Applied Animal Science 36:91–99
https://doi.org/10.15232/aas.2019-01920

PRODUCTION AND MANAGEMENT: *Original Research*

Mastitis control in bred dairy heifers using dry cow therapy and teat sealant to prevent new infections and to cure existing ones

S. C. Nickerson,[1]* PAS, F. M. Kautz,[1] A. E. Harding,[1] L. O. Ely,[1] and D. J. Hurley[2]
[1]Department of Animal and Dairy Science, and [2]Department of Population Health, University of Georgia, Athens 30602

ABSTRACT

Objective: Is use of dry cow therapy, teat sealant, or dry cow therapy + teat sealant more effective in controlling heifer mastitis and decreasing SCC than no treatment?

Materials and Methods: At 2 mo prepartum, 304 mammary quarters of 76 pregnant Holstein heifers were randomly assigned to (1) dry cow therapy, (2) teat sealant, (3) dry cow therapy + teat sealant, or (4) untreated control. Before treatment, mammary secretion samples were collected and stored for bacteriological analysis. After calving, infection data were compared with data collected prepartum, and treatment means were separated using SAS 9.3.

Results and Discussion: Compared with cure rate in untreated controls (55.2%), treatment with dry cow therapy (100%), teat sealant (85.7%), or dry cow therapy + teat sealant (96.1%) resulted in greater cure rates and lower SCC in quarters infected prepartum with *Staphylococcus aureus* or coagulase-negative staphylococci. Prevention rates against all new intramammary infections (IMI) were similar for control quarters (95.9%), dry cow therapy (92.2%), teat sealant (97.9%), and dry cow therapy + teat sealant (95.9%).

Implications and Applications: Although the IMI prevention rates were similar across treatments, the majority of heifers did have at least one quarter infected with *Staph. aureus* or coagulase-negative staphylococci, which dry cow therapy has been shown to be very effective in curing as well as decreasing future SCC. In problem herds, an udder health program should incorporate treating all quarters with dry cow therapy to cure existing IMI plus teat sealant to prevent new IMI, under supervision of the herd veterinarian.

Key words: antibiotic cure rate, somatic cell count, *Staphylococcus aureus*, coagulase-negative staphylococci

INTRODUCTION

Because of the importance of bred heifers to the future milk production of dairy operations, it is critical that udder health be maximized to ensure that these animals calve free of IMI with low SCC. During a heifer's first gestation, the presence of clinical and subclinical mastitis can compromise the development of milk-producing tissues. In the case of *Staphylococcus aureus*, milk yield may be reduced up to 10% over the first lactation (Owens et al., 1991; Nickerson, 2009). Milk quality is also reduced due to an increase in 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 permanently blind (nonfunctional) quarter.

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

Use of nonlactating or dry cow antibiotic infusion products in dairy heifers has 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 *Staph. aureus* IMI ranged from 67 to 100% and were greater than the spontaneous cure rate (25%) of 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 at calving by 93%.

The 2- to 3-mo prepartum period is recognized as the time frame during which the majority of mammary growth and development occurs in heifers; mammary growth dur-

The authors have not stated any conflicts of interest.
*Corresponding author: scn@uga.edu

ing this time is exponential in nature (Swanson and Poffenbarger, 1979). Moreover, development of the lobules and alveoli takes place during the last third of pregnancy (Akers et al., 2014). Unfortunately, the presence of mastitis leads to mammary cellular and tissue damage, which is associated with reductions in milk yield (see review by Enger, 2019).

Thus, use of nonlactating cow therapy has been shown to be effective in both curing existing IMI and preventing new cases of mastitis. Studies have also shown that successful treatment 8 to 12 wk prepartum with dry cow therapy leads to improved milk yield (e.g., if bred heifers infected with *Staph. aureus* were left untreated, they produced 10% less milk in early lactation than those receiving intramammary nonlactating cow therapy during gestation; Trinidad et al., 1990b; Owens et al., 1991). Moreover, research has shown that *Staph. aureus* mastitis in heifers resulted in significant production losses during the first lactation, which carried over into the subsequent lactation, even if infected quarters were successfully treated in the first lactation (Woolford et al., 1983).

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 sealant approximately 1 mo before calving in heifers reduced the risk of new IMI by 74% and prevalence of postcalving 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 sealants, or (3) the combination of the 2 products? The purpose of this study was to determine what product or combination of products was most effective in curing existing IMI and preventing the development of new IMI in pregnant dairy heifers when administered 2 mo prepartum compared with an untreated control.

MATERIALS AND METHODS

Animals

Seventy-six pregnant Holstein heifers (304 mammary quarters) were enrolled in this trial and housed in a far-off pasture at the University of Georgia Teaching Dairy. Animals were fed a TMR once daily containing 2.7 kg of concentrate mixed with 11.3 kg of wheat silage. Heifers had access to free-choice bermudagrass hay and pasture. The mix provided 73% TDN, 13% CP, and 41% NDF. Approximately 2 mo before 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. During the horn fly season (April through September), teat scabs caused by these blood-sucking flies were observed, and pour-on fly treatment (Ultra Boss, Merck Animal Health, Summit, NJ) was initiated and administered to all heifers every 14 to 21 d to minimize fly density and allow teats to heal.

At approximately 2 to 3 wk prepartum, heifers were relocated to a close-up pasture, and the TMR described above was top-dressed and mixed with approximately 0.2 kg/head per day of DCAD mix. Random heifers were selected twice a week (Monday and Thursday) to check urine pH. Adjustments were made to the amount of DCAD fed depending on the results of the urine test. All husbandry procedures were carried out according to the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 2010).

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

Intramammary Treatments

After the mammary-secretion sample collection was performed 2 mo prepartum, 4 treatments were administered as follows: (1) untreated control, (2) dry cow therapy (Spectramast DC, ceftiofur hydrochloride, Zoetis, Florham Park, NJ), (3) teat sealant (Teatseal, bismuth subnitrate, Zoetis), and (4) dry cow therapy + teat sealant. Treatments were distributed in such a manner that a different pattern of quarters was allotted the 4 treatments for each heifer to account for any dependency among quarter location with respect to incidence of mastitis. After treatments were administered, teats were sprayed using a postmilking teat germicide to eliminate any bacterial contaminants inadvertently placed on the teat end via the sampling and treatment processes.

Dry cow therapy was chosen over lactating cow therapy because the cure rate is greater than that achieved using lactating cow therapy. Particularly for *Staph. aureus*, a greater antibiotic dose can be safely used, retention time of the antibiotic in the udder is longer, and any tissue damage caused by mastitis may be regenerated before freshening (NMC, 2010).

Sample Processing for Bacteriology, SCC, Differential Leukocyte Counts, and Antimicrobial Residues

Mammary secretions from each quarter that were collected prepartum and milk samples collected postpartum were mixed by vortexing and plated on trypticase soy agar with 5% sheep blood using sterile, flamed 10-μL loops. Plates were incubated for 48 h at 37°C and then visu-

ally 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 and as mannitol positive or negative by plating on mannitol-salt agar. 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 Slidex test and the API Strep Test (bioMerieux Inc.).

The culture of the same bacterial species in both the 3- and 10-d postpartum milk samples qualified as an infection. If the 2 postpartum samples did not agree, a third sample was collected, and the infection status was based on the results of 2 out of 3 samples. The percentages of heifers and quarters diagnosed with IMI were determined at both prepartum and postpartum samplings, and prevalence of each bacterial species was calculated. Of the 304 quarters available, 9 (2.96%) were determined to be nonfunctional or blind, 5 diagnosed prepartum and 4 postpartum.

The SCC of mammary secretions and milk samples were determined using a Direct Cell Counter (DeLaval). Differential leukocyte counts of heifer mammary secretion prepartum were determined as follows: for the preparation of the differential smear, 50 μL of 7.5% 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 160 × *g* at 23°C. 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 1,000× under an oil immersion lens, and percentages of lymphocytes, macrophages, and neutrophils were recorded. A total of 100 cells per slide was counted to determine the population distribution.

To ensure that antibiotic residues were not present in milk of heifers, all of which were treated with dry cow therapy in 2 quarters (except for blind quarters), the 3-d composite postpartum milk samples were analyzed using the Delvotest (Royal Gist-brocades NV, Delft, the Netherlands). Every heifer freshened with no antibiotic residues detected in their 3-d postpartum milk samples.

Statistical Analyses

The individual mammary quarter was the experimental unit and considered an independent variable. To analyze results, 2 data sets were created: one for quarters that were initially diagnosed as uninfected prepartum that either developed new IMI or remained uninfected at calving, and another data set for quarters that were diagnosed as infected prepartum that either cured or failed to cure at calving. After calving, quarter infection data collected on d 3 and 10 were compared with quarter 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 quarter secretions collected at time of treatment (2 mo prepartum) and for d 3 and 10 postpartum. 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 Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Prevalence of Mastitis, Cure Rates of Existing IMI, and Prevention of New IMI

Overall prevalence of IMI among heifers at the time of treatment 60 d prepartum was 63.2%, and prevalence among quarters was 35.9%. Species prevalences are presented in Table 1. The vast majority of IMI at this time were caused by staphylococci (82.0%), which included *Staphylococcus hyicus* (26.4%), *Staphylococcus chromogenes* (25.5%), *Staph. aureus* (24.5%), *Staphylococcus xylosus* (2.0%), *Staphylococcus saprophyticus* (0.9%), *Staphylococcus epidermidis* (0.9%), *Staphylococcus capitis* (0.9%) and unspeciated *Staphylococcus* spp. (0.9%). Other IMI that comprised the remaining 18% of infections included *Streptococcus dysgalactiae* (15.1%), *Pseudomonas* spp. (2.0%), and *Trueperella pyogenes* (0.9%).

The prevalence of IMI among quarters prepartum (35.9%) was similar to that observed by Fox et al. (1995) in a national survey, during which a 34.4% prevalence was found; likewise, these later researchers found that CNS and *Staph. aureus* predominated.

Overall prevalence of IMI among heifers postpartum based on d-3 and -10 microbial culture data was 22.4%, and prevalence among quarters was 6.1%. The vast majority of IMI at this time were caused by staphylococci (89.5%) and included *Staph. hyicus* (31.6%), *Staph. chromogenes* (26.3%), *Staph. aureus* (26.3%), and *Staph. xylosus* (5.3%). Remaining IMI were *Strep. dysgalactiae* (10.5%). See Table 1.

The prevalence of IMI among quarters postpartum (6.1%) was less than that observed by Fox et al. (1995) in a national survey, during which a 36% prevalence was found; however, none of quarters were treated with dry cow therapy or teat sealant in the later trial. As in the present study, Fox et al. (1995) found that the CNS and *Staph. aureus* predominated postpartum.

Compared with prevalence of IMI among heifers prepartum (63.2%), the level of IMI postpartum (22.4%) was reduced by 64.6%, and compared with prevalence of IMI

Table 1. Percentages of bacterial infections among infected quarters of heifers sampled prepartum[1] and postpartum[2]

Isolate	Prepartum	Postpartum
Staphylococcus hyicus	26.4	31.6
Staphylococcus chromogenes	25.5	26.3
Staphylococcus aureus	24.5	26.3
Staphylococcus xylosus	2.0	5.3
Staphylococcus saprophyticus	0.9	—
Staphylococcus epidermidis	0.9	—
Staphylococcus capitis	0.9	—
Staphylococcus spp.	0.9	—
Streptococcus dysgalactiae	15.1	10.5
Pseudomonas spp.	2.0	—
Trueperella pyogenes	0.9	—

[1]Overall prevalences of intramammary infection among heifers and quarters prepartum were 63.2 and 35.9%, respectively.

[2]Overall prevalences of intramammary infection among heifers and quarters postpartum were 22.4 and 6.1%, respectively.

among quarters prepartum (35.9%), the level of IMI postpartum (6.1%) was reduced 83.0%. One quarter per heifer was left as an untreated control, and the reduction in level of IMI postpartum likely would have been greater if all quarters had been treated with dry cow therapy or teat sealant.

Because the vast majority of IMI both at time of treatment (82.0%) and postpartum (89.5%) were caused by staphylococci (CNS and *Staph. 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 presented in Table 2.

Untreated control quarters exhibited a cure rate of 55.2% (Table 2), 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. In contrast, significantly greater cure rates ($P < 0.001$) were observed in quarters that received dry cow therapy (100%), teat sealant (85.7%), and dry cow therapy + teat sealant (96.1%). All treatments were equally effective in preventing new IMI, ranging from 92.2% for dry cow therapy to 97.9% for teat sealant.

The cure rates observed in the present study are within the range observed by others that treated infected heifer quarters with dry cow therapy products, as were the new IMI prevention rates of uninfected quarters (see review by Nickerson, 2009). Conversely, the 97.7% prevention rate against new IMI for teat sealant is greater than that (74%) observed by Parker et al. (2008).

Treatment Effects on SCC of Uninfected Quarters Prepartum

As outlined in the Materials and Methods section, 2 data sets were created to analyze results: (1) uninfected quarters prepartum that developed new IMI or remained uninfected at calving (Table 3), and (2) infected quarters prepartum that either cured or failed to cure at calving (Table 4).

The SCC data before treatment (2 mo prepartum) and after calving in quarters initially diagnosed as uninfected are found in Table 3. Pretreatment SCC were typical of those found in mammary secretions of uninfected quarters of bred heifers and ranged from $1{,}052 \times 10^3$ to $3{,}207 \times 10^3$/mL among the 196 quarters. Among the 9 quarters that were diagnosed with new IMI postpartum, SCC on d 3 were highest in control quarters and quarters treated with dry cow therapy ($P < 0.001$). On d 10, SCC were highest in control quarters ($P < 0.001$), with the exception of those treated with dry cow therapy + teat sealant. In fact, SCC were numerically highest in control quarters on d 3 and 10, which suggests that despite the failure to prevent new IMI among these 9 quarters, treatment with any of the infused products resulted in lower SCC postpartum.

Among the 187 quarters that were diagnosed as uninfected at time of treatment and remained uninfected at calving, SCC on d 3 ranged from 519×10^3 to 677×10^3/mL, and on d 10, SCC were lower as the volume of milk increased and diluted the leukocytes in milk, ranging from 207×10^3 to 274×10^3/mL. No SCC differences were observed among treatments within day for these quarters that remained uninfected.

Table 2. Cure rate of existing intramammary infection (IMI) and prevention rate against new IMI across the 4 treatments

Variable	Control	Dry cow therapy (DC)	Teat sealant (TS)	DC + TS	SE	*P*-value
Cure rate (%)	55.2[a]	100.0[b]	85.7[b]	96.1[b]	6.4	0.001
Prevention rate (%)	95.9[a]	92.2[a]	97.9[a]	95.9[a]	3.1	0.590

[a,b]Values in a row with different superscripts are different at $P < 0.05$.

Table 3. The SCC values (× 10^3/mL) before treatment (prepartum) and on d 3 and 10 postpartum across treatments for quarters that were uninfected prepartum and did or did not develop new intramammary infections (IMI) postpartum

Treatment	n	Prepartum	d 3	d 10
New IMI				
Control	2	3,207	3,468[a]	2,738[a]
Dry cow therapy (DC)	4	2,760	2,617[a]	541[c]
Teat sealant (TS)	1	NSS[1]	658[b]	713[bc]
DC + TS	2	NSS	535[b]	1,350[ab]
No new IMI				
Control	47	1,209	519[b]	244[c]
DC	47	1,442	658[b]	274[c]
TS	46	1,331	677[b]	266[c]
DC + TS	47	1,052	476[b]	207[c]
SE		383.2	152.9	75.3
P-value		0.284	0.001	0.001

[a–c]Values in a column with different superscripts are different at $P < 0.05$.

[1]NSS = no secretion sample was available, or secretion was too viscous to process for SCC.

Treatment Effects on SCC of Infected Quarters Prepartum

The SCC before treatment and those found postpartum in quarters initially diagnosed as infected are presented in Table 4. Among the 88 quarters that were diagnosed as cured postpartum, SCC on d 3 were highest in control quarters ($P = 0.047$) compared with other treatments. Importantly, treatment with any of the products lowered the SCC on d 3 compared with the control. Although SCC on d 10 were numerically greatest in control quarters compared with other treatments for cured quarters, the difference was not significant ($P = 0.094$). As was observed in Table 3, SCC were numerically greatest in control quarters, again suggesting that treatment with any of the infused products resulted in lower SCC postpartum.

Among quarters treated (with dry cow therapy, teat sealant, or the combination) that cured, prepartum SCC were 3,245, 2,650, and 2,129 × 10^3/mL, respectively, and

Table 4. The SCC values (× 10^3/mL) before treatment (prepartum) and on d 3 and 10 postpartum across treatments for quarters that were infected prepartum and had an intramammary infection (IMI) status of cured or failed postpartum

Treatment	Postpartum IMI status	n	Prepartum	d 3	d 10
Control	Cured	16	3,278	1,638[a]	567[ab]
Dry cow therapy (DC)	Cured	23	3,245	914[b]	399[b]
Teat sealant (TS)	Cured	24	2,650	587[b]	343[b]
DC + TS	Cured	25	2,129	534[b]	335[b]
Control	Failed	13	1,791	825[b]	1,020[a]
DC	Failed	0	NV[1]	NV	NV
TS	Failed	4	1,891	1,515[ab]	1,427[a]
DC + TS	Failed	1	TV[2]	B[3]	B
SE			412.1	231.9	174.6
P-value			0.259	0.047	0.094

[a,b]Values with different superscripts within a column are different at $P < 0.05$.

[1]NV = no value.

[2]Secretion sample was too viscous to process for SCC.

[3]B = quarter was nonfunctional or blind at both postpartum samplings, most likely as a result of the *Streptococcus dysgalactiae* IMI diagnosed at the prepartum sampling, so it was considered a treatment failure.

decreased to 914, 587, and 534 × 10^3/mL, respectively, postpartum (Table 4). Such decreases are in line with those of others after successful treatment with dry cow therapies (Owens et al., 1991; Owens et al., 1994).

Among the 18 quarters that were diagnosed as infected at the time of treatment and failed to cure postpartum, no SCC differences were observed among treatments on d 3 or 10 (Table 4). It is noteworthy that there were no treatment failures for the dry cow therapy treatment and only 1 treatment failure for the dry cow therapy + teat sealant treatment; the latter failure resulted in a blind quarter at the time that the d-3 and -10 postpartum samples were collected.

Comparison of Pre- and Postpartum SCC by Treatment and by Pre- and Postpartum IMI Status

Comparisons of SCC taken 2 mo prepartum with SCC taken on d 3 and 10 after calving by treatment and infection status are presented in Table 5. Results are presented below by quarter treatment.

Control. Untreated control quarters that were uninfected at the time of treatment but developed a new IMI by the postpartum sampling exhibited elevated SCC both pre- and postpartum. However, if the uninfected control quarters remained uninfected at the postpartum sampling, then SCC decreased significantly from the prepartum sampling on d 3 and 10 postpartum ($P = 0.003$) and were least on d 10.

Untreated control quarters that were infected at time of treatment but were spontaneously cured by the postpartum sampling, showed a significant reduction in SCC from the prepartum sampling through d 3 and 10 ($P < 0.001$). However if control quarters were infected and failed to cure, SCC remained elevated at all sampling times postpartum.

Dry Cow Therapy. Uninfected quarters that were treated with dry cow therapy and developed new IMI by the time of calving exhibited elevated SCC postpartum, although SCC were appreciably less by d 10 postpartum (Table 5). Among dry cow therapy–treated uninfected quarters that remained uninfected at calving, SCC decreased ($P = 0.003$) from the prepartum sampling to d 3 and 10 postpartum.

Infected quarters treated with dry cow therapy that were cured at calving exhibited reductions ($P < 0.001$) in SCC from the prepartum sampling through d 3 and 10. There were no infected quarters treated with dry cow therapy that failed to cure.

Teat Sealant. Uninfected quarters that were treated with teat sealant and developed new IMI by the time of calving had no secretions available or secretion was too viscous to process prepartum, so prepartum SCC were not conducted. However, d-3 and -10 SCC were 658 × 10^3 and 713 × 10^3/mL, respectively (Table 5). Among teat seal-

Table 5. Comparison of prepartum and postpartum (d 3 and 10) SCC (× 10^3/mL) by treatment and by prepartum and postpartum intramammary infection (IMI) status

Quarter treatment	Prepartum IMI status	Postpartum IMI status	Prepartum	d 3	d 10	SE	*P*-value
Control	Uninfected	New IMI	3,207	3,468	2,738	1,955	0.965
Control	Uninfected	Uninfected	1,209[a]	519[b]	244[b]	111.7	0.003
Control	Infected	Cured IMI	3,278[a]	1,638[b]	567[c]	358.6	0.001
Control	Infected	Failed to cure	1,791	825	1,020	397.1	0.288
Dry cow therapy (DC)	Uninfected	New IMI	2,760	2,617	541	847.8	0.258
DC	Uninfected	Uninfected	1,442[a]	658[b]	275[b]	144.8	0.003
DC	Infected	Cured IMI	3,245[a]	914[b]	399[b]	247.1	0.001
DC	Infected	Failed to cure	NV[1]	NV	NV	NV	NV
Teat sealant (TS)	Uninfected	New IMI	NSS[2]	658	713	NV	NV
TS	Uninfected	Uninfected	1,331[a]	677[b]	266[c]	121.2	0.001
TS	Infected	Cured IMI	2,650[a]	587[b]	343[b]	239.1	0.001
TS	Infected	Failed to cure	1,891	1515	1,427	787.1	0.929
DC + TS	Uninfected	New IMI	NSS	536	1,350	845.1	0.566
DC + TS	Uninfected	Uninfected	1,052[a]	476[b]	207[c]	65.10	0.001
DC + TS	Infected	Cured IMI	2,129[a]	534[b]	335[b]	181.3	0.001
DC + TS	Infected	Failed to cure	NSS	B[3]	B		

[a–c]Values in a row with different superscripts are significantly different at $P < 0.05$.

[1]NV = no value.

[2]NSS = no secretion sample available or secretion was too viscous to process for SCC.

[3]B = quarter was nonfunctional or blind at both postpartum samplings, most likely as a result of *Streptococcus dysgalactiae* IMI, so it was considered a treatment failure.

Table 6. Differential leukocyte counts (% ± SD) for uninfected and infected quarters at the prepartum sampling

Quarter infection status	Lymphocytes	Macrophages	Neutrophils
Uninfected	43.2 ± 23.9	41.1 ± 20.6	15.7 ± 18.2
Infected	27.1 ± 14.7	30.8 ± 15.1	42.1 ± 18.4

ant–treated uninfected quarters that remained uninfected at the postpartum samplings, SCC decreased ($P < 0.001$) from the prepartum sampling to d 3 and 10 postpartum and were least on d 10.

Infected quarters treated with teat sealant that were cured at the postpartum samplings exhibited reductions ($P < 0.001$) in SCC from the prepartum sampling through d 3 and 10. However, if teat sealant–treated quarters were infected and failed to cure, SCC remained elevated at all sampling times.

Dry Cow Therapy + Teat Sealant. For uninfected quarters treated with dry cow therapy + teat sealant that developed a new IMI at calving, no prepartum SCC were available, so a comparison pre- and postpartum could not be made. Day-3 and -10 SCC were 536 × 10^3 and 1,350 × 10^3/mL, respectively (Table 5). For uninfected quarters treated with dry cow therapy + teat sealant that remained uninfected at the postpartum samplings, SCC decreased ($P < 0.001$) from the prepartum sampling to d 3 and 10 postpartum and were least on d 10.

Regarding infected quarters treated with dry cow therapy + teat sealant that were cured at calving, SCC decreased ($P < 0.001$) from the prepartum sampling to d 3 and 10 postpartum. Concerning infected quarters treated with dry cow therapy + teat sealant that failed to cure at calving, there were no data available for comparison because the prepartum secretion sample of the one quarter was too viscous to process for SCC and it was blind at both postpartum samplings.

Differential Leukocyte Counts in Uninfected and Infected Quarters

Examination of the differential leukocyte slides illustrated differences in the distributions of macrophages, lymphocytes, and neutrophils in mammary secretions between uninfected and infected quarters (Table 6). Infected quarters exhibited a greater mean percentage of neutrophils (42.1%) and lower mean percentages of lymphocytes (27.1%) and macrophages (30.8%) than uninfected quarters (15.7, 43.2, and 41.1%, respectively) but differences were not significant ($P > 0.05$). Although a basal population of neutrophils may be present in uninfected quarters that serves as a surveillance mechanism for bacteria entering the gland, the proportion of neutrophils increases in infected quarters, and their purpose is to identify and kill invading bacterial pathogens (Paape et al., 2000). In this case, the percentage of neutrophils in infected quarters was elevated approximately 2.7 fold over the percentage found in uninfected quarters (42.1 vs. 15.7%). 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.

A correlation of the prepartum SCC with the leukocyte differential count showed that SCC was negatively correlated with the percentages of lymphocytes (−0.349, $P < 0.003$) and macrophages (−0.082, $P < 0.494$) and positively correlated with the percentage of neutrophils (0.393, $P < 0.001$; Table 7). Thus, as the percentage of neutrophils increased (e.g., in response to bacterial infection), the SCC also increased and the percentages of lymphocytes and macrophages decreased.

Compared with previous trials using the University of Georgia Teaching Dairy herd, this trial (conducted in 2014–2015) experienced a lower prevalence of IMI among heifers 2 mo prepartum. In a survey conducted in 2012 to 2013, 85.7% of heifers had some sort of IMI 2 mo prepartum (Ryman et al., 2013) compared with the present trial's infection rate of 63.2%. Regarding the infection rate with *Staph. aureus*, the present trial observed a prepartum rate among heifers of 24.5%, which is also low compared with the previous findings of 59.5% (Ryman et al., 2013).

At calving, heifers in the present trial experienced an overall infection rate of 22.4%, which is lower than the infection rate of 37.7% among cows that calved in the herd over the same time period. Likewise, the incidence of *Staph. aureus* IMI at calving among heifers in the present trial was low (2.6%) compared with the mature cows that calved with *Staph. aureus* IMI (7.5%). Several trials have been carried out on the heifer herd at the University of Georgia Teaching Dairy in the past several years in attempts to reduce infection rates, especially for *Staph. au-*

Table 7. Correlation of the prepartum SCC with the leukocyte differential count

Variable	Leukocyte	Correlation	*P*-value
Prepartum SCC	Lymphocyte	−0.349	0.003
Prepartum SCC	Macrophage	−0.082	0.494
Prepartum SCC	Neutrophil	0.393	0.001

reus, including vaccination (Nickerson et al., 2017), feeding of the immunostimulant OmniGen (Phibro Animal Health Corp., Teaneck, NJ; Nickerson et al., 2019), and implementing fly control (Nickerson, 2017). It is possible that the rate of *Staph. aureus* IMI as well as the overall infection rates are decreasing due to these trials.

Results confirm that dry cow therapy is an effective management tool for curing existing IMI over the nonlactating period and lowering SCC at the time of calving. An unexpected benefit was that treatment with teat sealant resulted in an 85.7% cure rate compared with untreated control quarters (52.2%, $P < 0.001$). Teat sealant, being an inert physical barrier, functions to prevent bacteria from entering the teat canal and causing a new IMI infection. This product does not provide antimicrobial activity; thus, the elevated cure rate using this product is of interest. A possible explanation for this high cure rate is that the teat sealant is recognized as foreign by the cow's immune system. This may result in an increase in the mammary secretion SCC as a result of a greater influx of neutrophils in response to foreign material, which in turn, are able to clear the infection.

It is important to emphasize that this study focused on the use of nonlactating cow therapy, teat sealant, and both to control mastitis in heifers when administered 2 mo prepartum. This is the period during which the majority of mammary growth and development occurs (Swanson and Poffenbarger, 1979), and presence of IMI leads to tissue damage and is associated with reductions in future milk yield (Enger, 2019). Thus, the successful use of dry cow therapy at this time may allow the tissue to develop normally over the next 2 mo, resulting in minimum SCC and maximum production.

Others have used lactating cow therapy administered 7 to 21 d prepartum. For example, Oliver et al. (1992, 2003) evaluated the use of 2 lactating cow products (sodium cloxacillin, and cephapirin sodium) after infusion at 7 to 14 d prepartum and observed significant cure rates in treated heifers compared with untreated controls. They also found a significant reduction in somatic cell score and an increase in milk production over the first lactation. Middleton et al. (2005) infused a lactating cow product (pirlimicin) 10 to 14 d prepartum into heifers and observed an overall greater cure rate compared with untreated controls, but reduction in SCC was not consistent and no effect on milk yield was observed. Likewise, Borm et al. (2006) infused a lactating cow product (cephapirin) 10 to 21 d prepartum into heifers and observed greater cure rates among treated heifers compared with controls but found no effects on SCC or milk production. The later 2 studies concluded that routine prepartum therapy of heifers with lactating cow products may not benefit all herds.

Cure rates in this study as well as others using nonlactating cow therapy in heifers 2 mo prepartum (see review by Nickerson, 2009) are generally greater than those cited above using lactating cow therapy 7 to 21 d prepartum. The reason for this difference may include the following. Use of lactating cow therapy 7 to 21 d prepartum to cure chronic IMI in heifers that may have been present as early as 6 mo of age (Nickerson, 2009) is not likely to be as effective as using nonlactating cow therapy administered 2 mo prepartum (when the majority of mammary growth and development occurs), especially against *Staph. aureus*. In addition, nonlactating cow therapy contains a greater dose of antibiotic, is retained longer in mammary tissue, and historically results in greater cure rates than lactating cow therapy (NMC, 2010).

APPLICATIONS

Results suggest that if quarters are uninfected 2 mo prepartum, leaving them untreated is as effective as treatment with dry cow therapy, teat sealant, or the combination of dry cow therapy + teat sealant. However, the majority of heifers do have at least one quarter infected with *Staph. aureus* or CNS, which dry cow therapy has been shown to be highly effective in curing as well as lowering the SCC. So, our recommendation would be to pay attention to the SCC of heifers in early lactation. If SCC are elevated ($>1{,}500 \times 10^3$/mL), they are most likely freshening with mastitis that may have been acquired during gestation. It would be beneficial to implement an udder health program with their herd veterinarian that incorporates treating heifers with dry cow therapy for treatment of existing infections and following that with a teat sealant to assist in preventing new infections.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Zoetis (Florham Park, NJ) for providing the Spectramast DC and Teatseal used as treatments in this trial.

LITERATURE CITED

Akers, R. M., A. V. Capuco, and S. C. Nickerson. 2014. Invited Review. Bovine mammary anatomy and function. Chapter 5, Pages 51–56 in Ecology, Evolution and Behaviour of Wild Cattle: Implications for Conservation. M. Melletti and J. Burton, ed. Cambridge Univ. Press. Cambridge, UK.

Borm, A. A., L. K. Fox, K. E. Leslie, J. S. Hogan, S. M. Andrew, K. M. Moyes, S. P. Oliver, Y. H. Schukken, D. D. Hancock, C. T. Gaskins, W. E. Owens, and C. Norman. 2006. Effects of prepartum intramammary antibiotic therapy on udder health, milk production, and reproductive performance in dairy heifers. J. Dairy Sci. 89:2090–2098. https://doi.org/10.3168/jds.S0022-0302(06)72279-2.

Enger, B. D. 2019. Invited Review: Reevaluating how mastitis reduces milk yield: Discussion of competitive substrate utilization. Appl. Anim. Sci. 35:408–415. https://doi.org/10.15232/aas.2019-01876.

FASS. 2010. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Fed. Anim. Sci. Soc., Champaign, IL.

Fox, L. K., S. T. Chester, J. Hallberg, S. C. Nickerson, J. W. Pankey, and L. D. Weaver. 1995. Survey of intramammary infections in dairy heifers at breeding age and first parturition. J. Dairy Sci. 78:1619–1628. https://doi.org/10.3168/jds.S0022-0302(95)76786-8.

Middleton, J. R., L. L. Timms, G. R. Bader, J. Lakritz, C. D. Luby, and B. J. Steevens. 2005. Effect of prepartum intramammary treatment with pirlimycin hydrochloride on prevalence of early first-lactation mastitis in dairy heifers. J. Am. Vet. Med. Assoc. 227:1969–1974. https://doi.org/10.2460/javma.2005.227.1969.

National Mastitis Council. 2004. Microbial Procedures for the Diagnoses of Bovine Udder Infection and Determination of Milk Quality. 4th ed. Natl. Mastitis Counc. Inc., Verona, WI. Pages 9–41.

National Mastitis Council. 2010. Dry Cow Therapy. Natl. Mastitis Counc. Inc., Verona, WI. https://articles.extension.org/pages/11528/dry-cow-therapy.

Nickerson, S. C. 2009. Control of heifer mastitis: Antimicrobial treatment—An overview. Vet. Microbiol. 134:128–135. https://doi.org/10.1016/j.vetmic.2008.09.019.

Nickerson, S. C. 2017. Horn fly control to increase productivity in dairy heifers. Extension Bulletin (B1474). Accessed May 10, 2019. http://extension.uga.edu/publications/detail.cfm?number=B1474.

Nickerson, S. C., C. L. Hall, L. O. Ely, F. M. Kautz, and D. J. Hurley. 2017. Review article. Use of a *Mycobacterium* adjuvant to enhance the antibody response to vaccination against *Staphylococcus aureus* mastitis in dairy heifers. J. Vaccines Immunol. Immunopathol. 2:1–3.

Nickerson, S. C., F. M. Kautz, L. O. Ely, A. D. Rowson, D. J. Hurley, J. D. Chapman, and D. J. McLean. 2019. Effects of an immunomodulatory feed additive on intramammary infection prevalence and somatic cell counts in a dairy herd experiencing major health issues. Res. Vet. Sci. 124:186–190. https://doi.org/10.1016/j.rvsc.2019.03.013.

Oliver, S. P., M. J. Lewis, B. E. Gillespie, and H. H. Dowlen. 1992. Influence of prepartum antibiotic therapy on intramammary infections in primigravid heifers during early lactation. J. Dairy Sci. 75:406–414. https://doi.org/10.3168/jds.S0022-0302(92)77776-5.

Oliver, S. P., M. J. Lewis, B. E. Gillespie, H. H. Dowlen, E. C. Janicke, and R. K. Roberts. 2003. Prepartum antibiotic treatment of heifers: Milk production, milk quality and economic benefit. J. Dairy Sci. 86:1187–1193. https://doi.org/10.3168/jds.S0022-0302(03)73702-3.

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. J. Dairy Sci. 84:814–817. https://doi.org/10.3168/jds.S0022-0302(01)74538-9.

Owens, W. E., S. C. Nickerson, P. J. Washburn, and C. H. Ray. 1991. Efficacy of a cephapirin dry cow product for treatment of experimentally induced *Staphylococcus aureus* mastitis in heifers. J. Dairy Sci. 74:3376–3382. https://doi.org/10.3168/jds.S0022-0302(91)78527-5.

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. Zentralbl Veterinarmed B 41:90–100. https://doi.org/10.1111/j.1439-0450.1994.tb00212.x.

Paape, M. J., K. Shafer-Weaver, A. V. Capuco, K. Van Oostveldt, and C. Burvenich. 2000. Immune surveillance of mammary tissue by phagocytic cells. Adv. Exp. Med. Biol. 480:259–277. https://doi.org/10.1007/0-306-46832-8_31.

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. J. Dairy Sci. 93:2989–2997. https://doi.org/10.3168/jds.2009-2886.

Parker, K. I., C. 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. J. Dairy Sci. 91:169–181. https://doi.org/10.3168/jds.2007-0212.

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. Res. Vet. Sci. 95:343–346. https://doi.org/10.1016/j.rvsc.2013.04.017.

Swanson, E. W., and J. I. Poffenbarger. 1979. Mammary gland development of dairy heifers during their first gestation. J. Dairy Sci. 62:702–714. https://doi.org/10.3168/jds.S0022-0302(79)83313-5.

Trinidad, P., S. C. Nickerson, and R. W. Adkinson. 1990a. Histopathology of staphylococcal mastitis in unbred dairy heifers. J. Dairy Sci. 73:639–647. https://doi.org/10.3168/jds.S0022-0302(90)78715-2.

Trinidad, P., S. C. Nickerson, T. K. Alley, and R. W. Adkinson. 1990b. Efficacy of intramammary treatment in unbred and primigravid dairy heifers. J. Am. Vet. Med. Assoc. 197:465–470.

Woolford, M. H., J. H. Williamson, A. M. Day, and P. J. A. Copeman. 1983. An identical twin study of milk production losses due to subclinical mastitis. Pages 115–119 in Proc. Ruakura Farmers' Conf. Ruakura Farmers' Counc. Inc., Ruakura, New Zealand.

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