

Contents lists available at ScienceDirect

Animal

The international journal of animal biosciences



Internal teat sealants alone or in combination with antibiotics at dry-off – the effect on udder health in dairy cows in five commercial herds



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ARTICLE INFO

Article history: Received 13 July 2021 Revised 17 December 2021 Accepted 20 December 2021

Keywords: Antimicrobial Mastitis Selective dry cow therapy Somatic cell count Staphylococcus aureus

ABSTRACT

In the dairy industry, the dry period has been identified as an area for potential reduction in antibiotic use, as part of a one health approach to preserve antibiotic medicines for human health. The objective of this study was to assess the impact of dry cow treatment on somatic cell count (SCC), intramammary infection (IMI) and milk yield on five commercial Irish dairy herds. A total of 842 cows across five spring calving dairy herds with a monthly bulk tank SCC of < 200 000 cells/mL were recruited for this study. At dry-off, cows which had not exceeded 200 000 cells/mL in the previous lactation were assigned one of two dry-off treatments: internal teat seal (ITS) alone (Lo_TS) or antibiotic plus ITS (Lo_AB + TS). Cows which exceeded 200 000 cells/mL in the previous lactation were treated with antibiotic plus ITS and included in the analysis as a separate group (Hi_AB + TS). Test-day SCC and lactation milk yield records were provided by the herd owners. Quarter milk samples were collected at dry-off, after calving and at mid-lactation for bacteriological culture and quarter SCC analysis. Cow level SCC was available for 789 cows and was log-transformed for the purpose of analysis. Overall, the log SCC of the cows in the Lo_TS group was significantly higher than the cows in Lo_AB + TS group and not statistically different to the cows in the Hi_AB + TS group in the subsequent lactation. However, the response to treatment differed according to the herd studied; the log SCC of the cows in the Lo_TS group in Herds 3, 4 and 5 was not statistically different to the cows in Lo_AB + TS group, whereas in the other two herds, the log SCC was significantly higher in the Lo_TS when compared to the Lo_AB + TS group. There was a significant interaction between dry-off group and herds on SCC and odds of infection in the subsequent lactation. The results of this study suggest that the herd prevalence of IMI may be useful in decision-making regarding the treatment of cows with ITS alone at dry-off to mitigate its impact on udder health.

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Implications

A reduction in antibiotic use is required on dairy farms. This trial examined the effect of using internal teat seal alone compared to antibiotics plus internal teat seal at dry-off, on somatic cell count and level of infection in the following lactation in five commercial dairy herds. Cows treated with internal teat seal alone had higher somatic cell count and were more likely to have intramammary infection in the subsequent lactation. The efficacy of internal teat seals differed across herds, suggesting that the effects depend on herd-level factors such as herd infection level and the implementation of mastitis control measures.

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Introduction

Blanket antimicrobial dry cow therapy was developed and recommended as part of a five-point herd mastitis control plan (Smith et al., 1967). Its purpose includes curing existing infections and preventing new infections during the dry period (Dodd et al., 1969). This practice consists of infusing all teats of the cow with antimicrobials at the end of lactation, with or without the additional use of an internal teat seal (ITS). The effectiveness of antimicrobial dry cow therapy in eliminating existing infections and reducing new infections over the dry period and at calving is widely accepted. A study conducted by Bradley et al. (2010) showed that using dry cow therapy at dry-off resulted in an apparent dry period cure rate of over 90% in intramammary infections (IMIs) caused by major pathogens. Berry and Hillerton (2002a) reported that dry cow therapy reduced the rate of new IMI by

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approximately 80% and eliminated more existing IMI than spontaneous cure

In Ireland, blanket dry cow therapy with or without ITS is widely used, with most recent estimates showing that blanket dry cow therapy reached 100% usage in Irish dairy herds in 2015 (More et al., 2017). However, public concern over the use of antibiotics, and its implications for antimicrobial resistance, has led to the development of regulation 2019/6 on veterinary medicines by the European Union (European Parliament and the Council of the European Union, 2019). This regulation will come into force on 28 January 2022 and will include a regulation on the preventative use of antimicrobials in groups of animals. An alternative strategy to blanket dry cow therapy is to treat cows that demonstrably have IMI quarters or are at higher risk of IMI during the dry period, with antibiotics, while the remaining cows are treated with ITS alone.

Research comparing the effects of treating cows with ITS alone at dry-off versus antibiotic plus ITS on new IMI has mostly shown non-significant differences (Bradley et al., 2010; Vasquez et al., 2018). The sustained improvement in annual average somatic cell count (SCC) in Irish dairy herds (Animal Health Ireland, 2018) along with a need to reduce the use of antibiotics have led to exploring strategies for the treatment of low SCC cows with ITS alone at dry-off. However, McParland et al. (2019) reported that cows with low SCC across lactation (< 200 000 cells/mL) treated with ITS alone at dry-off had significantly higher SCC in the following lactation compared to cows treated with antibiotics plus ITS, in three research herds with similar bulk tank SCC. The differences found in the McParland et al. (2019) study compared to previous studies are likely due to differences in the predominant bacterial challenge that cows are exposed to. In Ireland, Staphylococcus aureus is the predominant pathogen (Egan and O'Dowd, 1982; Keane et al., 2013). International studies on treatment with ITS alone have reported a greater incidence of environmental major pathogens such as Streptococcus uberis or minor pathogens such as Coagulase-negative Staphylococci (CNS; Bradley et al., 2010; Vasquez et al., 2018). Irish dairy herds are seasonal calving and therefore large numbers of cows are dried off over a relatively short period of time at a point in the lactation when bulk tank SCC is at its highest (O'Connell et al., 2015). The primary objective of the current study was to quantify the effect of the administration at dry-off of ITS alone compared to antibiotics plus ITS on SCC, IMI, and milk production in the subsequent lactation of commercial cows with a low SCC (< 200 000 cells/mL). A secondary objective was to compare the SCC, IMI and milk production of cows with a high SCC (>200 000 cells/mL), following dry-off treatment with antibiotic plus ITS, to that of low SCC cows treated either with antibiotics plus ITS or ITS alone.

Material and methods

Herd selection

Five commercial dairy herds located in the south of Ireland were recruited for this study, which ran from October 2018 to December 2019, inclusive. All herds were spring calving (whereby the majority of cows calved between early February and mid-April) pasture-based systems of milk production. Herd size ranged from 114 to 244 cows and mostly comprised Holstein-Friesian genetics, with some Jersey crossbreds (Table 1). Bulk tank milk was collected and analysed every one to three days by one milk processing company, Kerry Agribusiness (Tralee Road, Castleisland, Co. Kerry, Ireland; https://www.kerryagribusiness.ie). All herds had a monthly bulk tank SCC of < 200 000 cells/mL for their 2018 lactation and conducted regular whole-herd milk recording (minimum of 4 test

dates) throughout the lactation (Table 1). Milk recording data were provided by the herd owners and bulk tank SCC data were provided by the milk processor, Kerry Agribusiness.

Treatment assignment

Herds undertook between four and nine test-day milk recordings during the 2018 lactation (Table 1). The cow level SCC (CSCC) obtained from these milk recordings was used to categorise cows within each herd. Cows with no CSCC recording greater than 200 000 cells/mL throughout the lactation of 2018 were categorised as low SCC cows. Cows which had one or more CSCC recordings greater than 200 000 cells/mL were classified as high SCC cows. Cows in the low SCC category were sorted on a perherd basis according to lactation number, proportion of Holstein-Friesian genetics, average SCC and expected week of calving in spring 2019. They were sequentially assigned to one of two groups: the first group received ITS (Boviseal, Bimeda) alone at dry-off (Lo_TS), while the second group (control) received a Cephalonium- based antibiotic (Cepravin Dry Cow Intramammary Suspension, MSD Animal Health) plus ITS (Lo_AB + TS) at dry-off. Cows categorised as high SCC were considered as a third group (Hi_AB + TS) and were administered the same antibiotic and ITS at dry-off as the Lo_AB + TS group. A total of 297, 294 and 251 cows were allocated to Lo_TS, Lo_AB + TS and Hi_AB + TS groups, respectively (Table 1).

Drying-off procedure and dry cow management

Cows were dried off in batches determined by the herd manager, depending on expected calving date and end of lactation milk production. The drying-off procedure was carried out by trained Teagasc research personnel across all five participating herds. Gloves were worn for the duration of the dry-off procedure and both gloves and teat ends were disinfected with cotton swabs soaked in methylated spirits before treatment was administered. The teat end was cleaned with swabs until the dirt was no longer visible on the swab. Front teats were disinfected first followed by the rear teats. Administration of the treatments was started in the opposite direction to avoid contamination of disinfected teats; rear teats were infused with treatment first, followed by front teats. For the cows in both the Lo_AB + TS and Hi_AB + TS groups, the nozzle of the antibiotic syringe was inserted into the teat canal and the contents infused. The antibiotic was then massaged up into the gland. Immediately after antibiotic administration and teat massage, ITS was infused to the same teat by pinching the base of the teat and inserting the nozzle into the teat canal. The ITS was not massaged, to ensure it remained in the teat cistern. For cows that were assigned to the Lo_TS group, teats were disinfected and ITS was infused following the same procedure as outlined previously. All teats were sprayed immediately after treatment with postmilking teat spray and cows were left to stand for 20 minutes. On the day of dry-off, cows were either housed in cubicles or let out to bare paddocks. Subsequently, cows were all housed in cubicles for the remainder of the dry period. Cubicles were swept and lime was added daily for the first 2-3 weeks of the dry period and automatic scrapers were run on passageways a minimum of once per day for the duration of housing. Cows were housed on clean straw beds the day of calving and were housed in cubicles postcalving. Postcalving, cubicles were swept and limed a minimum of twice daily until full-time grazing commenced.

Quarter level sampling

Quarter milk samples were aseptically collected prior to milking from all cows by trained Teagasc research personnel at three

Table 1
Total herd size in 2018 (pretrial), total number of dairy cows included in trial from 2018 to 2019, total number of dairy cows treated with Lo_TS¹, Lo_AB + TS² and Hi_AB + TS³, percentage of dairy cows Jersey crossbred⁴, mean (SD) 305 day milk yield 2018 (pretrial), mean bulk tank SCC October to November 2018 (pretrial), mean (range) parity in 2019, total number of test-day milk recordings carried out in 2018 and 2019, mean number of days between final test-day milk recording and dry-off 2018 (pretrial) and mean (SD) length of dry period for Herds 1–5.

	Herd				
Item	1	2	3	4	5
Count (no. of cows)					
Herd size 2018	220	221	244	125	114
Included in trial 2018–2019	199	207	229	105	102
Lo_TS ¹	73	75	67	42	40
Lo_AB + TS ²	75	68	72	41	38
Hi_AB + TS ³	51	64	90	22	24
Jersey crossbred ⁴ , %	39	49	19	2	0
305 day milk yield 2018, kg	6 031 (1 010)	6 719 (983)	6 009 (926)	7 104 (989)	5 841 (814)
Bulk tank SCC Oct-Nov 2018 ('000)	119	192	121	111	111
Parity 2019	3.5 (2-9)	4.0 (2-11)	3.2 (2-8)	4.1 (2-10)	3.3 (2-4)
No. of test-day milk recordings in 2018	5	4	9	7	7
No. of test-day milk recordings in 2019	6	5	8	7	7
Days between final test-day milk recording and dry-off 2018	23	40	30	26	31
Length of dry period days	90 (21)	101 (18)	86 (16)	96 (17)	101 (18)

Abbreviation: SCC = somatic cell count.

- 1 Lo TS = cows with cow somatic cell count < 200 000 cells/mL for all the 2018 lactation dried off with teat seal alone.
- ² Lo_AB + TS = cows with cow somatic cell count < 200 000 cells/mL for all the 2018 lactation dried off with antibiotic plus teat seal.
- ³ Hi_AB + TS = cows with ≥ one cow somatic cell count > 200 000 cells/mL during the 2018 lactation dried off with antibiotic plus teat seal.
- ⁴ Jersey crossbred defined as at least 25% Jersey.

time points across the lactation (Supplementary Table S1). Aseptic collection of quarter milk samples was achieved by disinfection of the teat ends with cotton swabs soaked in methylated spirits; teat ends were cleaned with swabs until the dirt was no longer visible on the swab. Front teats were disinfected first followed by the rear teats. Fore strips were discarded and milk collected on a perquarter basis in the opposite direction to avoid contamination of disinfected teats; rear teats were stripped and collected first, followed by front teats (Adkins et al., 2017). The first quarter milk sample was collected the day of dry-off, before the final milking. The second quarter milk sample was collected after calving (average 8 days in milk (**DIM**); SD = 3); sampling was conducted once a week on each farm during the calving period. The third quarter milk sample was collected at mid-lactation (average 100 DIM; SD = 21). The quarter milk samples were collected in 30 ml bottles with a different colour lid to identify each quarter and labelled. Sample bottles were brought to the laboratory immediately after sampling and refrigerated at 4 °C until processing. Quarter milk samples were processed within 72 h of collection.

Quarter level somatic cell count and bacteriological analysis

Somatic cells from quarter milk samples were counted using a Bentley Somacount 300 (Bentley Instrument Inc., Chaska, Minnesota 55318, USA). In order to identify the pathogens causing IMI, a non-selective media Blood Agar Base No 2 (OXOID) was used to isolate and identify bacteria from aseptic foremilk samples. Blood agar allows good differentiation between colonies of Streptococcus spp., Staphylococcus spp. and Micrococcus spp. To improve differentiation between Streptococcus spp., 0.1% aesculin was added to the media. The plates were divided into four equal quadrants, one for each quarter of the same cow. Samples were plated using 10 µL aseptic disposable loops. If samples had been refrigerated, they were warmed to 16-18 °C before mixing. Plates were incubated at 37 °C and examined 24-48 h after incubation, and colony morphology was assessed. Growth morphologic features (colony size, shape, colour, haemolytic characteristics) were used to identify and quantify bacterial colonies present. Staphylococcus aureus produces creamy, greyish-white and occasionally golden-yellow colonies on blood agar, 3-5 mm in diameter with

typical zones of haemolysis. Streptococcus spp. usually produce small (1–3 mm diameter) colonies that are smooth, translucent, cone shaped on blood agar. *Escherichia coli* has large grey colonies after 24 hr incubation and frequently produce mucoid colonies on blood agar (Adkins et al., 2017). Infection was defined as the isolation of at least six CFU of the same pathogen in the plated quarter milk sample (600 CFU/mL). A sample was considered contaminated if more than two different types of colonies grew on the plate; contaminated samples were discarded. If there were two types of colonies on the same plate, the predominant colony was considered the main cause of infection. Quality assurance of the described methodology is included in Supplementary Material S1.

Data analysis

We analysed the effect of dry-off group on CSCC in both early lactation and over the total lactation in 2019 for all cows available to the study. We also analysed the effect of dry-off group on CSCC for cows that were bacteriologically negative at dry-off. The effect of dry-off group on quarter SCC was analysed at dry-off (2018), calving (2019) and mid-lactation (2019). The effects of dry-off group on the odds of an IMI (defined as a bacteriologically positive quarter milk sample) at calving (2019) and at mid-lactation (2019) were analysed. The effects of dry-off group on the odds of an IMI on quarters with no IMI at dry-off (defined as bacteriologically negative) were also analysed. In addition, the odds of new IMI over the dry period and cured IMI over the dry period were analysed.

Somatic cell count (CSCC and quarter SCC) was transformed to log somatic cell count (CLogSCC and QLogSCC for cow and quarter level, respectively), as the logarithm to the base 10 of SCC (after adding a shift parameter of 1). Parities greater than five were grouped together. Twelve late-January calving cows (January 26–January 31) were grouped with February calving cows. Fig. 1 shows the number of cows/quarters enrolled in the trial and the different exclusions depending on the analysis conducted.

Quarter log somatic cell count analysis

Quarter milk samples were collected from 836 cows at dry-off (2018). Quarter milk samples obtained at calving were edited for analysis to remove samples taken <4 DIM or >15 DIM (n = 73,

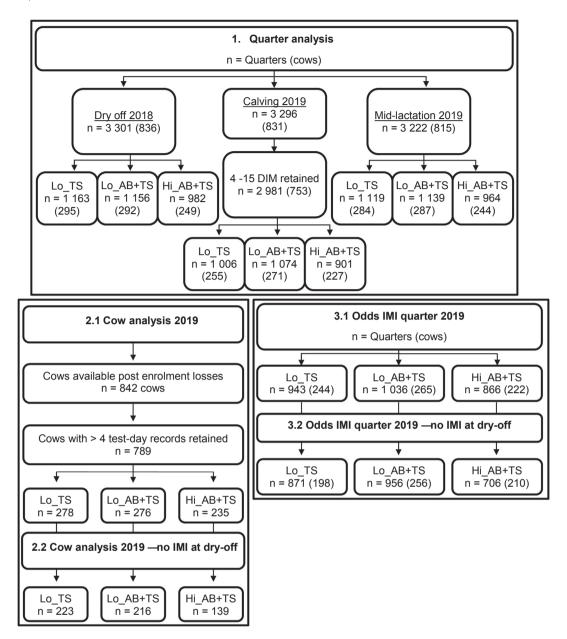


Fig. 1. Enrolment of dairy cows and quarters and generation of datasets used for analysis. Lo_TS = cows with cow somatic cell count < 200 000 cells/mL for all the 2018 lactation dried off with teat seal alone; Lo_AB + TS = cows with cow somatic cell count < 200 000 cells/mL for all the 2018 lactation dried off with antibiotic plus teat seal; Hi_AB + TS = cows with \geq one cow somatic cell count > 200 000 cells/mL during the 2018 lactation dried off with antibiotic plus teat seal; DIM = days in milk; IMI = intramammary infection.

Fig. 1). Each farm was visited once a week during the calving season for quarter milk sampling, which resulted in duplicate quarter milk samples for some cows. If this occurred, the first quarter milk sample collected was retained for analysis. For the analysis of QLogSCC in mid-lactation, there were quarter milk samples from 815 cows available. The breakdown of quarters and cows retained within each group across the three sampling points are detailed in Fig. 1 (dataset "1. Quarter analysis"). The effect of group (Lo_TS, Lo_AB + TS or Hi_AB + TS) on QLogSCC was tested using a repeatability linear mixed model (SAS 9.4, SAS Institute Inc., Cary, NC, USA) adjusted for the random effect of cow and the fixed effects of month of calving (February, March, April), parity (2, 3, 4 and 5 or greater), herd (1, 2, 3, 4, 5), proportion of Jersey genetics (continuous), the length of the dry period (continuous) and an interaction between time of testing (dry-off, calving, mid-lactation) and group (Lo_TS, Lo_AB + TS, Hi_AB + TS). The repeated effect of quarter

(front right, hind right, front left, hind left) and time of testing (calving, mid-lactation) nested within cow was tested. An autoregressive covariance structure was fitted. Model details are supplied in Supplementary Material S2.

Test-day cow log somatic cell count analysis

Cows with less than four test-day records available in 2019 (n = 53, Fig. 1) and records obtained after 305 DIM (n = 5) were removed from the dataset ("2.1 Cow analysis 2019"; Fig. 1). Early lactation records consisted of the first record from each cow up to 60 DIM (n = 787). Cows with an IMI at dry-off (n = 211) identified through bacteriology of the quarter milk samples were removed to create an additional dataset ("2.2 Cow analysis 2019 – no IMI at dry-off"; Fig. 1). The effect of the group the cows were assigned to (Lo_TS, Lo_AB + TS or Hi_AB + TS) on 2019 CLogSCC was analysed by considering 1) all cows in the dataset ("2.1 Cow

analysis 2019"; n = 789), 2) early lactation records (n = 787), and 3) cows with no IMI at dry-off (dataset "2.2 Cow analysis 2019 – no IMI at dry-off"; n = 578).

A repeatability linear mixed model (SAS 9.4, SAS Institute Inc., Cary, NC, USA) adjusted for the random effect of cow and the fixed effects of group (Lo_TS, Lo_AB + TS or Hi_AB + TS), parity (2, 3, 4 and 5 or greater), DIM (continuous) and herd (1, 2, 3, 4, 5) was tested. Cow was included as a repeated effect except when considering the effect of group in early lactation. The effect of dry period length was considered as a covariate; however, removed from final analyses as it had a non-significant effect. Model details are supplied in Supplementary Material S2, and the residual influence diagnostics component of the PROC MIXED is included in Supplementary Fig. S1. In a separate series of analyses, the interaction of herd with dry-off group (Lo_TS, Lo_AB + TS or Hi_AB + TS) was tested. The effect of dry-off group (Lo_TS, Lo_AB + TS or Hi_AB + TS) on the odds of CSCC exceeding 100, 200 or 400 × 1 000 cells/mL at any point in lactation (dataset "2.1 Cow analysis 2019") was quantified using a logistic regression model (PROC GENMOD; SAS 9.4, SAS Institute Inc., Cary, NC, USA) adjusted for the same fixed effects as the CLogSCC mixed model. Model details are provided in Supplementary Material S3.

Quarter bacteriological analysis

Bacteriological results were used to quantify the effect of dryoff group (Lo_TS, Lo_AB + TS, Hi_AB + TS) on the odds of an IMI quarter, 1) at calving and 2) in mid-lactation. Only quarters with results for all three sampling periods were included in the analysis (n = 2.845; 3.1 Odds IMI quarter 2019; Fig. 1). Quarters which had bacteria present at dry-off were removed from the dataset (n = 312) and the odds of an IMI in the 2019 lactation were analysed as separate dataset ("3.2 Odds IMI quarter 2019 - no IMI at dry-off"; Fig. 1). Quarter IMI was categorised as present or absent and was analysed using logistic regression (PROC GENMOD; SAS 9.4, SAS Institute Inc., Cary, NC, USA) adjusted for the same fixed effects as the model to predict QLogSCC with the exception of dry period length, month of calving and proportion of Jersey genetics. In a separate series of analysis, the interaction of herd with dryoff group (Lo_TS, Lo_AB + TS or Hi_AB + TS) was tested; month of calving was included as a fixed effect in this analysis. Model details are provided in Supplementary Material S3 and the receiver operating characteristic (ROC) curve estimating the goodness of fit and calibration curve for each decile of the predicted probability of the logistic regression is included in Supplementary Fig. S2.

The odds of a cured quarter (defined as IMI at dry-off but not at calving) and the odds of a new IMI quarter (defined as no IMI at dry-off but IMI at calving) were also investigated. Due to the small number of cured quarter observations available (n = 312), the logistic regression model included only cow, group and quarter as fixed effects; quarter nested within cow was included as a repeated effect (Supplementary Material S3).

Milk yield analysis

Records for 305d milk kg, fat kg and protein kg yield from 2019 were available for 836 cows. After biological extremes were removed, 833, 833 and 835 records were available for milk, fat and protein yield, respectively. The effect of dry-off group (Lo_TS, Lo_AB + TS or Hi_AB + TS) on milk, fat and protein yield was quantified using a linear mixed model adjusted for the same fixed effects as for CLogSCC; DIM was not included as a fixed effect, and the proportion of Jersey genetics was included only in milk and fat yield models (Supplementary Material S2).

Power calculation

Using SCC records from 2018 of the cows in the recruited herds, considering the availability of approximately 300 cows per group

and using the sample size calculation formula from Snedecor and Cochran (1989), we estimated that our detectable differences for CLogSCC with a power of 80% were less than 0.1 on the log scale. This calculation was also conducted for each herd separately resulting in the same detectable difference for CLogSCC. A similar power calculation using data from quarter sample results at dry-off showed that our detectable differences for QLogSCC were 0.2 on the log scale. We also tested our detectable differences ex post by randomly sampling 25% of the cows recruited in the trial and ran the mixed model again with that dataset. Results showed that with that dataset, we were still able to detect differences between the treatments at $\alpha = 5\%$ level and therefore we concluded that our study was adequately sized.

Results

Table 1 shows the mean 305d milk yield for 2018 (pretrial), mean bulk tank SCC October to November 2018 (pretrial), parity in 2019, number of test-day milk recordings in 2019, mean days between final test-day milk recording and dry-off and dry period length for each herd. The percentage of cows in the Hi_AB + TS group ranged from 21% in Herd 4 to 39% in Herd 3.

Quarter log somatic cell count analysis

Table 2 shows the LS means of the QLogSCC by group (dataset "1. Quarter analysis"; Fig. 1). The QLogSCC of the cows in the Lo_TS group was higher than the cows in the Lo_AB + TS group at calving (2019; P < 0.001) and mid-lactation (2019; P < 0.001).

Cow log somatic cell count analysis

In 2019 (dataset "2.1 Cow analysis 2019"; Fig. 1), the cows in the Lo_TS group had a significantly higher CLogSCC (P < 0.001) than cows in the Lo_AB + TS group. The CLogSCC of the Lo_TS group was not significantly different to the cows in the Hi_AB + TS group (Table 3). In early lactation (<60DIM), cows in the Lo_TS group had a significantly higher CLogSCC (P < 0.001) than cows in the Lo_AB + TS group. However, there was a non-significant treatment difference between the CLogSCC of cows in the Lo_TS group and those in the Hi_AB + TS group. The raw unadjusted mean (median) lactation SCC in 2019 was 125 213 (43 500) cells/mL, 75 753 (31 000) cells/mL and 167 080 (48 000) cells/mL for the cows in the Lo_TS, Lo_AB + TS and Hi_AB + TS groups, respectively.

In Herds 1 and 2 in 2019, cows in the Lo_TS group had a significantly higher CLogSCC (P < 0.001) than cows in the Lo_AB + TS group. However, there was a non-significant difference in CLogSCC between the Lo_TS and Lo_AB + TS groups in Herds 3, 4 and 5 (Table 3). In Herd 2, the CLogSCC of the cows in the Lo_TS group

Table 2Least square means (SE) of quarter level log somatic cell count (dataset "1. Quarter analysis") for each group of dairy cows across the three sample periods.

	Group			
Sample Period	Lo_TS ¹	Lo_AB + TS ²	Hi_AB + TS ³	
Dry-off	4.3(0.03) ^B	4.3(0.04) ^B	4.7(0.04) ^A	
Calving	$4.3(0.04)^{A}$	$4.1(0.04)^{B}$	$4.2(0.04)^{A}$	
Mid-lactation	$3.6(0.04)^{A}$	$3.4(0.04)^{B}$	$3.6(0.04)^{A}$	

 $^{^{1}\,}$ Lo_TS = cows with cow somatic cell count < 200 000 cells/mL for all the 2018 lactation dried off with teat seal alone.

A,B Different letters within a row represent significant differences at P < 0.001.

 $^{^2}$ Lo_AB + TS = cows with cow somatic cell count < 200 000 cells/mL for all the 2018 lactation dried off with antibiotic plus teat seal.

 $^{^3}$ Hi_AB + TS = cows with \geq one cow somatic cell count > 200 000 cells/mL during the 2018 lactation dried off with antibiotic plus teat seal.

Table 3

Least square mean estimates (SE) for each group of dairy cows, of test-day cow log somatic cell count for the full dataset (" $2.1\ Cow\ analysis\ 2019$ ") and individual herds and for cows without infection at dry-off (dataset " $2.2\ Cow\ analysis\ 2019\ -\ no\ intramammary\ infection\ at\ dry-off")$ for all herds and for individual herds for the 2019 lactation.

	Group						
Item	Lo_TS ¹	Lo_AB + TS ²	Hi_AB + TS ³				
2.1 Cow analysis 2019							
All Herds	$4.7(0.02)^{A}$	$4.5(0.02)^{B}$	$4.8(0.02)^{A}$				
Herd 1	$4.7(0.04)^{A}$	$4.5(0.04)^{B}$	$4.7(0.05)^{A}$				
Herd 2	$5.1(0.04)^{A}$	4.6(0.04) ^C	$4.8(0.05)^{B}$				
Herd 3	$4.8(0.04)^{AB}$	$4.7(0.04)^{B}$	$4.9(0.03)^{A}$				
Herd 4	4.6(0.05)	4.5(0.05)	4.6(0.07)				
Herd 5	$4.5(0.05)^{ab}$	$4.4(0.05)^{b}$	$4.6(0.06)^{a}$				
2.2 Cow analysis 2019 – no intramammary infection at dry-off							
All Herds	$4.7(0.02)^{A}$	$4.5(0.02)^{B}$	$4.7(0.03)^{A}$				
Herd 1	$4.6(0.04)^{A}$	$4.5(0.04)^{B}$	$4.6(0.06)^{A}$				
Herd 2	$4.9(0.04)^{A}$	$4.6(0.05)^{B}$	$4.6(0.08)^{B}$				
Herd 3	$4.8(0.04)^{B}$	$4.7(0.03)^{B}$	$4.9(0.03)^{A}$				
Herd 4	4.5(0.05)	4.5(0.05)	4.6(0.08)				
Herd 5	4.5(0.05)	4.4(0.05)	4.5(0.07)				

 $^{^{1}\,}$ Lo_TS = cows with cow somatic cell count < 200 000 cells/mL for all the 2018 lactation dried off with teat seal alone.

significantly exceeded (P < 0.001) that of cows in the Hi_AB + TS group (Table 3). The raw unadjusted mean (median) SCC for the 2019 lactation, in Herds 1–5 in the Lo_TS group, was 126 033 (32 000), 221 095 (97 000), 113 831 (55 500), 61 771 (34 000) and 99 739 (24 000) cells/mL, respectively. The corresponding raw unadjusted mean (median) SCC for the 2019 lactation in the Lo_AB + TS group was 46 957 (28 000), 114 330 (28 500), 82 221 (44 000), 69 919 (29 000) and 71 600 (23 000) cells/mL, respectively.

When cows identified with an IMI at drying-off (dataset "2.2 Cow analysis 2019 - no IMI at dry-off"; Fig. 1) were excluded from the analysis, the overall effect of ITS alone on CLogSCC in 2019 across the five herds was similar to that found for the entire dataset (Table 3). However, in Herds 4 and 5, there was a non-significant difference in CLogSCC between the cows in any of the three groups. In Herd 2, the CLogSCC in the Lo_TS group was still significantly higher than both the Lo_AB + TS and Hi_AB + TS groups.

The percentage of records with a CSCC of <50, 51–100, 101–200, 201–400, and >400, $\times 1$ 000 cells/mL within each group and herd in 2019 (dataset "2.1 Cow analysis 2019") are presented in Fig. 2. Across herds, the odds of a cow exceeding a CSCC of 100, 200 or 400, $\times 1$ 000 cells/mL during lactation 2019 were 2.8 (CI: 2.0–3.8), 3.1 (CI: 2.1–4.7) and 4.4 (CI: 2.5–7.8) higher in Lo_TS when compared to Lo_AB + TS cows.

Herd bulk tank somatic cell count for 2018 and 2019

Fig. 3 shows the monthly Kerry Agribusiness bulk tank SCC readings, weighted by milk volume for each month for each of the five herds for both 2018 (pretrial) and 2019 (year of trial). The mean milk volume weighted bulk tank SCC (cells/mL) for Herds 1–5 in 2018 was 123, 149, 107, 150 and 105, \times 1 000 cell/mL, respectively; the corresponding bulk tank SCC in 2019 was 112, 180, 94, 75 and 75, \times 1 000 cells/mL, respectively. While Herds

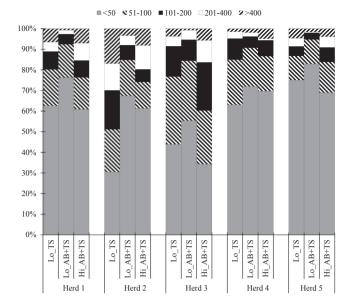


Fig. 2. Percentage of cow somatic cell count records in 2019 that were <50, 51–100, 101–200, 201–400, and >400, $\times 1$ 000 cells/mL per herd during the entire lactation for each group of dairy cows (dataset "2.1 Cow analysis 2019"). Lo_TS = cows with cow somatic cell count < 200 000 cells/mL for all the 2018 lactation dried off with teat seal alone; Lo_AB + TS = cows with cow somatic cell count < 200 000 cells/mL for all the 2018 lactation dried off with antibiotic plus teat seal; Hi_AB + TS = cows with \geq one cow somatic cell count > 200 000 cells/mL during the 2018 lactation dried off with antibiotic plus teat seal.

1, 3, 4 and 5 had numerically lower bulk tank SCC in 2019 compared to 2018, the bulk tank SCC of Herd 2 was higher.

Quarter bacteriology analysis

Table 4 shows the number of cows (quarters) with IMI at dry-off, calving and mid-lactation for each of the three groups of cows (dataset "3.1 Odds IMI quarter 2019"). At dry-off, the percentage of cows (quarters) with IMI in the Lo_TS, Lo_AB + TS and Hi_AB + TS group of cows was 20.0% (7.6%), 22.3% (7.7%), and 42.3% (18.5%), respectively. At calving, the percentage of cows (quarters) with IMI was 18.9% (7.1%), 3.4% (1.1%), and 5.4% (1.3%) for the Lo_TS, Lo_AB + TS and Hi_AB + TS groups, respectively, while in mid-lactation, it was 20.5% (7.3%), 4.2% (1.2%), and 15.3% (4.6%), respectively. The percentage of IMI cows (quarters) with IMI cured at calving were 81.6% (76.4%), 98.3% (97.5%) and 94.7% (96.3%) for the Lo_TS, Lo_AB + TS and Hi_AB + TS groups, respectively. The percentage of new IMI were 14.3% (5.3%), 2.6% (0.9%), 2.3% (0.6%) for the Lo_TS, Lo_AB + TS and Hi_AB + TS groups, respectively.

The odds (confidence interval in parentheses) of a quarter with an IMI at calving were 6.9 (3.6-13.3) and 5.4 (2.8-10.4) times higher for cows in the Lo_TS group compared to the Lo_AB + TS and Hi_AB + TS groups, respectively (dataset "3.1 Odds of IMI quarter 2019"). In mid-lactation, the odds of a quarter with an IMI in the Lo_TS cows were 6.6 (3.5-12.2) times higher than the Lo_AB + TS group. There was a non-significant difference in the odds of an IMI in mid-lactation between the Lo_TS and Hi_AB + TS groups. When quarters that were identified with an IMI at dry-off were removed (dataset "3.2 Odds IMI quarter 2019 – no IMI at dry-off"), the odds of a quarter with an IMI at calving was 6.1 (2.9-12.6) and 7.2 (2.8-18.5) times higher for cows in the Lo_TS group compared to the Lo_AB + TS and Hi_AB + TS groups, respectively. In mid-lactation, the odds of a quarter with an IMI in the Lo_TS cows were 8.5 (3.6-20.2) times higher than the Lo_AB + TS group. There was a non-significant difference in the odds of an IMI in midlactation between the Lo_TS and Hi_AB + TS groups.

 $^{^2\,}$ Lo_AB + TS = cows with cow somatic cell count < 200 000 cells/mL for all the 2018 lactation dried off with antibiotic and teat seal.

 $^{^3~}$ Hi_AB + TS = cows with \geq one cow somatic cell count > 200 000 cells/mL during the 2018 lactation dried off with antibiotic and teat seal.

a.c Different letters within a row represent significant differences at P < 0.05.

A,B Different letters within a row represent significant differences at P < 0.001.

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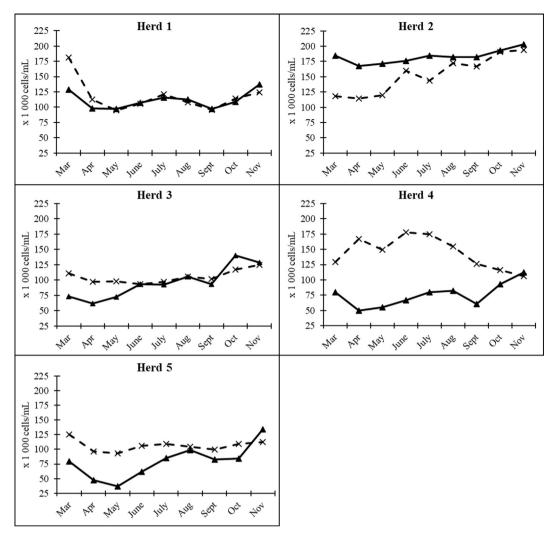


Fig. 3. Monthly weighted bulk tank somatic cell count for each of the five dairy herds for both pretrial 2018 (dashed line with cross) and trial 2019 (solid line with triangle) years.

Table 5 shows the percentage of IMI quarters in each herd in each group at the different sampling times (dataset "3.1 Odds IMI quarter 2019"). For Herds 2 and 4, the odds of a quarter IMI in the following lactation (confidence interval in parenthesis) were 16.5 (7.0–38.6), and 6.4 (1.3–31.7) times higher, respectively, for the Lo_TS group compared to the Lo_AB + TS group. The odds of an infected quarter in the following lactation in the Lo_TS group when compared to the Hi_AB + TS were 2.8 (1.6–4.9) times higher for Herd 2. There was a non-significant difference in the odds of an infected quarter between the Lo_TS and Hi_AB + TS groups for the remaining herds.

The odds of a new IMI quarter after calving (no infection at dry-off but infected at calving) in Lo_TS cows were 6.2 (3.0–12.6) and 7.6 (3.0–19.2) times higher than Lo_AB + TS and Hi_AB + TS cows, respectively (dataset "3.1 Odds IMI quarter 2019"). The odds of a cured IMI (infected at dry-off and not infected at calving) were 8.5 (3.1–23.1) and 12.1 (2.5–57.6) times higher in the Hi_AB + TS and Lo_AB + TS groups, respectively, compared to the Lo_TS group (dataset "3.1 Odds IMI quarter 2019").

Of the 359 cows that were recorded with having an IMI present across the study, 65.7% had an infection present in one quarter, 25.6% had an infection present in two quarters, 6.1% had an infection in three quarters, and 2.5% had an infection present in all four quarters. Of the 522 infected quarters identified in this study, 92.1% of infections were caused by *Staphylococcus aureus*, 4.4% by

Streptococcus uberis, 2.5% by coagulase-negative Staphylococci, 0.6% by non-haemolytic *Escherichia coli* and 0.4% by *Streptococcus dysgalactiae*. Supplementary Table S2 shows the percentage of pathogens isolated from infected quarters over the three sampling periods for each of the three treatment groups.

Table 4Dairy cows (quarters)¹ infected at dry-off, calving and mid-lactation and calving infection status for each group (dataset "3.1 Odds intramammary infected quarter 2019").

	Group			
Item	Lo_TS ²	Lo_AB + TS ³	Hi_AB + TS ⁴	
Total	244(943)	265(1036)	222(866)	
Infected at dry-off	49(72)	59(80)	94(160)	
Infected at calving	46(67)	9(11)	12(11)	
Infected at mid-lactation	50(69)	11(12)	34(40)	
Cured at calving	40(55)	58(78)	89(154)	
New infection at calving	35(50)	7(9)	5(5)	

 $^{^{\}rm 1}$ Only cows and quarters with bacteriological results for dry-off, calving and mid-lactation included.

 $^{^2\,}$ Lo_TS = cows with cow somatic cell count < 200 000 cells/mL for all the 2018 lactation dried off with teat seal alone.

 $^{^3}$ Lo_AB + TS = cows with cow somatic cell count < 200 000 cells/mL for all the 2018 lactation dried off with antibiotic plus teat seal.

 $^{^4}$ Hi_AB + TS = cows with \geq one cow somatic cell count > 200 000 cells/mL during the 2018 lactation dried off with antibiotic plus teat seal.

Table 5Percentage of quarters infected¹ in each of the individual herds at each sampling period for each of the individual groups of dairy cows (dataset "3.1 Odds intramammary infected quarter 2019").

	Dry-off		Calving			Mid-lactation			
Herd	Lo_TS ²	Lo_AB + TS ³	Hi_AB + TS ⁴	Lo_TS ²	Lo_AB + TS ³	Hi_AB + TS ⁴	Lo_TS ²	Lo_AB + TS ³	Hi_AB + TS ⁴
1	3.3	3.7	5.6	1.6	0.9	0.5	1.7	0.6	1.4
2	4.5	5.5	12.7	6.2	0.3	0.9	5.8	0.6	3
3	0.6	0.8	1.5	0.8	0.3	0.1	0.8	0.4	0.6
4	2.8	1.3	3.3	1.3	0.3	0.3	1.8	0.3	0.5
5	1.2	2.1	3.3	0.9	0	0	1.5	0	0.9

- ¹ Only quarters with bacteriological results for dry-off, calving and mid-lactation included.
- ² Lo_TS = cows with cow somatic cell count < 200 000 cells/mL for all the 2018 lactation dried off with teat seal alone.
- 3 Lo_AB + TS = cows with cow somatic cell count < 200 000 cells/mL for all the 2018 lactation dried off with antibiotic plus teat seal.
- ⁴ Hi_AB + TS = cows with ≥ one cow somatic cell count > 200 000 cells/mL during the 2018 lactation dried off with antibiotic plus teat seal.

Milk yield analysis

Cows in the Lo_TS group had 8 kg lower (P < 0.001) 305 day fat yield compared to the cows administrated with Lo_AB + TS (296 kg). There was a non-significant difference between the groups in milk or protein yield.

Discussion

This study examined the udder health status of cows in five commercial Irish herds throughout lactation, after treatment with ITS alone at dry-off compared to antibiotics plus ITS. The success of using ITS alone should be measured considering the prevention of new IMI during the dry period, the reduction in antimicrobial usage, and udder health and milk production in the subsequent lactation.

Quarter and cow log somatic cell count

A number of studies have shown that using ITS alone at dry-off reduces the level of new IMI during the dry period and in the following lactation when compared to an untreated control (Woolford et al., 1998; Berry and Hillerton, 2002b). Most recent studies have shown non-significant treatment differences on udder health by treating cows with ITS alone compared to antibiotics plus ITS in low SCC cows (Cameron et al., 2014; Vasquez et al., 2018; Kabera et al., 2020). This is contrary to what was found in the present study. The results of the current study are similar to those found by Scherpenzeel et al. (2014) and McParland et al. (2019) with a significantly higher risk of new IMI and elevated SCC in cows receiving ITS alone compared to antibiotics plus ITS.

In the current study, the prophylactic efficacy of using ITS alone at dry-off differed between herds. In Herds 4 and 5, the median raw CSCC of the cows in the Lo_TS group was only 5 000 and 1 000 cells/mL greater, respectively, than the cows in the Lo_AB + TS group. In contrast, in Herd 2, the CSCC of the cows in Lo_TS group was 69 000 cells/mL greater than in the Lo_AB + TS group. Despite all herds having a bulk tank SCC < 200 000 cells/mL for the 2018 lactation (pretrial; Fig. 3), the bulk tank SCC at dry-off of Herd 4 and Herd 5 in 2018 was much lower than in Herd 2 (Table 1). This was also associated with a lower level of IMI in Herds 4 and 5 compared to Herd 2.

For treatment allocation at dry-off, it is important to correctly assess the IMI status of each individual cow. Antimicrobial therapy at dry-off presents an important opportunity for curing existing IMI (Dodd et al., 1969) and ITS alone is only effective in preventing new infections during the dry period (Bradley and Green, 2004). Huxley et al. (2002) stated that the success of treating cows with ITS alone was strongly influenced by the ability to determine correctly the cow IMI status so that the appropriate treatment is

applied at dry-off. In the current study, using 200 000 cells/mL as a criterion to select cows eligible to be treated with ITS alone resulted in misclassification of some infected cows. Of the five herds, Herd 2 had the highest percentage of quarters with an IMI in cows categorised as low SCC at dry-off. This high level of misclassification of cows as low SCC in Herd 2 may have contributed to the higher CLogSCC of the ITS only cows in this herd. Djabri et al. (2002) showed that Staphylococcus aureus elicits a less pronounced increase in quarter SCC, which could lead to misclassification of infection when using CSCC as a determinant of IMI at dryoff. Lipkens et al. (2019) showed that the sensitivity to predict IMI at dry-off of using at least one SCC recordings over 200 000 cells/mL out of the last three consecutive SCC recordings was 58%. Additionally, Lipkens et al. (2019) showed that herd-level prevalence of subclinical mastitis, cow milk yield and parity need to be considered when differentiating infected from uninfected cows based on SCC data. Bradley et al. (2010) suggested that in herds with a high prevalence of gram-positive pathogens, blanket use of antibiotics at dry-off could be considered to decrease the prevalence of IMI.

Since the introduction of ITS alone as a dry-off treatment, different methods for identifying cows with IMI have been used, with previous lactation SCC alone or combined with the history of clinical mastitis, the most common selection methods (Scherpenzeel et al., 2014; Vasquez et al., 2018). According to the guidelines of the Royal Dutch Veterinary Council, primiparous cows with a SCC < 150 000 cells/mL and multiparous cows with a SCC < 50 000 cells/mL in the last milk recording in the 6-weeks before dry-off should be treated with ITS alone (Vanhoudt et al., 2018). McParland et al. (2019) showed that a significant difference between cows treated with ITS alone compared to antibiotic plus ITS on CLogSCC remained, even when the threshold for selection of cows was reduced from 200 000 cells/mL to 100 000 cells/mL. However, the results of the current study indicate that the overall level of IMI in the herd should also be taken into account when choosing a threshold to assign treatment to individual animals.

In the current study, the effect of using ITS alone at dry-off on CSCC extended throughout the following lactation. This can be explained by the type of bacteria most commonly infecting the herds in this study (*Staphylococcus aureus*), which has the ability to develop chronic infections of the gland (Bolte et al., 2020).

Diagnostics of the mixed model procedure presented in Supplementary Fig. S1 showed that, even with the log 10 transformation of raw SCC values, the residuals were non-normally distributed. This departure from the model assumption could result in underestimating the differences in CLogSCC between the different dry-off groups because of larger standard errors or changes to the estimates. A box cox transformation was applied to SCC data, and residuals from the mixed models tested on the box cox transformed data are presented in Supplementary Fig. S1. The outcomes of box cox transformed and log-transformed data were the same

across all models, with the exception of the quarter sample SCC analysis (dataset "1. Quarter Analysis", Fig. 1), where there was a non-significant treatment difference between Lo_TS and Lo_AB + TS at calving when analysed with box cox transformed data (Supplementary Table S3). Results from the log SCC transformation are presented here for ease of interpretation. The area under the curve shown in Supplementary Fig. S2 for the logistic regression analysis showed that this model was a good fit for the data. However, the receiver operating curves sometimes might be influenced by the prevalence of a certain condition and therefore we added calibration curves that confirm the appropriate fit of the model (Supplementary Fig. S2).

Table 2 shows that the Lo_TS and Lo_AB + TS groups had similar QLogSCC before dry-off, showing that our randomisation was successful in balancing the data between the two groups. The outlier SCC milk records from 2019, which contribute to the non-normal distribution of log SCC data, were cow SCC milk records greater than 830 000 cells/mL (milk records greater than three SDs from the mean) in the 2019 lactation. Of the 78 milk records over 830 000 cells/mL, 27%, 28%, 22%, 9% and 14% were in Herds 1-5, respectively, and 39%, 19%, and 42% of records were in Lo_TS, Lo_AB + TS and Hi_AB + TS groups, respectively. This is in line with the higher level of infection identified in Herd 2 compared to Herds 4 and 5, and the higher risk of infection in the Lo_TS group compared to the Lo_AB + TS group. Additionally, since 92.1% of infections across the five herds were attributed to Staphylococcus aureus, it is unlikely that acute infections with Escherichia coli were the cause of these extreme observations. The large number of cows and milk records (Table 1) should have reduced the impact of an extreme observation on data variability, caused by an acute case of Escherichia coli mastitis. Clinical cases of mastitis were not included in the analysis of cow SCC, as cows that may have been affected with clinical mastitis at the time of a milk recording would not have been milk recorded. Cows that had previously been treated for clinical mastitis may have been included in the milk recording and may have been represented in the data. Therefore, the extreme data are more likely due to the effect of dry-off group and herd.

Quarter bacteriology

Studies have shown large variation in the predominant pathogens causing mastitis depending on geographical location, production system and local mastitis control programmes (Zadoks and Fitzpatrick, 2008). A study carried out on Irish dairy herds from 1978 to 1980 identified Staphylococcus aureus as the most common pathogen present at dry-off, isolated in over 60% of the positive samples (Egan and O'Dowd, 1982). Based on bacteriology analysis of 630 clinical mastitis samples from 30 Irish herds, Keane et al. (2013) identified Staphylococcus aureus and Streptococcus uberis as the mastitis-causing pathogens in 38 and 29% of the culture-positive samples, respectively. In the current study, over 90% of IMIs were caused by Staphylococcus aureus, similar to that observed by Gleeson et al. (2018). However, further biochemical analysis to support the confirmation of bacterial species would have been beneficial, but due to the large volume of samples received in a short period of time, this was not possible.

In the current study, infection at dry-off was defined as more than six CFU of the same pathogen in the plated quarter milk sample collected at the last milking of lactation. Our data suggest that by not including cows with an IMI at dry-off in the analysis, the differences between using ITS alone and ITS plus antibiotic could be reduced, especially in Herds 4 and 5 (Table 3), highlighting the importance of proper identification of the bacterial status of the cow at dry-off. The sensitivity of a single milk sample for *Staphylo*-

coccus aureus can largely vary depending on the criteria used for classifying the quarters as having an IMI (Dohoo et al., 2011; Cameron et al., 2014). Dohoo et al. (2011) reported that for an IMI definition similar to the one used in this study, sensitivity was close to 70% compared to a gold standard of three samples taken on three consecutive weeks. Buelow et al. (1996) observed that one quarter sample had a sensitivity of 91% to detect Staphylococcus aureus infection versus a gold standard of two culture-positive quarter milk samples from six consecutive days of sampling. Consecutive quarter milk samples could help better detect cows with an IMI at dry-off; however, this method is not practical or economical for commercial dairy farms. PCR technique has proven useful in detecting bacteria, especially where a bacterial culture produces a "no growth" result (Gillespie and Oliver, 2005) and should also be considered as an alternative for diagnosis of IMI.

The apparent IMI cure rates of Lo TS. Lo AB + TS and Hi AB + TS cows are in line with the target set out by Green et al. (2007) of >80% during the dry period. However, these high apparent cure rates could be a result of undetected bacteria in a bacterial culture testing due to the cyclical shedding pattern of Staphylococcus aureus (Sears et al., 1990). In quarter milk samples, PCR has shown higher sensitivity for detection of Staphylococcus aureus and Streptococcus uberis (Svennesen et al., 2018). In the current study, the level of apparent new IMI during the dry period was significantly higher in the cows treated with ITS alone as compared to the cows treated with antibiotics plus ITS. This is in contrast to studies by Vasquez et al. (2018) and Cameron et al. (2014), where there was a non-significant difference in the number of new infections when cows were treated with or without antibiotic. The average dry period lengths for those studies were 55 and 59 days, respectively. In the current study, the average dry period length was 94 days (median = 90 days). Robert et al. (2008) showed that cows not treated with antibiotics during the dry period were 1.6 times more likely to have a new infection in the following lactation when the length of the dry period was greater than 65 days compared to cows with a shorter dry period. The longer dry period in the current study is a result of the seasonal pasture-based system, where milk production follows grass growth pattern (Dillon et al., 2008). Special attention should be paid to dry period management practices in herds with longer dry period lengths, as they could have a major influence on new IMIs.

In our study, the largest proportion of IMIs were caused by *Staphylococcus aureus*. Antimicrobial therapy at the end of lactation has been shown to be the optimum strategy to cure existing infections of *Staphylococcus aureus* (Dodd et al., 1969). Antimicrobial therapy also could prevent infection soon after calving and in particular IMIs caused by *Staphylococcus aureus*, which are likely to establish more new IMIs in herds where they are prevalent (Berry and Hillerton, 2002a). Given the medium sensitivity of a single quarter milk sample, long dry periods and predominance of infections with *Staphylococcus aureus*, we believe that the impact of dry-off group on SCC was a result of suboptimal IMI detection combined with high dry period/early lactation new IMI rates.

Milk yield

The relationship between an increase in SCC and a decrease in milk yield has been shown previously (Forsbäck et al., 2009; Hand et al., 2012) and is primarily attributed to a reduction in the synthetic capacity of the gland (Harmon, 1994). The current study showed a non-significant treatment difference in total milk yield. Hand et al. (2012) reported that the negative effect of increased SCC on milk yield increased with increasing SCC level and milk yield of the cows. The relatively low SCC and milk production levels in the cows used in this study could explain the absence

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of significant differences. The negative effect of SCC on milk fat has been related to the reduction in milk yield (Harmon, 1994); however, Forsbäck et al. (2009) reported a lower fat yield in quarters with high SCC compared to quarters with low SCC, with non-significant differences on milk yield.

In the current study, cows treated with ITS alone compared to antibiotics plus ITS during dry-off had a higher CLogSCC in the following lactation and a higher odds of an IMI at calving. *Staphylococcus aureus* was the predominant mastitis-causing pathogen identified in the current study. Additionally, this study showed that the efficacy of ITS differed across herds, suggesting that effects depend on herd-level factors such as herd infection level and the implementation of mastitis control measures, especially control measures related to the dry period. These measures should ensure that the level of *Staphylococcus aureus* infection in the herd is low enough so that ITS can be successfully used at dry-off to reduce the use of antimicrobials on dairy farms. Milk and protein yield were not affected by dry-off treatment, with a slight reduction in fat yield in cows treated with ITS alone compared to cows to antibiotic plus ITS at dry-off.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100449.

Ethics approval

This study was approved by the Teagasc Animal Ethics Committee (License No. 1542017), and all procedures were authorised and carried out in accordance with the Health Products Regulatory Authority (HPRA) of Ireland.

Data and model availability statement

None of the data were deposited in an official repository. The data are available for sharing upon request to the authors.

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- **C. Clabby** conducted fieldwork, statistical analysis and drafted manuscript.
- **S. McParland** methodology, statistical analysis and review and editing.
 - **P. Dillon** assisted in design, review and editing.
- **S. Arkins** university supervisor, assisted in design, review and editing.
 - J. Flynn laboratory analysis, assisted in design.
 - J. Murphy assisted in design and project management.
- **P. Silva Boloña** project management, statistical analysis, review and editing.

Declaration of interest

None.

Acknowledgements

The authors thank Kerry Agribusiness for their support in this study. A special thanks to the five farm owners who partook in this study. Many thanks to Katie Sugrue, Chloe Millar, Noel Byrne, Aoife McDonald, Jake Canniffe and Victoria Aublet (Teagasc Moorepark) for their contribution in the collection and processing of quarter milk samples. Financial support from the Dairy Research Ireland Dairy Levy Trust is greatly acknowledged.

Financial support statement

This work was financially supported by Dairy Research Ireland and the Teagasc Walsh Scholarship Programme.

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