Mastitis Prevention and Cure Rates in Heifers Treated with Spectramast Dry Cow Therapy and/or Orbeseal Dry Cow Teat Sealant

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Introduction:

Dairy cows are vital for milk production, cheese, cream, and so much more. Healthy udders and teats are necessary to keep production levels at their highest and to keep the number of cows being culled to the lowest levels. Inflammation of the mammary gland is called mastitis, which is typically caused by bacteria that invade the teat canal. This disease is most often transmitted by contact with contaminated milking equipment, through contaminated hands or udder wash cloths, or by overexposure to bacteria in the cows' environment. Mastitis is the most economically important disease of adult dairy cattle, and annual losses average approximately $1.7-2 billion (Jones and Bailey, 2009), which are attributed to decreases in milk yield and quality, and costs due to medications, veterinary services, and culling of affected animals.

Losses to mastitis can be minimized by sound lactating and dry cow management practices, such as using proper milking time hygiene, providing a clean and dry environment, use of functionally adequate milking machines, treatment of clinical mastitis cases, and use of proper dry-off procedures including dry cow therapy and teat seals (Philpot and Nickerson, 2000). In addition to adult cows, this disease is also prevalent in bred heifers; however, less information is available on management procedures to treat and prevent this disease in younger dairy animals. The most prevalent and contagious mastitis pathogens among heifers are *Staphylococcus aureus* and the coagulase-negative staphylococci (CNS) (Nickerson, 2009).

The purpose of this study was to test the efficacies of using dry cow therapy, teat sealant, or a combination of dry cow therapy and teat sealant in bred heifers to determine the optimal management tool for reducing the prevalence of mastitis with staphylococcal mastitis when the heifers calved.

Materials and Methods:

Experimental Animals

Thirty-eight bred Holstein heifers from the University of Georgia Teaching Dairy were used for this study, each due to calve 30 to 60 days from the date of initial treatment. The heifers were housed on a grass pasture and fed a total mixed ration (TMR) once daily based on wheat or sorghum silage and 2.3 kg of dry cow grain mix. The grain mix contained the following in kg/t: rolled corn (790), soybean meal (100), dicalcium phosphate (7.8), salt (3.8), a trace mineral pack (4.4), vitamins A, D, and E (4.4), Zinpro$^{®}$ performance minerals (3.4), and limestone (2.0). Water and Bermudagrass hay were available to the heifers ad libitum. 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 lime-

stone (Harding, 2015). All animal care and research practices were in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

Spectramast Dry Cow Treatment

Two different intramammary infusion products were used in this study. One product was Spectramast Dry Cow (DC) Therapy, (Zoetis, Florham, NJ), which is ceftiofur hydrochloride, a third-generation cream-colored cephalosporin antibiotic. Spectramast DC comes in a 10-ml syringe with an applicator tip to accommodate proper infusion of the teat canal. One tube of Spectramast DC was infused per quarter when assigned to treatment groups.

Orbeseal Treatment

The other infusion product used was Orbeseal Dry Cow Teat Sealant (Zoetis, Florham, NJ). Orbeseal is not an antibiotic but is an off-white sealant paste composed of 65% bismuth subnitrate in a mineral oil vehicle, which is administered into the teat canal. Each syringe is filled with 4 g of Orbeseal and has an applicator tip for easy administration. Orbeseal is infused into the teat cistern via the teat canal and remains in the distal teal cistern and teat canal, serving as a physical barrier to bacterial penetration (Figure1.)

Figure 1. Example of teat sealant in each quarter of an udder.

Secretion Sample Collection and Treatment

At approximately 30 to 60 days before projected calving date, each heifer's mammary secretions were obtained by first sanitizing the teat ends with 70% isopropyl alcohol followed by sample collection of approximately 2-3 ml. After the secretion samples were obtained, each teat was randomly assigned a treatment. The treatments were as follows:

1. Control (no treatment)
2. Spectramast DC
3. Orbeseal
4. Spectramast DC plus Orbeseal

For the fourth treatment, the antibiotic was infused prior to infusion of the teat sealant. After treatments were administered, all four teats were sprayed with a betadine solution to kill any possible contaminating bacteria at the teat orifice.

Secretion Scoring

After collection, lacteal secretions were scored (from 1-3) based on increasing degrees of viscosity. A score of 1 was given for a secretion that was thin and had the consistency of water or whey. A score of 2 was given for a secretion that was more viscous than water and had the consistency of cream or milk. A score of 3 was given for secretions that were thick like honey.

Teat Scoring

At the time of sampling, each teat was scored (from 1 to 3, Figure 2.) based on the

outward appearance of the health of the teat to determine any fly damage. A score of 1 indicated that the teat skin was in a healthy condition with no signs of fly damage, e.g., no abrasions or scabbing. A score of 2 was given for any teat that had residual damage, e.g., exhibiting healing scabs and abrasions. A score of 3 was given for teats that were unhealthy that had fresh scabs and or open bleeding abrasions.

Figure 2. Teat scores of 1, 2, and 3.

Secretion Sample Plating

Secretion samples were plated onto Trypticase Soy Agar sheep blood agar plates, placed in an incubator at 37 C, and read 48 hours later. Bacterial colonies were identified presumptively based on color characteristics, densities, hemolytic patterns, and gram-staining of the isolates. Staphylococci were differentiated from streptococci using the catalase test; staphylococci being catalase positive.

If *Staph. aureus* was presumptively identified, a coagulase test was conducted and a mannitol salt agar plate was processed to confirm the isolate as *Staph. aureus* (Figure 4). The coagulase test was considered positive if the coagulase plasma clumped 4 to 24 hours after incubation, and was considered negative if the coagulase plasma remained liquid. The mannitol salt agar (MSA) plate test was considered positive if the original

pink color indicator turned bright yellow due to the fermentation of mannitol. These were incubated at 37 C and read 24 hours later. To be identified as *Staph. aureus*, both the coagulase test and MSA test must read positive.
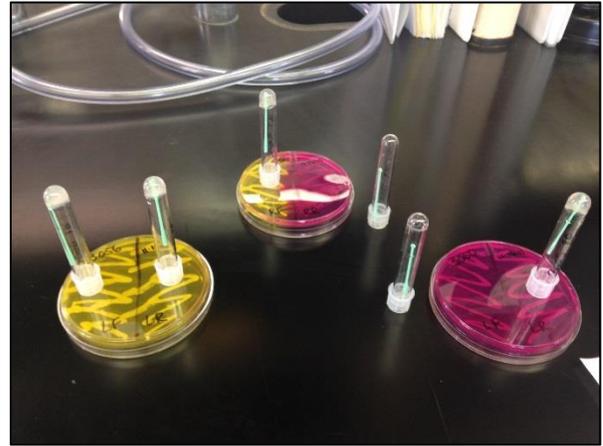

Figure 4. Coagulase and MSA Testing.

If either or both MSA or coagulase tests were negative, an Analytical Profile Index (API) test was conducted to identify the genus and species of bacteria. A sample of colonial growth was obtained from the blood agar plate and was used to fill each microtube of the test strip containing a dehydrated substrate. The API strip was then incubated for 18 to 24 hours, and then additional reagents were incorporated to complete the test. Each microtube would turn a specific color based on the biochemical reaction with the bacteria (Figure 5). These colors, based on the biochemical reaction that took place, would then be further specified as positive or negative, and test results were recorded into a database. The software indicated a genus and species based on the numerical index from the number of positive versus negative biochemical tests (Biomerieux, 2002).
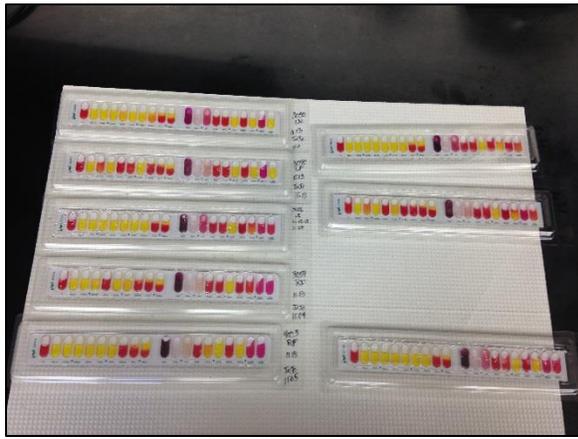
Figure 5. Analytical Profile Index.

Somatic Cell Counts

Somatic cell counts (SCC) were performed on secretions on the same day that samples were obtained using a DeLaval Direct Cell Counter (DCC) (Figure 6) to determine the concentration of leukocytes per ml of secretion. Not all secretions could be processed as some were too thick to flow properly through the DCC, such as those with a secretion score of 3.

In addition, when volume and consistency of secretions allowed, differential leukocyte counts were performed using a cytospin followed by Wright's Stain. Once the slides were stained, each was placed on the stage of a Meiji microscope and examined at 1000x. Leukocytes were enumerated from representatively chosen microscope fields until 100 cells had been counted. Leukocytes were differentiated as either macrophages, lymphocytes or neutrophils. Once 100 cells had been counted, the number for each was used as an overall average for the entire slide.

Figure 6. DeLaval Direct Cell Counter.

Postpartum Sample Collection

Upon calving, milk samples were obtained 3 and 10 days post calving. Samples were processed to 1) determine absence or presence of antimicrobial residues remaining from the Spectramast DC, 2) determine infection status, and 3) conduct SCC.

Antibiotic Residue Testing

In order to ensure that antibiotic residues were not present in milk from treated heifers, the 3-day postcalving samples were tested with Delvo testing procedures. A Delvotest is a broad spectrum antibiotic residual testing kit, which detects the presence of antibiotic residues with a high degree of accuracy (DSM, 2011). Each quarter was sampled and tested according to the Delvotest recommended procedures; every heifer freshened with no antibiotic residues detected in their 3- day postcalving milk samples (Figure 7).
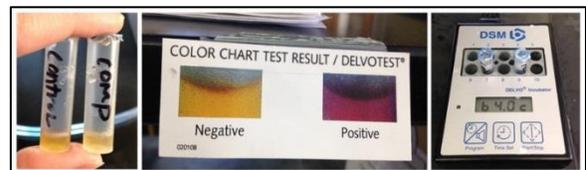

Figure 7. Delvo Testing Procedure.

Results:

The cure rates after calving were the highest in the Spectramast DC therapy treatment group (100%), with the lowest cure rate being the control at 56.2% (Table 1). The cure rates for both the Orbeseal alone and the combination of Spectramast DC therapy and Orbeseal were at 93.8%.

The prevention rates were all very high at greater than 90% for all 4 treatments, with the control being at 95.6%, Spectramast DC therapy at 90.9%, Orbeseal at 100%, and the combination of treatments at 100% prevention. Cure rates and prevention rates are shown graphically in Figures 8a and 8b, respectively.
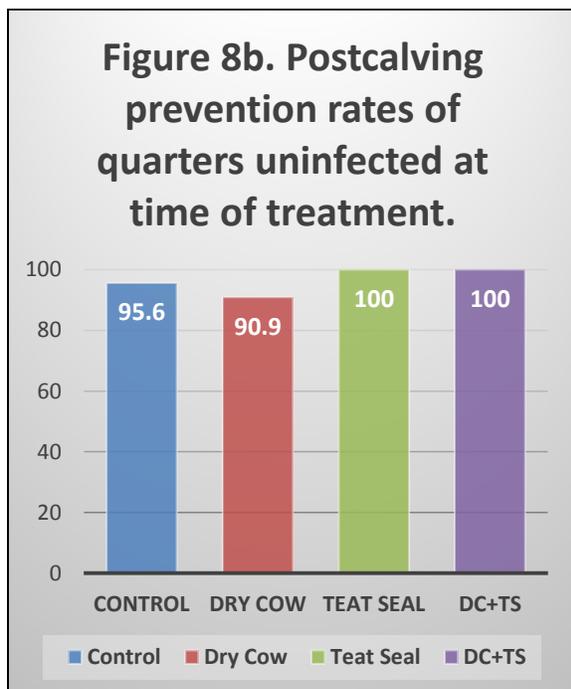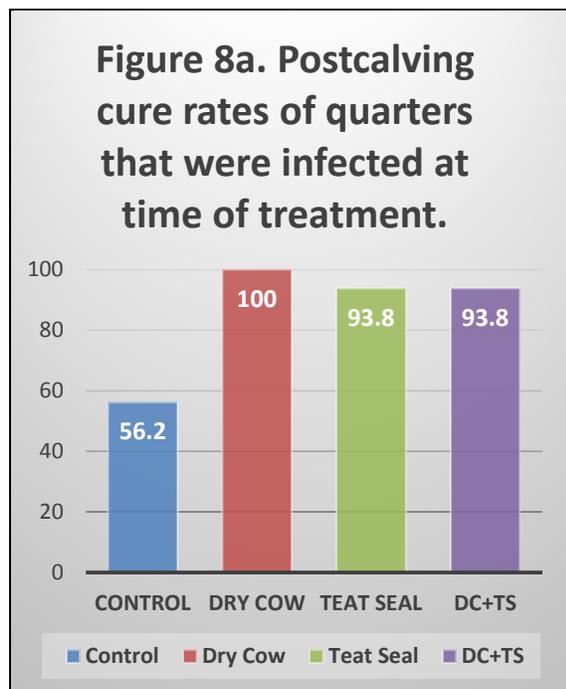
Table 1. Cure rates (%) for quarters infected at the initial sampling and prevention rates (%) against new IMI for quarters uninfected at the initial sampling across treatments.

	Control	DC[1]	TS[2]	DC + TS	SE	*P*
Cure (%)	56.2[b]	100.0[a]	93.8[a]	93.8[a]	7.9	0.001
Prevention (%)	95.6	90.9	100.0	100.0	4.1	0.310

[1] DC= Spectramast DC.
[2] TS = Teat sealant.
[a, b] Values in a row with different letters are significantly different at *P*<0.05.

Figure 8a. Postcalving cure rates of quarters that were infected at time of treatment.

Figure 8b. Postcalving prevention rates of quarters uninfected at time of treatment.

The SCC data for infected quarters at the initial sampling and on Day 3 and Day 10 postpartum for quarters that cured postcalving are presented in Table 2 and Figure 9. Across treatments, SCC in infected quarters that cured postcalving significantly decreased from the initial sampling through days 3 and 10 postpartum. The SCC in control quarters exhibited the least reduction in SCC from the precalving sampling through Day 10 postpartum (3,809,000/ml → 1,755,000/ml → 664,000/ml); whereas SCC in quarters treated with Spectramast DC and Orbeseal exhibited the greatest reduction in SCC (2,283,000/ml → 518,000/ml → 193,000/ml). By Day 10

post calving, SCC in Control quarters had decreased from 3,809,000/ml at the initial sampling to 664,000/ml; whereas the average SCC of all treated quarters had decreased from 2,799,000/ml at the initial sampling to 351,000/ml on Day 10, which is approximately half of the SCC of Control quarters (664,000/ml). This decrease emphasizes the role of successful treatment in lowering SCC at calving and early lactation. So, not only does treatment cure IMI, it also reduces the SCC and improves milk quality. SCC are shown graphically in Figure 9.

Table 2. SCC values ($x10^3$) for infected quarters at the initial sampling (Precalving), Day 3, and Day 10 across treatments for quarters that cured postcalving.

Treatment	Precalving	Day 3	Day 10	SE	P
Control	3809[a]	1755[b]	664[b]	523	0.012
Spectramast DC	3306[a]	1118[b]	417[b]	331	0.002
Orbeseal (TS)	2809[a]	826[b]	441[b]	354	0.001
DC+TS	2283a	518[b]	193[b]	201	0.001

Figure 9. SCC x 10^3 of infected quarters precalving that were cured postcalving on days 3 and 10.
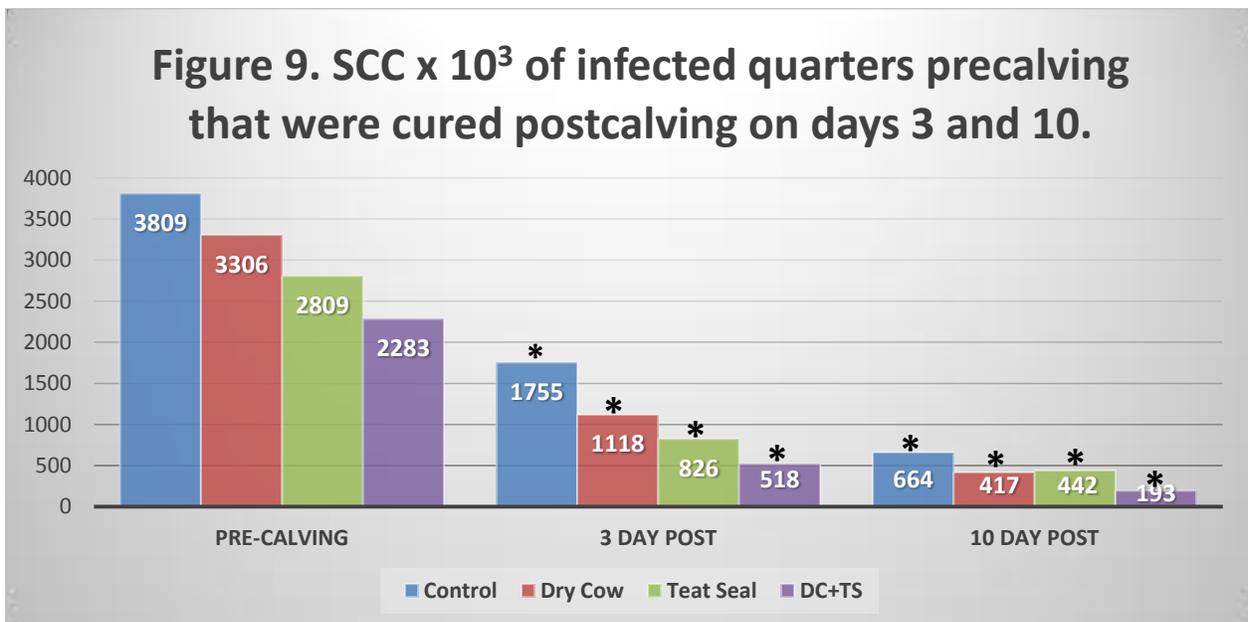
* Values are significantly lower than precalving values ($P<0.05$).

Discussion:

Overall, either intramammary treatment with Spectramast DC, Orbeseal, or the combination of both was very beneficial in treating mastitis in bred heifers. Being able to cure infections precalving greatly reduces the incidence of postcalving infections as well as numbers of quarters going blind (nonfunctional). Blind quarters are due to atrophy of the secretory tissues caused by the infecting bacteria, resulting in little or no milk production in that quarter. At the time that this study was initiated, several quarters had already presented blind.

Interestingly, even though Orbeseal does not have any antimicrobial properties, there were still significant numbers of quarters (n=15) that were cured (93.8%) of their infections after treatment with this product. A possible explanation for this is that the teat seal is recognized as foreign by the cow's immune system. This results in an increase in the mammary secretion SCC as a result of a higher influx of white blood cells in response to foreign material, which in turn, are able to clear the infection. Orbeseal is also beneficial in that scenario as this teat sealant is helping to prevent bacteria from entering the teat canal and causing a new infection.

While treatment of quarters with either Spectramast DC, Orbeseal, or the combination of both was expected to help prevent new infections from occurring, there was no statistical significance among these treatments and the untreated control in regard to prevention. This would suggest that it would be acceptable to leave these uninfected quarters without treatment. However, the majority of heifers do have at least one quarter infected with

Staph. aureus or CNS against which Spectramast DC has been shown to be very effective in curing as well as in lowering the SCC.

So, recommendations to dairymen would be to pay attention to the SCC of heifers in early lactation. If SCC are elevated, it can be assumed that they are freshening with mastitis. It would be beneficial to implement an udder health program with their herd veterinarian that incorporates treating heifers with both dry cow therapy for treatment of current infections as well as a teat sealant to assist in preventing new infections. The treatment of these heifers would need to take place no later than 30 days pre-calving to prevent any antibiotic residues.

Studies have shown that heifers with *Staph. aureus* mastitis produce 10% less milk than uninfected herd mates over their first lactation (Harding, 2015), and considering the high prevalence of mastitis among heifers, the total loss per year would be an economical burden to the dairy producer.

Conclusions:

Treatment and prevention of mastitis is vital for the future of the dairy industry. Using either dry cow therapy, teat sealant, or the combination of both has over a 94% cure rate in treating existing intramammary infections. Ideally, prevention of mastitis can be realized with proper handling, maintaining a clean environment, fly control, and vaccination, but if an infection does occur, timely treatment of the heifer improves the chances for a successful cure rate and decreases the chances of a quarter going blind. Ultimately, an udder

health program will result in greater milk yield and enhanced milk quality for the dairy producer, resulting in higher profits for their farm operations.

References:

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