Mastitis control in bred heifers: Use of dry cow therapy and teat sealant for curing existing intramammary infections and preventing new ones

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Abstract

Presence of mastitis in pregnant as well as unbred 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 existing IMI and preventing new IMI 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. To examine this, 4 quarters of each of 76 pregnant heifers were treated randomly 30 to 60 days prepartum as follows: 1) untreated control, 2) dry cow therapy, 3) teat sealant, or 4) dry cow therapy + teat sealant. Results demonstrated that compared to the spontaneous 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 ($P < 0.001$) cure rates in quarters infected prepartum with *Staphylococcus aureus* or CNS. The reason for the 85.7% cure rate in teat sealant-treated quarters remains unclear. Additionally, SCC 3 d after calving were lower ($P < 0.05$) in quarters previously infected and cured, that were treated with dry cow therapy (914 x 10^3/mL), teat sealant (587 x 10^3/mL), and dry cow therapy + teat seal (534 x 10^3/mL), compared with untreated controls (1638 x 10^3/mL). Although the prevention rate against new IMI was similar for control quarters (95.9%) and those receiving dry cow therapy (92.2%), teat sealant (97.9%), and the combination of dry cow therapy + teat sealant (95.9%), it is recommended to implement an udder health program that incorporates treating all quarters with dry cow therapy to cure existing IMI plus a teat sealant to prevent new IMI.

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 these animals freshen free of IMI with low SCC. During a heifer's first gestation, the presence of mastitis can compromise the development of milk-producing tissues, and in the case of *Staph. 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 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 the coagulase-negative staphylococci (CNS) and *Staph. aureus*, and up to 30% is caused by *Staph. aureus* alone (Nickerson 2009). Such IMI induce a chronic inflammation, which is associated with elevated SCC (as high as 10 x 10^6/ml) and damage to the developing milk-producing tissues (Trinidad et al., 1990b). Thus, an udder health care program should be in place for bred heifers to

eliminate existing IMI and prevent new ones 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 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 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 at calving by 93%.

Thus, use of nonlactating cow therapy was effective in both curing existing IMI and preventing new cases of mastitis. Studies have also shown that successful treatment 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 (Owens et al., 1991; Trinidad et al., 1990a). Other 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 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? 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.

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 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, 2010).

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 as described below.

Intramammary treatments:

After the mammary secretion sample collection was performed 30 to 60 days 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 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 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 treating processes.

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 plates using sterile, flamed 10-µL loops. Plates were incubated for 48 h at 37°C and then 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, and 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-day 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 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, 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.

In order to ensure that antibiotic residues were not present in milk of heifers, all of which were treated with dry cow therapy in 2 quarters, the 3-day 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-day postpartum milk samples.

Statistical analyses:

To analyze results, 2 data sets were created: one for quarters that were diagnosed as uninfected prepartum that either developed new IMI or remained uninfected at calving, and another dataset for quarters that were diagnosed as infected prepartum that either cured or failed to cure at calving. 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 (prepartum) and for days 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, 2013).

Results and Discussion

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

Overall prevalence of IMI among heifers at time of treatment 30 to 60 days prepartum was 63.2%, and prevalence among quarters was 35.9%. The vast majority of IMI at this time were caused by the staphylococci (82.0%), which included *Staphylococcus hyicus* (26.4%), *Staphylococcus chromogenes* (25.5%), *Staphylococcus aureus* (24.5%), *Staphylococcus xylosus* (2.0%), *Staphylococcus saprophyticus* (0.9%), *Staphylococcus epidermidis* (0.9%), *Staphylococcus capitis* (0.9%) and *Staphylococcus* spp. (0.9%). Other IMI that contributed to the remaining 18% of infections included *Streptococcus dysgalactiae* (15.1%), *Pseudomonas* spp. (2.0%), and *Trueperella pyogenes* (0.9%). See Table 1.

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 the CNS and *Staph. aureus* predominated.

Table 1. Percentages of bacterial infections among infected quarters of heifers sampled prepartum* and postpartum**.

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	---

*Overall prevalences of IMI among heifers and quarters prepartum were 63.2% and 35.9%, respectively.

** Overall prevalences of IMI among heifers and quarters postpartum were 22.4% and 6.1%, respectively.

Overall prevalence of IMI among heifers postpartum, based on days 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 the 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 lower 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 seal 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 among quarters prepartum (35.9%), the level of IMI postpartum (6.1%) was reduced 83.0%. It must be kept in mind that one quarter per heifer was left as an untreated control, and the reduction in level of IMI postpartum likely would have been greater.

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 found 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 higher cure rates ($P < 0.001$) were observed in quarters that received dry cow therapy (100%), teat sealant (85.7%), and dry cow therapy plus 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.

Cure rates are within the range found by others when treating infected quarters of heifers with dry cow therapy, and prevention rates in uninfected quarters are also within the range found by others (see review by Nickerson, 2009). The 97.9% prevention rate against new IMI for teat sealant is greater than that (74%) observed by Parker et al. (2008).

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

Variable	Control	Dry Cow (DC)	Teat Seal (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 row with different superscripts are different at the *P*-values indicated.

Treatment effects on SCC of uninfected quarters prepartum:

The SCC data before treatment (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 1052 x 10^3/mL to 3207 x 10^3/mL among the 196 quarters. Among the 9 quarters that were diagnosed with new IMI postpartum, SCC on day 3 were highest in control quarters and quarters treated with dry cow therapy ($P < 0.001$), and on day 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 days 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 day 3 ranged from 519 x 10^3/mL to 677 x 10^3/mL, and on day 10, SCC were lower as the volume of milk increased and diluted the leukocytes in milk, ranging from 207 x 10^3/mL to 274 x 10^3/mL. No SCC differences were observed among treatments within day.

Table 3. SCC values ($x10^3$) prior to treatment (Prepartum) and on days 3 and 10 postpartum across treatments for quarters that were uninfected prepartum that did (Yes) or did not (No) develop new IMI postpartum.

Treatment	New IMI	n	Prepartum	Day 3	Day 10
Control	Yes	2	3207	3468[a]	2738 [a]
Dry Cow	Yes	4	2760	2617[a]	541 [c]
Teat Seal	Yes	1	---[*]	658[b]	713[b,c]
DC+TS	Yes	2	---	535[b]	1350[a,b]
Control	No	47	1209	519[b]	244[c]
Dry Cow	No	47	1442	658[b]	274 [c]
Teat Seal	No	46	1331	677[b]	266[c]
DC+TS	No	47	1052	476[b]	207[c]
SE			383.2	152.9	75.3
P			0.284	0.001	0.001

[a, b, c] Values in a column with different letters are different the *P*-values indicated.
[*]--- No secretion sample available or secretion was too viscous to process for SCC.

Treatment effects on SCC of infected quarters prepartum:

The SCC before treatment and postpartum in quarters initially diagnosed as infected are found in Table 4. Among the 88 quarters that were diagnosed as cured postpartum, SCC on day 3 were highest in control quarters ($P < 0.047$) compared with other treatments. Thus, along with improved cure rates, treatment with any of the products lowered the SCC on day 3 compared with the control. Although SCC on day 10 were numerically highest in control quarters compared with other treatments for cured quarters, the difference was not significant ($P < 0.094$). As observed in Table 3, SCC were numerically highest 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 3245, 2650, and 2129 x 10^3/mL, respectively, and decreased to 914, 587, and 534 x 10^3/mL, respectively postpartum. 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 time of treatment and failed to cure postpartum, no SCC differences were observed among treatments on day 3 or 10. 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 seal treatment; the latter failure resulted in a blind quarter at the time that the day 3 and 10 postpartum samples were collected.

Table 4. SCC values ($x10^3$) prior to treatment (Prepartum) and on days 3 and 10 postpartum across treatments for quarters that were infected prepartum that had an IMI status of cured or failed postpartum.

Treatment	IMI status	n	Prepartum	Day 3	Day 10
Control	Cured	16	3278	1638[a]	567[e,f]
Dry Cow	Cured	23	3245	914[b]	399[f]
Teat Seal	Cured	24	2650	587[b]	343[f]
DC+TS	Cured	25	2129	534[b]	335[f]
Control	Failed	13	1791	825[b]	1020[e]
Dry Cow	Failed	0			
Teat Seal	Failed	4	1891	1515[a,b]	1427[e]
DC+TS	Failed	1	---[*]	---[**]	---[**]
SE			412.1	231.9	174.6
P			0.259	0.047	0.094

[a,b]Values in a column with different letters are different the *P*-value indicated.
[e,f]Values in a column with different letters are different the *P*-value indicated.
[*]Secretion sample was too viscous to process for SCC.
[**]Quarter was nonfunctional or blind at both postpartum samplings, most likely as a result of the *Strep. dysgalactiae* IMI diagnosed at the prepartum sampling, so it was considered a treatment failure.

A comparison of SCC taken 30 to 60 days prepartum with SCC taken on days 3 and 10 post calving by treatment and infection status is presented in Table 5. Untreated control quarters that were uninfected at time of treatment, which 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 to days 3 and 10 postpartum ($P < 0.003$), and were lowest on day 10. Untreated control quarters that were infected at time of treatment, which spontaneously cured by the postpartum sampling, showed a significant reduction in SCC from the prepartum sampling through days 3 and 10 ($P < 0.001$); however if control quarters were infected and failed to cure, SCC remained elevated at all sampling times.

Uninfected quarters that were treated with dry cow therapy, which developed new IMI by the time of calving exhibited elevated SCC both pre- and postpartum, although SCC were lowered appreciably by day 10 postpartum. Among dry cow therapy-treated uninfected quarters that remained uninfected at calving, SCC decreased ($P < 0.003$) from the prepartum sampling to days 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 days 3 and 10; there were no infected quarters treated with dry cow therapy that failed to cure.

Uninfected quarters that were treated with teat sealant, which 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 day 3 and 10 SCC were 658 x 10^3/mL and 713 10^3/mL, respectively. Among teat sealant-treated uninfected quarters that remained uninfected at the postpartum samplings, SCC decreased ($P < 0.001$) from the prepartum sampling to days 3 and 10 postpartum, and were lowest on day 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 days 3 and 10; however, if teat sealant-treated quarters were infected and failed to cure, SCC remained elevated at all sampling times.

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 x 10^3/mL and 1350 x 10^3/mL, respectively. 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 days 3 and 10 postpartum, and were lowest on day 10. For infected quarters treated with dry cow therapy + teat sealant that were cured at calving, SCC decreased ($P < 0.001$) from the prepartum sampling to days 3 and 10 postpartum. For 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.

Table 5. Comparison of prepartum and postpartum (days 3 and 10) SCC by treatment and by prepartum and postpartum IMI status.

Quarter treatment	Prepartum IMI status	Postpartum IMI status	Prepartum	Day 3	Day 10	SE	*P*
Control	Uninfected	New IMI	3207	3468	2738	1955	0.965
Control	Uninfected	Uninfected	1209[a]	519[b]	244[b]	111.7	0.003
Control	Infected	Cured IMI	3278[a]	1638[b]	567[c]	358.6	0.001
Control	Infected	Failed to cure	1791	825	1020	397.1	0.288
Dry Cow	Uninfected	New IMI	2760	2617	541	847.8	0.258
Dry Cow	Uninfected	Uninfected	1442[a]	658[b]	275[b]	144.8	0.003
Dry Cow	Infected	Cured IMI	3245[a]	914[b]	399[b]	247.1	0.001
Dry Cow	Infected	Failed to cure					
Teat Seal	Uninfected	New IMI	---[*]	658	713	---	---
Teat Seal	Uninfected	Uninfected	1331[a]	677[b]	266[c]	121.2	0.001
Teat Seal	Infected	Cured IMI	2650[a]	587[b]	343[b]	239.1	0.001
Teat Seal	Infected	Failed to cure	1891	1515	1427	787.1	0.929
DC+TS[**]	Uninfected	New IMI	---	536	1350	845.1	0.566
DC+TS	Uninfected	Uninfected	1052[a]	476[b]	207[c]	65.10	0.001
DC+TS	Infected	Cured IMI	2129[a]	534[b]	335[b]	181.3	0.001
DC+TS	Infected	Failed to cure	---[*]	---[***]	---[***]		

a, b, c values in row with different letters are significantly different at the *P*-values indicated.
*No secretion sample available or secretion too viscous to process for SCC.
**Dry cow therapy + teat sealant.
***Quarter was nonfunctional or blind at both postpartum samplings, most likely as a result of *Strep. dysgalactiae* IMI, so it was considered a treatment failure.

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 higher mean percentage of neutrophils (42.1%), and lower mean percentages of lymphocytes (27.17%), 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 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 also observed a 2.7-fold increase in the percentage of neutrophils in secretions of infected quarters in heifers compared with uninfected quarters.

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

Quarter infection status	Lymphocytes	Macrophages	Neutrophils
Uninfected	43.2	41.1	15.7
Infected	27.1	30.8	42.1

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.

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

Compared to previous trials using the UGA Teaching Dairy herd, this trial (conducted in 2014-2015) experienced a lower prevalence of IMI among heifers 30 to 60 days prepartum. In a

survey conducted in 2012-2013, 85.7% of heifers had some sort of IMI 30 to 60 days 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 to 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 UGA Teaching Dairy in the past several years in attempts to reduce infection rates, especially for *Staph. aureus*, including vaccination, feeding of the immunostimulant OmniGen®, and implementing fly control. 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 important management tool for curing existing IMI over the nonlactating period and lowering SCC at 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 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 higher influx of neutrophils in response to foreign material, which in turn, are able to clear the infection.

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; treatments ranged from 92.2% to 97.9% effective in preventing new IMI (Table 2). Thus, results suggest that if quarters are uninfected at 30 to 60 days prepartum, leaving them untreated is as effective as treatment with dry cow therapy, teat seal, or the combination of dry cow therapy + teat seal. In fact, SCC values (Table 3) were not different across all 4 treatments on day 3 in uninfected quarters that remained uninfected (range: 519 x 10^3 to 677 x 10^3/mL) as well as on day 10 (range: 207 x 10^3 to 274 x 10^3/mL) postpartum. However, the majority of heifers do have at least one quarter infected with *Staph. aureus* or CNS against which dry cow therapy 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 (>1500 x 10^3/mL), 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 existing infections and following that with a teat sealant to assist in preventing new infections.

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