goal of mastitis control is to prevent new infections, which means a constant war must be waged against the multitude of different microorganisms that are always poised to invade the udder and cause damage to milk-secreting tissues.

Some transmission of mastitis pathogens will occur within every dairy herd, even under the best of hygienic circumstances, and it will not be possible to keep teats completely free of potentially harmful microorganisms. Anything that comes into contact with an infected udder and subsequently touches another udder is a means for transmitting microorganisms. If transmission is broken, or substantially reduced, there will be a decrease in incidence of new infections. A significant amount of transmission often occurs during the milking process via: (1) milkers' hands; (2) udder clothes or sponges; and (3) teat cup liners. Microorganisms most likely to be transmitted at this time are contagious pathogens such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma* species.

Some transmission will occur during the interval between milkings. Possible methods of transmission include: (1) contaminated bedding or soil; (2) contact of teats with rear legs; (3) tail switch; (4) licking of teats and udder; (5) flies; and (6) wetting cows excessively with sprinklers, which results in water running down the flanks and teats of the cows. Microorganisms most frequently transmitted during the intermilking period are environmental streptococci and coliforms.

Disinfection of Teats Prior to Milking

Because the rate of new infection is a function of the number of microorganisms present on teats, the primary objective of pre-milking udder preparation and teat sanitation is to achieve an acceptable level of decontamination. This aids in: (1) reducing the spread of microorganisms and the incidence of mastitis; and (2) minimizing the number of organisms that find their way into the bulk tank and raw milk supply. The process of preparing teats for milking has several other advantages including: (1) promotion of milk letdown; (2) speeding up milking; and (3) helping to ensure that a maximum amount of available milk is harvested without causing damage to sensitive teat tissues.

The primary objective of good milking hygiene should always be to milk teats that are sanitized and dry, which is important for both prevention of mastitis and the production of high quality milk. If teats are relatively clean and free of bedding materials, mud, and manure when cows enter the milking center, it is recommended that milking personnel simply: (1) forestrip; (2) pre-dip with a disinfectant; (3) wait 20 to 30 seconds; and (4) dry each teat carefully with a paper towel or recently laundered cloth towel. Strategic washing is sometimes used on selected teats, but the amount of water used must be minimized.

With the decrease in mastitis caused by contagious mastitis organisms, such as *Staphylococcus aureus* and *Streptococcus agalactiae*, concern has increased about environmental pathogens such

as *Streptococcus uberis* and coliforms. Though these organisms have not increased in overall prevalence, they have become relatively more important to producers simply because they now constitute a larger portion of the mastitis problem as a consequence of having reduced contagious mastitis. This concern has led to the widespread use of pre-dipping by a majority of dairy producers in the United States and Canada, and the number using the practice continues to increase as the benefits for controlling environmental organisms have become more widely known.

Scientific research on pre-dipping has shown that: (1) the incidence of new infections caused by environmental streptococci and coliforms is reduced by about 50%; (2) clinical mastitis is reduced; and (3) the incidence of *Staphylococcus aureus* is often reduced. Producers must exercise care, however, when using pre-dipping in order to prevent residues of the germicide from contaminating the milk supply. Fortunately, when teats are dried carefully after pre-dipping, the amount of additional residue present in the milk will be negligible.

Post-milking Teat Dipping

The transfer of some mastitis organisms is inevitable at milking time. If the incidence of mastitis is to be reduced, it is important that the vast majority of organisms present on teats be destroyed after machine detachment. The dipping of teats after milking in a suitable germicidal product is regarded by practically every specialist in the international dairy industry as being the most important single practice producers can follow to prevent new intramammary infections in lactating dairy cattle. This is attested to by the fact that the vast majority of producers in North America, Europe, and other countries with a developed dairy industry now utilize the practice in almost all dairy herds.

The concept of teat dipping after milking is not new. Indeed, its use dates back to 1916 when a dilute pine oil solution was used in an effort to reduce the spread of *Streptococcus agalactiae*. The practice was not adopted widely for several decades because supporting research data were not available on existing teat dip products. Interest in post-dipping was revived in the 1950s when researchers demonstrated reductions in staphylococcal populations on teat cup liners after teat dipping was employed. This work was validated by researchers at the National Institute for Research in Dairying in England where milking hygiene programs were evaluated in large field trials involving commercial dairy herds. The hygiene programs were successful in reducing the rate of infection, and teat dipping was shown to be a highly effective component of the programs.

Since the 1960s, a large number of studies on teat dipping have confirmed the value of teat dipping for reducing new infections, especially against contagious pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae*. It is now accepted that the correct application of a good teat dip after milking machine detachment will reduce the rate of new infection by about 50%. Teat dipping has probably had a greater economic impact on the dairy industry than any other single technique used for mastitis control.

Teat Dipping vs. Teat Spraying

Some persons associated with the dairy industry have expressed concern that teat dip cups can become contaminated with bacteria and may actually serve to transmit mastitis organisms from

teat to teat and from cow to cow. Additionally, the practice of dipping teats may be less efficient time wise than spraying. These concerns have motivated them to recommend teat spraying rather than teat dipping. The only mastitis pathogens likely to grow in teat dips are *Pseudomonas* species and *Serratia* species, both of which are incredibly rare as causes of mastitis. Thus, the suggestion that teat dipping causes mastitis is clearly wrong.

Both research and practical field experience have shown that teat spraying is as effective as teat dipping — if it is done properly. To be as effective as teat dipping, the entire barrel of the teat touched by the teat cup liner must be covered with teat dip, but, unfortunately, this is rarely accomplished because producers and their employees usually apply teat spray to only one side of the teats rather than to the entire surface of the teats. Moreover, to do an excellent job of teat spraying will require more time and more teat disinfectant than teat dipping. For these reasons, it is recommended to dip rather than spray teats.

Types of Teat Dips

Teat dip manufacturers were quick to recognize the economic potential of the teat dip market and developed a wide variety of products. Many varied only slightly from products that had been tested under research conditions and shown to be highly effective. Other products utilized novel disinfectants and imaginative formulations that were marketed without prior testing to determine effectiveness. Subsequent testing confirmed that most of the products were effective, but some were shown not to be effective, and a few products actually increased the infection rate.

The two basic types of teat dips are germicidal and barrier products. The germicidal products enjoy the vast majority of the teat dip market in the United States. These products are highly effective in reducing populations of mastitis organisms remaining on teats at the end of milking. The persistency of germicidal activity is limited, however, because it is partially neutralized by milk remaining on teats at the end of milking and by organic matter in the environment. Fortunately, most products exert their activity very rapidly and this does not affect their ability to kill contagious organisms remaining on teats after milking.

The theory behind barrier teat dips is that they form a physical obstruction between teats and the environment, thus reducing infections during the intermilking period, especially with microorganisms of environmental origin. Included in this group are: (1) latex; (2) acrylic; and (3) polymer-based products.

When selecting a teat dip, producers should require manufacturers to provide evidence of efficacy based on one or more of the National Mastitis Council (NMC) protocols discussed below, and also provide evidence that the manufacturer is following Good Manufacturing Practices as required by the Food and Drug Administration. Only products on which these data are available should be used.

Evaluation of Teat Dips

Practically all of the procedures used throughout the world to evaluate teat dip efficacy in reducing the new intramammary infection rate were developed by the NMC in the United States. Five

procedures have been used. Three of the more commonly used methods of efficacy testing are discussed briefly, along with an in vitro procedure to evaluate germicidal activity against common mastitis pathogens.

1. Protocol for Determining Efficacy of a Teat Dip after Experimental Exposure of Teats to Mastitis Microorganisms

In most instances, this protocol has been used in university research herds. The protocol evaluates the ability of a teat dip to reduce the incidence of new infections compared with undipped control teats when all teats are challenged experimentally with mastitis microorganisms to enhance the rate of new infection. The enhanced infection rate reduces the amount of time required to reach a statistically valid proof point in terms of numbers of new infections. All four teats of each experimental cow are challenged by immersion in a suspension of specific mastitis microorganisms immediately after removal of the milking machine. A few seconds later, two teats are immersed in the teat dip under test while the two remaining teats serve as undipped controls. An alternate procedure that is used is to dip all teats on half of the cows in the herd with a teat dip and leave the teats of the other half of the cows to serve as undipped controls.

2. Protocol for Determining Efficacy of a Teat Dip Based on Reduction of Naturally Occurring New Infections

Usually, the herd is divided into two treatment groups by either a split-udder or split-herd design as described immediately above. The number of new infections occurring in the dipped versus undipped quarters is measured to determine efficacy of the teat dip being evaluated.

3. Protocol for Comparing an Experimental Teat Dip with a Product of Known Efficacy Based on Incidence of Naturally Occurring New Infections

Teats on half of the quarters are dipped with an experimental dip, and half are dipped with a product of known efficacy in either a split-udder or split-herd design. The product of proven efficacy should have been tested previously under conditions outlined under the protocols above. Comparisons are made between the experimental product and the proven product as outlined in Protocol 2 above.

4. In Vitro Testing of Germicidal Activity

A modification of the Kirby-Bauer method as an indirect measure of product germicidal activity is used. Absorbent paper disks impregnated with test products are placed on nutrient agar plates that have been swirl-plated with a culture of bacteria to provide a lawn of microbial growth. Plates with adhered disks are incubated for 48 hours, and then the zones of microbial growth inhibition are measured in millimeters and compared to a product of known efficacy.

Evaluation of Forticept pre-dip and Forticept post-dip using Protocol 3

Experimental design

The efficacy testing of Forticept pre- and post-dip products (Lidan, Inc., NY, NY) was conducted in a trial following the 3rd protocol described above by comparing the 2 experimental teat dips with a product of known efficacy. This trial took place at a University dairy herd (UGA), and efficacy was based on incidence of naturally occurring new intramammary infections over a 6-month period.

A split-udder design was used on 120 lactating cows enrolled in the UGA Teaching Dairy Herd in which the right front (RF) and left rear (LR) quarters of each cow served as experimental quarters and were dipped in the product to be tested (Forticept), and the left front (LF) and right rear (RR) quarters were dipped in the positive control (Bovidine). More specifically, RF and LR quarters were pre-dipped in Forticept pre-dip (0.13% benzalkonium chloride) and post-dipped in Forticept postdip (0.1% benzalkonium chloride), and LF and RR quarters were pre-dipped and post-dipped in Bovidine. Bovidine – a 1% iodine product – was used as the pre-dip as well as the post-dip for the positive control quarters.

Milking procedures and application of teat dip products

Prior to each twice-a-day milking and as soon as cows were secured in the milking parlor, teats were dry-wiped with single-service paper towels to remove loose sand, dirt, and manure. Teats were then fore-stripped to stimulate milk down, to flush teat canals, and to detect any abnormal milk. After fore-stripping, the RF and LR teats were immersed in Forticept pre-dip, and the LF and RR teats were immersed in Bovidine. After 30 seconds of exposure time to the pre-dips, the germicidal residues were removed and teat skin was dried using single service paper towels. The milking units were attached to udders, and after milk was removed, the units detached automatically. Post-dipping was carried out as follows: RF and LR teats were immersed in Forticept post-dip, and the LF and RR teats were immersed in Bovidine. After applying post-dips, cows were released from milking stalls and exited the parlor.

Milk sampling, microbiological procedures, somatic cell counts, and teat scoring

Quarter milk samples were collected aseptically using standard procedures recommended by the National Mastitis Council as follows. Immediately after the pre-dipping step described above (e.g., after 30 seconds of exposure time, germicidal residues were removed and teat skin was dried using single service paper towels), teat ends were sanitized using cotton balls soaked in 70% ethyl alcohol, and milk samples were collected into 10-ml disposable polypropylene test tubes pre-labelled with cow and quarter identification numbers, placed in test tube racks, then stored in an ice chest until transport to the laboratory for processing. At the laboratory, racks were placed in a refrigerator at 4C for overnight storage for processing the next day.

At the beginning of the trial, 2 consecutive bi-weekly samples were collected to establish the infection status of each mammary quarter, after which time quarters were sampled every 2 weeks for a 6-month period to diagnose new infections. Also, at the beginning of the trial, teat end condition was scored at 2 consecutive bi-weekly samplings to establish the condition of teat ends, after which time teat ends were scored once a month for a 6-month period to monitor any changes in teat end condition after exposure to the teat dips.

For microbiological analyses, quarter milk samples were plated on blood agar containing 5% ovine blood and incubated at 37C for 48 hr. After incubation, plates were read and bacteria were presumptively identified based on colony morphology/color, hemolytic patterns, and aromatic characteristics using standard NMC procedures. New intramammary infections were confirmed when the same microorganism was isolated from a quarter from 2 consecutive samples (every 2 wk). Somatic cell counts (SCC) on quarter milk samples were performed using a DeLaval Cell Counter (DCC) to determine the concentration of leukocytes per ml of milk.

For teat end condition scoring, each teat was assigned a score ranging from 1 to 4 using the following system:

Score 1 – No ring, the teat end is smooth with a small even orifice.
Score 2 – Smooth or slightly rough ring with a raised ring around the orifice. The raised area may be smooth or slightly rough. No keratin is present.
Score 3 – Rough ring with raised, roughened ring of keratin extending 1-3 mm from the orifice.
Score 4 – Very rough ring with keratin extending more than 4 mm from the orifice. The rim of the ring may be cracked.

Results

Results of the 6-month trial demonstrated a new infection rate of 10.5% among mammary quarters pre-dipped and post-dipped in Forticept Udder Wash (pre-dip) and Forticept Udder Forte (post-dip), which was greater but not significantly higher ($P < 0.06$) in comparison with the rate of 5.8% among quarters pre-dipped and post-dipped in the positive control iodine product (Bovidine). See Table 1.

Average SCC was 304,000/ml among mammary quarters pre-dipped and post-dipped in Forticept products, and 239,000/ml among quarters pre-dipped and post-dipped in the positive control product; the difference was not significant ($P < 0.06$). See Table 1.

Average teat condition scores were very similar ($P < 0.96$) among quarters pre-dipped and post-dipped in Forticept products (1.40), and quarters pre-dipped and post-dipped in the positive control (1.46). See Table 1.

Table 1. Effect of Control and Forticept teat dips on the new infection rate, somatic cell count (SCC), and teat scores over a 6-month period*.

	Control		Forticept			
Variable	No	%	No	%	SEM	P-value
% New Infection P1	241	2.07	239	4.60	1.16	0.123
% New Infection P2	243	3.70	239	5.86	1.38	0.268
% New Infection	**243**	**5.77**	**239**	**10.46**	**1.81**	**0.066**
SCC-2	135	185.67	134	184.88	40.11	0.989
SCC-1	135	187.41	135	217.30	41.84	0.614
SCC0	21	445.33	21	261.00	109.20	0.240
SCC1	198	227.67	197	267.79	33.82	0.401

SCC2	205	207.14	203	333.43	38.71	0.630
SCC3	201	185.34	199	249.95	30.91	0.139
SCC4	201	148.32	199	317.41	41.36	0.237
SCC5	203	357.26	201	465.88	51.82	0.138
SCC6	215	249.33	212	277.46	36.93	0.589
SCC7	218	303.73	216	366.59	49.53	0.369
SCC8	229	209.82	226	257.47	32.18	0.294
SCC9	231	170.98	228	229.13	30.13	0.181
SCC10	235	190.13	234	285.74	32.04	0.035
SCC11	231	205.44	230	312.46	41.16	0.066
SCC12	226	204.49	224	262.84	28.78	0.151
SCC P0	147	219.32	147	213.94	32.91	0.908
SCC P1	239	261.30	235	315.68	27.92	0.167
SCC P2	236	191.99	234	273.30	29.54	0.052
SCC avg	**242**	**238.67**	**239**	**304.07**	**26.38**	**0.079**
Teat score 1	170	1.53	171	1.50	0.06	0.701
Teat score 2	205	1.50	203	1.46	0.05	0.571
Teat score 3	201	1.52	199	1.55	0.04	0.564
Teat score 4	215	1.52	213	1.50	0.04	0.761
Teat score 5	225	1.49	222	1.50	0.04	0.970
Teat score 6	226	1.37	224	1.37	0.04	0.955
Teat score avg	**240**	**1.40**	**238**	**1.46**	**0.03**	**0.960**

*****Forticept Trial – Dec 04, 2017 through June 26, 2018.

Pre Period (P0) is samples -2, -1 and 0.
Period 1 (P1) is samples 1 through 8 (first formulations).
Period 2 (P2) is samples 9 through 12 (second formulations).
Average value is samples 1 through 12.

Findings suggest that under the conditions of this 6-month study and the statistical analysis used, the new mammary quarter infection rate, SCC, and teat condition scores were similar among quarters dipped in Forticept products and quarters dipped in the positive control product. No significant differences were found among the three parameters measured. Therefore, the test product (Forticept) was as good as the positive control product (Bovidine) when used as a pre and post-dip to reduce the new infection rate in the University herd used in the trial. However, because the ultimate goal is to bring the new infection rate of 10.5% among mammary quarters dipped in Forticept closer to the rate of 5.8% for the positive control quarters, further in vitro testing was carried out on 3 new formulations designed to improve germicidal activity (see below).

Quality evaluation of Forticept pre- and post-dips from the milkers' perspectives

Student milkers found Forticept pre-dip easy to dispense into teat dip cups, and easy to apply to teats prior milking. The product did not stain clothing, and was nonirritating to milkers' skin. It

was suggested that some type of coloring agent be added to the pre-dip, which is a clear liquid, so that it could be more easily observed on the teat skin surfaces after dipping to ensure that all teats were pre-dipped. Students commented that the viscosity and blue color of the post-dip was pleasing to the eye and really stood out on teats after dipping, which was beneficial in ensuring that all teats were dipped after the milking process.

Evaluation of Forticept post-dip using Protocol 4

Three Forticept formulations (1, 2 and 3) were tested for germicidal activity against 2 *S. aureus* isolates, 2 Gram-negative isolates (*Klebsiella* spp. and *E. coli*), 2 streptococcal isolates (*Streptococcus* spp. and *Str. uberis*), and 2 coagulase-negative species (CNS) also known as *Staph*. spp.

A modification of the Kirby-Bauer method was used. The bacterial isolates listed above were swirl-plated onto 5% ovine blood agar plates. Absorbent paper disks impregnated with the 3 test products were then placed on inoculated plates followed by incubation at 37C for 48 hours. Zones of microbial growth inhibition exhibited by the 3 formulations were then measured in millimeters and compared to a product of known efficacy (FS104X, 0.5% iodine). See Table 2 below.

Inhibition of microbial growth against the 2 strains of *S. aureus* tested was greatest for Forticept Formulation 3, which was similar to the positive control teat dip. Inhibition of microbial growth against *Klebsiella* spp. was similar among all 3 Formulations, ranging from 8.75 to 10.0 mm, which was slightly lower than the positive control at 12.0 mm. Inhibition of microbial growth against *E. coli* was similar among all 3 Formulations, ranging from 6.75 to 8.25 mm, which was similar to the positive control at 7.0 mm. Inhibition of microbial growth against the 2 strains of streptococci tested was greatest for Forticept Formulation 3, which was lower than that for the positive control (20 mm). Inhibition of microbial growth against the 2 strains of staphylococci (CNS) tested was greatest for Forticept Formulation 3, which was similar to the positive control teat dip.

Compared with initial in vitro testing of Forticept Post-Dip in December of 2017 (Table 3), the more recent testing revealed that germicidal activity against *S. aureus* appeared superior when using Formulation 3 (Table 2), as zones of inhibition were very similar to the positive control. Likewise, germicidal activity against *Staph.* spp. appeared superior when using Formulation 3, as zones of inhibition were slightly above those of the positive control. Zones of inhibition were lowest for *E. coli*; however, zones for Formulation 3 were more similar to those of the positive control, whereas in the initial testing, Forticept Post-Dip did not perform as well as the positive control. As observed with the initial testing, Formulation 3 did not perform as well as the positive control against *Strep*. spp.

Table 2. Zone of Inhibition Assay to Determine Antimicrobial Activity of Three Forticept Teat Dip Formulations

*See footnote for interpretation of results

Dates: 10/05/2018, 10/18/18 & 10/22/18

Incubation Period: 48 Hr

PRODUCT	Plate 1 (mm)	Plate 3 (mm)	Plate 5 (mm)	Plate 6 (mm)	Plate 9 (mm)	Plate 4 (mm)	Plate 7 (mm)	Plate 8 (mm)
Bacterial species tested:	**S. aureus**	**S. aureus**	**Klebsiella spp.**	**E. coli**	**Strep spp.**	**Str. uberis**	**Staph spp.**	**Staph spp.**
Forticept Dip 1	9	11	11	9	8	8	10	10
Forticept Dip 1	11	11	9	7	10	11	11	11
Forticept Dip 1	9	10	11	10	9	7	10	11
Forticept Dip 1	11	12	8	7	9	10	12	12
Ave zone diam. (mm) =	***10***	***11***	***9.75***	***8.25***	***9***	***9***	***10.75***	***11***

PRODUCT	Plate 1 (mm)	Plate 3 (mm)	Plate 5 (mm)	Plate 6 (mm)	Plate 9 (mm)	Plate 4 (mm)	Plate 7 (mm)	Plate 8 (mm)
Bacterial species tested:	**S. aureus**	**S. aureus**	**Klebsiella spp.**	**E. coli**	**Strep spp.**	**Str. uberis**	**Staph spp.**	**Staph spp.**
Forticept Dip 2	12	13	10	10	9	11	8	9
Forticept Dip 2	11	14	8	7	10	10	10	9
Forticept Dip 2	12	12	10	11	9	12	8	8
Forticept Dip 2	11	13	7	6	9	11	10	7
Ave zone diam. (mm) =	***11.5***	***13***	***8.75***	***8.5***	***9.25***	***11***	***9***	***8.25***

PRODUCT	Plate 1 (mm)	Plate 3 (mm)	Plate 5 (mm)	Plate 6 (mm)	Plate 9 (mm)	Plate 4 (mm)	Plate 7 (mm)	Plate 8 (mm)
Bacterial species tested:	**S. aureus**	**S. aureus**	**Klebsiella spp.**	**E. coli**	**Strep spp.**	**Str. uberis**	**Staph spp.**	**Staph spp.**
Forticept Dip 3	15	15	10	7	11	14	15	12
Forticept Dip 3	15	15	9	8	13	12	14	12
Forticept Dip 3	15	15	11	6	12	13	15	12
Forticept Dip 3	15	15	10	6	14	11	14	12
Ave zone diam. (mm) =	***15***	***15***	***10***	***6.75***	***12.5***	***12.5***	***14.5***	***12***

PRODUCT	Plate1 (mm)	Plate 3 (mm)	Plate 5 (mm)	Plate 6 (mm)	Plate 9 (mm)	Plate 4 (mm)	Plate 7 (mm)	Plate 8 (mm)
Bacterial species tested:	**S. aureus**	**S. aureus**	**Klebsiella spp.**	**E. coli**	**Strep spp.**	**Str. uberis**	**Staph spp.**	**Staph spp.**
Teat Dip*	15	14	12	7	19	21	14	12
Teat Dip	15	15	12	7	20	22	13	11
Ave zone diam. (mm) =	***15***	***14.5***	***12***	***7***	***19.5***	***21.5***	***13.5***	***11.5***

*FS-104X.

*Inhibition of microbial growth against the 2 strains of *S. aureus* tested was greatest for Forticept Formulation 3, which was similar to the positive control teat dip.

Inhibition of microbial growth against *Klebsiella* spp. was similar among all 3 Formulations, ranging from 8.75 to 10.0 mm, which was slightly lower than the positive control at 12.0 mm.

Inhibition of microbial growth against *E. coli* was similar among all 3 Formulations, ranging from 6.75 to 8.25 mm, which was similar to the positive control at 7.0 mm.

Inhibition of microbial growth against the 2 strains of streptococci tested was greatest for Forticept Formulation 3, which was lower than that for the positive control (19.5 mm).

Inhibition of microbial growth against the 2 strains of staphylococci (CNS) tested was greatest for Forticept Formulation 3, which was similar to the positive control teat dip.

Table 3. Zone of Inhibition Assay to Determine Antimicrobial Activity of Forticept Teat Dip

Date 12.11.17

Incubation Period 24 hr

PRODUCT	PLATE 1 (mm)	PLATE 2 (mm)	Plate 3 (mm)	Plate 4 (mm)
Bacterial species tested:	***Staph*. spp**	***Staph. aureus***	***E. coli***	***Strep. spp.***
1 Forticept Active Complex	20	17	10	15
2 Forticept Active Complex	19	16	10	15
3 Forticept Active Complex	20	15	15	13
4 Forticept Active Complex	20	16	11	16
5 Forticept Active Complex	18	16	11	14
Ave. zone diam. (mm) =	19.4	16	11.4	14.6
1 Iodine Teat Dip (1%)	17	15	9	15
2 Iodine Teat Dip (1%)	17	16	10	14
Ave. zone diam. (mm) =	17	15.5	9.5	14.5
1 Forticept Post Dip	16	13	8	9
2 Forticept Post Dip	14	12	10	10
Ave. zone diam. (mm) =	15	12.5	7	9.5

Evaluation of Blue Butter Antimicrobial Gel

Blue Butter Antimicrobial Gel (Blue Butter) was applied to various lesions, abscesses, and abrasions that were observed on the teats, udders, and legs of the UGA milking herd. As cows entered the milking parlor and were being prepped for milking, any of the skin abnormalities listed above were noted, and after the milking process was terminated, Blue Butter was topically applied, using a gloved hand in a liberal fashion, by the individual doing the milking. This was repeated at subsequent milkings (about every 12 hours until the wound visually appeared to be healed. If the abnormality was present on a teat, the Blue Butter was applied first, then the teat was dipped in the appropriate post-dip for that teat. As long as the lesion, abscess, or abrasion was visible, the milkers continued to apply Blue Butter at subsequent milkings until the skin abnormality was no longer visible.

Blue Butter also was used on cows and heifers that had recently calved. Many of such animals had swollen and raw teats because of milk accumulation, and when the milking unit was applied, the mouth of the liner exerted pressure at the base of the teat, causing irritation to the teat skin. So, after the cow/heifer was milked out, and the milking unit was removed, Blue Butter was applied to the area of irritation to soothe the affected skin and promote healing.

Results suggest that the treated lesions, abscesses, abrasions, and areas of irritation healed quite nicely. The positive attributes of Blue Butter are 1) The product color: This is the only blue ointment on the market for treating skin abnormalities in dairy cows, and the blue color shows up

very well on cows skin/hair; 2) The viscosity of the product: It is very thick and does not drip or run off the teat, udder, or leg where it is applied and often remains in place until the next milking; and 3) The odor of the product: It has a medicinal smell that is not objectionable.

Although untreated control wounds were not included in this study, milking personnel and researchers believed that use of Blue Butter on such abrasions and lesions was far superior to not applying any type of antimicrobial or healing product.

Conclusions

Results of in vitro and in vivo testing of Forticept pre- and post-dips suggest that the products are as efficacious as the positive control iodine product in reducing the microbial growth and development of new IMI, respectively, caused by the common mastitis-causing pathogens. Use of Blue Butter Antimicrobial Gel was beneficial in the healing of lesions, abscesses, abrasions, and areas of irritation on cows' teats, udder, and legs.

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