Use of Dry Cow Therapy and Teat Seal in Managing Mastitis in Bred Holstein Heifers

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Introduction

Because of the importance of bred heifers to the future milk production of any dairy operation, it is critical that udder health be maximized to ensure that animals freshen free of intramammary infection (IMI). During the heifer's first gestation, the presence of mastitis can compromise the development of milk-producing tissues, and in the case of *Staphylococcus aureus*, milk yield may be reduced up to 10% over the first lactation (Nickerson, 2009; Owens, 1991). Milk quality is also reduced due to an increase in the somatic cell count (SCC) for the duration of the lactation (Paradis et al., 2010). In some of the worst cases, mammary tissue is replaced with scar tissue, causing the heifer to calve with a blind or nonfunctional quarter.

Greater than 90% of breeding age and bred heifers may have IMI caused by the coagulase-negative staphylococci (CNS) and *S. aureus*, and up to 30% is caused by *S. aureus* alone (Nickerson 2009). Such IMI induce a chronic inflammation, which is associated with elevated SCC ($>10x10^6$/ml) and damage to the developing milk-producing tissues (Trinidad et al., 1990). Thus, an udder health care program should be in place to eliminate existing IMI and prevent new ones in bred heifers so that they freshen free of mastitis with low SCC and the potential for maximum yield.

Use of nonlactating or dry cow antibiotic infusion products have been successful in curing existing IMI that develop during pregnancy and preventing new cases that occur in late gestation. For example, Owens et al. (2001) evaluated the efficacy of 5 different nonlactating cow antimicrobial products administered 8 to 12 wk prepartum and found that cure rates for *S. aureus* IMI ranged from 67 to 100%, and were higher than the spontaneous cure rate (25%) observed in untreated control quarters. In another study (Owens et al., 1994), the infusion of nonlactating cow therapy into uninfected quarters 8 to 12 wk prepartum reduced new environmental streptococcal IMI by 93%. Thus, use of nonlactating cow therapy was effective in both curing existing IMI and preventing new cases of mastitis.

Other studies have tested the efficacy of internal teat sealant barriers (bismuth subnitrate) in preventing the development of new IMI by physically impeding bacterial entry to the teat canal and distal teat cistern. Parker et al. (2008) found that the placement of a teat seal approximately 1 mo prior to calving in heifers reduced the risk of new IMI by 74% and prevalence of post-calving IMI by 65%.

The question becomes, from a heifer management standpoint, which tool is most beneficial for mastitis control: 1) infusion of nonlactating cow therapy, 2) placement of teat seals, or 3) the combination of the two products?

Materials and Methods

Animals:

To answer this question, 38 bred Holstein heifers were enrolled in the trial and housed in a far-off pasture at the UGA Teaching Dairy. Animals were fed a total mixed ration (TMR) once daily based on wheat or sorghum silage and 2.3 kg/head/day of dry cow grain mix. Between 30 and 60 days prior to the expected calving date, mammary secretion samples were collected aseptically from each quarter of each heifer and processed for bacteriological analysis, SCC, and differential leukocyte counts as described below.

At approximately 2 to 3 weeks prepartum, heifers were relocated to a close-up pasture, and the TMR was top-dressed with approximately 0.8 kg/head/day of dietary cation anion diet (DCAD) mix, 2.7 kg/head/day of dry cow grain mix, and 0.11 kg/head/day of limestone. All husbandry procedures were carried out according to the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

Heifers calved in maternity paddocks, and within 24 hours of parturition, they began twice daily milking in a double-6 herringbone parlor using a DeLaval system equipped with automatic milking unit takeoffs, milk volume meters, and electronic cow identification. Quarter milk samples were collected on days 3 and 10 postpartum, analyzed bacteriologically, and processed to determine SCC. A third milk sample was collected if culture results on days 3 and 10 did not agree. A composite 3-day milk sample from each heifer was tested for the presence of antibiotic residues before milk was added to the bulk tank.

Intramammary treatments:

After sample collection 30 to 60 days prepartum, 4 treatments were administered as follows: 1) untreated control; 2) nonlactating cow antibiotic (Spectramast® DC, ceftiofur hydrochloride, Zoetis, Florham Park, NJ); 3) teat sealant (Teatseal®, bismuth subnitrate, Zoetis); and 4) nonlactating cow antibiotic plus teat seal. Treatments were distributed in such a way that a different pattern of quarters was allotted the 4 treatments for each heifer to account for any dependence among quarters with respect to incidence of mastitis. After treatment, teats were sprayed using a postmilking teat germicide to eliminate any bacterial contaminants.

Sample processing for bacteriology, SCC, and differential leukocyte counts:

Mammary secretions collected prepartum and milk samples collected postpartum were mixed by vortexing and plated on trypticase soy agar with 5% sheep blood plates using sterile, flamed 10-µL loops. Plates were incubated for 48 h at 37°C and visually inspected for presence of colonial growth and hemolysis. Presumptive identification of microbial growth was performed following procedures outlined by the National Mastitis Council (2004). After presumptive identification, bacteria were further identified as follows: Staphylococci were differentiated from streptococci by means of the catalase test. Staphylococci were differentiated as coagulase positive or negative by conducting the coagulase test. Final identification of the staphylococcal species was performed using the API Staph test (bioMerieux, Inc., Marcy l'Etoile, France). Identification of *Streptococcus* spp. was verified by means of the API Strep Test (bioMerieux, Inc.). The culture of bacteria in 2 out of 3 postcalving milk samples, when necessary, qualified as an infection.

The SCC of mammary secretions and milk samples were determined using a Direct Cell Counter (DeLaval, Tumba, Sweden). Differential leukocyte counts of heifer mammary secretion prepartum were determined as follows: For the preparation of the differential smear, 50 µL of 7.5% bovine serum albumin (BSA) and 25 µL of secretion sample were added to a cytospin well. After being secured in a metal holder with a clean microscope slide, the prepared secretion sample was placed in a Cytospin 2 Centrifuge (Shandon, Pittsburgh PA) and operated for 2 min at 1200 rpm. After the slide was removed and air-dried, the smear was stained using the Wright stain method (Wright, 1902). Once dry, the sample was examined at 1000x under an oil immersion lens, and percentages of lymphocytes, macrophages, and neutrophils were recorded. A total of 100 cells/slide was counted to determine the population distribution.

Statistical analyses:

After calving, infection data collected on days 3 and 10 were compared with infection data collected prepartum, and results were used to determine 1) the percentage cure of existing IMI at time of treatment and 2) the percentage of IMI that were prevented across all 4 treatments. The SCC means among treatments were calculated for secretions collected at time of treatment and for days 3 and 10 postpartum as well as the average of days 3 and 10. Mean percentages among differential leukocyte populations (lymphocytes, macrophages, neutrophils) between infected and uninfected quarters were also determined. Means, expressed on a per treatment basis, were separated using SAS 9.3 Proc Glm for Windows (SAS, 2013).

Results

Prevalence of mastitis, cure rates of existing IMI, and prevention of new IMI:

Overall prevalence of IMI among quarters at time of treatment 30 to 60 days prepartum was 27.0%. The vast majority of IMI at this time were caused by the staphylococci (23.7%), which included the CNS (17.8%) and *S. aureus* (5.9%). Other IMI included *Streptococcus dysgalactiae* (2.0%) and *Pseudomonas* spp. (1.3%). Among the CNS, *S. hyicus* predominated (9.9% of all IMI) followed by *S. chromogenes* (5.9%), *S. xylosus* (1.32%), and *S. saprophyticus* (0.7%).

Overall prevalence of IMI among quarters postpartum, based on days 3 and 10 microbial culture data, was 8.6%. Compared with the prevalence of mastitis prepartum (27.0%) the level of infection decreased by 63.8% shortly after calving. The vast majority of IMI at this time were caused by the staphylococci (7.9%), which included the CNS (6.6%) and *S. aureus* (1.3%). Other IMI included *Streptococcus dysgalactiae* (0.7%). Among the CNS, *S. hyicus* predominated (3.3% of all IMI) followed by *S. chromogenes* (2.6%) and *S. xylosus* (0.7%), similar to trends observed prepartum.

Because the vast majority of IMI both at time of treatment (87.8%) and postpartum (92.3%) were caused by staphylococci (CNS and *S. aureus*), all infection data were combined to determine the cure and prevention rates for each treatment. The percentage cure (cure rate) of existing IMI at time of treatment and the percentage of IMI that were prevented (prevention rate) for each treatment are found in Table 1.

Untreated control quarters exhibited a cure rate of 58.3% (Table 1), typically referred to as the spontaneous cure rate because the IMI cured as the result of the heifer's immune system without the aid of antibiotic therapy. This spontaneous cure rate is relatively high compared with previous trials (25%) (Owens et al., 2001). In contrast, 100% cure rates were observed in quarters that received dry cow therapy or dry cow therapy plus teat seal, which were greater ($P < 0.05$) than the cure rate in control quarters (58.3%). Quarters treated with teat seal exhibited an average cure rate of 75%, which was numerically higher than but not different from the control value. All treatments were equally effective (> 90%) in preventing new IMI, ranging from 92.8% for dry cow therapy to 96.2 for the control and teat seal. Thus, treatment had no effect on prevention of new IMI in this group of heifers.

Table 1. Cure rate of existing IMI and prevention rate against new IMI across treatments.

Variable	Control	Dry Cow (DC)	Teat Seal (TS)	DC+TS	SE	*P* value
Cure rate (%)	58.3^{b}	100.0^{a}	75.0^{ab}	100.0^{a}	0.10	0.028
Prevention rate (%)	96.2^{a}	92.8^{a}	96.2^{a}	93.1^{a}	0.50	0.916

$^{a, b}$ Values in row with different superscripts are different ($P < 0.05$).

Treatment effects on SCC of uninfected quarters precalving:

The SCC data before treatment and after calving in quarters initially diagnosed as uninfected are found in Table 2. Overall average SCC across all 109 uninfected quarters prior to treatment (Pre-tmt) was 1172×10^3. Among the 6 quarters that were diagnosed with new IMI at calving, average SCC across treatments were 2281×10^3 on day 3 and 1098×10^3 on day 10. Among these 6 infected quarters on day 3, control quarters exhibited the greatest SCC (5374×10^3), which were elevated ($P < 0.05$) over all other treatments. This suggests that despite the failure to prevent new IMI, the treatment with any of the infused products significantly lowered SCC at calving. No SCC differences were observed among treatments on day 10, but an examination of the day 3 and day 10 average showed that among the 6 quarters that were diagnosed with new IMI at calving, quarters treated with teat seal exhibited lower ($P < 0.05$) SCC than untreated control quarters.

Among the 103 quarters that were diagnosed as uninfected at time of treatment and remained uninfected at calving, average SCC across treatments were 1315×10^3 precalving, 747×10^3 on day 3, and 336×10^3 on day 10. No SCC differences were observed among treatments on any sampling time.

Table 2. SCC values (x10^3) prior to treatment (Pre-tmt), day 3, day 10, and the average of days 3 and 10 (Ave SCC) across treatments for uninfected quarters precalving that did (Yes) or did not (No) develop new IMI postcalving.

Treatment	**New IMI**	**n**	**Pre-tmt**	**Day 3**	**Day 10**	**Ave SCC**
Control	Yes	1	598	5374^a	1723	2453^a
Dry Cow	Yes	2	---*	2557^b	607	1124^{abc}
Teat Seal	Yes	1	---	658^{bc}	713	686^{bc}
DC+TS	Yes	2	---	536^{bc}	1350	1955^{ab}
		∑= 6	μ=598	μ=2281	μ=1098	μ=1554
Control	No	25	1432	571^c	322	445^c
Dry Cow	No	26	1052	918^c	381	554^c
Teat Seal	No	25	1308	854^c	389	565^c
DC+TS	No	27	1469	647^c	253	441^c
		∑= 103	μ=1315	μ=747	μ=336	μ=501
Overall ∑		∑= 109				
Overall μ			μ=1172	μ=1514	μ=717	μ=1028
SE			433	238	131	126
P			0.914	0.004	0.185	0.004

[a, b, c] Values in a column with different letters are significantly different at $P < 0.05$.

*--- No secretion sample available or secretion too viscous to process for SCC.

Treatment effects on SCC of infected quarters precalving:

The SCC before treatment and postcalving in quarters initially diagnosed as infected are found in Table 3. Average SCC/ml across all 43 quarters prior to treatment was 2397x10^3. Among the 35 quarters that were diagnosed as cured at calving, average SCC across treatments were 720x10^3 on day 3 and 380x10^3 on day 10. Among these cured quarters on day 3, control quarters exhibited the greatest SCC (1488x10^3), which were elevated ($P < 0.1$) over all other treatments. No SCC differences were observed among treatments on day 10 or for the day 3 and day 10 average. Curiously, infected quarters treated with teat seal that cured exhibited the lowest numerical SCC on days 3 and 10 among all treatments.

Among the 8 quarters that were diagnosed as infected at time of treatment and did not cure at calving, average SCC across treatments were 2076x10^3 precalving, 1181x10^3 on day 3, and 1454x10^3 on day 10. No SCC differences were observed among treatments on any sampling times. It is noteworthy that there were no treatment failures for the dry cow therapy treatment or for the dry cow therapy plus teat seal treatment.

Table 3. SCC values (x10^3) prior to treatment (Pre-tmt), day 3, day 10, and the average of days 3 and 10 (Ave SCC) across treatments for infected quarters precalving that cured (Yes) or failed (No) treatment 30 to 60 days precalving.

Treatment	Cure?	n	Pre-tmt	Day 3	Day 10	Ave SCC
Control	Yes	7	2747	1488^{ab}	442	900
Dry Cow	Yes	10	3183	596^{c}	371	479
Teat Seal	Yes	9	2299	189^{c}	180	203
DC+TS	Yes	9	1994	607^{c}	548	571
		∑= 35	µ=2556	µ=720	µ=380	µ=538
Control	No	5	2260	846^{abc}	1481	1158
Dry Cow	No	0	---*	---	---	---
Teat Seal	No	3	1891	1515^{a}	1427	1183
DC+TS	No	0	---	---	---	---
		∑= 8	µ=2076	µ=1181	µ=1454	µ=1171
Overall ∑		∑=43				
Overall µ			µ=2397	µ=873	µ=742	µ=749
SE			1165	503	573	407
P			0.808	0.061	0.168	0.120

$^{a, b, c}$ Values in a column with different letters are significantly different at $P < 0.1$.
*--- No secretion sample available or secretion too viscous to process for SCC.

Differential leukocyte counts in uninfected and infected quarters:

Examination of the differential leukocyte slides illustrated marked differences in the distributions of macrophages, lymphocytes, and neutrophils in mammary secretions between uninfected and infected quarters (Table 4). Infected quarters exhibited a higher ($P<0.05$) mean percentage of neutrophils, and lower mean percentages of lymphocytes and macrophages than uninfected quarters. Although a basal population of neutrophils is present in uninfected quarters that serves as surveillance mechanism for bacteria entering the gland, the proportion of neutrophils increases dramatically in infected quarters as their purpose is to identify and kill invading bacterial pathogens. In this case, the percentage of neutrophils in infected quarters was elevated approximately 3.5 fold over the percentage found in uninfected quarters (32.1% vs. 9%). The percentage of neutrophils present in mammary secretions in heifers may be used to predict the likelihood of a quarter being infected. Our findings are in agreement with those of Ryman et al. (2013) who observed a 2.7 fold- increase in the percentage of neutrophils in secretions of infected quarters in heifers compared with uninfected quarters.

Table 4. Differential leukocyte counts (%) for uninfected and infected quarters.

Quarter infection status	Lymphocytes	Macrophages	Neutrophils
Uninfected	45.3	45.9	9
Infected	34.7*	33.1*	32.1*

*Different from uninfected quarters ($P < 0.05$).

Discussion

Compared to previous trials using the UGA Teaching Dairy herd, this trial (conducted in 2014-2015) experienced a much lower prevalence of IMI among heifers 30 to 60 days prepartum, which may have dampened the effects of the various treatments. In 2012-2013, 85.7% of heifers had some sort of IMI 30-60 days precalving (Ryman et al., 2013) compared with the present trial's infection rate of 50%. Regarding the infection rate with *S. aureus*, the present trial observed a precalving rate among heifers of 21.0%, which is also very low compared to the previous findings of 59.5% (Ryman et al., 2013). Thus, in the present trial, the lower percentages of existing IMI as well as the lower number of new IMI that developed over the 30-60 days prepartum may have made it more challenging to observe any treatment differences than if there was a much higher infection rate.

At calving, heifers in the present trial experienced an overall infection rate of 31.6%, which is slightly lower than but similar to the infection rate of 37.7% among cows that calved in the herd over the same time period. Likewise, the incidence of *S. aureus* IMI at calving among heifers in the present trial was low (2.6%) compared with the mature cows that calved with *S. aureus* IMI (7.5%). Several trials have been carried out on the heifer herd at the UGA Teaching Dairy in the past several years in attempts to reduce infection rates, especially for *S. aureus*, including vaccination, feeding of the immunostimulant OmniGen®, and fly control. It is possible that the rate of *S. aureus* IMI as well as the overall infection rate are decreasing due to these trials.

Heifers exhibited SCC comparable with those of cows that calved over the same period based on the 3-day and 10-day SCC data (heifers: $541 x 10^3$ for day 3 and $451 x 10^3$ for day 10, and cows: $626.4 x 10^3$ for day 3 and $404.5 x 10^3$ for day 10).

Conclusions

Compared to untreated control quarters (58.3%), treatment with dry cow therapy (100%) or dry cow therapy plus teat seal (100%) resulted in greater ($P < 0.05$) cure rates in quarters that had been infected prepartum with CNS or *S. aureus* (Table 1). Along with improved cure rates, SCC on day 3 after calving (Table 3) were lower ($P < 0.05$) in quarters treated with dry cow therapy ($596 x 10^3$), teat seal ($189 x 10^3$), and dry cow therapy plus teat seal ($607 x 10^3$) compared with control quarters ($1488 x 10^3$). Results confirm that dry cow therapy is an important management tool for curing existing IMI over the nonlactating period and lowering SCC at time of calving. Although treatment with teat seal resulted in a 75% cure rate compared with untreated control quarters (58.3%), the difference was not significant. Teat seal, being an inert physical barrier, does not provide antimicrobial activity, and its use resulted in a higher number of infected quarters that failed to cure (n=3) compared with use of dry cow therapy (n=0) or dry cow therapy plus teat seal (n=0) (Table 3).

Although it was hypothesized that treatment with dry cow therapy, teat seal, and the combination of dry cow therapy plus teat seal would help to reduce prevalence of new IMI at calving, no differences were observed compared with untreated controls; all 4 treatments ranged from 93.1% to 96.2% effective in preventing new IMI (Table 1). Thus, results suggest that if quarters are uninfected at 30 to 60 days precalving, leaving them untreated is as effective as treatment with dry cow therapy, teat seal, or the combination of dry cow therapy plus teat seal. In fact, SCC values (Table 2) were not different across all 4 treatments on day 3 in quarters that remained uninfected (range: $571x10^3$ to $918x10^3$) and day 10 (range: 253 $x10^3$ to $389x10^3$) postcalving.

Mean SCC for uninfected quarters that developed new IMI (Table 2) were numerically lower across postcalving sampling times (day 3, day 10, and the average of days 3 and 10) for all 3 infused treatments compared with untreated control quarters. This suggests that although infections may not have been prevented, the infusion of some form of treatment exerted a beneficial effect in lowering SCC. However, for uninfected quarters at the time of treatment that remained uninfected postcalving, treatment had no discernable effect on SCC across postcalving sampling times (Table 2). Curiously, infected quarters treated with teat seal that cured exhibited the lowest numerical postcalving SCC measurements among all of the treatments.

The small number of heifers (n=38) available for this trial coupled with the low infection rate and high spontaneous cure rate may have limited the ability to detect greater differences among treatments, especially the effect on the new IMI rate. A larger sample size may provide a greater incidence of infection and improve the power for a more valid statistical analysis.

Summary

Presence of mastitis in bred dairy heifers can adversely affect the development of milk-producing tissues, leading to less than maximal milk production and increased SCC during their first lactation. Use of nonlactating cow therapy or teat sealants have been beneficial in curing infections and preventing new ones from developing. When used together, the combination of the two products may be more effective than either alone in controlling mastitis in these young dairy animals. The 4 quarters of 38 bred heifers were treated randomly 30 to 60 days prepartum as follows: 1) untreated control; 2) nonlactating cow antibiotic; 3) teat sealant; or 4) nonlactating cow antibiotic plus teat seal. Results demonstrated that compared to untreated controls (58.3% cure), treatment with dry cow therapy (100% cure) or dry cow therapy plus teat seal (100% cure) resulted in greater ($P < 0.05$) cure rates in quarters infected prepartum with CNS or *S. aureus*. Treatment with teat seal resulted in a 75% cure rate but the difference was not significant. Additionally, SCC after calving were lower ($P < 0.05$) in quarters treated with dry cow therapy ($596x10^3$), teat seal ($189x10^3$), and dry cow therapy plus teat seal ($607x10^3$) compared with controls ($1488x10^3$). Although it was hypothesized that treatment with dry cow therapy, teat seal, and the combination of dry cow therapy plus teat seal would help to prevent new IMI at calving, no differences were observed compared with untreated controls; all 4 treatments ranged from 93.1% to 96.2% effective in preventing new IMI.

References

FASS. 1999. Federation of Animal Sciences Societies. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Federation of Animal Sciences Societies, Champaign, IL, USA.

National Mastitis Council. 2004. Microbial procedures for the diagnoses of bovine udder infection and determination of milk quality. Fourth Edition. National Mastitis Council, Inc. Verona, WI. Pages 9-41.

Nickerson, S. C. 2009. Control of heifer mastitis: Antimicrobial treatment – An Overview. Veterinary Microbiology. 134:128-135.

Owens, W. E., S. C. Nickerson, R. L. Boddie, G. M. Tomita, and C. H. Ray. 2001. Prevalence of mastitis in dairy heifers and effectiveness of antibiotic therapy. Journal of Dairy Science 84:814-817.

Owens, W.E., S.C. Nickerson, P.J. Washburn, and C.H. Ray. 1994. Prepartum antibiotic therapy with a cephapirin dry cow product against naturally occurring intramammary infections in heifers. Vet. Med. B. 41:90-100.

Owens, W.E., Nickerson, S.C., Washburn, P.J., Ray, C.H., 1991. Efficacy of a cephapirin dry cow product for treatment of experimentally induced *Staphylococcus aureus* mastitis in heifers. J. Dairy Sci. 74:3376-3382.

Paradis, M. E., E. Bouchard, D. T. Scholl, F. Miglior, and J. P. Roy. 2010. Effect of nonclinical Staphylococcus aureus or coagulase-negative staphylococci intramammary infection during the first month of lactation on somatic cell count and milk yield in heifers. Journal of Dairy Science 93:2989-2997.

Parker, K. I., C. W. Compton, F. M. Anniss, C. Heuer, and S. McDougall. 2008. Quarter-level analysis of subclinical and clinical mastitis in primiparous heifers following the use of a teat sealant or an injectable antibiotic, or both, precalving. Journal of Dairy Science 91:169-181.

Ryman, V. E., S. C. Nickerson, D. J. Hurley, R. D. Berghaus, and F. M. Kautz. 2013. Influence of horn flies (Haematobia irritans) on teat skin condition, intramammary infection, and serum anti-S. aureus antibody titres in holstein heifers. Research in Veterinary Science 95:343-346.

SAS. 2013. Business and analytical software. SAS Institute, 100 SAS Campus Drive, Cary, NC. USA.

Trinidad, P., S.C. Nickerson, and R.W. Adkinson. 1990. Histopathology of staphylococcal mastitis in unbred dairy heifers. Journal of Dairy Science 73:639-647.

Wright, J.H. 1902. A rapid method for differential staining of blood films and malarial parasites. J. Med. Res. 7:138-144.