



Retention of internal teat sealants over the dry period and their efficacy in reducing clinical and subclinical mastitis at calving

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ABSTRACT

Internal teat sealants (ITS) reduce the risk of new intramammary infections over the dry period by forming a physical barrier to pathogen ingress. As the first and last 2 wk of the dry period are high-risk periods for new infections, maintaining an effective barrier in this period is a key requirement. Few studies have systematically examined sealant retention and none have done so under New Zealand pastoral conditions, where cows frequently move to separate grazing for dry periods, typically 80 to 90 d long. This multi-herd study was a split-udder equivalence trial comparing 2 ITS formulations for retention and efficacy in preventing periparturient clinical and subclinical mastitis. Both ITS contained 65% (2.6 g) bismuth salts, which contribute to the barrier within the teat canal, emulsified in ≤ 1.4 g of mineral oil. However, one ITS additionally contained $< 10\%$ amorphous silica. At dry-off, treatment was randomly allocated to diagonal teat-pairs within 409 cows on 4 farms. All cows met industry best practice criteria for ITS treatment alone. The study unit was quarter within cow and farm. Outcomes included clinical mastitis (CM) incidence for the last 7 d of the dry period and first 42 d of lactation, subclinical mastitis (SCM) incidence 96 h after calving, and quantity of residual after centrifuging 50 mL of colostrum collected from each quarter within 24 h of calving. Proportional outcomes were analyzed using Bayesian mixed models with a binomial distribution and logit link function, whereas the quantity of residual was analyzed using Bayesian finite mixture models and cluster bootstrapping. We set a region of probable equivalence (ROPE) of $\pm 2.5\%$ between proportions and ± 0.2 g for residual weight. Records were available for 1,596 quarters (399 cows). We detected no meaningful difference in inci-

dence of CM or SCM attributable to differences in sealant: the model predicted treatment differences of 0.00 with a 95% highest density interval (HDI) of $\pm 1.00\%$. Across all cows and farms, the marginal difference in the percentage of quarters with CM was 0.11% (95% HDI: -2.11 to 2.49%), and for SCM 0.00 (95% HDI: -1.98 to 1.94%). Including the quantity of residual recovered at calving did not improve fit or predictive ability of the models predicting CM or SCM, and the coefficient spanned the null value. The distribution of the weight of material recovered at calving was multi-modal; for 25% of quarters, more residual was recovered than inserted. When the residual weight was less than or equal to the median residual weight (2.06 g; range: 0.19–6.03 g), there was a $\geq 90\%$ probability that any treatment difference in residual was ≤ 0.2 g. When the residual weight was between the median and 75th percentile (4.40 g; 95% HDI: 4.00 to 4.75 g), there was no clear difference in residual between products. Above the 75th percentile, there was a 90% probability that the residual from quarters differed by product type (difference = 0.36 g, 90% HDI: 0.20 to 0.54 g). In conclusion, both products had equivalent efficacy for SCM and CM. As the quantity of residual increased, the difference in residual weight recovered increased but this may represent increases in debris rather than indicating a more effective barrier.

Key words: internal teat sealant, mastitis, dry period, cattle

INTRODUCTION

In pastoral dairy systems, the dry period is a high-risk time for establishment of new IMI and clinical mastitis (CM; Woolford et al., 1998), especially from environmental pathogens such as *Streptococcus uberis* (Compton et al., 2014). In herds in the Waikato region of New Zealand, 55% of cows and 18% of glands not treated with an antibiotic dry-cow therapy (ADCT) or internal teat sealant (ITS) at the end of lactation devel-

Received November 15, 2021.

Accepted February 10, 2022.

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oped a new IMI over the dry period (McDougall, 2010). In a further study in the Canterbury-Otago region of New Zealand, cows with no history of elevated SCC or CM and not treated with ADCT or ITS at drying-off had twice the incidence of CM between drying-off and 84 d after calving and 1.5 times the prevalence of elevated SCC ($\geq 200,000$ cells/mL at the first herd test after calving) compared with treated cows (Bates and Saldias, 2018).

Some form of dry-cow therapy is thus part of dairy industry standard recommendations (Dairy NZ, 2012). For cows with a low risk of IMI at dry-off, 2 meta-analyses have shown that the use of ITS alone is equivalent or superior to no treatment or the use of ADCT alone (Rabiee and Lean, 2013; Dufour et al., 2019). Consequently, as part of efforts to reduce antimicrobial usage on dairy farms in New Zealand, ADCT is frequently restricted to cows with evidence of an IMI, with uninfected cows receiving ITS alone (New Zealand Veterinary Association, 2015; Dairy NZ, 2019).

With increasing reliance on ITS alone, persistence of the ITS barrier is perceived as an important metric of efficacy by farmers and veterinarians. Few studies have evaluated the persistency of ITS over the dry period and the proportion of quarters at calving where the sealant barrier is still present. In confinement systems, Kabera et al. (2018) reported that ITS was present at calving in 83% of quarters treated with ITS alone at dry-off. Similarly to Bradley et al. (2010), these researchers found that retention was reduced in cows receiving both ADCT and ITS and in front quarters compared with rear quarters but was unaffected by length of dry period (up to 75 d) or level of milk production on the day of dry-off. No studies have been published on ITS retention under pastoral, seasonal dairy farming conditions in which drying-off is used as a strategic tool to manage body condition and pasture cover and dry periods are frequently ≥ 90 d (McDougall, 2010; Bryan et al., 2011; Bates and Dohoo, 2016). Moreover, within these systems, cows calve in large groups at pasture and may remain within the group after calving for up to 24 h before milking. This increases the risk of ITS loss from calf suckling and milk leakage.

All teat sealants marketed within New Zealand contain 65% (2.6 g) bismuth salts, which contribute to the barrier within the teat canal, emulsified in ≤ 1.4 g of mineral oil. However, one product (Teatseal; Zoetis Animal Health Ltd.) additionally contains $< 10\%$ amorphous silica. Differences in formulation are apparent as differences in viscosity at usage. This raises questions about the effect of formulation on retention and what is the most appropriate formulation for New Zealand's farming systems. Using commercial farms under New Zealand pastoral conditions, the present study was

designed as an equivalence study to identify risk factors for the retention and efficacy in preventing periparturient clinical and subclinical mastitis of 2 brands of ITS differing in their formulation and viscosity, as represented by Duraseal (Norbrook Laboratories Ltd.) and Teatseal.

MATERIALS AND METHODS

Animal Use

The trial was conducted between May 2020 and February 2021 on a convenience sample of 4 commercial, spring-calving (July to October) pastoral dairy farms serviced by Vetlife Ltd., New Zealand. All procedures were preapproved by Massey University Animal Ethics Committee, Palmerston North, New Zealand (approval reference 20/28).

Housing and Management

The main feed source for milking cows was grazed ryegrass (*Lolium perenne*) pasture with supplementary grass silage at 2 to 10 kg/cow per day and 1 to 2 kg/cow per day of milled wheat (*Triticum aestivum*) depending on stocking rate and pasture supply.

Over winter, cows were managed on separate grazing blocks of fodder beet (*Beta vulgaris*) with ad libitum access to ryegrass straw without mineral supplementation. Two to 3 wk before calving, cows returned to the milking platform and were fed a grass-based diet supplemented with 60 g of $MgCl_2$, 60 g of MgO , and 50 g of $CaHPO_4$ /cow per day mixed into perennial ryegrass silage and the same mineralized water supply as before drying off. All cows calved at pasture in groups of 50 to 200 from July 23 to October 1, 2020. Fresh-calved cows were separated from this group once daily and milked, although calves were picked up multiple times per day.

Enrollment

All farms had electronic animal identification and automatic drafting facilities at the milking shed (Pro-track; MSD/Merck) and recorded cases of CM and their treatment in the national electronic database (Livestock Improvement Corp.). On each farm, the sample frame consisted of mixed-aged cows (4–8 yr) due for dry-off between May 15 and May 31, 2020, expected to calve within ± 7 d of the mid-point of calving (August 11, 2020), with no record of CM in the preceding lactation and an SCC $\leq 150,000$ cells/mL recorded ≤ 42 d before dry-off date and so conforming to industry best practice guidelines for treatment at drying off with ITS alone (Dairy NZ, 2012).

Experimental Groups

On each farm, the sample frame was drawn up before dry-off using the herd's computerized records to identify cows meeting the selection criteria. The sample was randomly drawn from the sample frame by selecting every $n/100$ th cow where n was the number of animals in the sample frame. These cows (with 10% surplus for misidentification, misdrafting, or exclusion) were then drafted at dry-off for the trial.

Exclusions

Any cows with rough or very rough teat ends using the field scoring system suggested by Mein et al. (2001), signs of clinical disease [lack of ruminal fill, abnormal milk, changes in the quarter (swelling or redness) or milk (clots, watery milk, and changes in consistency; Lago et al., 2011)], or with fewer than 4 functional quarters were excluded. After the last milking, all enrolled quarters were subjected to a rapid mastitis test (RMT; Shoof Direct NZ) and the results recorded on a scale of 0 to 4, where 0 = no reaction, 1 = trace reaction, 2 = weak positive, 3 = distinct positive, and 4 = strong positive (Bhutto et al., 2012). Cows with any quarters scoring ≥ 3 were excluded, and an aseptic milk sample (>5 mL) was collected from any quarter with a score of 1 or 2 into a clean polyethylene sample vial. These were stored at 0 to 4°C and transported to the practice laboratory for culture and antimicrobial sensitivity testing within 24 h.

Administration of Product

Immediately after RMT, teats of eligible enrolled cows were individually sanitized with cotton wool impregnated with 70% ethanol before treatment with the appropriate ITS. Sealant was administered by veterinary technicians and veterinarians employed by Vetlife Ltd. All farm staff were blinded to treatment status but there was no blinding to treatment among those administering the products. Cows were treated in the farm's milking shed following industry standard best practice guidelines, including insertion of ≤ 2 mm of the tip of the intramammary syringe into the teat canula and occlusion of the teat canal proximal to the udder to maximize sealant placement within the teat canal. All staff undertook training according to the Vetlife standard operating procedure for the administration of internal sealants and were under veterinary supervision. After insertion, all quarters were sprayed with the normal teat spray for that herd, and cows exited the milking shed and had access to clean pasture for at least 30 min.

Treatment was assigned at the quarter level using a split-udder model within the same cow. Within each cow, product A (Duraseal) was applied to a diagonal pair of quarters (one front and one back quarter), and product B (Teatseal) was applied to the diagonally opposite pair of quarters. For each cow, all staff inserted ITS in the same order: front left, front right, back right, and back left. Four intramammary syringes of sealant were provided for each cow and were preloaded into a clip worn by the operator. For each cow, the 2 products (A and B) were used according to 1 of 2 ordering options: either A:B:A:B or B:A:B:A. This was determined by a randomized list of ordering options (Excel, Microsoft Corp.) and facilitated administration in the appropriate but randomized order to each diagonal pair of quarters.

Collection of Data and Laboratory Procedures

Clinical and Subclinical Mastitis Monitoring.

Electronic treatment records for CM were accessed via the national Livestock Improvement Corp. database and included affected cow identity, age, breed, calving date, diagnosis date, and, for CM, quarter affected.

During the dry period, cows were observed daily for signs of inappetence, udder enlargement, or discoloration. However, lack of robust handling facilities at the winter grazing blocks meant that accurate attribution of CM to quarters was not reliable with treatment administered systemically to the affected animal. Consequently, CM diagnosed during this period did not form part of the study outcomes. However, on return to the milking platform before calving, all cows were observed twice daily through the milking shed. Clinical mastitis was diagnosed by farm staff trained in detection of CM using changes in the quarter (swelling or redness) or milk (clots, watery milk, and changes in consistency; Lago et al., 2011). All cases of CM diagnosed in enrolled cows from 7 d before to 42 d after calving were recorded for the study.

At the eighth milking, 96 h after calving, all quarters were subject to an RMT, and an aseptic milk sample was collected from any quarter scoring ≥ 1 , indicating subclinical mastitis (SCM).

All milk samples were stored on farm at 0 to 4°C before collection within 24 h by visiting veterinary technicians for bacterial identification and antimicrobial sensitivity testing at the practice laboratory. All milk samples were analyzed using the Mastatest system (Mastaplex Ltd., <https://www.mastaplex.com/mastatest>). This is a diagnostic system for bovine mastitis that indicates the pathogen and its *in vitro* antibiotic sensitivity using the microdilution technique (Jones et al., 2019); further details can be found in Supplemental Tables S1–S6,

Figures S1–S4, and File S1 (<https://data.mendeley.com/datasets/hss6cbfz4w/2>; Bates, 2022).

Teat Sealant Recovery. The weight of sealant (g) recovered immediately after expulsion from 33 tubes of Teatseal and 33 tubes of Duraseal was recorded. This represented the weight of sealant administered to each quarter. In addition, the weight of residue was recorded from 66 milk samples (50 mL per sample), half of which had been individually mixed with a tube of Teatseal and half individually mixed with a tube of Duraseal before being centrifuged at $3,000 \times g$ for 5 min. This represented the weight of sealant plus extraneous residue (Bradley et al., 2010).

At the first milking and within 24 h of calving, veterinary technicians collected 50 mL of colostrum from each quarter of all enrolled cows. All personnel were instructed to include all foremilk, and all samples were frozen and transported once per week to the Department of Microbiology and Immunology, University of Otago (Dunedin, New Zealand).

Following the procedure reported by Bradley et al. (2010), colostrum samples were thawed before centrifugation at $3,000 \times g$ for 5 min. The supernatant was decanted, leaving the remnants of teat sealant and other solids in the base of the tube. Tubes were inverted and allowed to drain fully before weighing to establish the mass of retrieved solids.

Statistical Analysis

Details of the sample size calculation for the dependent variables using Bayesian simulation are given in Supplemental Table S1 and Supplemental Figures S1 and S2 (Bates, 2022).

For each quarter, the presence of farmer-diagnosed CM from 7 d before to 42 d after calving and the occurrence of quarters with an RMT score ≥ 1 at the eighth milking after calving were recorded as categorical binomial variables, indicating presence or absence in each quarter. The distribution of the weight (g) of sealant administered per intramammary tube and the weight of sealant plus milk residue recovered immediately after mixing milk and sealant were skewed (skew factors -0.43 and 0.33 , respectively), and medians and 95% highest density intervals (HDI; Kruschke, 2018; Makowski et al., 2019) are presented. The distribution of the weight (g) of material recovered at calving from each quarter infused at dry-off with a single tube of either sealant was right skewed (skew factor 0.57) and multimodally distributed. Quarters from which the weight of material recovered within 24 h of calving was less than the median weight of product infused at dry-off and the occurrence of quarters where the weight of recovered material was less than the median weight of

residue plus debris were recorded as categorical binomial variables.

For all models, variables at the quarter level were treatment group and front or back quarter. For the occurrence of CM and SCM, 2 alternative models were constructed, one where treatment group was included and one where it was replaced by the weight of material recovered from each quarter at calving. These 2 variables were not included together because we suspected that residual weight might be an intermediary variable in any pathway between sealant type and disease risk. The possibility of cow-level variables acting as confounders or colliders (Janszky et al., 2010; Sjölander and Zetterqvist, 2017) was assessed before the study using directed acyclic graphs and reduced by the split-udder design. A full list of the cow level variables is included in Supplemental Table S3 (Bates, 2022), but none were incorporated into the final multivariable models.

The aim of the study was to establish that the clinical outcomes (cases of quarter mastitis from 7 d before calving to 42 d after calving), RMT score at 4 d after calving, and weight of residual recovered from quarters were not different (within an acceptable margin) between the 2 treatment groups. In this context, the “acceptable margin” was defined by the investigators as that difference between the treatment groups that was effectively still zero, when contextualized against the range of factors that can affect the outcome and the lack of biological significance in small differences. This range around a mean difference of zero has been defined as the region of probable equivalence (ROPE; Kruschke, 2018). Thus, if the mean difference—and the associated probability interval (PI) for that difference—between product A and product B falls within the ROPE, we can be confident (to that level of probability) that the difference is effectively zero. For ease of comparison with frequentist statistics, we have chosen the 95% HDI as the PI best expressing uncertainty about our estimation of parameters.

We defined a ROPE for each dependent variable, based on previously published data (detailed within Supplemental Tables S1 and S2; Bates, 2022) on the incidence rate of mastitis and sealant retention. For comparison of the binomial dependent variables, we expressed these as percentages and used a ROPE of $\pm 2.5\%$ for equivalence; for comparison of residual sealant weight, we used a ROPE of ± 0.2 g for equivalence.

To model the prevalence of CM and SCM, we used a mixed multivariable model with a binomial distribution and a logit link function, and a random intercept for cow and farm to account for clustering of quarters within cows and cows within farms. Comparison of residual sealant weight recovered for each product, from

the injectors and from milk samples immediately after mixing with sealant, was made by bootstrapping using 1,000 samples, repeated 100 times. A finite mixture model with a random intercept for cow and farm was used to quantify the effect of product on the residue weight recovered at calving, and the distribution of difference in weight of material recovered at calving across the range of values of residue recovered was investigated using clustered bootstrapping with 1,000 samples repeated 100 times (Huang, 2018).

The percentage of quarters where the weight of material recovered within 24 h of calving was less than the median weight of product infused at dry-off was compared between sealant types using a mixed multivariable model with a binomial distribution and a logit link function, and a random intercept for cow and farm. This was repeated for the percentage of quarters for which the weight of material recovered at calving was less than the median weight of residue plus debris.

Structural control of cow-level confounders from the design of the experiment meant fixed variable selection was restricted to the 2 categorical quarter-level variables: treatment and quarter (front or back). The experimental design also ensured there was no uneven distribution between quarter position and sealant type; therefore, quarter front or back could not be a confounder for the effect of sealant type on the outcome (Aly et al., 2010). Competing models were assessed in terms of their widely applicable information criteria (WAIC; Watanabe, 2010). Starting from a model containing both fixed variables (and with random effects for cow and farm), predictor variables were retained if their coefficient values had 95% HDI excluding the null or if the WAIC for the full model was closer to zero and the addition of the variable made biological sense. Once the most parsimonious model had been established, the effects of any 2-way interactions on the WAIC and coefficient estimates were assessed.

A variety of distributions were used within the finite mixture model to represent pellet weight, including normal, log-normal, skew-normal, and gamma. The number of latent classes and their distributions are set by the investigator although how the data are partitioned between the latent classes is determined by maximizing the fit of the model. We adopted a conservative approach to the number of possible latent classes to avoid over-fitting and to reduce the risk that a small number of data points were used to parameterize a distribution. Within this constraint, we compared models combining gamma and normal distributions against models combining multiple normal distributions. Values for the WAIC, parsimony in terms of the number of distributions, and ease of interpretation guided final distribu-

tion choice. Details of the priors and model equations are given in the statistical analysis (Supplemental File S1; Bates, 2022).

All models were fit using the Bayesian regression “brms” package (Bürkner, 2017) and implemented in R (R Core Team, 2013). For all models, the effective sample size was 40,000 and convergence of 4 Monte Carlo Markov chains, autocorrelation, and comparison of the observed data with a random sample of replicates were assessed visually (Gelman et al., 2004). Values of the Gelman and Rubin potential scale statistic (maximum tolerated value 1.1) and the standard error of the mean of the posterior draws (maximum tolerated value 10% of the posterior standard deviation) were assessed (Brooks and Gelman, 1998). The assumptions inherent in regression were assessed using standardized residual plots (Hartig, 2020) for homoskedasticity, linearity, normality, and under- and over-dispersion.

For the final models, sensitivity to priors was assessed by comparing coefficient values and WAIC when the model was rerun with regularizing priors [Normal (0,1)] for estimating the coefficients. Further, as all models involved a nonlinear link function and hierarchical priors, we ran simulations for the induced priors to confirm all potential prior values were within expected ranges (Seaman et al., 2012).

In cluster bootstrapping, the standard bootstrapping procedure with regard to the resampling process is slightly modified. Instead of drawing a random sample of n observations from each treatment group, for cluster bootstrapping, the level of sampling is set at the lowest cluster level. For this data set with n cows in total, this equates to $n/2$ “front quarter” or “back quarter” pairs (1 left quarter and 1 right quarter in each pair) within cow, within farm. The first step is to randomly select $n/2$ of these clusters with replacement (Davison and Hinkley, 1997). For each cluster selected (with some clusters selected more than once and others not selected at all), all observations within that cluster (one pellet weight for each product) are included in the bootstrapped sample. Then the desired statistics (median or other quartile) by treatment group are computed using the bootstrapped sample and the process is repeated (in our case) 1,000 times.

RESULTS

Teat sealant was administered at dry-off to 1,636 quarters from 409 eligible cows on 4 farms. One cow aborted during the dry period and failed to develop an udder, whereas 9 trial cows were not identified for colostrum sampling within 24 h of calving (3 from farm A, 4 from farm C, and 2 from farm D). These 10 cows

were excluded from further analysis. Summary statistics by farm are presented in Supplemental Table S3 (Bates, 2022).

Percentage of Quarters with CM

From 7 d before to 42 d after calving, there were 35 cases of CM from 1,596 quarters = 2.19% (exact binomial confidence interval: 1.53 to 3.04%). There were 17 cases in 799 quarters treated with Teatseal (2.13%; 95% HDI: 1.24 to 3.38%) and 18 cases in 797 quarters treated with Duraseal (2.26%; 95% HDI: 1.34 to 3.55%). At the univariate level, the data supported no difference in relative risk of CM in quarters infused with Teatseal compared with Duraseal (relative risk = 0.94; 95% HDI: 0.49 to 1.81).

After assessment for confounding, the final variables in the model were farm, cow, and treatment group with no 2-way interactions (Supplemental Table S4; Bates, 2022). The differences in WAIC between fixed and mixed models were all small, but the WAIC for the mixed model was closer to zero. The coefficient for the weight of residue recovered at calving spanned the null value whether as a linear, smooth spline, or categorical variable, and inclusion did not lower the model's WAIC. The final model explained 27% of the variance in the data (95% PI: 0.14 to 0.40).

The intraclass correlation (ICC) was estimated through simulation (Goldstein et al., 2002). For the intercept-only model, ICC = 0.02 for the correlation between farms and 0.25 between cows, reflecting that of 35 quarter cases, 14 were contemporaneous cases within 4 cows. All post hoc assessments of model diagnostics, posterior predictive checks, assessment of residuals, and sensitivity to prior formulation were satisfactory.

From the mixed logistic regression model, the estimated difference in probability of CM in a quarter 7 d before to 42 d after calving resulting solely from the difference between Duraseal and Teatseal was 0.00% (95% HDI: -0.74 to 0.92%). Averaging the effect across all cows and farms, the marginal mean predicted probability for CM in quarters treated with Duraseal was 2.43% (95% HDI: 0.00 to 10.06%) and 2.31% (95% HDI: 0.00 to 10.00%) if treated with Teatseal, giving a marginal difference of 0.12% (95% HDI: -2.15 to 2.49%). So, 95% of the most likely values for the difference were within the ROPE, indicating no meaningful difference in risk of CM between products.

Percentage of Quarters with SCM at Eighth Milking

At the eighth milking after calving, there were 24 cases of SCM from 1,596 quarters, or 1.50% (exact binomial confidence interval: 0.97–2.22%). There were 12

cases in 799 quarters treated with Teatseal (1.50; 95% HDI: 0.78 to 2.61%) and 12 cases in 797 quarters treated with Duraseal (1.51; 95% HDI: 0.78 to 2.62%). At the univariate level, the data supported no difference in the relative risk of SCM in quarters infused with Teatseal compared with quarters infused with Duraseal (relative risk = 1.00; 95% HDI: 0.45 to 2.21).

Independent variables retained and model structure were identical to the model for CM (Supplemental Table S5; Bates, 2022). The final model explained 31% of the variance in the data (95% PI: 0.14 to 0.44). The ICC estimated through simulation indicated zero correlation between farms and 0.28 between cows, reflecting the fact that of the 24 quarter cases, 10 were contemporaneous cases within 3 cows. All post hoc assessments of model diagnostics, posterior predictive checks, assessment of residuals, and sensitivity to prior formulation were satisfactory.

From the mixed logistic regression model, the estimated difference in probability of SCM in a quarter at 8 milkings after calving resulting solely from the difference between Duraseal and Teatseal was 0.00 (95% HDI: -0.20 to 0.20%). Averaging the effect across all cows and farms, the marginal mean predicted probability for SCM in quarters treated with Duraseal was 1.65% (95% HDI: 0.00 to 6.88%) and that for Teatseal was 1.65% (95% HDI: 0.00 to 6.87%), giving a marginal difference of 0.00 (95% HDI: -1.98 to 1.94%). So, 95% of the most likely values for the difference were within the ROPE, indicating no difference risk of SCM between products.

Bacteriology Results

The results of bacterial identification from samples collected from quarters diagnosed with SCM at sealant insertion (dry-off) or at the eighth milking after calving are shown in Table 1. Given the small number of positive cultures, no further analysis was undertaken.

Sealant Recovery

The expected weight of sealant per intramammary tube was 4 g (2.6 g of bismuth subnitrate plus 1.4 g of mineral oil for Duraseal; 2.6 g of bismuth subnitrate, <0.4 g of amorphous silica, and 0.8–1.2 g of mineral oil for Teatseal). Bootstrapped estimates for the median weight of sealant recovered after expulsion from the tubes was 3.96 g (95% HDI: 3.85 to 4.09 g) for Teatseal (n = 33), and 4.08 g (95% HDI: 3.90 to 4.16 g) for Duraseal (n = 33), with a median difference of 0.11 g (95% HDI: 0.08 to 0.14 g), entirely within the ROPE of 0.00 ± 0.20 g, and thus, for the purposes of this study, effectively zero.

Table 1. Bacteriology results from quarter samples testing positive with the rapid mastitis test (RMT) either at dry-off (sealant insertion) or at the eighth milking (96 h) after calving¹

Treatment group	Bacteriology from quarters with SCM ² at sealant insertion				
	No growth	Coliforms	<i>Streptococcus uberis</i>	Other streptococci	CNS
Duraseal	3	0	0	2	0
Teatseal	2	0	1	3	2
Treatment group	Bacteriology from quarters with SCM at milking 8 (96 h) after calving				
	No growth	Coliforms	<i>Streptococcus uberis</i>	Other streptococci	CNS
Duraseal	8	0	2	2	0
Teatseal	8	1	3	0	0

¹Quarters were infused at dry-off with a single syringe of Duraseal (Norbrook Laboratories Ltd.) or Teatseal (Zoetis Animal Health Ltd.).

²Subclinical mastitis.

The median weight of residue recovered from individual milk samples ($n = 33$) from cows that had been infused with a tube of Teatseal was 4.59 g (95% HDI: 4.10 to 4.98 g) and 4.46 g (95% HDI: 4.08 to 4.77 g) for milk samples ($n = 33$) infused with one tube of Duraseal. The median difference was 0.13 g (95% HDI: 0.02 to 0.27 g), with the difference being >0.2 g 17% of the time.

Initial summary statistics for the weight of residue recovered from quarters within 24 h of calving are presented in Table 2. Our preliminary data suggested there was no difference between products in the weight of sealant administered. Consequently, where the weight of material recovered at calving was <4.00 g, we can be confident that some sealant is missing. However, sealant migration away from the teat canal or expulsion from the udder can coexist with accumulation of debris. We cannot assume the difference represents only loss of sealant. Similarly, where the quantity of residue recovered was >4 g, we cannot assume this means no sealant loss, as debris accumulation could be in excess of sealant loss. Accordingly, we analyzed the weight of residue on an “as is” basis, without subtraction of a

nominal quantity to represent sealant administered or sealant plus debris.

The distribution of the recovered weights is shown in Figure 1. By simple bootstrapping, the median weight recovered for quarters infused with Teatseal was 2.07 g (95% HDI: 0.19 to 6.15 g) and with Duraseal 2.05 g (95% HDI: 0.19 to 5.90 g). The unadjusted, crude difference in the median weight of the residue recovered by product was 0.02 g (95% HDI: -0.30 to 0.45 g).

After assessment for confounding, the final variables in the model were farm and cow as random effects, with fixed effects for treatment group and front or back quarter and no 2-way interactions (Supplemental Table S6; Bates, 2022). All finite mixture models tested had a WAIC $>10\%$ lower than any of the unimodal models. The final finite mixture model was based on 3 Normal distributions; further details are given in the statistical analysis (Supplemental File S1; Bates, 2022). The ICC was estimated using the variance decomposition method to estimate the ratio between the variance of the posterior draws not conditional on the group-level terms (farm and cow) relative to the variance of the posterior draws conditional on the group-level terms.

Table 2. Summary statistics for the weight (g) of residue recovered within 24 h of calving from quarters infused with teat sealant at dry-off¹

Group	Number	Mean	SD	Median	25th percentile	75th percentile	Range (minimum–maximum)
No grouping	1,596	2.64	1.93	2.06	0.89	4.70	0.19–8.20
Farm							
A	388	2.90	1.88	2.64	1.08	4.75	0.27–6.61
B	400	2.22	2.06	1.18	0.54	4.06	0.19–7.99
C	392	2.62	1.95	1.96	0.88	4.11	0.19–8.07
D	416	2.82	1.76	2.39	1.27	4.17	0.35–8.21
Quarter							
Back	798	2.74	2.01	2.22	0.88	4.50	0.19–8.07
Front	798	2.53	1.84	1.92	0.92	4.00	0.23–8.21
Sealant							
Duraseal	798	2.57	1.86	2.05	0.91	4.11	0.19–7.94
Teatseal	798	2.70	2.00	2.07	0.88	4.50	0.19–8.21

¹Quarters were infused at dry-off with a single syringe of Duraseal (Norbrook Laboratories Ltd.) or Teatseal (Zoetis Animal Health Ltd.).

For the intercept-only model, the ICC for cow was 0.11 (95% PI: 0.04 to 0.18) and for farm 0.01 (95% PI: 0.01 to 0.13), indicating little effect of clustering for the weight of residue material recovered. All post hoc assessments of model diagnostics, posterior predictive checks, assessment of residuals, and sensitivity to prior formulation were satisfactory.

The finite mixed model separated the data into 3 latent classes, each with an average posterior predicted weight of residual sealant recovered per quarter across all farms and cows, adjusted for sealant type and front versus back quarters. For each latent class, the intercepts and their predictive intervals in Supplemental Table S6 (Bates, 2022) represent the mean values and their 95% HDI for each of these latent classes and are, respectively, the 24th, 44th, and 72th percentiles of the distribution of residual sealant weight.

Graphically, the effect of treatment on the weight of residue recovered is shown in Figure 2. For the first latent class, the extra weight recovered from quarters treated with Teatseal was 0.00 g (95% HDI: -0.04 to 0.05 g), which lies entirely within the ROPE of ± 0.2 g; thus, we are $>95\%$ confident that there was no meaningful difference between the 2 products. For the second latent class, the extra weight recovered was 0.07 g (95% HDI: -0.10 to 0.25 g). In this case, we can only be 90% confident of no meaningful difference (90% HDI: -0.08 to 0.20 g). For the third latent class, the extra weight was 0.36 g (95% HDI: 0.16 to 0.56 g). In this case, we are 90% confident (90% HDI: 0.20 to 0.54 g) that the weight of residue recovered from Teatseal quarters was >0.2 g more than that recovered from Duraseal quarters. The effect of quarter position (front vs. back)

and farm are discussed in Supplemental Figures S3 and S4 (Bates, 2022).

Bootstrapping was used to estimate the distribution of the difference in residue weight over the range of residue weight recovered, allowing for clustering within front and back quarters, cows, and farms.

Quantiles of the difference were estimated across the distribution of residue weights to determine where changes in the difference in residue recovered occurred. The results of this analysis are presented in Figure 3.

The pattern of difference in residue weights across the distribution of residue weights was very similar to the results from the finite mixture model, with 95% (vs. 90%) of the HDI for the 44th percentile lying entirely within the ROPE. The residue weight at which 90% and 75% of differences exceeded a ROPE of ± 0.2 g is illustrated in Figure 4.

Figure 4 suggests that for the lower 50% of the distribution of residue weights (up to a mean weight of 2.10 g), the probability that the absolute difference in residue weight between quarters infused with Teatseal and quarters infused with Duraseal is less than 0.2 g is $\geq 90\%$. Correspondingly, for a further 12% of samples (where the mean residue weight is $> 2.10 \leq 3.20$ g), the probability that the absolute difference in residue weights is less than 0.2 g is $\geq 75\%$. Above the 62nd percentile, the difference in residual was greater and the probability that it was within the ROPE decreased: above the 75th percentile, there was a 90% probability that the residual from Teatseal quarters was greater than that for Duraseal quarters (difference = 0.36 g; 90% HDI: 0.20 to 0.54 g).

For quarters infused with Teatseal, the percentage where less material was recovered at calving than was infused at dry-off was 70.0% (95% HDI: 67.0 to 73.0%), whereas for quarters infused with Duraseal, the equivalent figure was 74.0% (95% HDI: 71.0 to 77.0%). The median unadjusted difference in the percentage of quarters where the amount of material recovered at calving was less than that infused at dry-off was -5.0% (95% HDI: -8.0 to -1.0%). In contrast, the Bayesian regression model predicted that the subject-specific difference due to treatment alone would be -5.0% (95% HDI: -9.0 to -1.0%), with 85% of the differences being more extreme than the lower ROPE limit of $\pm 2.5\%$.

For quarters infused with Teatseal, 76.0% (95% HDI: 73.0 to 79.0%) of the time less material was recovered at calving than the amount of sealant and debris that could be recovered when sealant was mixed with milk samples, centrifuged, and weighed, whereas for quarters infused with Duraseal, the equivalent value was 79.0% (95% HDI: 76.0 to 82.0%). The median difference in the percentage of quarters where the amount of material recovered at calving was less than the debris plus

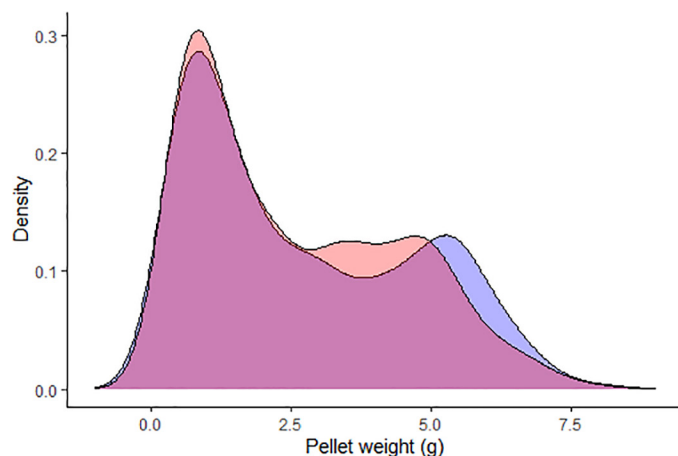


Figure 1. Distribution of the weight in grams of the residue recovered after centrifuging a 50-mL colostrum sample collected within 24 h of calving from quarters infused either with Teatseal (blue; Zoetis Animal Health Ltd.) or Duraseal (red; Norbrook Laboratories Ltd.) at dry-off.

sealant recovered after infusion was -3.0% (95% HDI: -6.0 to -1.0%). In contrast, the Bayesian regression model predicted that the subject-specific difference due to treatment alone would be -3.0% (95% HDI: -7.0 to 1.0%).

Forty-three percent of predicted values lay within the ROPE of $\pm 2.5\%$, with 57% of the predicted differences in percentage being more extreme than the lower ROPE limit.

DISCUSSION

The evidence from this trial suggests that for CM over the last 7 d of the dry period and first 42 d of lactation and SCM assessed at the eighth milking, we are $>95\%$ certain that any difference in the percentage of affected quarters due solely to the difference between the 2 products is $<1.0\%$ (subject-specific difference). When averaging over the effect from different cows and herds, we are $>95\%$ confident that the difference is $<2.5\%$ (marginal difference). We have presented an absolute measure of effect (risk difference) because we believe this gives the most useful information on the likely difference in risk between the 2 products (Irwig et al., 2008; Noordzij et al., 2017).

We cannot be certain how the 2 products would compare when the incidence of mastitis differs from that reported here. McDougall et al. (2022) reported 3.1% (exact binomial CI: 2.1 to 4.4%) CM for the first 60 d of lactation in cows treated with ITS alone, and Bates and Saldias (2018) found 5.7% (95% CI: 4.0 to 8.0%) CM in cows treated with ITS alone for the period from 30 d before to 84 d after calving. Despite the longer follow-up in those studies compared to the present study, the majority of cases occurred within 14 d of calving, suggesting the level of CM recorded in the present study is typical of other studies from New Zealand pastoral systems. The sensitivity of farmer-recorded CM differs between herds (Bates and Saldias, 2018), but quarter treatment status was blinded to farming personnel. Moreover, as treatments were equally distributed across quarters on farms, there is no a priori reason for diagnostic sensitivity to differ between treatments.

Previous studies have identified parity, breed, length of dry period, yield, and SCC history for the previous lactation as risk factors for CM and SCM (McDougall, 2003; Berry and Meaney, 2005; Bryan et al., 2011; Bates and Dohoo, 2016). However, none of these factors was found to be associated with disease in the present model. Cows recruited for this study were purposively

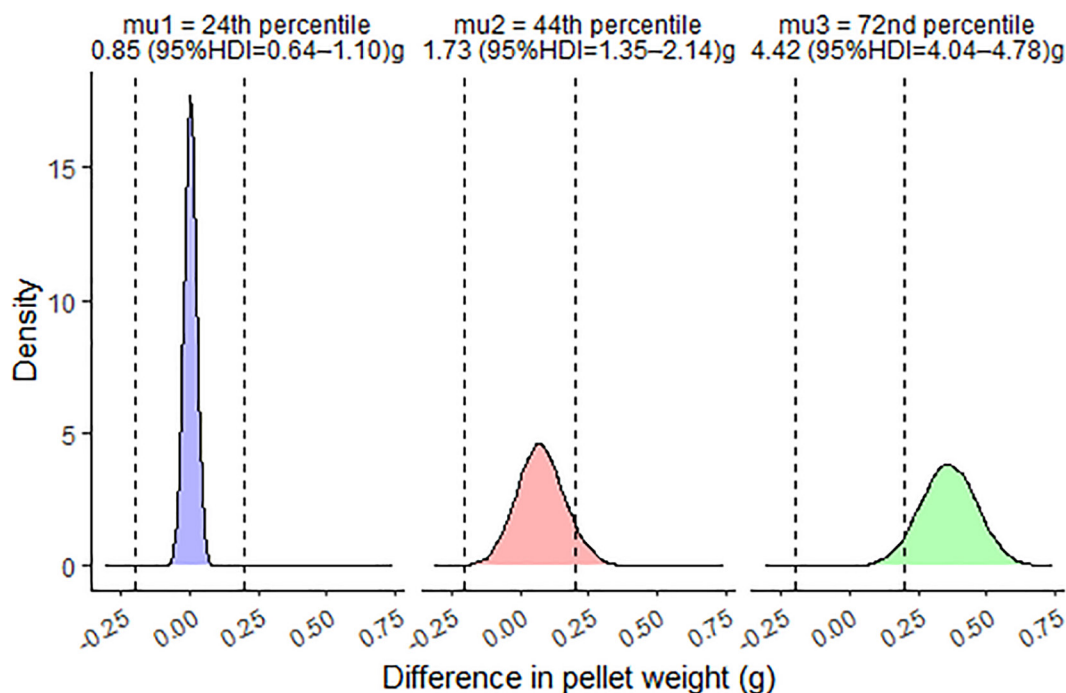


Figure 2. Distribution of the difference in sealant residue recovered within 24 h of calving from quarters infused with Teatseal (Zoetis Animal Health Ltd.) compared with Duraseal (Norbrook Laboratories Ltd.) at dry-off as predicted by a finite mixed model where the distribution of recovered residual sealant weights is modeled as 3 Gaussian distributions with means (μ) and standard errors (SE) of 0.85 g (0.12 g), 1.73 g (0.20 g), and 4.42 g (0.19 g) equivalent to the 24th, 44th, and 72nd percentile of the distribution of recovered residual sealant weights. Vertical dotted lines indicate the region of probable equivalence of ± 0.2 g. HDI = highest density interval.

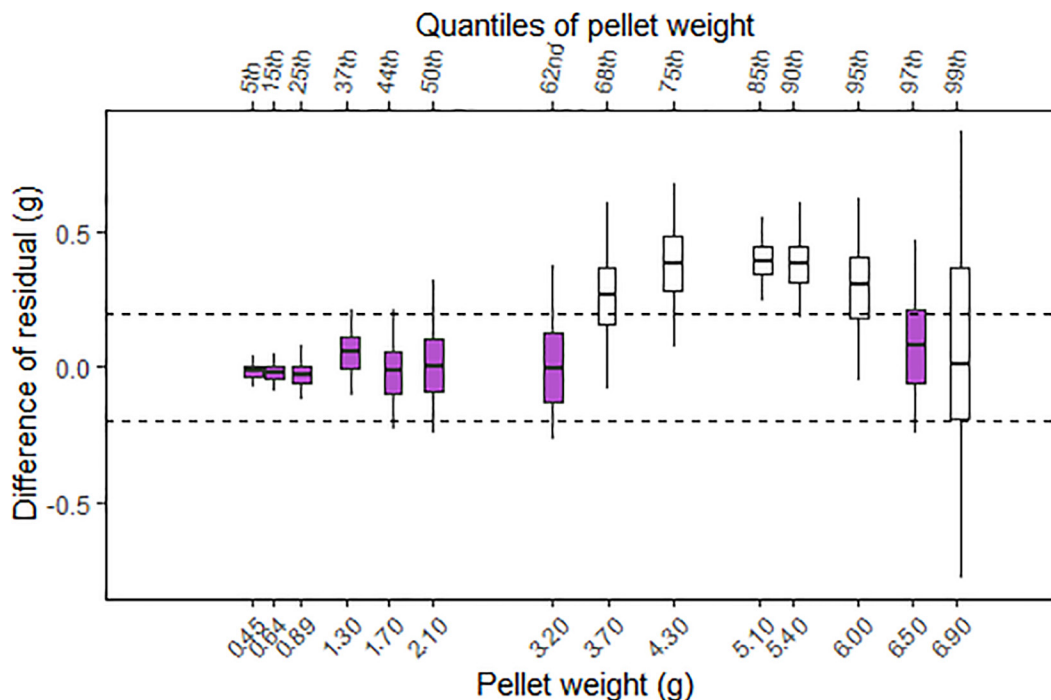


Figure 3. The plot shows the distribution (as box plots) of the differences [Teatseal (Zoetis Animal Health Ltd.) – Duraseal (Norbrook Laboratories Ltd.)] in the equivalent quantiles of the residue recovered (in grams). Within each box, the median is marked by the horizontal black line, the interquartile range is indicated by the upper and lower limits of the box [shaded purple when within the region of probable equivalence (ROPE)], and maximum and minimum values are indicated by the whiskers. Each box represents the distribution of the differences for a particular summary statistic for the residue weights. The dotted horizontal lines represent the upper and lower region of the ROPE.

selected as being suitable for treatment with ITS alone, thus excluding cows with a history of CM or elevated SCC. This may additionally have biased the sample toward younger animals, with the percentage of animals >7 yr being $<5.0\%$ for all herds. Similarly, the 4 herds were predominantly Friesian, and there may have been insufficient variation within these predictors to demonstrate an association with the outcome, particularly as the mastitis prevalence was low.

The quantity of sealant recovered was generally greater than the mean of 1.27 g, median of 0.38 g, and range of 0.00 to 6.34 g reported by Bradley et al. (2010). This may reflect lower milk volumes at calving in the present study (with less dilution or flushing of sealant), differences in milk solids composition, or differences in the insertion technique at dry-off. In both studies, some samples yielded more residue material than had been infused and some less, but when the weight recovered is greater than the weight of sealant administered, some of the residue must consist of extraneous material.

Recovery immediately after mixing into milk samples of more material than inserted suggests that at least some of the residue is not purely the result of accretion of cellular debris, keratin, and precipitation from the dry gland. Rather, with the expectation that centrifuga-

tion will precipitate the heavier milk components (Patton and Huston, 1986), the precipitate recovered may contain aggregated (3–100 μm) milk proteins and cells, as well as the components of the sealant (Dumpler et al., 2017). Our results on the recovery of the residue directly after mixing sealant into milk suggest that although $\sim 90\%$ of the time, difference in recovered material was <0.2 g, we are $>95\%$ certain that the residue weight recovered was greater in milk from Teatseal-infused cows than from Duraseal-infused cows. This suggests a difference in the response to centrifugation between samples infused with Teatseal and samples infused with Duraseal, with Teatseal samples yielding more extraneous material. However, although some of the difference in the weight of recovered residue may be due to greater precipitation of extraneous material in Teatseal samples, the evidence from the clustered bootstrapping and third latent class of the finite mixture model suggest this cannot fully explain the difference between the products, which we are 90% certain is >0.2 g for this upper quartile.

Numerically, for the top 38% of residue weights (≥ 3.20 g), the probability that the difference exceeded the ROPE of ± 0.2 g increased from 25% to a maximum of 99% for residue weights of ≥ 5.10 g but was driven

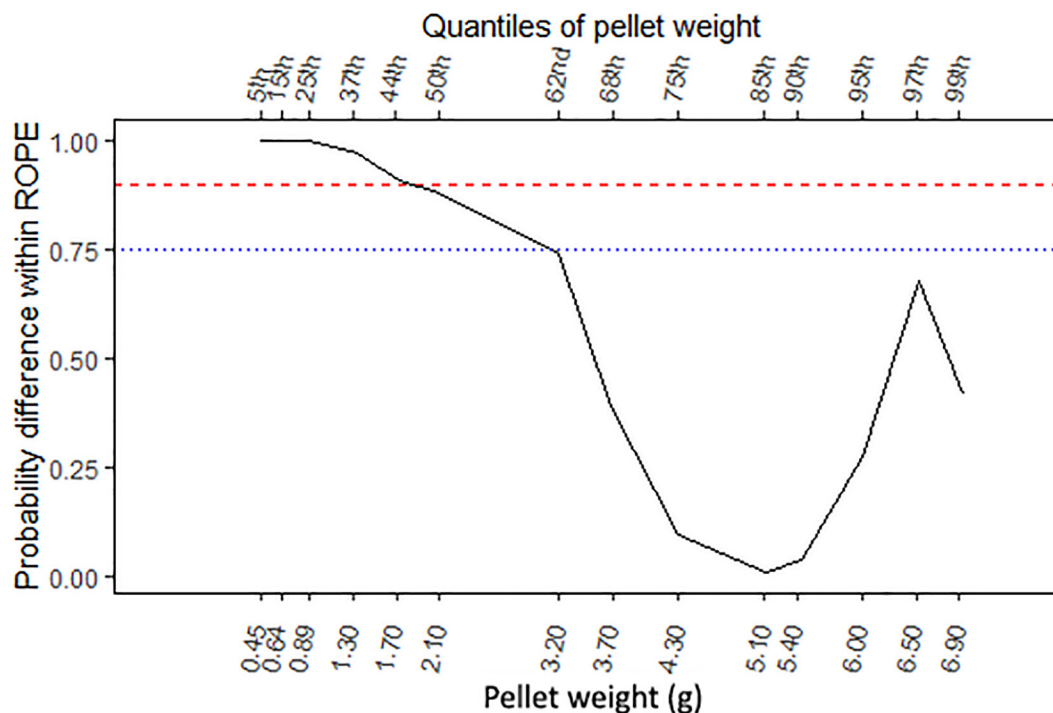


Figure 4. Estimated probability that the difference for any particular quantile of the distribution of residue weights is within the region of probable equivalence (ROPE) of ± 0.2 g. The horizontal dashed (red) and dotted (blue) lines indicate 90 and 75% probability, respectively.

by increasingly small numbers of datapoints and should not be overinterpreted.

We were unable to assess suckling by calves before sample collection or collect reliable data on the presence of an intact teat plug because of variation between operators in assessing teat plug integrity. Nevertheless, it is likely some cows did not have intact teat plugs at sampling, and all staff reported wide variation in the ease of stripping and the perception of sealant residue within the teat canal. It is possible, and consistent with the division of the 3 latent classes by the finite mixture model, that the population of quarters with a mean predicted residue of 0.85 g represents quarters in which there was no teat plug and major loss of sealant had occurred. The lack of difference observed for this population in the quantity of residue between products is consistent with most or all of the material having been removed in this way.

For the present data set, it is probable that quarters with an intact teat plug are found with increasing frequency within the upper 3 quartiles of the distribution, giving an estimated upper limit for the percentage of quarters with an intact teat plug of approximately 75%. This is comparable to the 83% of quarters with an intact teat plug at the first milking after calving reported by Kabera et al. (2018). These workers found no evidence for a protective association between the

presence of an intact teat plug and the subsequent risk of mastitis, and our analysis also failed to find any association between the weight of material recovered and the risk of CM or SCM.

Bacteriology was only performed on positive RMT samples at insertion or at the eighth milking after calving. The limited nature of the sampled population means that no inferences can be drawn about the etiological agents for the CM diagnosed by the farmers. As expected for seasonal calving systems, the RMT-positive quarter samples yielded predominantly environmental bacteria (Tucker et al., 2009) or no growth, reflecting the poor specificity of the RMT as an indicator of the presence of bacteria (Wesen et al., 1968).

A major limitation in quantifying the amount of residue present after calving is the difficulty in assessing retention and presence of an effective bacterial barrier over the full-time course of the study. Sealant losses are commonly associated by farmers with stock movement shortly after dry-off, and the loss of sealant in this study could have occurred at any time from insertion to colostrum sample collection. Similarly, the length of time from calving to colostrum collection, risk of milk leakage, suckling by calves, and sealant expulsion were uncontrolled and varied between cows. Thus, in quantifying the efficacy of sealant in reducing the risk of new IMI at the beginning and end of the dry period

(Bradley and Green, 2004), our method lacks specificity: for quarters where less residue was recovered, the new IMI risk depends on when the sealant was lost. Similarly, our protocol lacked sensitivity: we could not know how much of the residue was sealant and whether the balance of material had any value in preventing new IMI.

In this data set, above the 75th percentile of residual weight, there was a 90% probability that the residual was greater from Teatseal quarters than from Duraseal quarters (difference = 0.36 g; 90% HDI: 0.20 to 0.54 g). Nevertheless, the equivalence for the probability of CM and SCM in quarters treated with Teatseal or Duraseal suggests there was no difference in the clinical efficacy of these 2 products, irrespective of the amount of material recovered and notwithstanding the delay between calving and first milking. This is an important outcome because this clinical equivalence reflects the key role of the product to prevent new IMI. Recent patent applications for new formulations of sealants feature greatly reduced (1.0–2.5 g) amounts of sealant per intramammary syringe and report radiographic evidence of comparable retention in the teat canal with less fragmentation and ascension into the cistern and udder (Smith, 2019). This suggests that the quantity of material recovered may be less significant for efficacy than the persistence of product within the teat canal. This agrees with our finding that including the amount of residual material recovered at calving did not improve fit or predictive ability of the models predicting CM or SCM, and the coefficient spanned the null value.

CONCLUSIONS

Both ITS products had equivalent efficacy in preventing SCM and CM. As the quantity of residual increased, Teatseal quarters tended to yield more residual than Duraseal quarters, but this was not reflected in a decrease in mastitis risk and may represent an increase in debris rather than a more effective barrier. Nevertheless, our work raises multiple questions about the assessment of risk associated with the persistence of ITS, the factors that influence this, and the risk of new IMI.

ACKNOWLEDGMENTS

We thank all the Vetlife colleagues, farmers, and staff who helped make this study possible and Vetlife management for their ongoing commitment to practice-based trial and research work. The authors acknowledge the financial support for this project from Norbrook NZ Ltd., in the form of a one-time payment. They acknowledge that CK and MD are employees of Norbrook Ltd.

All analysis was conducted independently by AJB. The authors have not stated any other conflicts of interest.

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