Evaluation of internal teat sealant persistence in the mammary gland during the dry period

Michelle P. Buckley,[1] MS, DVM; ****Jenna Bayne**,[2] DVM, MS, DACVIM; **Tiago Tomazi**,[3] DVM, MS, PhD; **Brian E. Miller**,[3] DVM; **Gustavo S. Silva**,[2] DVM, MS, PhD; and ***Patrick J. Gorden**,[2] DVM, PhD, DABVP, DACVCP

[1]Department of Veterinary Microbiology and Preventative Medicine, Iowa State University, Ames, IA 50011
[2]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA 50011
[3]Dairy Technical Services, Merck Animal Health, Lenexa, KS 66219

***Corresponding author:** Dr. Patrick J. Gorden, **pgorden@iastate.edu**
ORCID: **https://orcid.org/0000-0002-6096-0965**
[**]Dr. Bayne's current address: Auburn College of Veterinary Medicine, Auburn, AL 36849

Abstract

The aim of this study was to characterize the behavior of 2 commercially available internal teat sealants containing bismuth subnitrate (SO[a] and ORB[b]) during the dry- and post-fresh periods in dairy cattle. Digital radiography was used to monitor location and percentage of teat cistern fill at dry-off and during the dry period. Teat-level health parameters including infection status at dry-off and post-fresh as well as incidence of clinical mastitis were also assessed. Radiographic imaging revealed that 21.1% of teats had sealant present in the gland cistern at dry-off (SO= 23.6%, ORB = 18.5%) which increased to 55.9% (SO = 59.6%, ORB = 52.2%) at pre-fresh imaging. Quarters with sealant that had migrated into the gland shed noticeable amounts of sealant for twice as long (mean 14.34 milkings), compared to quarters that did not have sealant in the gland (mean: 7.12, $P < 0.001$). There was no correlation between sealant location and intramammary infection at dry-off or post-fresh, nor did sealant location impact clinical mastitis in the first 120 days in milk. Milk leakage after dry-off also was not correlated with an increased risk of intramammary infection during the dry or post-fresh periods. There was no difference between treatments in any of the parameters evaluated. Prolonged sealant shedding is a common reason that producers cite for not utilizing internal teat sealant products in their dry-off protocols. Further research is indicated to determine the optimal amount of sealant required to provide protection while minimizing post-fresh shedding.

Key words: internal teat sealant, dry cow therapy, milk quality

Introduction

Internal teat sealant use is integral to many effective dry cow therapy programs in preventing an intramammary infection (IMI) during the dry period.[1,2] Previous research has noted that keratin plug formation occurs in response to sustained fluid build-up within the mammary gland resulting from the cessation of milking.[3] There is a wide variation in the amount of time that is required for keratin plug formation, ranging from 7 to 45+ days dry.[4] Due to the variation in timing of keratin plug formation, internal teat sealant (ITS) have been utilized to form a synthetic barrier within the teat canal and cistern during the dry period.

Research supports the claim that the use of ITS products can reduce IMI development within the dry period.[5,6,7] Though the benefits of ITS are believed to be mainly due to its mechanical function, one study demonstrated an inhibitory effect on bacterial growth of *Streptococcus uberis, Staphylococcus aureus* and *Escherichia coli* on in-vitro culture in the presence of bismuth subnitrate, indicating that chemical properties may also be of significance.[8] Current dry treatment recommendations in the United States call for either blanket or selective antimicrobial therapy followed by bismuth subnitrate-based ITS use on all cows at dry-off.[1,2,9] Despite these recommendations, the USDA NAHMS Dairy 2014 survey indicated that only 36.9% of the farms in the survey used an ITS on at least some cows and 33.9% of surveyed farms used ITS on all cows at dry-off.[10]

Although a significant amount of ITS is visibly eliminated during the first milking after calving, there are potential issues associated with its use that limit more widespread adoption of the product by dairy producers. Many farmers have noted that ITS residues persist for several days into the lactation, depositing in milking equipment and filters, thus requiring more aggressive cleaning and more frequent replacement of equipment. One study identified the major ingredient in commercially available ITS, bismuth subnitrate, in defects (i.e., black spot defect) found in aged Cheddar cheese, rendering them unsalable under USDA procurement programs.[11] Those authors hypothesized that this may be due to incomplete removal of ITS from the udder, but no data supporting this assertion was provided. This has led to some producers citing ITS residue persistence in their milking system or the potential association between ITS use and "black spot defect" as reasons for not incorporating ITS into their dry-off protocol (author's personal communications with dairy producers). Therefore, further research is needed to elucidate this relationship and its prevalence within the cheese industry.

To better evaluate the importance of physical location of ITS, Meaney et al. utilized radiography to track sealant location within the teat cistern and streak canal throughout the dry period.[12] Postmortem examinations were also performed on a subset of animals which revealed migration of sealant into the gland in some individuals. This anatomic location was not captured on radiographs, however. Sealant shedding was also described in this study and was noted to occur for up to 21 DIM. However, the sealant utilized in this study was formulated slightly differently than those available commercially today (7.5 g of 25% bismuth subnitrate vs. 4g of 65% bismuth subnitrate in currently available ITS products in the United States). In another study, ITS shedding patterns were evaluated at the first milking and for each subsequent milking, up to 12 DIM.[13] This study did not find a significant association between the presence of a sealant plug at freshening and

presence of a new intramammary infection (NIMI) in the post-calving period. These findings suggest that sealant location may change during the dry period and that sealant location may not be as significant at the end of the dry period as it is in the early dry period. It should be noted that neither of these studies evaluated products currently on the U.S. market at the time of publication.

This study aimed to describe the behavior of 2 commercially available ITS (SO[a] and ORB[b]) administered at dry-off regarding fractionation, location and shedding in the post-calving period.

Materials and methods

This study was a secondary objective of a larger project evaluating the efficacy of SO compared to ORB, which is reported elsewhere.[14] Prior to initiation of the study, protocol was approved by the Iowa State University's (ISU) Institutional Animal Care and Use Committee (protocol number 21-068). The trial was conducted at the ISU Dairy Farm in the summer of 2021. At the time of the study, the dairy had 372 cows (350 Holstein, 22 Jersey) producing 84 pounds (38 kg) of milk per day, 4.2% fat, 3.3% protein, and a 237,000 cells/mL at the DHIA test before initiation of the trial. Most of the cows on this farm were housed in free stalls. The lactating cows were bedded with green manure solids while the far-off dry cows were bedded with new sand. At 21-28 days ahead of expected calving, the cows in this trial were housed on deep bedded corn stalks that were completely removed one time per week.

Inclusion criteria

To accomplish the objective, cows were enrolled if they had a planned dry period of 30-90 days, at least 3 functional quarters, a body condition score > 2.0 out of 5,[15] and a lameness score < 4 out of 5.[16] Cows that farm management had designated as an early-lactation cull in the subsequent lactation were ineligible for enrollment. Cows were also excluded if they received any antimicrobial treatment within 14 days of expected dry-off. Eligible animals were identified the week before they were due to be dried off and quarters were randomized in a cross-udder design, with one set of quarters on each cow receiving one ITS treatment (SO or ORB) and the opposite set of quarters receiving the other ITS treatment. To achieve the cross-udder design, quarters were assigned by fore quarter, with the left front assignment including the right rear quarter, while the right front assignment included the left rear quarter. Treatment assignment was determined using the "rand()" function in a spreadsheet program[c] with treatments assigned at the time of enrollment, 1 week before dry-off.

Dry-off procedure

On the day each cow was to be dried-off, study personnel evaluated farm records and animals were observed to ensure that eligible cows still met enrollment criteria. To compare intramammary (IMM) infection dynamics before and after the dry period, aseptic, duplicate, quarter level milk samples were collected before and after the dry period and milk culture procedures were undertaken as described by Buckley et al.[14] After milking machine detachment, all cows were administered 500 mg of cloxacillin benzathine[d] per quarter followed by the assigned bismuth subnitrate sealant (SO and ORB). To ensure proper placement of ITS in the teat, after the ITS teat cannula was placed in the teat orifice by the study technician, the base of the teat at the junction with the udder was pinched between the thumb and index finger while infusing the entire contents of ITS tube. If the teat cistern became over-full, pressure was maintained at the top of the teat until the applicator tip was removed. Excess sealant was allowed to discharge from the teat orifice.

Positioning of ITS

To assess ITS positioning following administration, radiographic images were collected by study personnel utilizing portable digital radiograph equipment[e] immediately following ITS administration. Images were captured from the rear of each cow by positioning the digital image capture plate directly cranial to the udder for the first image, allowing visualization of all 4 teats, and then between the cranial and caudal halves of the udder to better isolate the rear teats. The x-ray generator was held approximately 3 feet from the plate using settings of 70 kVp and 3.5 mAs for image capture. Teats were then post-dipped, and the cows were moved to the farm's dry cow facilities and managed per farm protocols. Repeat radiographic images were collected on all cows in the study on the same date. As such, the days between DO and PC image capture were not the same for all cows. The same radiographic procedures were used for pre-calving (PC) image capture as those used at dry-off (DO). Dry-off and PC images were compared to assess sealant location within the udder (streak canal [1], teat cistern [2], gland cistern [3]). Two novel scoring methods were created and utilized to describe sealant location. The first was a dichotomous system (yes or no) that evaluated whether sealant was present at both time points in each of 3 locations: streak canal, teat cistern and gland. The second method, which accounted for variation in magnification due to positioning of plate and generator, determined the percent teat cistern fill, by comparing internal teat cistern length (denominator, Figure 1 'A') with length of sealant within the cistern (numerator, Figure 1 'B'). Internal teat cistern length was determined using calipers to measure the distance from the teat dorsal aspect of the streak canal to the junction of the gland cistern. Sealant fill within the teat cistern was also measured with calipers in a similar manner.

Milk leakage

Milk leakage at the quarter level was visually assessed for 3 days following dry-off. This was accomplished by locking cows in stanchions 3 times per day and observing cows for 15 minutes for the presence of milk leakage.

Post-calving sealant shedding

Following parturition, assessment of sealant fractionation was determined at colostrum collection and at each milking following calving until the cow was determined to have cleared the sealant from all 4 quarters or she reached 21 days post-calving, whichever came first. Following parturition, each quarter had the first 50 mL of colostrum collected into conical shaped tubes, which were immediately placed in a refrigerator post-collection. The following day, these tubes were collected from the farm and centrifuged at room temperature for 20 minutes at 3,000 rpm.[j] The supernatant was then poured off and the remaining solids were weighed inside the collection tube. The net weight of the sealant was determined by subtracting off the empty tube weight, which was determined using several tubes in the same lot. Then at each subsequent milking a quarter milk sample was collected before milking unit attachment. To accomplish this, each teat was sanitized with pre-dip and

wiped with a cloth towel before collection and then each teat was hand stripped 10 times into a mastitis detection cup[f] lined with a brown, flat-bottomed coffee filter. These filters were later examined for the presence of sealant and a fractionation score developed by the authors was assigned based on size and quantity of sealant present. The scoring system and unenrollment criteria are shown in Table 1. The system was designed to differentiate the presence of no sealant in foremilk (category 1) from sealant flakes that would likely be missed by milk harvest technicians while pre-stripping teats (denoted by categories 2 and 3) or sealant flakes that could be mistaken as mastitis garget (denoted by categories 4 and 5) based on the size of the flakes.

Intramammary infection dynamics

Dry-off and post-fresh (PF) milk culture results were compared to evaluate IMI dynamics during the dry period. Sample collection schedules, culturing techniques, and factors to determine infection status are described in our companion publication.[14] In summary, cured infections were defined as quarters with a confirmed IMI at DO which either cleared the infection on the PF sample or a different bacterial species was isolated. A new IMI was classified as either a quarter with no infection at DO and positive for an IMI at PF sampling or infected with a different pathogen at DO and PF sampling.

Data management

Study personnel collecting milk samples and applying treatments were not blinded to treatment, as they needed to know treatment assignment to treat animals correctly. Farm staff and laboratory personnel responsible for culturing milk samples were blinded to treatment. The author responsible for all radiographic evaluations (JEB) and those scoring sealant fractionation were also blinded to treatment assignments.

Statistical analysis

A generalized linear mixed model (logistic) was used to build a multivariable model to assess the differences between treatments for each explanatory variable: IMI at enrollment, IMI at post-calving, cured intramammary infection (CIMI) risk, and NIMI risk. The multivariable models were built including treatment as a fixed effect. Interactions between the treatment and potential covariates were also assessed, but no interactions were identified as significant ($P > 0.05$) in any of the models, and thus were excluded from the final models.[17] Quarters within cows were included as random intercept in each model. ANOVA was performed to assess the statistical difference between groups, Tukey's test was used for posthoc comparison to assess statistical differences, and the least square means were used to estimate the marginal means for

Figure 1a: Radiograph at dry-off (DO) demonstrating sealant within streak canals and teat cistern with no gland contamination (Cow 11887). Anatomic landmarks evaluated include the streak canal (1), teat cistern (2), and gland cistern (3). Red lines (A) denote measurements utilized for calculating teat cistern volume while green lines (B) denote sealant volume. These measurements were then utilized to calculate percent of cistern fill. **Figure 1b:** Radiograph at mid-dry period (PF) demonstrating sealant movement during dry period with contamination of the gland evident (Cow 11887).

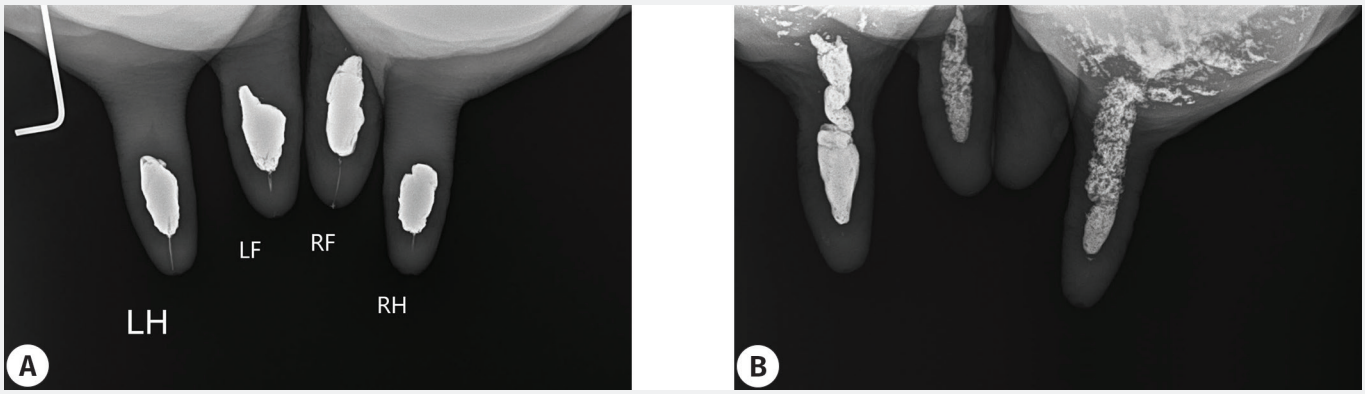


Table 1: Fractionation scoring and unenrollment* system developed for description of sealant shedding patterns that may be confused with mastitis garget (score 4 & 5) vs. easily identified sealant residue (scores 2 & 3).

Score	Definition
1	No sealant present
2	5 or less small (3 mm or less) sealant particles – would not be mistaken for mastitis
3	> 5 small (3 mm or less) sealant particles – would not be mistaken for mastitis
4	5 or less easily visible flakes/globules (> 3 mm) of sealant – could easily be mistaken for mastitis
5	Large number (> 5) of flakes/globules of sealant > 3 mm – could easily be mistaken for mastitis

* Unenrollment Criteria: A cow acquired a sealant score of 1 for 2 consecutive milkings on all quarters, OR had no sealant score higher than a 3 for 6 consecutive milkings on all quarters, OR reached 21 days in milk, OR culled from the herd before any of the other criteria were reached. All cows remained on the trial for a minimum of 4 milkings after calving.

each predictor. The risk difference and confidence intervals were obtained from the output of the generalized linear mixed models (odds ratio) and converted into risk ratio as previously described in our companion paper.[14] This method was used to obtain the risk ratio since the odds ratio will overestimate risk when risk is greater than 10%. Spearman's r coefficient was used to assess the correlation between sealant weight at freshening and the number of milkings to unenrollment.[18]

Results

Figure 2 displays the transition through the study regarding cow and quarter numbers at each observation and animals that were lost to follow up. There were 32 cows enrolled in the study, with 2 cows having 1 blind teat each. Therefore, there were 63 quarters (126 total) treated with each sealant product. Two cows were removed from the study during the dry period. One cow aborted and 1 cow calved after 25 days dry due to an apparent error in her records. As a result, 30 cows (118 quarters) remained in the study and were evaluated post-calving. One cow was culled from the herd before being unenrolled from the sealant fractionation observations and 4 cows were culled between 21 and 120 DIM and not included in the mastitis by 120 DIM statistics. In the following results summary, the number of quarters included may be different due to loss to follow up because culture results that were contaminated or failure to collect usable radiographic images.

As stated previously, this work was part of a larger study[14] for which cows outside the work cited here were assigned sealant treatment at the cow-level as compared to quarter-level. Within the population of quarters used in the current study, the prevalence of IMI at dry-off was 29.3% (34/116) with no difference detected between treatment groups (prevalence in SO quarters 31.0% [95% CI 20.2, 41.9] versus ORB quarters 27.6% [95% CI 17.0, 38.2]). The current study also established a CIMI risk difference of -6.5 [95% CI -33.54, 20.53] for SO versus ORB quarters. These results were similar to our larger study (see Discussion).[14]

Repeat radiographic imaging for cows ranged between 21-55 days after dry-off with a mean of 39 days dry and 24 days before calving (range: 0-57 days). Table 2 shows the results of the dry-off and post-calving radiographic sealant assessment and Figure 3 shows the comparison of sealant location after dry treatment and during the dry period. At dry-off, 1.6% (2/126) of quarters did not have sealant present in the teat cistern while 2.3% (3/126) of streak canals did not have sealant present. It should be noted that the 2 quarters that did not have sealant present in the teat cistern did have it in the streak canal as well as the gland and the 3 quarters that did not have sealant present in the streak canal at dry-off imaging did have sealant present in the teat cistern, indicating inaccurate sealant placement. None of these quarters had bacterial infections present on PF cultures, nor did they develop clinical mastitis within the first 120 DIM. At dry-off 21.1% (23/109) of the quarters had sealant present in the gland cistern. That increased during the dry period to 56% (52/93 quarters) at pre-calving imaging (Figure 3). On pre-calving imaging, 22.6% (26/115) of teats did not have sealant in the teat cistern. Analysis of radiographic images determined that there was no difference in cistern fill between treatments at dry-off (mean percent fill: SO = 66.9% vs ORB = 67.5%, $P = 0.32$) or pre-calving (mean percent fill: SO = 43.2% vs. ORB = 41.0%, $P = 0.41$).

Milk leakage in the 3 days following dry-off

Milk leakage was noted in 12 of 126 (9.5%; SO = 6 of 72, ORB = 6 of 71) quarters post dry-off and no quarters leaked at more than 1 monitoring time point. No correlation was found between milk leakage and intramammary infection on PF culture ($P = 0.67$), risk of clinical mastitis in the first 120 DIM ($P = 0.26$), and sealant weight at freshening ($P = 0.67$).

Sealant strip weight at colostrum collection

Sealant strip weights at colostrum collection ranged from 0 to 9.5 g. No difference by treatment was detected ($P = 0.15$) between ORB (mean 1.92g, SD ± 2.01) and SO (mean 2.26, SD ± 2.35). The linear mixed effects model revealed a negative correlation between sealant volume removed at colostrum collection and number of milkings required to achieve unenrollment (r = -0.86; $P < 0.0001$, 95% CI -0.9480, -0.6465) (Figure 4). However, no relationship was identified between pre-calving percent of cistern fill and PF infection status ($P = 0.2911$).

Sealant shedding during the fresh period

Figure 5 shows the persistence of sealant shedding in cows and teats that received a sealant score of either 4 or 5 over the first 10 days in milk. This figure demonstrates that, despite aggressive stripping of quarters at colostrum collection, approximately 54% of quarters (SO =51%, ORB = 58%) encompassing 90% of cows, had sealant present at the first milking after colostrum collection. The presence of sealant decreased during the first 10 days in milk; however, at day 10, 10% of cows still had at least 1 quarter shedding noticeable amounts of sealant (score 4 or 5). Additionally, 11.0% (13/118) of quarters had noticeable sealant flakes from 11-15 DIM while 3.3% (4/118) of quarters continued to shed sealant from 16-20 days post-calving (score 4 or 5, data not shown).

Figure 6 depicts number of milkings to unenrollment based on whether quarter did or did not have sealant present in the gland at pre-fresh imaging. Teats with sealant that had migrated into the gland shed noticible amounts of sealant for twice as long (mean 14.34 milkings, [95% CI 11.99, 17.16]) compared to quarters that did not have sealant in the gland (mean: 7.12, [95% CI 5.67, 8.95], $P < 0.001$).

During the first 120 DIM, 6.1% of the SO quarters developed clinical mastitis compared to 8.2% of ORB quarters (Table 2). Percent cistern fill was not associated with development of clinical mastitis in the first 120 DIM ($P = 0.7778$). There was no correlation between sealant present in the streak canal ($P = 0.91$, r = -0.09), teat cistern ($P = 0.91$, r = -0.20), or gland ($P = 0.89$, r= -0.08) on PC imaging and mastitis in the first 120 days.

Discussion

This work was part of a larger study for which cows outside the work cited here were assigned sealant treatment at the cow-level as compared to quarter-level. The outcome of that work indicated that the crude overall IMM infection prevalence at DO was 32.8% and 31.8% at PF. The new intramammary infection (NIMI) risk difference for SO treated quarters compared to ORB quarters (NIMI risk SO – NIMI risk ORB) was -1.60% [95% confidence interval (CI) -5.62, 2.42%], while the risk difference for CIMI difference was -0.83% [95% CI -1.96, 3.62%] for SO versus ORB.[14]

Figure 2: Loss to follow-up summary for cows treated with two internal teat sealants in a cross-udder design after application of a dry cow antimicrobial.

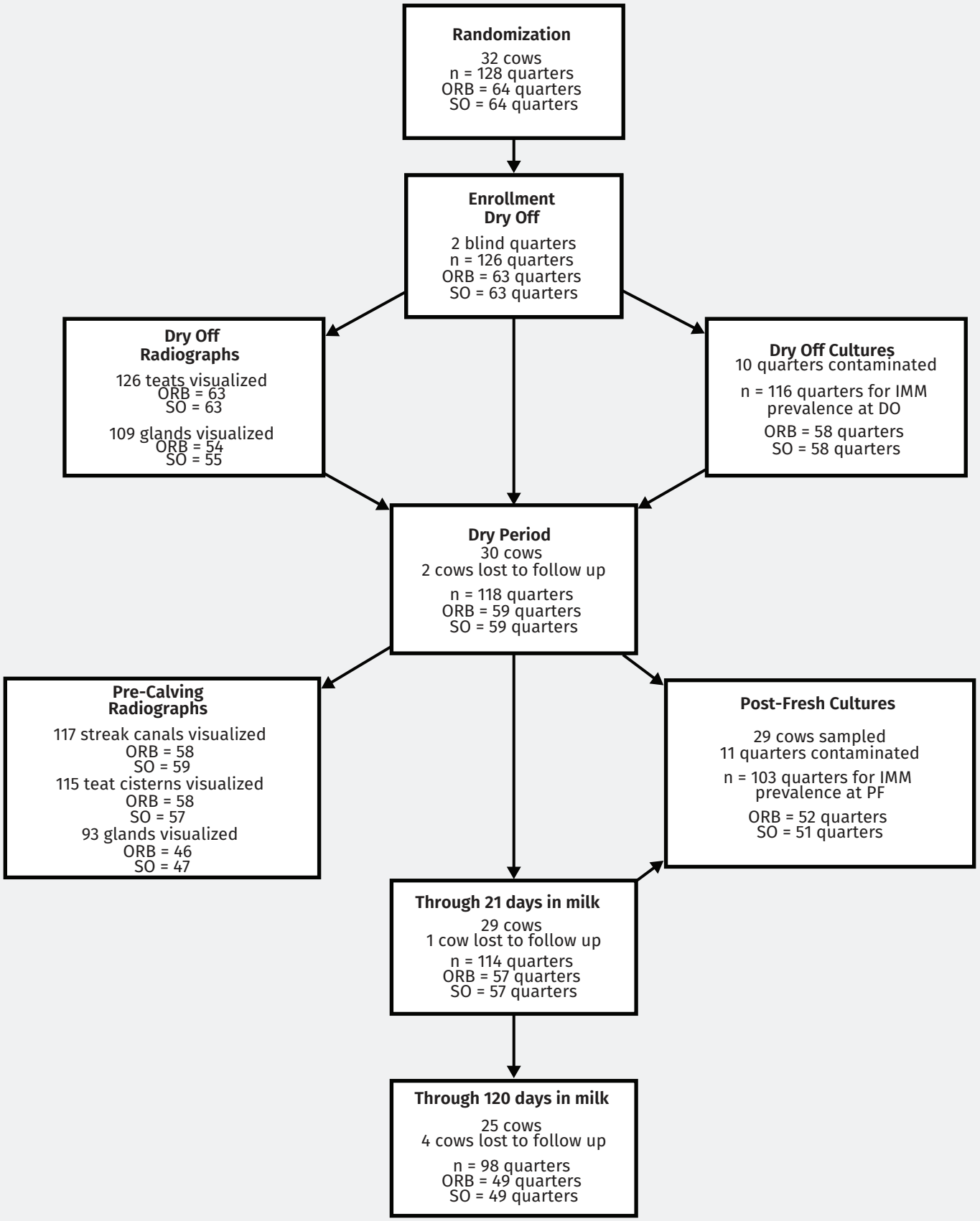


Table 2: Summary descriptive data for all enrolled quarters for cows treated with two internal teat sealants in a cross-udder design after application of a dry cow antimicrobial. As this was a descriptive study of sealant fractionation, statistical comparisons were limited.

	ShutOut®	Orbeseal®	Total
Cured IMI (CIMI)*	93.3% (14/15)	90.0% (9/10)	92.0% (23/25)
New IMI (NIMI)**	18.8% (9/48)	17.9% (7/39)	18.4% (16/87)
Sealant present in streak canal at DO	96.8% (61/63)	98.4% (62/63)	97.6% (123/126)
Sealant present in teat cistern at DO	98.4% (62/63)	98.4% (62/63)	98.4% (124/126)
Sealant present in gland at DO	23.6% (13/55)	18.5% (10/54)	21.1% (23/109)
Sealant present in streak canal at PC	23.7% (14/59)	22.4% (13/58)	23.1% (27/117)
Sealant present in teat cistern at PC	74.1% (43/58)	80.7% (46/57)	77.4% (89/115)
Sealant present in gland at PC	59.6% (28/47)	52.2% (24/46)	55.9% (52/93)
Average sealant strip weight (g)	2.3	1.9	2.1
Average milkings to unenrollment	10.8	10.8	10.8
Cases of clinical mastitis in first 120 DIM	6.1% (3/49)	8.2% (4/49)	7.1% (7/98)

DO – Dry-off

PC – Pre-calving

*Definition of a cured intramammary infection - a quarter with a confirmed IMI at DO which either cleared the infection on the PF sample, although it may have been infected with a different pathogen at that time point

** Definition of a new intramammary infection - a quarter with no infection at DO and positive for an IMI at PF sampling, or infected with a different pathogen at the DO and PF sampling

Figure 3: Comparison of sealant location from radiographs at dry-off (DO; grey) and at pre-fresh imaging (PF; red).

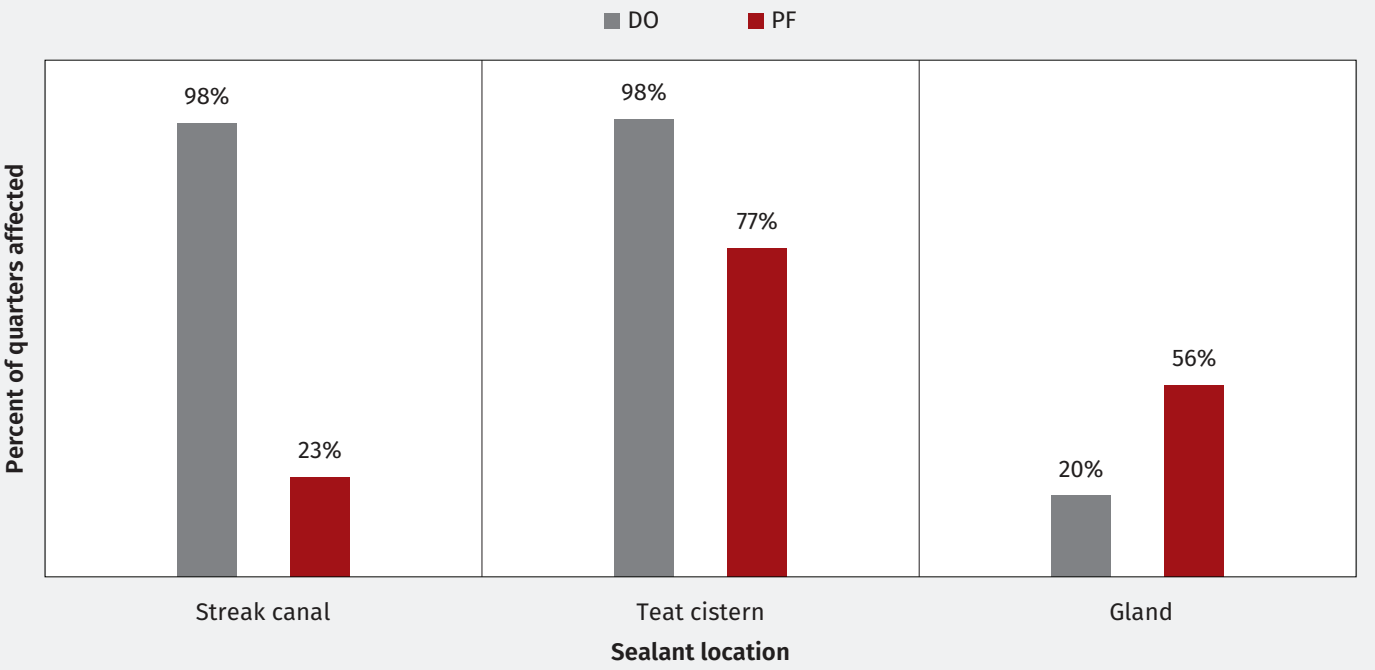
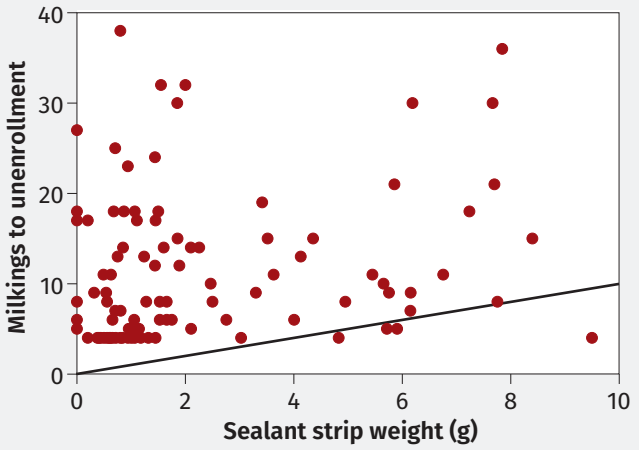
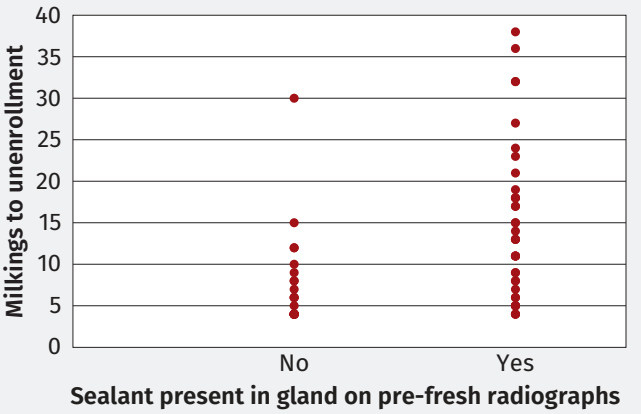


Figure 4: Sealant strip weight at colostrum collection compared with number of milkings required to achieve unenrollment criteria.* There was a correlation noted between amount of sealant collected and duration of sealant shedding (*P* = 0.0139).



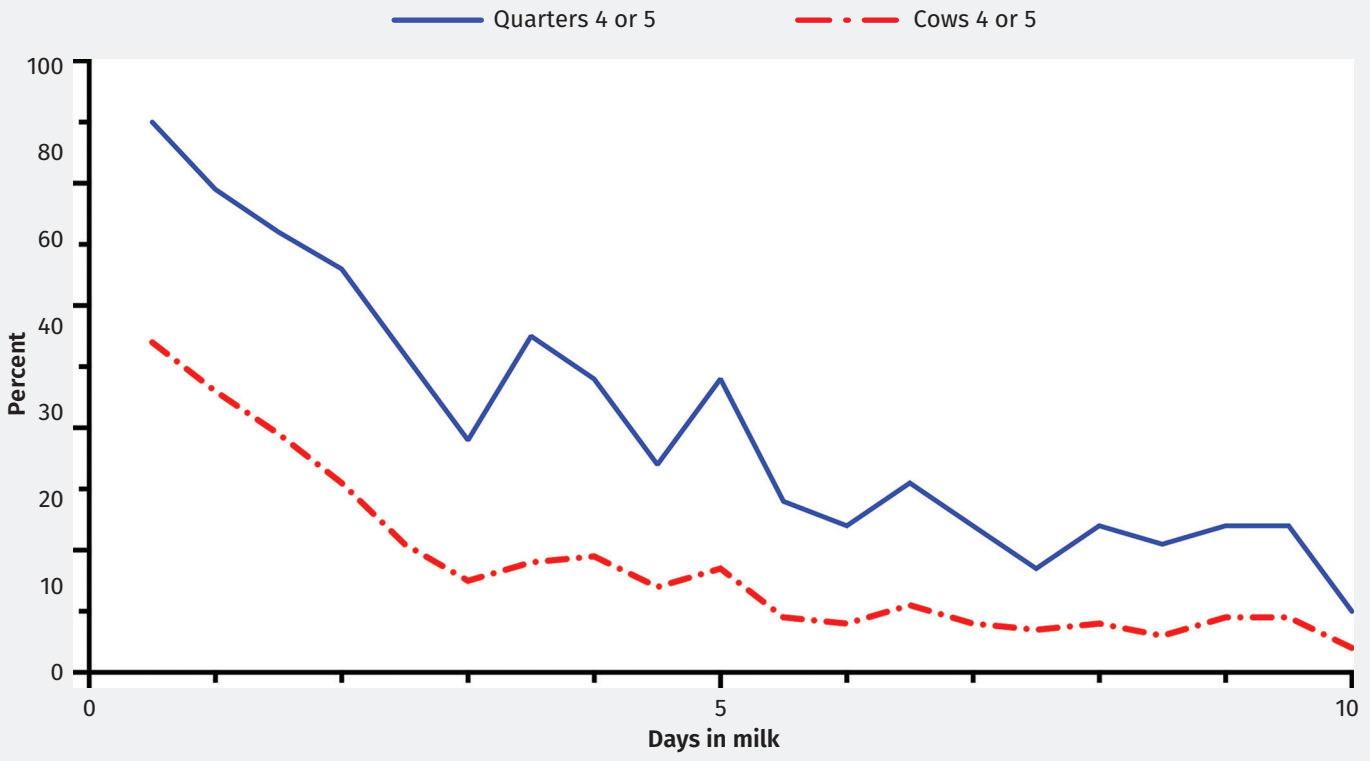
* Cows were unenrolled from the PC sealant shedding portion of the study if they: acquired a score of 1 for 2 consecutive milkings, OR had no score higher than a 3 for 6 consecutive milkings, OR reached 21 days in milk, OR were culled from the herd before any of the other criteria were reached. No cow was unenrolled before the 4th milking after calving.

Figure 6: Comparison of time to unenrollment based on whether sealant was present in the gland on pre-fresh imaging. Quarters with sealant present in the gland took an average of 14.34 milkings (95% CI 10.55, 18.1) to achieve unenrollment criteria* compared with quarters with no sealant present in the gland which required an average of 7.57 milkings (95% CI 3.36, 11.8).



* Cows were unenrolled from the PC sealant shedding portion of the study if they: acquired a score of 1 for 2 consecutive milkings, OR had no score higher than a 3 for 6 consecutive milkings, OR reached 21 days in milk, OR were culled from the herd before any of the other criteria were reached. No cow was unenrolled before the 4th milking after calving.

Figure 5: Percent of cows and quarters that had a sealant score of 4 or 5 over the first 10 days in milk when using a novel scoring system as described in Table 1.



Previous work looking at sealant behavior only characterized sealant location via radiography or shedding during the post-fresh period. The findings from the work reported here are consistent with Meaney's[12] description of a subset of the population demonstrating prolonged PF shedding and movement of the sealant based on radiographic findings. In the Meaney study, which used 7.5 g of 25% bismuth subnitrate, they radiographed the teats 1 time per week until the sealant was missing for 2 consecutive weeks. They determined that the sealant "remained as a unit and in position in the teats for 3 to 4 weeks after infusion." However, based on disappearance of the sealant on radiographic images and post-mortem findings on 2 of their trial cows, they suggested that the sealant migrated out of the teat after 3 to 4 weeks. The current study using 4 g per tube of 65% bismuth subnitrate noted migration of sealant into the gland in 52 of 93 quarters evaluated on pre-calving imaging that occurred 21-55 days dry.

Manufacturer's recommendations[19] for administration suggests that the base of the teat should be pinched off between the thumb and index finger while infusing the sealant to prevent the sealant from entering the gland cistern. These recommendations imply that failure to sequester sealant in the streak canal can lead to contamination of the gland with sealant causing persistence of sealant shedding in milk during early lactation. This work suggests that movement of sealant into the gland is not solely due to user error as sealant movement into the gland cistern was noted over the course of the dry period, despite proper administration technique as verified by dry-off radiographs. Our data also confirms the assumption that sealant localizing in the gland results in prolonged shedding during the PF period with affected quarters shedding sealant twice as long on average when compared to unaffected quarters.

The data generated by this project also demonstrates an indirect relationship between sealant volume removed at colostrum collection and duration of sealant shedding, however this is counterintuitive as one would expect that larger amounts of sealant removed initially would result in a shorter duration of shedding. One possible explanation for this relationship is that cows with more sealant present at colostrum collection may also have more total sealant present in the gland. It is also of note that more than 4 grams of material was harvested from several quarters at colostrum collection despite both sealant products only containing 4 grams of product. This phenomenon has also been previously described by Bradley et al., who suggested this may be due to an interaction between the teat sealant and foreign matter in the milk and the carrier for dry cow antimicrobial products.[20] Unfortunately, available product labeling is not sufficient regarding the carrier to determine if the carrier is similar between the dry cow antimicrobials used in the different studies. In a more recent study, Larsen et al.[21] also reported increased sealant weight and suggested that administration of ITS products containing bismuth subnitrate may result in a localized inflammatory response in which immune products may be incorporated into the sealant product. Further research needs to be completed to more completely understand the cause of increased sealant harvested after calving and the influence on its persistence into lactation.

This study found no correlation between amount of sealant present in the teat cistern and bacterial infection during the PF period or clinical mastitis within the first 120 DIM. The outcome of this work and previously published work[12,14] challenge the dogma that maintenance of ITS in the teat cistern throughout the dry period is a significant factor in preventing mastitis during the dry period. However, failure to do so likely contributes to contamination of the gland cistern with sealant and extended sealant shedding during the PF period.

While this study was able to provide new insights regarding teat sealant behavior, it is not without its limitations. A more regular and standardized schedule for radiography would allow for a more thorough evaluation of sealant behavior throughout the dry period instead of only 2 time points for each cow. This would also expand the data set for each time point and give more power for the assessment. The sample size for evaluation of milk leakage was also quite limited (12 quarters total) which may have limited our ability to accurately detect associations with sealant behavior. Additional work should be considered to focus on tracking sealant movement during the dry period as well as the effects of utilizing different volumes of ITS on udder health.

Internal teat sealant usage is described as an integral aspect of the success of selective dry cow therapy programs which can help to significantly decrease antimicrobial usage within the U.S. dairy industry. This study is the first known attempt at evaluating internal teat sealant behavior within the mammary gland during the dry and post-fresh period utilizing products currently marketed in the U.S. We believe that the technique utilized here, along with the availability of portable, digital radiographic equipment, could make these types of evaluations useful to dairy clients looking to improve compliance with recommended administration techniques. Based on the results from this study, we conclude that further research is needed to investigate the effects of changing the volume of sealant administered, altering the administration technique, and investigating products with different chemical characteristics that limit the movement of sealant into the gland. Decreasing sealant shedding may increase use of sealant products by dairy producers and help to prevent mastitis during the dry period, thereby decreasing antimicrobial use in dairy cattle.

Funding

This project was funded by Merck & Co., Inc., Rahway, NJ 07065.

Conflicts of interest

The study investigators (MPB, JB, GSS, and PJG) have no financial interest to declare. Co-authors TT and BEM are employees of Merck & Co.

Acknowledgements

The authors would like to thank the student workers who assisted in carrying out this study, as well as the staff of the veterinary diagnostic laboratories at both participating universities.

Author contributions

Author roles were: MPB was involved in labor coordination, fieldwork, laboratory work, data management, statistical analysis and manuscript preparation. JB was responsible for radiographic imaging, image evaluations and manuscript editing. TT and MEB were involved in study conceptualization, data capture and manuscript editing. GSS was involved in study conceptualization, statistical analysis and manuscript editing. PJG was involved in study conceptualization, supervision of fieldwork, data management, statistical analysis and manuscript editing.

Endnotes

[a]ShutOut®, Merck & Co., Inc., Rahway, NJ

[b]Orbeseal®, Zoetis, Inc, Parsipany, NJ

[c]Excel, Microsoft Corporation, Redmond, WA

[d]Orbenin DC®, Merck & Co, Madison, NJ

[e]Next II Digital Radiograph machine & TR9030 Sound Generator, Sound Technologies, Carlsbad, CA

[f]Ambic Equipment Limited, Witney, UK

[g]R Statistical Programing Environment, v.4.1.0, R Core Team (2022)

[h]GraphPad Prism, v 9.5.1 (2022)

[i]Falcon 50 mL High Clarity Conical Centrifuge Tubes, ThermoFisher Scientific, Waltham, MA

[j]Sorvall ST 16R Centrifuge, ThermoFisher Scientific, Waltham, MA

Abbreviations

IMI	Intramammary infection
ITS	Internal teat sealant
DO	Dry-off
PC	Pre-calving
PF	Post-fresh
ORB	Orbeseal®
SO	ShutOut®

References

1. Vasquez AK, Nydam DV, Foditsch C, et al. Use of a Culture-Independent On-Farm Algorithm to Guide the Use of Selective Dry-Cow Antibiotic Therapy. *J Dairy Sci.* 2018;101:5345-5361. **http://doi.org/10.3168/jds.2017-13807.**

2. Winder CB, Sargeant JM, Kelton DF, et al. Comparative Efficacy of Blanket Versus Selective Dry-Cow Therapy: A Systemic Review and Pairwise Meta-Analysis. *Anim Health Res Rev.* 2020;20:217-228. **http://doi.org/10.1017/S1466252319000306**.

3.vSmith KL, Todhunter DA, Schoenberger PS. Environmental Pathogens and Intramammary Infection During the Dry Period. *J Dairy Sci*. 1985;68:402-417. **http://doi.org/10.3168/jds.S0022-0302(85)80838-9**.

4. Dingwell RT, Leslie KE, Timms LL, Kelton DF, Schukken YH. The Importance of Teat Canal Closure on Other Risk Factors on Dry Period Intramammary Infections. Abstract in: *2003 Proc Am Assoc Bov Pract Conf.* 2003:161. **http://doi.org/10.21423/aabppro20035299.**

5. Huxley JN, Green MJ, Bradley AJ, Green LE. Evaluation of the Efficacy of an Internal Teat Sealer During the Dry Period. *J Dairy Sci*. 2002;85:551-561. **http://doi:10.3168/jds.S0022-0302(02)74108-8.**

6. Godden S, Rapnicki P, Stewart S, et al. Effectiveness of an Internal Teat Sealant in the Prevention of New Intramammary Infections During the Dry and Early-Lactation Periods in Dairy Cows when used with a Dry Cow Intramammary Antibiotic. *J Dairy Sci.* 2003;86:3899-3911. **http://doi:10.3168/jds.S0022-0302(03)73998-8.**

7. McParland S, Dillon PG, Flynn J, Ryan J, Arkins S, Kennedy A. Effect of using internal teat sealant with or without antibiotic therapy at dry-off on subsequent somatic cell count and milk production. *J Dairy Sci*. 2019;102:4464-4475. **http://doi.org/10.3168/jds.2018-15195.**

8. Notcovich S, Williamson NB, Flint S, Yapura J, Schukken YH, Heuer C. Effect of bismuth subnitrate on in vitro growth of major mastitis pathogens. *J Dairy Sci*. 2020;103:7249-7259. **http://doi.org/10.3168/jds.2019-17830**.

9. Berry EA, Hillerton JE. The Effect of Selective Dry Cow Treatment on New Intramammary Infections. *J Dairy Sci*. 2002;85:112-121. **http://doi.org/10.3168/jds.S0022-0302(02)74059-9.**

10. USDA. 2016. "Dairy 2014, Milk Quality, Milking Procedures, and Mastitis in the United States, 2014" USDA–APHIS–VS–CEAH–NAHMS. Fort Collins, CO, #704.0916.

11. Lay AM, Kolpin KM, Sommer DA, Rankin SA. Hot Topic: Black Spot Defect in Cheddar Cheese Linked to Intramammary Teat Sealant. *J Dairy Sci*. 2007;90:4938-4941. **http://doi.org/10.3168/jds.2007-0385.**

12. Meaney WJ. Effect of a Dry Period Teat Seal on Bovine Udder Infection. *Irish J Ag Res*. 1977;16: 293-299. **http://www.jstor.org/stable/25555880.**

13. Kabera F, Dufour S, Keefe G, Roy JP. An observational cohort study on persistency of internal teat sealant residues in milk after calving in dairy cows. *J Dairy Sci*. 2018;101:6399-6412. **http://doi:10.3168/jds.2017-13986.**

14. Buckley, MP, Bayne, J, Tomazi, T, Miller, BE, Godden, SM, Silva, GSS, Gorden, PJ. A randomized equivalence study evaluating the efficacy of two commercially available teat sealants in dairy cows. *Bov Pract*, 2023;57(2):36-50.

15. Wildman EE, Jones GM, Wagner PE, Bowman RI, Trout HF, Lesch TN. A dairy cow body condition scoring system and its relationship to selected production variables in high-producing Holstein dairy cattle. *J Dairy Sci.* 1982;65(3):495-501. **http://doi.org/10.3168/jds.S0022-0302(82)82223-6.**

16. Sprecher DJ, Hostetler DE, Kaneene JB. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. *Theriogenology*. 1997;47(6):1179-1187. **http://doi: 10.1016/S0093-691X(97)00098-8s**.

17. Greenland S, Pearce N. Statistical Foundations for Model-Based Adjustments. *Annu Rev Public Health*. 2015;36:89-108. **http://doi:10.1146/annurev-publhealth-031914-122559**.

18. MT, Neuhäser M, Ruxton GD. Effective us of Spearman's and Kendall's correlation coefficients for association between two measured traits. *Anim Behav,* 2015;102:77-84. **http://doi.org/10.1016/j.anbehav.2015.01.010**.

19. Orbeseal: Bismuth Subnitrate. Zoetis. Accessed 19 June 2022. **https://www.zoetisus.com/products/dairy/orbeseal-teat-sealant**.

20. Bradley AJ, Breen JE, Payne B, Williams P, Green MJ. The use of a cephalonium containing dry cow therapy and an internal teat sealant, both alone and in combination. *J Dairy Sci.* 2010;93:1566–1577. **http://doi: 10.3168/jds.2009-2725.**

21. Larsen LR, Baker PH, Enger KM, et al. Administration of internal teat sealant in primigravid dairy heifers at different times of gestation to prevent intramammary infections at calving. *J Dairy Sci*. 2021;104:12773-12784. **http://doi.org/10.3168/jds.2021-20819.**

