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Postpartum excretion of internal teat sealant after selective dry cow treatment of dairy cows

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ABSTRACT

To comply with antibiotic restriction policies in the European Union, internal teat sealants (TS) are increasingly used at dry off (DO) in selective dry cow treatment protocols to maintain udder health. Postcalving TS residue attachment to milking equipment and associated cleaning difficulties is a reason some farmers stay away from blanket TS use. Our objective was therefore to improve insight into TS excretion visibility and to compare quantity, pattern, and presence versus absence of TS excretion postcalving between the typical 2 cow categories at DO: high (H)- and low (L)-SCC cows, treated with antibiotic (AB) plus TS (H-ABTS) or TS only (L-TS), respectively. In herds in the Netherlands (n = 3), and Germany (n = 4), cows were enrolled at DO, and categorized as H-ABTS (n = 93), or L-TS (n = 99). Postcalving, quarter-level TS visibility, quantities, patterns, and percentage of TS infused and excreted postcalving were recorded from 50 mL of premilk of every quarter at each of the first 15 or 16 milkings. Udder quarter health status was determined by bacteriological culture and SCC of quarter milk samples taken at DO and at d 3 postcalving and by clinical mastitis incidence from DO until 30 DIM. Univariable and multivariable models were created to explore associations of TS excretion presence versus absence at the first 3 milkings. Irrespective of SCC category, both laboratory personnel and farmers saw TS residues at the first milking in an equal 72% of quarters. Compared with laboratory as the gold standard, farmer sensitivity to spotting TS in premilk was 74.5% at the first milking and decreased to a maximum of 8.3% at the last 3 milkings. At the first milking, TS excretion quantities showed a bimodal distribution pattern and the mean percentage of TS infused (3.83 g) that was excreted in premilk at the first milking, was higher in the L-TS cows (45.5%) compared with the H-ABTS cows (32%). At the second and third milking, mean-adjusted TS percentage excreted was higher in the H-ABTS cows (8.5% and 1.8%, respectively) compared with the L-TS cows (4.6% and 0.4%, respectively). The multivariable model of the first 3 milkings showed parity at both the first and second milking, and the study group at both the second and third milking was significantly associated with TS presence. The univariable model showed no association between TS presence at the first milking and udder health. In conclusion, in premilk of the first milking, TS residue excretion was bimodal, higher in L-TS cows, more likely to be present in multiparous cows, and not associated with udder health. At the second and third milking, excretion was higher in H-ABTS cows and TS presence was only more likely in multiparous cows at the second milking.
Key words: selective dry cow treatment, internal teat sealant, residue, intramammary infection

INTRODUCTION

The dry period of dairy cows is important for udder health as many new IMI in dairy cows occur during this time, potentially causing a loss of milk production during the first months of the following lactation (Green et al., 2002; Rowe et al., 2021). Antibiotic (**AB**) treatment in all quarters of all cows at dry off (**DO**), is traditionally performed to both cure existing infections and to prevent new IMI. To comply with restrictions on prophylactic use of antibiotics (**AB**) in the EU (European Union, 2019), farmers increasingly use alternative applications to prevent new IMI, such as internal teat sealants (**TS**). Teat sealants compensate for the delay of natural keratin plug formation because up to 23% of quarters may still be "open" at 6 wk after DO (Dingwell et al., 2004). In preventing new IMI during the dry period, TS are shown to

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be equally (Vanhoudt et al., 2018; Kabera et al., 2020) or more effective (Newton et al., 2008; Golder et al., 2016; Dufour et al., 2019) compared with AB. As a result, in selective dry cow treatment protocols, the blanket use of TS is becoming routine and a mainstay of good veterinary practice (Weber et al., 2021).

Teat sealant is an inert viscous paste that is aseptically infused as a plug in the teat canal at DO and is physically removed through premilking each quarter before the first milking postcalving. Although TS should be able to stay in the teat canal for at least 100 d (Woolford et al., 1998), Kabera et al. (2018) reported that in 17% of quarters no TS plug was present at first milking, and Buckley et al. (2023) demonstrated that prefresh, separate small pieces of TS were present in the alveolar tissue of 56% of quarters. Absence of TS at the first milking postcalving, does not necessarily mean absence of TS in subsequent milkings, but was associated with a shorter average duration of TS excretion in subsequent milkings after calving to 1.2 d, when compared with presence of TS (4.5 d), when visually detected by milkers (Kabera et al., 2018), and TS may even be seen for up to 21 d postcalving (Berry and Hillerton, 2002). The presence of TS in milk, especially after the regulated colostrum period, is an issue for dairy farmers because TS residues stick to the internal surface of rubber liners, milk pipes, and automated cleaning machines, causing obstruction and cleaning difficulties (Haldemann, 2021, Lely, 2023). Also, when TS residues pass through milk filters, they enter the bulk milk tank and could cause black spots in aged cheddar cheese, if dairy companies do not use raw milk clarification technologies to physically remove the relatively dense internal TS from cheese milk (Lay et al., 2007).

Currently, data on TS excretion are predominantly based on visual detection by milkers. Few studies objectively measure TS residues, especially in cows treated with TS and AB and for longer than the first milking. Weighed quantities of TS excreted at every quarter of every premilking beyond the first milking could provide insight in TS excretion patterns and could reveal differences in excretion between the 2 categories at DO typical for selective dry cow treatment protocol: high- and low-SCC cows treated with TS plus AB and TS only, respectively. Such insights could support better postcalving management of TS excretion to control TS attachment to milking equipment, prevent TS from entering the bulk tank, and, potentially, serve as a basis for future TS product improvement.

Therefore, the primary objective of this study was to compare quantities of TS excreted in premilk in the first 7 d postcalving, between high- and low-SCC cows at DO, in a selective dry cow treatment protocol, using TS in all cows and only adding AB in high-SCC cows. Secondary objectives were to explore associations of the 2 cow categories, TS excretion and udder health dynamics.

MATERIALS AND METHODS

This study was performed in accordance with local national animal welfare law, local regulatory requirements, and EU guideline 2010/63 (European Commission, 2010).

Herd Selection

Seven herds were selected based on proximity to either Hannover, Germany (n = 4) or Deventer, the Netherlands (n = 3), routine monthly DHI testing, a conventional milking system, milking 2 times per day, and willingness to participate in and comply with the study protocol. Cows were managed according to normal husbandry practices on the farm, and farm-specific data such as bulk milk somatic cell count (**BMSCC**), clinical mastitis (**CM**) incidence, and milk production were collected.

Cow Selection

Cows were enrolled before their last milking in lactation and assessed for suitability from April to November 2021. They were eligible for enrollment if they were pregnant, had 4 functional quarters, a milk yield of more than 5 L during the last day before DO, and were in good general health. Cows were not considered eligible for enrollment if systemic or intramammary AB or anti-inflammatory treatment or surgery within 30 d before DO had occurred, or when the expected dry period was less than 32 d, as indicated on the label of the intramammary dry cow AB as the minimal withdrawal time. Cows were withdrawn due to concomitant AB or anti-inflammatory treatment due to any abnormalities (other than mastitis) during the dry period or in the first 30 d after calving, lack of treatment administration, or when the farmer wanted to do so for whatever reason.

Study Protocol

For the 3 Dutch herds, A, B, and C, the farmers were the milkers and executed the protocol. A week before the start of the trial they were trained by experienced technicians on how to execute the protocol and how to take quarter milk samples aseptically, and were visited weekly to check compliance, collect samples, and answer any questions they would have. For the 4 German herds, D, E, F, and G, the same protocol was executed by both farmers and experienced technicians who visited the farms 4 times per week.

At DO. Cows were checked for general health, and quarter milk samples were collected for bacteriology and SCC. Cows were categorized as high or low SCC based on their SCC levels from the 3 most recent monthly DHI tests before DO. Cows with any of these 3 tests showing a SCC ≥200,000 cells/mL were considered as high SCC and cows with all 3 tests showing a cow SCC <200,000 cells/mL were considered low SCC.

High-SCC cows received intramammary AB (CEFA-SAFE, MSD Animal Health) in all 4 quarters, and TS (SHUTOUT, MSD Animal Health), containing 2.6 g of bismuth subnitrate, a white paste in an oily base in a 4 g syringe (**H-ABTS**). Low-SCC cows only received TS in all 4 quarters (**L-TS**). All treatments were administered following strict asepsis according to predefined and trained protocols.

TS Quantity Infused. After intramammary TS infusion, the empty TS tube was labeled with a preprinted barcode sticker to allow for association with the corresponding animal number and quarter location and stored on farm until transport to the laboratory to determine the weight. Farmers were asked to report spilling of TS for each quarter, and if this happened, to estimate the percentage of spilling. If spilling occurred, data of TS quantity infused were adapted accordingly. If spilling was reported as "unknown" or if spilling was reported but the associated estimation of percentage that was spilled was lacking, the associated quarters were excluded from the analysis.

The quantity of TS infused into each quarter was determined by the difference in the average weight of the full TS tube before infusion (the average weight of 24 full tubes from a random commercial TS carton pack of the tubes used in the trial), and the weight of the empty tube for each quarter after infusion into the teat canal, as determined in the laboratory.

After DO. After DO, cows were managed according to their normal husbandry practices, were checked at least once a day for CM and twice a day for their general health status, and any concurrent treatments were recorded. In case of CM, a milk sample from the affected quarter was taken for bacteriology, and frozen at −18°C until transport to the laboratory.

On the first day after DO, BCS, according to the Dairy-Co Guide (available at https://ahdb.org.uk/knowledge-library/body-condition-scoring-flow-chart), and teat end callosity (**TEC**) score (Neijenhuis et al., 2001) was determined at the quarter level. At 24, 48, and 72 h, each ± 4h, after the last milking before DO, in the far-off dry cow pen, milk leakage was determined visually for each cow at quarter level by experienced technicians (Germany) or trained students (the Netherlands). Milk leakage was defined as the observation of leaking milk at any of these time points, by using a mirror below the teat to observe leakage of milk from the teat end, twice in a row, for 30 s. Additionally, farmers were asked if they had seen milk leakage at least once precalving in the close-up pen.

Postcalving. Teat sealant excretion was investigated from the day of calving (d 0) until d 7 after calving. During these 8 d, at each milking, 50 mL of premilk of every single quarter of each cow was collected by the farmer, filling the tube until the maximum line indicated on the outside of a 50-mL plastic centrifuge tube with a free-standing conical bottom (VWR, Avantor, Amsterdam, the Netherlands). At d 0, the first sample was taken as soon as possible after calving and if a cow calved early enough in the day, a second sample was taken at the regular afternoon milking. If cows were sampled twice on d 0, a total of 64 quarter premilk samples were collected per cow from 16 subsequent milkings in 8 d. If cows were milked once on d 0, 60 premilk quarter samples from 15 subsequent milkings in 7.5 d were collected per cow. On d 3 postcalving, a quarter milk sample was taken aseptically by the farmer for bacteriology and quarter SCC, using a 10-mL tube. After milking, all tubes were frozen on farm at −18°C, collected every 2 to 3 mo, and transported by car under cooled conditions (2°C–8°C) to the laboratory (GD Animal Health, Deventer, the Netherlands) for TS excretion, bacteriology, and SCC measurements.

Dairy herd improvement data, specifically milk production and individual cow SCC, from the first 3 monthly tests after calving, were collected for enrolled cows. The SCC from the first monthly DHI test was only included in the dataset when sampling occurred after 5 DIM. From the day of calving until 30 DIM, all cows were checked twice a day for general health, occurrence of CM, or any other disease or concurrent treatments, by the same pre-trained technicians described earlier. In the case of CM, a milk sample just before treatment was taken aseptically by the milkers from the affected quarter for bacteriology.

TS Residue Visibility and Quantity. Without being specifically trained for it, to stay close to the practical situation, farmers were asked to judge premilk samples cowside on TS residue visibility (yes or no), and afterward, the same samples were judged on visibility in the laboratory (see "Laboratory Methods"). The quantity of excreted TS plus extraneous solids, such as, for example, cellular debris and milk solids, was determined from premilk of each quarter in each cow by first determining the total weight (in grams) of the 50-mL plastic tubes including the substance (TS residue + extraneous solids) at the tube base, after centrifuging, decanting the supernatant, and draining and drying the tube upside down for 30 min. Second, to separate the TS residues from the extraneous solids at the base of the tube, we assumed only extraneous solids and no TS residue were left at the final milking(s), milking 15 and 16 (when present). We estimated the net quantity of TS excreted during milkings 1 through 14 by deducting the mean, plus 2× the SD of the total weight of all tubes from the last 2 milkings (milkings 15 and 16) from the total weight of each tube of the previous milkings, assuming only extraneous solids were present in almost all samples from milkings 15 and 16. If the adjusted weight was <0 g it was set at 0 g. Teat sealant presence in premilk was defined by the percentage of quarters for which the net adjusted weight of excreted TS was >0 g.

Excreted Percentage. The estimated weight percentage of TS excreted at each premilking in each of the 2 SCC categories was calculated by dividing the weight of TS excreted in premilk in each quarter by the weight of TS infused into the same quarter. The total cumulative percentage excreted in each of the 2 SCC categories was calculated from quarters with the complete set of data combinations of the amount infused and the amount excreted in 15 consecutive premilkings postcalving.

Udder Health. The udder health–related dry period outcome was defined by the quarter-level bacteriological and SCC status at DO and at d 3 postcalving and CM occurrence at the cow level during the dry period and the first 30 DIM. A case of CM was defined at the cow level by the occurrence of visible changes in the milk of a quarter, such as watery milk, clots, or flakes, with or without changes and swelling or redness of the affected quarter.

A new IMI based on bacteriology was defined as the quarter-level presence of a pathogen postcalving, that was not present at DO. Therefore, a quarter infected with a pathogen at DO was eligible to acquire a new infection with a different pathogen. No new IMI was defined by the absence of any pathogen in a quarter both at DO and postcalving. Bacteriological cure was defined as the absence at quarter level of a pathogen postcalving that had been present at DO. No bacteriological cure was defined as the presence at quarter level of pathogens postcalving, that had also been present at DO.

A new IMI based on quarter SCC was defined by a quarter SCC being <200,000 cells/mL at DO and ≥200,000 cells at d 3 postcalving. No new IMI was defined by quarter SCC being <200,000 cells/mL at DO and remaining <200,000 cells postcalving. SCC cure was defined by quarter SCC being ≥200,000 cells/mL at DO and <200,000 cells at d 3 postcalving. No SCC cure was defined by a quarter SCC being ≥200,000 cells/mL at DO and postcalving.

Laboratory Methods

TS Visibility. In the laboratory, 2 students were asked to record visibility of TS in the tube containing the 50 mL of premilk. They did so after thawing and centrifuging the sample for 5 min at 3,000 rotations/minute (±2,100 × *g*), subsequently removing the cap of the plastic 50-mL tube, decanting the supernatant, and putting the tube upside down to allow draining and drying of the substance (TS plus extraneous solids) in the base of the tube for approximately 30 min. TS was recorded as visible when any typical white substance attached to the base of the tube was macroscopically visible. The 2 students judged all samples together and were able to clearly distinguish the white TS at the tube base. Teat sealant was considered not visible when the base of the tube was clean.

Udder Health. Udder health–related parameters were both SCC and bacteriology, and CM incidence, all at quarter level, from DO until 30 DIM. Milk SCC was determined using fluorescence flow cytometry (SomaScope *Smart*, PerkinElmer, Rodgan, Germany), ISO 13366–2, IDF 148–2 (ISO, 2006). Bacteriological culture was performed according to National Mastitis Council (NMC, 2017) recommendations. For routine samples 0.01 mL of milk was inoculated on 6% sheep blood agar (bioTRADING, Mijdrecht, the Netherlands). Aerobic growth of mastitis-causing pathogens was examined at 18 to 24 h at 37°C and again after 48 h. Pathogen identification was performed by MALDI-TOF MS using the MALDI Biotyper Sirius system (Bruker Daltonics GmbH, Germany; Barreiro et al., 2010). Milk samples that cultured negative with standard culture procedure and having a SCC above 200,000 cells/mL were cultured again onto 6% sheep blood agar following a combination of freezing and preincubation (Sol et al., 2002). An IMI was defined as a pure culture or predominance of one or 2 types of mastitis-causing pathogens with growth of at least 6 (in case of a pure culture) or more than 10 (when more than one type was present) colonies on the plate. In case of growth of *Staphylococcus aureus*, *Streptococcus agalactiae*, or hemolytic streptococci, the presence of at least one colony was considered an IMI. Contamination was defined as growth of more than 2 phenotypically different colony types without a dominant mastitis-causing pathogen. In case of growth of *Staphylococcus aureus*, *Streptococcus agalactiae*, or hemolytic streptococci in such samples, the sample was not considered contaminated, and the putative mastitis-causing pathogen was identified by the MALDI Biotyper. Contaminated samples would be reported but would not be included into the udder health parameters.

Data Collection and Statistical Analysis. Standard laboratory data were collected and registered in the laboratory database, and additional data such as weights of tubes were registered using separate Excel files. All filled paper forms from the farms were digitized using an online data entry software program (Formdesk, Innovero Software Solutions, Wassenaar, the Netherlands), and were then exported to a Microsoft Excel format and imported and further analyzed in Stata version 17.0 (Stata Corp. LLC, College Station, TX), where they were merged with other imported datafiles to enable analysis.

This study was part of larger study with a primary focus on determining the efficacy of TS or a combination of TS and AB treatment in preventing and curing IMI. Therefore, the number of quarters and cows included in this study was based on this objective. Using 135 quarters, and assuming 1.5 quarters would be infected per cow, based on the average of 1.7 quarters infected per cow (Poelarends et al., 2001) and 1.3 quarters infected per cow (Dingwell et al., 2003), we planned to include 135/1.5 = 90 cows in each treatment category. Of the 135 quarters, only H-ABTS cows, so half the dataset (n = 67 quarters) would be suitable for the cure rate analysis and the other half (L-TS cows) for the new IMI rate analysis, assuming high-SCC cows were infected and low-SCC were not infected. Based on 67 quarters in the analysis, a power of 80% and a 95% CI, an expected error margin of ± 10% could be reached around the expected cure and new IMI prevalence.

Descriptive and graphical analyses were conducted to explore the data, specifically on the differences between the 2 categories (H-ABTS vs. L-TS). Potential differences in variables between both categories, associations between DO categories and TS excretion, and associations of TS excretion and udder health for both categories, were analyzed univariably using an appropriate statistical test, for example, proportion test, linear regression, or a nonparametrical alternative such as the Wilcoxon rank sum (Mann Whitney U) or median test. The level of significance of the formal tests was set at 5% and all tests were 2 sided.

The sensitivity of the farmer to spot teat seal residues in premilk was determined by comparing it to laboratory judgment as the gold standard. The farmer sensitivity was determined both overall and specifically for the first and last 3 milkings. To check if the sensitivity of the farmer was correlated within cows or farms, or both, an empty multilevel mixed-effects logistic model on farmers' judgment (absence or presence of TS residues) was performed considering only those observations where the laboratory indicated seeing TS residues. A random cow and farm effect was included to determine the respective intraclass correlation coefficients (including 95% CI).

To specifically investigate the association between the 2 categories and the presence of TS excretion in premilk of the first milking, we created a multivariable multilevel mixed-effects logistic model looking at binary presence (>0 g) versus absence (≤0 g) of TS in premilk of the first milking. A random farm and cow effect were included to correct for the possible correlation with cows and farms. The degree of correlation within the cow or farm, or both, was determined using the intraclass correlation coefficient. The following fixed covariates were also considered during model selection due to their potentially confounding role: BCS, milk leakage in the first 3 d after DO, milk leakage before calving, SCC at DO, length of the dry period, 24 h milk production before DO, parity, TEC score the day after DO, and quarter location (hind/front). Confounding was regarded to be present when a >20% change in the study group (L-TS and H-ABTS cows) covariate coefficient occurred. Possible effect modification was investigated as well. The final multivariable model was also performed for the second and third milking, to check if the association between the DO category and TS excretion in premilk differed compared with the first milking.

RESULTS

Key characteristics of the 7 study herds are shown in Table 1. From these herds, 192 cows were enrolled in the study at DO, 93 cows were categorized as H-ABTS and 99 cows as L-TS.

Univariable Analysis

At DO. The cow-level variables measured, including parity, milk yield in the last 24 h before DO, and lnSCC during the last 3 DHI tests before DO, and quarter-level variables, lnSCC, and percent >200,000 cells/mL, showed significant differences ($P < 0.001$) between the H-ABTS and L-TS cow categories (Table 2).

TS Quantity Infused. The mean weight (±SD) of 24 full TS tubes in a commercial TS product package was 12.94 g (±0.027 g). The mean weight (±SD) of 761 empty TS tubes after infusion into the teat canal of each quarter, was 9.11 g (±0.40 g). Therefore, the mean TS quantity infused into the teat canal was 3.83 g (12.94 minus 9.11). Estimation of spilling of TS at infusion was reported by farmers in 1.6% (12/761) of quarters.

After DO. Among the cow- and quarter-level variables measured after DO, only the TEC score and dry period length were significantly different between H-ABTS and L-TS cows ($P < 0.001$ and $P < 0.012$, respectively; Table 3), but BCS and milk leakage percent in the first days after DO were not different ($P = 0.446$ and $P = 0.477$, respectively; Table 3).

TS Residue Visibility. Laboratory (n = 10,605 samples) and farmer visibility (n = 9,913 samples) of the first 15 or 16 milkings is presented as the percentage of quarters with visible TS residues excreted, irrespective of dry cow category, in Figure 1.

At the first milking, using laboratory TS visibility judgment as the gold standard, farmer sensitivity was 74.5% (95% CI: 70.2%–78.5%; n = 633 comparisons), but quickly declined afterward to 29.1% (95% CI:

Table 1. Key characteristics of the 7 study farms in the Netherlands (herds A, B, and C) and Germany (herds D, E, F, and G)

	Netherlands			Germany			
Variable	A	B	C	D	E	F	G
Herd size (no. of cows)	206	260	143	365	295	522	167
No. of cows enrolled	36	35	19	39	20	15	29
Breed[1]	HF	HF	HF	HF	HF	HF	HF
12 mo geometric mean BMSCC[2]	153	353	172		215	138	138
CM (%) 12 mo before study start[3]	24	12	30	14	11	19	8
305-d milk yield (L) at study start[4]	10,245	9,880	9,512	14,195	9,902	12,965	10,944
Dry cow housing[5]	C	C	C	C	P	C	C
Dry cow bedding[6]	Sw, L	St, L, W	Sw, L	M, St, L	P	St, L, W	BG, L

[1]HF = Holstein Friesian.

[2]Calculated bulk milk SCC based on individual cow recording.

[3]Clinical mastitis (CM) incidence in cases per 100 cows per year.

[4]Cows and heifers.

[5]C = cubicles; P = pasture.

[6]Dry cow bedding in freestall. M = dried manure; Sw = sawdust; St = straw; L = lime; W = mixed with water; BG = biogas substrate; P = pasture. When substances are combined in the bedding, they are a mixture of the indicated ingredients.

Table 2. Summary of the mean (SD) and the median, or the proportion (%) of variables based on cow- or quarter-level data within each category at dry off (DO)[1]

	H-ABTS[2]		L-TS[3]	
Variable	Mean (SD), median	95% CI	Mean (SD), median	95% CI
Cow level at DO				
Number of enrolled cows[4]	93		99	
Parity[5]	3.6 (1.9) 3	3.2–4.0	2.2 (1.4) 2	1.9–2.5
Milk yield last 24 h (L)	22.4 (18.2) 19	18.7–26.2	28.7 (18.6) 23	25.0–32.4
Cow lnSCC 1 mo before DO	5.6 (1.0) 5.6	5.4–5.8	3.9 (0.8) 3.9	3.7–4.0
Cow lnSCC 2 mo before DO	5.6 (1.1) 5.5	5.3–5.8	3.7 (0.7) 3.7	3.6–3.8
Cow lnSCC 3 mo before DO	5.5 (1.1) 5.5	5.2–5.7	3.6 (0.7) 3.7	3.5–3.8
Quarter level at DO				
Number of enrolled quarters	372		396	
lnSCC	5.7 (1.5) 5.8	5.6–5.9	4.6 (1.7) 4.5	4.5–4.8
Percent >200,000 cells/mL[5]	61.2	56.0–66.4	33.6	28.8–38.4

[1]High- and low-SCC cows treated with antibiotic plus teat sealant (H-ABTS) or teat sealant only (L-TS), respectively, from a study in the Netherlands and Germany comparing excretion of internal teat sealant after selective dry cow treatment in dairy cows. The statistical difference between H-ABTS and L-TS categories at DO was based on linear regression or Wilcoxon rank sum (when assumptions regarding the model residual were not met: parity, milk yield) and was significant ($P < 0.001$) for all variables. Note: 61.2% of quarters in the cow-level high-SCC category (H-ABTS) had a quarter SCC >200,000 cells/mL, and 66.4% of the cow-level low-SCC category (L-TS) had a quarter SCC ≤200,000 cells/mL.

[2]H-ABTS = cows with any of the 3 most recent monthly DHI tests before DO showing a cow-level SCC ≥200,000 cells/mL and treated with antibiotic and teat sealant at DO.

[3]L-TS = cows with all 3 most recent DHI tests before DO showing a cow-level SCC <200,000 cells/mL and treated with teat sealant only at DO.

[4]Complete dataset was not achieved for all cows enrolled.

[5]Proportion test, $P < 0.001$.

Table 3. Descriptive results of the mean (SD) and the median, or the proportion (%) of variables measured after dry off (DO) and precalving, of both high- and low-SCC cows receiving different treatments[1]

	H-ABTS[2]		L-TS[3]		
Variable	Mean (SD), median	95% CI	Mean (SD), median	95% CI	*P*-value
Cow level after DO					
Number of enrolled cows	93		99		
BCS (d 1)	3.2 (0.5) 3	3.1–3.3	3.1(0.5) 3	3.0–3.2	0.446
Milk leakage (%; d 1–3)[4]	33.3	23.9–42.9	28.6	19.6–37.5	0.477
Dry period length (d)	56.0 (19.8) 50	51.8–60.2	49.2 (12.9) 47.5	46.6–51.8	0.012
Cow level precalving					
Milk leakage (%)	3.4	0.7–9.6	3.4	0.7–9.5	0.989
Quarter level after DO					
Number of enrolled cows	372		396		
TEC score 0–3 (d 1)[5]	0.7 (0.8) 0	0.6–0.8	0.5 (0.6) 0	0.4–0.5	<0.001
Milk leakage LF[6] d 1–3 (%)	8.6	2.9–14.3	3.1	0–6.5	0.100
Milk leakage RF d 1–3 (%)	4.3	0.2–8.4	4.1	0.2–8.0	0.940
Milk leakage LR d 1–3 (%)	12.9	6.1–19.7	14.3	7.4–21.2	0.781
Milk leakage RR d 1–3 (%)	28.0	18.9–37.1	20.4	12.4–28.4	0.223

[1]From a study in the Netherlands and Germany comparing excretion of internal teat sealant after selective dry cow treatment in dairy cows. Calculation of statistical difference is based on a proportion test, except for BCS, dry period length, and TEC score (Wilcoxon rank sum test). *P*-values and 95% CI were calculated for the interaction between the H-ABTS and L-TS categories at DO.

[2]H-ABTS = cows with any of the 3 most recent monthly DHI tests before DO showing a cow-level SCC ≥200,000 cells/mL and treated with antibiotic and teat sealant at DO.

[3]L-TS = cows with all 3 most recent DHI tests before DO showing a cow-level SCC <200,000 cells/mL and treated with teat sealant only at DO.

[4]Percentage of quarters with milk leakage at any of the daily measurements during the first 3 d after DO.

[5]The teat end callosity (TEC) score is a score of each teat end from 0 (normal) to 3 (very rough).

[6]LF = left front quarter; RF = right front quarter; LR = left rear quarter; RR = right rear quarter.

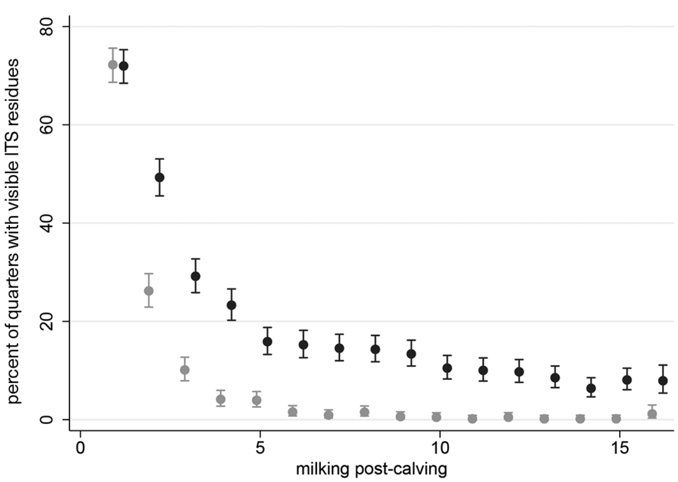

Figure 1. Percentage and 95% CI of quarters with binominal macroscopic visibility (yes or no) of teat sealant residues in premilk of the first 16 milkings postcalving, as judged by either farmers cowside (n = 9,913, gray dots) or by laboratory personnel (n = 10,605, black dots) in the laboratory, after centrifugation and discarding the supernatant, in the first 50 mL of quarter milk. Data were collected on farms in the Netherlands (n = 3) and Germany (n = 4) to study excretion of internal teat sealant (ITS) after selective dry cow treatment in dairy cows. The maximum number of farmer observations per milking was 668 (milking 2) and the minimum observations per milking was 592 (milking 15), not considering milking 16 because data were not present for all cows.

24.3%–34.4%) at milking 2 (n = 664), and 16.4% (95% CI: 11.5%–22.4%) at milking 3 (n = 652). At the last 3 milkings the farmer sensitivity was 2.7% (95% CI: 0.1%–14.2%), 0.0% (95% CI: 0.0%–8.4%) and 8.3% (95% CI: 1.0%–27.0%) at milkings 14, 15, and 16, respectively. Of all milkings (n = 9,840) farmer sensitivity was 25.9% (95% CI: 23.9%–27.9%). Using only observations of TS residues of laboratory personnel (n = 1,907 quarters from 174 cows on 7 farms), the correlation with farmers judgment within a cow and farm was determined. The intraclass correlation coefficient within the cow was 0.55 (95% CI: 0.31–0.77) and intraclass correlation coefficient within farm was 0.36 (95% CI: 0.12–0.71).

TS Residue Pattern and Quantity. The mean weight + 2 × SD of 50-mL laboratory tubes (n = 685) containing premilk of milking 15 and 16 (if present), after centrifugation, decanting the supernatant, and drying, was 12.16 + 2 × 0.15 g = 12.46 g. After deducting this weight from the laboratory tubes with premilk of milking 1 and setting the minimum weight at 0 g (negative weights are not possible), the adjusted weight of TS excreted in the premilk at the first milking showed a bimodal distribution (Figure 2). The 10th, 25th, 50th, 75th, and 90th percentiles of TS excretion weight were 0, 0, 0.45, 2.09, and 3.49 g in the

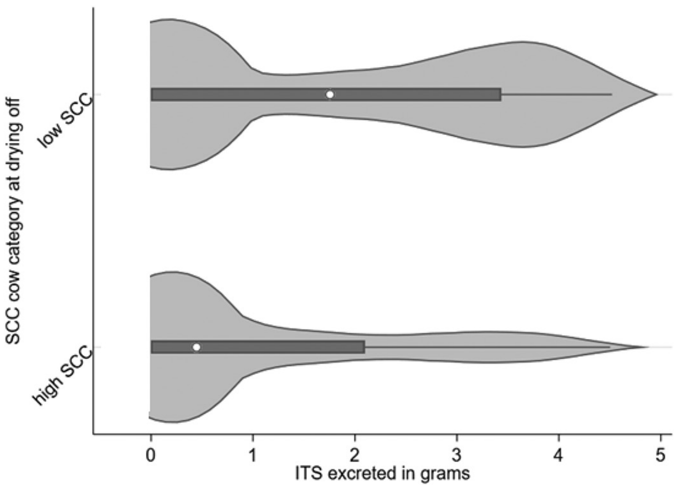

Figure 2. Violin plot of internal teat sealant (ITS) excretion in 50 mL of premilk of quarters at the first milking postcalving from farms in the Netherlands (n = 3) and Germany (n = 4) comparing excretion of ITS after selective dry cow treatment of dairy cows in high- (n = 324 quarters) and low-SCC (n = 341 quarters) cows at dry off (DO), treated with antibiotic plus teat sealant (H-ABTS) or teat sealant only (L-TS), respectively. The light gray area on the y-axis shows the kernel density estimation. The wider the area, the higher the probability of a net weight data point, as indicated on the x-axis. The x-axis shows the weight in grams of teat sealant residues left over after centrifugation and discarding the supernatant and adjusting for residual milk solids. The dark gray bar indicates the first-to-third interquartile range, and the open dot shows the median weight. The dark gray line represents the full distribution, except outliers. H-ABTS = cows with any of the 3 most recent monthly DHI tests before DO showing a cow-level SCC ≥200,000 cells/mL. L-TS = cows with all 3 most recent DHI tests before DO showing a cow-level SCC <200,000 cells/mL.

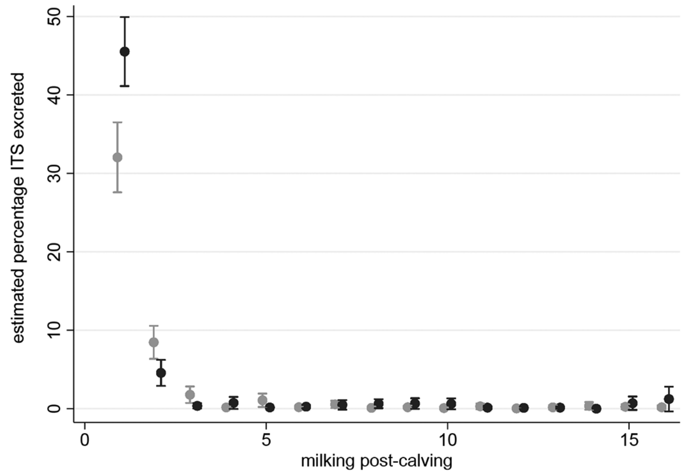

Figure 3. Estimated percentage and 95% CI of weighed teat sealant applied at dry off (DO) that was excreted in the first 50 mL of premilk of the first 16 milkings postcalving, both in the high- and low-SCC cows, treated with antibiotic plus teat sealant (H-ABTS; n = 4,354; gray dots) or teat sealant only (L-TS; n = 5,136; black dots), respectively, from farms in the Netherlands (n = 3) and Germany (n = 4) comparing excretion of internal teat sealant (ITS) after selective dry cow treatment in dairy cows. H-ABTS = cows with any of the 3 most recent monthly DHI tests before DO showing a cow-level SCC ≥200,000 cells/mL. L-TS = cows with all 3 most recent DHI tests before DO showing a cow-level SCC <200,000 cells/mL.

H-ABTS category and 0, 0, 1.75, 3.43, and 3.97 g in the L-TS category, respectively.

Percentage of TS Excreted. The percentage of TS infused into the teat canal that was excreted in premilk of the first 16 milkings was based on 5,136 and 4,354 quarter-milking combinations (maximum 16 milking moments per quarter and therefore maximum 16 × 4 = 64 observations per cow), in the L-TS and H-ABTS category, respectively, and is shown in Figure 3. When the percentage excreted exceeded 100%, it was set at 100%. The mean (median) percentage of the TS infused that was excreted in premilk of the first milking was 32.0% (12.1%) in the H-ABTS group and 45.5% (43.9%) in the L-TS group. The group median was significantly higher in the L-TS group compared with the H-ABTS group (median test, $P = 0.001$). In premilk of quarters at milkings 2 and 3, the mean (median) excreted TS percentage was 8.5% (0%), 1.8% (0%), respectively, in the H-ABTS group and 4.6% (0%), and 0.4% (0%), respectively, in the L-TS group. Although medians were the same (0%), the proportion of quarters that excreted >0%, was 23.7%, 42.5% (milking 2) and 4.9%, 14.7% (milking 3) for the L-TS and H-ABTS groups, respectively and were significantly higher in the H-ABTS group compared with the L-TS group for both milkings 2 and 3 (median test, $P < 0.001$). In premilk of the last 2 recorded milkings, 15 and 16, these mean (median) percentages were 0.2% (0%), 0.2% (0%) in the H-ABTS group versus 0.7% (0%), 1.2% (0%) in the L-TS group, respectively (median test, $P = 0.738$ and $P = 0.857$ for milking 15 and 16, respectively). Only 477 quarters had a complete dataset of quarter combinations of the first 15 milkings to be able to calculate the cumulative percentage excreted during the first 15 milkings. Complete TS excretion (≥100%) occurred in 12.2% (27/221) and 17.7% (44/256) of quarters in the H-ABTS and L-TS categories, respectively (proportion test, $P = 0.13$).

TS Residue Presence. The percentage of quarters with presence of TS residue (>0 g), based on a total of 10,566 quarter-milking combinations, in both H-ABTS (n = 5,170) and L-TS categories (n = 5,396) during the first week postcalving, is shown in Figure 4. In premilk of the first milking, 73.5% and 73.0% of quarters showed TS presence in H-ABTS versus L-TS categories, respectively, which was not significantly different (proportion test, $P = 0.90$). At milkings 2 and 3, the mean percent of quarters with TS presence in the H-ABTS was 42.1% and 16.1%, respectively, both being significantly higher than the 22.7% and 4.7%, respectively, in the L-TS cow category (proportion test: milking 2, $P < 0.001$; milking 3, $P < 0.001$). At the last recorded milkings, 15 and 16, the

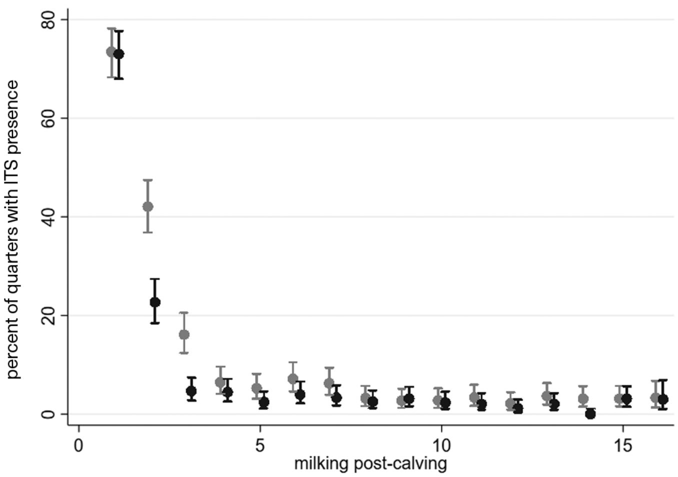

Figure 4. Percentage of quarters with teat sealant presence (adjusted weight >0 g), and their 95% CI, in the first 50 mL of premilk of the first 16 milkings postcalving, based on a total of 10,566 quarter-milking combinations, in both the high-SCC category, treated with antibiotic and teat sealant (H-ABTS; n = 5,170; gray dots) and low-SCC category, treated with teat sealant only (L-TS; n = 5,396; black dots) from farms in the Netherlands (n = 3) and Germany (n = 4) comparing excretion of internal teat sealant (ITS) after selective dry cow treatment in dairy cows. H-ABTS = cows with any of the 3 most recent monthly DHI tests before dry off (DO) showing a cow-level SCC ≥200,000 cells/mL. L-TS = cows with all 3 most recent DHI tests before DO showing a cow-level SCC <200,000 cells/mL.

mean percent of quarters with quantitative TS presence was 3.1% and 3.3% for the H-ABTS group, respectively, and 3.1% and 3.0% for the L-TS group, respectively, and difference between groups was not significant (proportion test: milking 15, $P = 0.98$; milking 16; $P = 0.86$).,

Udder Health. Mastitis etiology at DO and postcalving of both H-ABTS and L-TS categories is shown in Table 4. The most frequently isolated pathogens at DO were NAS (21%), followed by *Staphylococcus aureus* (11.5%), and *Lactococcus* spp. (3.2%); 19.6% was considered contaminated and 31.1% showed no growth. Treatment efficacy of H-ABTS versus L-TS cow categories both at the key pathogen and overall level is summarized in Tables 5 and 6. Bacteriological and SCC cure and new IMI, and CM incidence were not significantly different between the 2 categories (Table 6). There were no significant associations between TS presence probability and the tested udder health–related categories, neither within the H-ABTS group nor within the L-TS group ($P > 0.05$, Table 7).

Multivariable Analysis

The multilevel mixed-effects logistic model for the binary presence or absence of TS in premilk from the first 3 milkings, based on the quantitative measurement in which we corrected for clustering within cows and herds using a random effect, is shown in Table 8. Based on their individual association with TS excretion as the outcome parameter parity, quarter location and milk yield 24 h before DO needed to be investigated on their potentially confounding role, in corresponding order based on their univariable *P*-values when checking their association with TS excretion. The final model consisted of the H-ABTS and L-TS cow category and parity due to its confounding role. Interaction between parity and DO category was not present at the first ($P = 0.49$), second (P

Table 4. Prevalence (%) of infection at the quarter level of high- and low-SCC cows at dry off (DO), treated with antibiotic plus teat sealant (H-ABTS) or teat sealant only (L-TS), respectively, and at d 3 postcalving[1]

	At DO				Postcalving			
	H-ABTS		L-TS		H-ABTS		L-TS	
Pathogen	n	%	n	%	n	%	n	%
Staphylococcus aureus	40/347	11.5	4/381	1.1	9/315	2.9	4/343	1.2
Streptococcus uberis	4/347	1.2	0/381	0.0	1/315	0.3	1/343	0.3
Streptococcus dysgalactiae	0/347	0.0	2/381	0.5	0/315	0.0	1/343	0.3
Lactococcus spp.	11/347	3.2	6/381	1.6	2/315	0.6	2/343	0.6
Enterococcus spp.	5/347	1.4	7/381	1.8	0/315	0.0	2/343	0.6
Escherichia coli	3/347	0.9	2/381	0.5	4/315	1.3	2/343	0.6
Yeast spp.	3/347	0.9	0/381	0.0	11/315	3.5	0/343	0.0
NAS	73/347	21.0	83/381	21.8	29/315	9.2	49/343	14.3
Contaminated[2]	68/347	19.6	86/381	22.6	92/315	29.2	84/343	24.5
No growth	108/347	31.1	153/381	40.2	155/315	49.2	182/343	53.1

[1]From a study in the Netherlands and Germany comparing excretion of internal teat sealant after selective dry cow treatments: H-ABTS = cows with any of the 3 most recent monthly DHI tests before DO showing a cow-level SCC ≥200,000 cells/mL and treated with antibiotic and teat sealant at DO; L-TS = cows with all 3 most recent DHI tests before DO showing a cow-level SCC <200,000 cells/mL and treated with teat sealant only at DO.

[2]Contamination was defined as growth of more than 2 phenotypically different colony types without a dominant mastitis-causing pathogen.

Table 5. Summary of the dry period cure and new IMI rates for key mastitis pathogens within high- and low-SCC cows at dry off (DO) that were given different treatments[1]

	H-ABTS				L-TS			
	Cure		New IMI		Cure		New IMI	
Pathogen	No.	%	No.	%	No.	%	No.	%
Staphylococcus aureus	31/38	81.6	2/276	0.7	4/4	100	4/332	1.2
Streptococcus uberis	3/3	100	1/311	0.3	NA[2]	NA	1/336	0.3
Streptococcus dysgalactiae	NA	NA	0/314	0	1/1	100	1/335	0.3
Lactococcus spp.	10/10	100	2/304	0.7	5/6	83.3	1/330	0.3
Enterococcus spp.	5/5	100	0/309	0	4/5	80.0	1/331	0.3
Escherichia coli	2/2	100	4/312	1.3	2/2	100	2/334	0.6
Yeast spp.	2/3	66.7	10/311	3.2	0		0/336	0
NAS	56/64	87.5	23/250	9.2	60/74	81.1	35/262	13.4

[1]From a study in the Netherlands and Germany comparing excretion of internal teat sealant after selective dry cow treatment in dairy cows. H-ABTS = cows with any of the 3 most recent monthly DHI tests before DO showing a cow-level SCC ≥200,000 cells/mL and treated with antibiotic and teat sealant at DO; L-TS = cows with all 3 most recent DHI tests before DO showing a cow-level SCC <200,000 cells/mL and treated with teat sealant only at DO.

[2]NA = not applicable because not cultured.

= 0.78), nor at the third milking ($P = 0.58$). In the multivariable model, the odds of TS presence in the L-TS versus H-ABTS category in premilk at the first milking were 1.9, with a 95% CI of 1.0 to 4.0, and not significantly different ($P = 0.07$). At the second and third milkings the probability of TS presence was significantly higher in the H-ABTS versus L-TS category ($P < 0.01$). The intraclass correlation coefficient of the first 3 milkings is presented below Table 8. Only at the first 2 milkings was the probability of TS presence significantly increased with increasing parity ($P < 0.01$). A sensitivity analysis, not considering the 12 quarters reporting spilling during TS treatment, did not alter conclusions (data not presented).

DISCUSSION

Although TS is recommended and increasingly routinely performed in selective dry cow treatment protocols to maintain udder health (Kabera et al., 2021), residues of TS have been associated with problems with cheese manufacture and milking equipment cleaning. We therefore need more and better data on factors that influ-

Table 6. Summary of overall quarter-level bacteriological and SCC cure, new IMI, and CM incidence rate in high- or low-SCC cows receiving different treatments at dry off (DO)[1]

	H-ABTS			L-TS			
Item	%	No.	95% CI	%	No.	95% CI	*P*-value
Bact cure[2]	88.7	94/106	81.1–94.0	91.4	85/93	83.8–96.2	0.525
Bact new IMI[3]	25.3	44/174	19.0–32.4	24.9	50/201	19.1–31.4	0.927
SCC cure[4]	67.5	112/166	59.8–74.5	57.3	59/103	47.2–70.0	0.091
SCC new IMI[5]	22.9	22/96	15.0–23.3	28.7	62/216	22.8–35.2	0.288
CM incidence[6]	2.2	8/364	1.0–4.3	3.2	12/376	1.7–5.5	0.405

[1]From a study in the Netherlands and Germany comparing excretion of internal teat sealant after selective dry cow treatment in dairy cows. Statistical differences were determined using a univariable proportion test. H-ABTS = cows with any of the 3 most recent monthly DHI tests before DO showing a cow-level SCC ≥200,000 cells/mL and treated with antibiotic and teat sealant at DO; L-TS = cows with all 3 most recent DHI tests before DO showing a cow-level SCC <200,000 cells/mL and treated with teat sealant only at DO.

[2]Bact cure = the absence of a pathogen at d 3 postcalving that had been present at DO. Contaminated quarters at either DO or at d 3 postcalving were excluded from analysis.

[3]Bact new IMI = the presence of a pathogen at d 3 postcalving that had not been present at DO. A quarter infected with a pathogen at DO was eligible to acquire a new infection with a different pathogen. Contaminated quarters at either DO or at d 3 postcalving were excluded from analysis.

[4]SCC cure = high cow SCC (>200,000 cells/mL) at DO and a low cow SCC (≥200,000 cells/mL) after calving.

[5]SCC new IMI = low cow SCC (<200,000 cells/mL) at DO and a high cow SCC (≥200,000 cells/mL) after calving.

[6]CM incidence = clinical mastitis incidence from DO until 30 DIM.

Table 7. Univariable association (proportion test) between udder health parameters new IMI and cure, based on bacteriology, and probability (%) of teat sealant (TS) presence (adjusted TS weight >0 g) versus absence (adjusted weight ≤0 g) in premilk of quarters at the first milking postcalving[1]

	H-ABTS		L-TS	
Outcome	% TS present (n)	*P*-value	% TS present (n)	*P*-value
Total no. of quarters	96		82	
Cure[2]	70.2 (59/84)		68.4 (52/76)	
No cure[3]	66.7 (8/12)	0.801	66.7 (4/6)	0.929
Total no. of quarters	161		185	
New IMI[4]	75.6 (31/41)		71.4 (33/46)	
No new IMI[5]	72.5 (87/120)	0.698	71.9 (100/139)	0.979

[1]Data obtained using logistic regression, separately for the high- and low-SCC cows at dry off (DO), respectively, from a study in the Netherlands and Germany comparing excretion of internal teat sealant after selective dry cow treatment in dairy cows. The presence of internal teat sealant at the first milking postcalving was not significantly different between the categories "cure" versus "no cure" and "new IMI" versus "no new IMI," within both low- and high-SCC cow categories and their associated treatment protocol at DO. H-ABTS = cows with any of the 3 most recent monthly DHI tests before DO showing a cow-level SCC ≥200,000 cells/mL and treated with antibiotic and teat sealant at DO; L-TS = cows with all 3 most recent DHI tests before DO showing a cow-level SCC <200,000 cells/mL and treated with teat sealant only at DO.

[2]Cure = the absence of a pathogen at d 3 postcalving that had been present at DO. Contaminated quarters at either DO or at d 3 postcalving were excluded from analysis.

[3]No cure = the presence of a pathogen at d 3 postcalving that was also present at DO. Contaminated quarters at either DO or d 3 postcalving were excluded from the analysis.

[4]New IMI = the presence of a pathogen at d 3 postcalving that had not been present at DO. A quarter infected with a pathogen at DO was eligible to acquire a new infection with a different pathogen. Contaminated quarters at either DO or at d 3 postcalving were excluded from analysis.

[5]No new IMI = the absence of any pathogen at both DO and postcalving.

ence their presence. To study postcalving TS visibility, weighed quantities, patterns and presence, this study provides a unique dataset of more than 10,000 quarter premilk samples of weighed TS residues recovered from the first 16 milkings.

TS Visibility

At the first milking, we found TS residue to be visible in 72% of quarters (Figure 1) and a presence of >0 g in 73% of quarters (Figure 4). This is in line with the

Table 8. Outcomes of the multivariable multilevel mixed-effects logistic model at the quarter level from farms in the Netherlands (n = 3) and Germany (n = 4) comparing excretion of internal teat sealant after selective dry cow treatment in dairy cows with high and low SCC at dry off (DO)[1]

	First milking[2]			Second milking[3]			Third milking[4]		
Item	OR	95% CI	*P*-value	OR	95% CI	*P*-value	OR	95% CI	*P*-value
SCC category									
H-ABTS		1 (referent)			1 (referent)			1 (referent)	
L-TS	1.9	1.0–4.0	0.069	0.4	0.2–0.7	0.002	0.2	0.1–0.6	0.005
Parity									
1		1 (referent)			1 (referent)			1 (referent)	
2	4.9	1.9–12.2	0.001	4.4	1.8–10.6	0.001	1.4	0.3–7.0	0.675
3	5.5	2.0–14.7	0.001	5.7	2.3–14.5	<0.001	1.0	0.2–5.3	0.956
4	4.8	1.5–15.0	0.007	2.8	0.9–8.1	0.064	1.4	0.2–9.1	0.696
≥5	10.8	3.4–34.2	<0.001	3.8	1.4–10.5	0.010	2.1	0.4–12.4	0.402

[1]High-SCC cows were treated with antibiotic plus teat sealant (H-ABTS) and low-SCC cows were treated with teat sealant only (L-TS). The table shows the association (OR and 95% CI) between both categories at DO and teat sealant presence versus absence, based on adjusted teat sealant weight being either >0 g or ≤0 g, respectively, in 50 mL premilk of the first milking (n = 661 quarters, 173 cows), the second milking (n = 700 quarters, 176 cows), and the third milking (n = 695 quarters, 174 cows) postcalving. High SCC = cows with any of the 3 most recent monthly DHI tests before DO showing a cow-level SCC ≥200,000 cells/mL; low-SCC = cows with all 3 most recent DHI tests before DO showing a cow-level SCC <200,000 cells/mL.

[2]The intraclass correlation coefficient at the first milking was 0.40 at the cow level (95% CI; 0.28–0.55) and 0.02 at the herd level (95% CI; 0.00–0.34).

[3]The intraclass correlation coefficient at the second milking was 0.42 at the cow level (95% CI; 0.27–0.58) and 0.14 at the herd level (95% CI; 0.04–0.39).

[4]The intraclass correlation coefficient at the third milking was 0.58 at the cow level (95% CI; 0.41–0.74) and 0.05 at the herd level (95% CI; 0.00–0.56).

83% quarter-level TS visibility, as judged by farmers, in the study of Kabera et al. (2018). High farmer sensitivity (74.5%) versus the laboratory to see TS at the first milking decreased to 2.7%, 0.0%, and 8.3% at the last 3 milkings, suggesting that farmers can see TS residues cowside as accurately as laboratory personnel in the laboratory at the start, but afterward, when lower quantities of TS are excreted, this becomes more difficult. We have seen that if left to laboratory personnel to see TS residues, instead of farmers to judge visibility in premilk, the estimated duration of excretion may be longer than the maximum of 12 d estimated by farmers (Kabera et al., 2018), and agree with Berry and Hillerton (2002), it could even last until 21 d postcalving. Nevertheless, mean-adjusted quantities of TS residues excreted after the third milking were low and close to zero (Figure 3), and were excreted in only around 5% of quarters at each milking (Figure 4). If presented as TS presence at any time point after the third milking, 23.9% of all quarters and 51.4% of all cows showed TS presence, often intermittently, again suggesting that low quantities of TS residues can be excreted for quite some time after the first week postcalving. These small TS quantities could, over time, still contribute to the obstruction of milk pipes, milk meters, sensors and associated cleaning difficulties or end up in the bulk tank when able to pass through the milk filter.

Study Groups and Interpretation

In addition to comparing TS visibility in the laboratory versus cowside, this study's principal aim was to compare TS excretion between 2 study groups, high- and low-SCC cows. In these 2 groups, the level of SCC was inseparably connected to the treatment protocol because low-SCC cows were always treated with TS only and high-SCC cows were always treated additionally with AB. In addition to treatment protocol, the 2 groups also differed in parity and milk production (Table 2). Although the multivariable analysis corrected for these differences, no model is ever perfect, and the issue remained that the animals were different and not randomly assigned. Last, the 2 study groups were allocated at the cow level but most of the analysis was performed at the quarter level. This means, for example, that high-SCC quarters were from low-SCC cows and vice versa. We have chosen cow-level grouping because selection of dry cow therapy also usually occurs at the cow level, because treatment selection at the quarter level is not commonly done.

TS Excretion Quantities

At the first milking, L-TS cows excrete a significantly higher percentage (45.5%) of the total amount of TS infused, compared with H-ABTS cows (32.0%) in an equal percentage (73%) of quarters (Figures 3 and 4). The associated mean-adjusted quantities excreted at the first milking of 1.83 g and 1.15 g in the L-TS versus H-ABTS category, respectively, were higher than the 1.27 g and 0.65 g in L-TS and H-ABTS cow categories, respectively, as reported by Bradley et al. (2010), but lower than the 2.64 g in L-TS cows found in the study of Bates et al. (2022). Such differences in TS excretion quantities are expected due to variability between the studies in milk production, residual milk solids, and the presence or absence of adjustments for it, the TS product, or insertion techniques. However, a higher mean quantity excreted in L-TS versus H-ABTS quarters at the first milking was in line with the findings of Bradley et al. (2010). The latter study was a within-cow comparison, excluding between cow variation, allowing for attributing differences between categories in excretion to the difference in treatment. We could not do that because the 2 selective dry cow therapy study categories, H-ABTS and L-TS cows, were not the same because besides SCC and treatment, they also differed in milk yield, parity, TEC, and dry period length (Tables 2 and 3).

Teat sealant excretion quantities at the first milking varied widely among quarters and, interestingly, as in the study of Bates et al. (2022), showed a bimodal distribution. The bimodality was more pronounced in the L-TS category, showing a higher density of quarters excreting larger amounts of TS compared with the H-ABTS cow category (Figure 2). Our multivariable model was based on presence versus absence and not on TS quantities and therefore we have no further associations nor a clear explanation why TS residues are excreted bimodally, and why this is more pronounced in the L-TS than in the H-ABTS cow category at the first milking. Maybe, besides excretion quantity, as was previously suggested (Bradley et al., 2010), treatment protocol also directly or indirectly affects excretion pattern. Additionally, differences could also be due to the lower parity; higher milk production; and lower SCC, TEC, and dry period length in the L-TS versus the H-ABTS categories (Table 2).

At the second and third milkings, the TS percentage excreted reversed between the DO categories, becoming significantly higher in the H-ABTS versus the L-TS groups (Figure 3). These higher percentages of TS excretion in the H-ABTS category were accompanied by excretion in a significantly higher percentage of quarters compared with L-TS cows (Figure 4). As expected, at the second and third milkings, the H-ABTS category was catching up from the lower TS excretion at the first milking.

Associations of DO Category and TS Presence

The associations of TS presence versus absence between study categories, when corrected for parity, are shown in Table 8. In line with excreted TS percentage and quantities, the odds for TS presence at the first milking were higher in L-TS than in H-ABTS cows (odds ratio [**OR**] = 1.9), and the odds at the second (OR = 0.4) and third milkings (OR = 0.2), indicated the opposite: a higher probability of TS presence in H-ABTS versus L-TS cows.

An association of TS residues with treatment protocol has been suggested as an explanation for the difference in excretion between the DO categories at the first milking (Bradley et al., 2010). These authors looked at excreted TS quantities and hypothesized that oil-based AB-containing substance in dry cow tubes would mix with the oily substance in TS, thus facilitating TS dispersion into the udder, and therefore less TS would be retrieved at the first premilking. This finding also helps to explain the now-inversed significant difference in odds of presence between the DO categories at the second and third milkings (OR = 0.4 and 0.2, respectively), again suggesting that after the first milking, TS excretion of H-ABTS cows was catching up with that of L-TS cows.

Like Kabera et al. (2018), we found an association of TS presence and parity. In our model, the difference between older cows versus heifers was significant for the first 2 milkings only (Table 8), probably because at these milkings excreted TS quantities were high enough to be able to show a difference in presence versus absence (Figure 3) or because fewer cows were excreting TS after the first 2 milkings, the comparison lost the statistical power to show a difference. It is unclear why older cows would have higher odds for TS presence. An explanation could be that because older cows have both longer teats and a wider teat canal, they can hold more TS at the teat base, which is further away from the proximal teat cistern and opening to the gland cistern where milk is coming down into the teat canal to mix with the teat sealant and disperse into the udder (Meaney, 1977), leaving more TS in the distal teat canal and, as a result, a higher probability of any TS to be stripped out at the first premilking. To confirm this anatomy-based hypothesis further research, using regular x-rays to track TS location over time during the dry period, would be needed.

TS Presence and Udder Health

As with previous studies (Bradley et al., 2010; Kabera et al., 2018; Bates et al., 2022), we found no association between udder health status as defined by bacteriology and TS presence at the first premilking, confirming the low clinical relevance of differences in TS excretion between the 2 treatment groups. If a proportion of the administered TS is dispersed into the udder without having a negative effect udder health, less than the 4 g of content in each TS tube in each quarter may be sufficient (Smith, 2019). If this is true, lower amounts of TS administered would likely reduce TS residue excretion duration; lower the probability of contamination or obstruction of milk liners, pipes, and tubes; and potentially, and lower the residues passing through the milk filters, entering the bulk milk tank, and ending up in dairy products. However, if reducing the dose does not affect duration of excretion, the benefits will be limited.

Study Limitations

This work was conducted to study the excretion of TS and the prevalence of udder health–related dry period outcomes of the 2 study categories, but not to investigate associations, resulting in limited power to find associations between bacterial udder health status and TS excretion; it further suffered from the high average percentage of bacteriological contamination of milk samples (19.6%), ranging from 9% to 44% among the 7 farms, likely reflecting differences in ability among individuals taking samples. Sampling for bacteriology needs to be done slowly and precisely, and it is likely we underestimated the pressure milkers were under having to sample 8 quarters for each cow enrolled, each day, during each of the 7 to 8 d postcalving to recover the TS residues. We hypothesized this may have disturbed the milking routine to such an extent that, despite the extended prestudy training on asepsis, the relatively few samples for bacteriology were taken too quickly and less precisely, eventually resulting in elevated bacterial contamination rates.

Another study limitation was that we only looked at TS residues recovered in 50 mL of premilk, assuming all TS residues are present in premilk. It is currently not clear how the excretion of TS residues in premilk relates to the amount excreted during the rest of the milking. This could explain why the cumulative proportion of TS excreted in premilk of the first 3 milkings was only around 40% to 50%. Another explanation could be that after milking 3, part of the TS infused was still somewhere in the udder. Also, besides the 2.6 g of bismuth salts, TS tubes used in this study contain an additional 1.4 g of mainly liquid paraffin, which easily emulsifies with milk, and as a result would be difficult to identify as TS residue and likely would not cause issues for milking equipment nor for aged cheeses.

Although the study protocol prescribed to separate the calf from the mother directly after birth, farmers and milkers were given the opportunity to indicate if calves were left with the mother unattended. If so, they could potentially have suckled their mother, thereby removing TS residues and potentially resulting in an underestimation of TS residue excretion. Leaving the calf unattended was indicated to have occurred in 15.2% and 18.7% of calves in the H-ABTS and L-TS categories, respectively, and particularly occurred at farm E and F. Despite this percentage not being different between the DO categories (P = 0.53), quantities are unknown, and therefore could have influenced the outcome of the study group comparison. However, when eliminating calves who were left unattended with their mother from the data analysis, the conclusions from the multivariable model on the TS excretion at the first milking did not change.

CONCLUSIONS

In premilk of the first milking, TS residue excretion was bimodal, higher in L-TS cows, more likely to be present in multiparous cows, and not associated with udder health. At the second and third milkings, TS excretion was higher in H-ABTS cows and TS presence was only more likely in multiparous cows at the second milking.

NOTES

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Nonstandard abbreviations used: AB = antibiotics; BG = biogas substrate bedding; BMSCC = bulk milk SCC; C = cubicle; CM = clinical mastitis; DO = dry off; H-ABTS = high-SCC cows treated with AB and TS; HF = Holstein Friesian; ITS = internal teat sealant; L = lime bedding; L-TS = low-SCC cows treated with TS only; M = dried manure bedding; OR = odds ratio; P = pasture; St = straw bedding; Sw = sawdust bedding; TEC = teat end callosity; TS = teat sealant; W = bedding mixed with water.

REFERENCES

Barreiro, J. R., C. R. Ferreira, G. B. Sanvido, M. Kostrzewa, T. Maier, B. Wegemann, V. Böttcher, M. N. Eberlin, and M. V. dos Santos. 2010. *Short communication:* Identification of subclinical cow mastitis pathogens in milk by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J. Dairy Sci. 93:5661–5667. https://doi.org/10.3168/jds.2010-3614.

Bates, A. J., C. King, M. Dhar, C. Fitzpatrick, and R. A. Laven. 2022. Retention of internal teat sealants over the dry period and their efficacy in reducing clinical and subclinical mastitis at calving. J. Dairy Sci. 105:5449–5461. https://doi.org/10.3168/jds.2021-21585.

Berry, E. A., and J. E. Hillerton. 2002. The effect of an intramammary teat seal on new intramammary infections. J. Dairy Sci. 85:2512–2520. https://doi.org/10.3168/jds.S0022-0302(02)74334-8.

Bradley, A. J., J. E. Breen, B. Payne, P. Williams, and M. J. Green. 2010. The use of a cephalonium containing dry cow therapy and an internal teat sealant, both alone and in combination. J. Dairy Sci. 93:1566–1577. https://doi.org/10.3168/jds.2009-2725.

Buckley, M. P.,, J. Bayne, T. Tomazi, B. E. Miller, G. S. Silva, and P. J. Gorden. 2023. Evaluation of internal teat sealant persistence in the mammary gland during the dry period. Bov. Pract. (Stillwater) 57:51–59. https://doi.org/10.21423/bovine-vol27no2p51-59.

Dingwell, R. T., K. E. Leslie, Y. H. Schukken, J. M. Sargeant, and L. L. Timms. 2003. Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactation dairy cows. Can. Vet. J. 44:413–415.

Dingwell, R. T., K. E. Leslie, Y. H. Schukken, J. M. Sargeant, L. L. Timms, T. F. Duffield, G. P. Keefe, D. F. Kelton, K. D. Lissemore, and J. Conklin. 2004. Association of cow and quarter-level factors at drying-off with new intramammary infections during the dry period. Prev. Vet. Med. 63:75–89. https://doi.org/10.1016/j.prevetmed.2004.01.012.

Dufour, S., V. Wellemans, J. Roy, P. Lacasse, A. Ordonez-Iturriaga, and D. Francoz. 2019. Non-antimicrobial approaches at drying-off for treating and preventing intramammary infections in dairy cows. Part 1. Meta-analyses of efficacy of using an internal teat sealant without a concomitant antimicrobial treatment. Anim. Health Res. Rev. 20:86–97. https://doi.org/10.1017/S1466252319000070.

European Commission. 2010. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official J. EU 276:33–79.

European Union. 2019. Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products. https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:02019R0006-20220128&qid=1694511915171.

Golder, H. M., A. Hodge, and I. J. Lean. 2016. Effects of antibiotic dry-cow therapy and internal teat sealant on milk somatic cell counts and clinical and subclinical mastitis in early lactation. J. Dairy Sci. 99:7370–7380. https://doi.org/10.3168/jds.2016-11114.

Green, M. J., J. N. Huxley, and A. J. Bradley. 2002. A rational approach to dry cow therapy 1. Udder health priorities during the dry period. In Pract. 24:582–587. https://doi.org/10.1136/inpract.24.10.582.

Haldemann, J. 2021. Black spots in cheese caused by teat sealant. Agroscope Merkblatt, no. 138. https://bamos.ch/wp-content/uploads/2021/12/Agroscope-138-Schwarze-Flecken.pdf.

ISO. (International Organization for Standardization). 2006. ISO 13366-2, IDF 148–2. Milk: Enumeration of somatic cells—Part 2; Guidance on the operation of fluoro-opto-electronic counters. ISO.

Kabera, F., S. Dufour, G. Keefe, M. Cameron, and J.-P. Roy. 2020. Evaluation of quarter-based selective dry cow therapy using Petrifilm on-farm milk culture: A randomized controlled trial. J. Dairy Sci. 103:7276–7287. https://doi.org/10.3168/jds.2019-17438.

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

Kabera, F., J.-P. Roy, M. Afifi, S. Godden, H. Stryhn, J. Sanchez, and S. Dufour. 2021. Comparing blanket vs. selective dry cow treatment approaches for elimination and prevention of intramammary infections during the dry period: A systematic review and meta-analysis. Front. Vet. Sci. 8:688450. https://doi.org/10.3389/fvets.2021.688450.

Lay, A. M., K. M. Kolpin, D. A. Sommer, and S. A. Rankin. 2007. *Hot Topic:* Black spot defect in cheddar cheese linked to intramammary teat sealant. J. Dairy Sci. 90:4938–4941. https://doi.org/10.3168/jds.2007-0385.

Lely. 2023. Preparation for dry period pays off. Accessed Oct. 9, 2023. https://www.lely.com/farming-insights/preparation-dry-period-pays.

Meaney, W. J. 1977. Effect of a dry period teat seal on bovine udder infection. Isr. J. Agric. Res. 16:293–299.

NMC (National Mastitis Council). 2017. Laboratory Handbook on Bovine Mastitis. Rev. ed. National Mastitis Council, New Prague, MN.

Neijenhuis, F., H. W. Barkema, H. Hogeveen, and J. P. T. M. Noordhuizen. 2001. Relationship between teat-end callosity and occurrence of clinical mastitis. J. Dairy Sci. 84:2664–2672. https://doi.org/10.3168/jds.S0022-0302(01)74720-0.

Newton, H. T., M. J. Green, H. Benchaoui, V. Cracknell, T. Rowan, and A. J. Bradley. 2008. Comparison of the efficacy of cloxacillin alone and cloxacillin combined with an internal teat sealant for dry cow therapy. Vet. Rec. 162:678–683. https://doi.org/10.1136/vr.162.21.678.

Poelarends, J. J., H. Hogeveen, O. C. Sampimon, and J. Sol. 2001. Monitoring subclinical mastitis in Dutch dairy herds. Page 145– 149 in Proc. 2nd Int. Symp. Mastitis and Milk Quality, Vancouver, BC. National Mastitis Council Inc.

Rowe, S. M., S. M. Godden, E. Royster, J. Timmerman, and M. Boyle. 2021. Postcalving udder health and productivity in cows approaching dry-off with intramammary infections caused by non-*aureus Staphylococcus, Aerococcus, Enterococcus, Lactococcus*, and *Streptococcus* species. J. Dairy Sci. 104:6061–6079. https://doi.org/10.3168/jds.2020-19288.

Smith, B. G. 2019. Internal teat sealants and their use in the prevention of bovine mastitis in the dry cow. Zoetis Broomhill IP Ltd. US Pat. No. 16/249974. https://patents.google.com/patent/US20190343875A1/en.

Sol, J., O. C. Sampimon, E. Hartman, and H. Barkema. 2002. Effect of preculture freezing and incubation on bacteriological isolation from subclinical mastitis samples. Vet. Microbiol. 85:241–249. https://doi.org/10.1016/s0378-1135(01)00507-7.

Vanhoudt, A., K. van Hees-Huijps, A. T. M. van Knegsel, O. C. Sampimon, J. C. M. Vernooij, M. Nielen, and T. van Werven. 2018. Effects of reduced intramammary antimicrobial use during the dry period on udder health in Dutch dairy herds. J. Dairy Sci. 101:3248–3260. https://doi.org/10.3168/jds.2017-13555.

Weber, J., S. Borchardt, J. Seidel, R. Schreiter, F. Wehrle, K. Donat, and M. Freick. 2021. Effects of selective dry cow treatment on intramammary infection risk after calving, cure risk during the dry period, and antibiotic use at drying-off: A systematic review and meta-analysis of current literature (2000–2021). Animals (Basel) 11:3403. https://doi.org/10.3390/ani11123403.

Woolford, M. W., J. H. Williamson, A. M. Day, and P. J. A. Copeman. 1998. The prophylactic effect of a teat sealer on bovine mastitis during the dry period and the following lactation. N. Z. Vet. J. 46:12–19. https://doi.org/10.1080/00480169.1998.36044.