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Effect of omitting teat preparation on bacterial levels in bulk tank milk

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Abstract

The objective of this study was to investigate the effect of omitting teat preparation prior to milking on the bacterial levels in milk directly after milking and after a period of milk storage. Eighty Holstein–Friesian dairy cows were assigned to two pre-milking teat preparation treatments: (i) washing of teats and drawing of foremilk, followed by drying with paper towels and (ii) no teat preparation. Individual cow measurements included individual quarter somatic cell count (SCC) and teat swabs for the presence of *Bacillus cereus sensu lato*. On seven monthly occasions, all milk produced over a 24 h period from each treatment group was segregated into a separate tank and sampled. Sub-samples of this milk were stored at 4 °C for 0, 24, 48 and 72 h, and the milk was analysed for total bacterial count (TBC), thermoduric bacterial count and the presence of *B. cereus s. l.* Individual quarter SCCs were numerically higher for unprepared teats (159,000 cells/mL) compared with those for prepared teats (133,000 cells/mL; $P < 0.09$). A similar trend was observed for bulk tank SCC, with the unprepared teat treatment tending to have a higher SCC (155,857 cells/mL) compared to the prepared teat treatment (102,286 cells/mL; $P < 0.09$). The TBC was not significantly higher from unprepared teats (3,152 cfu/mL) compared with milk from prepared teats (1,678 cfu/mL) ($P < 0.10$). Milk TBC was significantly higher after storage for 72 h compared with that after 0, 24 and 48 h ($P < 0.01$). The results of this study indicate that under good hygienic conditions in an outdoor grazing situation, the omission of pre-milking teat preparation has a minimal effect on TBC and SCC.

Keywords

bacterial counts • dairy cows • somatic cell count • teats • teat preparation

Introduction

The total bacterial count (TBC) and thermoduric count of bulk tank milk reflects, or can be an indicator of, the hygienic condition of the farm environment, the cow and the milking equipment. The bacterial levels observed in milk may be influenced by teat preparation practices, sanitation of the milking equipment and the milk storage efficiency (Hayes *et al.*, 2001; Chambers, 2002; Ruegg and Reinemann, 2002; Elmoslemany *et al.*, 2009). Likewise, the presence of thermoduric bacteria in milk is used as an indicator of parlour and equipment hygiene (Jayarao and Wolfgang, 2003). While the European Union (EU) currently imposes a regulatory limit of <100,000 TBC colony-forming units (cfu)/mL (EEC, 1992; Council Directive 92/46/EEC), some milk processors in Ireland have implemented more stringent regulations on farmers for TBC (<30,000 cfu/mL) and thermoduric bacteria (<500 cfu/mL). Similarly, for the somatic cell count (SCC), there are a number of incentives at the processor level to achieve a level below 200,000 cells/mL, half the required EU regulatory limit (400,000 cells/mL). One strategy to help achieve these quality targets is the application of different teat preparation procedures prior to cluster application for milking. Washing cows' teats with water and drying with a paper towel just before cluster attachment is a common pre-milking teat preparation practice (Ingawa *et al.*, 1992). However, it is a common practice on seasonal farms in Ireland to omit teat preparation entirely prior to cluster application (Kelly *et al.*, 2009; O'Connell *et al.*, 2013). Many authors have demonstrated that among all pre-milking procedures, a wet cleaning treatment followed by manual drying with paper resulted in the lowest milk bacterial counts (Galton *et al.*, 1984; McKinnon *et al.*, 1990; Gibson *et al.*, 2008). The practice of dry wiping teats to remove debris prior to cluster attachment was also associated with lowering the presumptive *Bacillus cereus* count in bulk tank milk when animals were managed outdoors (O'Connell *et al.*, 2013). *B. cereus s. l.* are specific thermoduric bacteria that are resistant to pasteurisation (Granum, 2005) and have been isolated from pasteurised dairy products (Becker *et al.*, 1994; Lin *et al.*, 1998; Larsen and Jorgensen, 1999) and, therefore, are of particular interest to the Irish dairy industry. *B. cereus s. l.* is abundant in soil; therefore, removing dry soil and dirt from contaminated

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teats has the potential to lower the *B. cereus* count in milk. When cows are at pasture, contamination of teats with soil is the main route of spore entry into milk (Christiansson *et al.*, 1999). Sulphite-reducing *Clostridia* (SRC) are Gram-positive anaerobic spore-forming bacteria and the term describes a group of bacteria consisting of 12–14 different species (Donnelly and Busta, 1981). The spores can germinate when conditions are favourable. Growth temperatures for each species can vary from 3.3 to 80 °C. SRC are used as an indicator of faecal or soil contamination (Dodds, 1993). Different teat-cleaning methods have been evaluated to determine their effect on the presence of spores in milk. The most effective method (showing a reduction of 96%) included the use of a moist washable towel, followed by drying with a paper towel (Magnusson *et al.*, 2006). The probability of udder infection is correlated with the number of pathogens on the teat end (Pankey, 1989). Washing and drying of teats can reduce the colonisation load of staphylococcal and streptococcal bacteria on teat skin prior to cluster application (Gleeson *et al.*, 2009) as can the application of teat disinfectant followed with drying using paper (Fox, 1991; Fox and Norell, 1994). While the omission of pre-milking teat preparation may have implications for bacterial levels in milk and new infection rates, it is increasingly likely that teat preparation will be omitted due to labour constraints, especially with expanding dairy herds.

The hypothesis was that bulk tank milk quality from prepared and unprepared teats would be similar during an extended summer period, when historically bacterial levels are lowest. The objective of this study was to determine the effects of teat preparation on milk quality and to investigate whether the initial milk bacterial load affects total bacterial and thermoduric counts after different storage periods.

Materials and methods

This study was undertaken with licence under the Cruelty to Animals Act, 1876 (reference B100/445). Eighty spring-calving Holstein–Friesian dairy cows from the Moorepark Research Farm (Fermoy, County Cork, Ireland) were assigned to either of two pre-milking teat preparation treatments based on SCC, parity and calving date. One treatment comprised washing teats with running water, drawing of foremilk and subsequent teat disinfection with an iodine-based disinfectant (0.5%) (Deosan Super Iodip; Johnson Diversey (Ireland) Ltd, Dublin, Ireland), followed by drying using individual paper towels prior to attaching the milking cluster (reflecting practices on 16% of farms in Ireland) (O'Connell *et al.*, 2013). The second treatment involved no routine teat preparation prior to milking (representing practices on 46% of farms in Ireland) (Kelly *et al.*, 2009). However, teats with a hygiene score of 4 (<1% of teats) (>30% of surface area caked with dirt), based on the scorecard developed by Schreiner and Ruegg (2003), were washed and dried before cluster application. Once weekly, foremilk was drawn from teats that received no teat preparation, to check for abnormalities. Post-milking teat disinfection was carried out for both treatment groups using a spray application of the iodine-based disinfectant applied before milking for Treatment 1. All cows were managed as one herd outdoors on pasture for the duration of the study (220 d). Cows on the unprepared teat treatment were identified with leg bands to indicate to milking staff that teat preparation should be omitted. Cows were milked as one group in a 30-unit, 80° side-by-side milking parlour (Dairymaster, Causeway, County Kerry, Ireland). Clusters were automatically removed as directed by the electronic milk meters when milk flow rate dropped to 0.2 kg/min with a delay time of 20 s. Cows were milked twice daily at 07:30 h and 15:30 h.

Microbiological analysis – herd measurements

On seven (approximately monthly) occasions from May to October, all milk produced over a 24 h period (two milkings: 07:30 h and 15:30 h) from each treatment group was segregated into a separate milk tank. A sample of the bulk milk (1 L) was taken (when the milk tank temperature control reached 4 °C) and stored in a refrigerator at a controlled temperature of 4 °C. This sample is reported as 0 h sample, and sub-samples of this milk were subsequently taken at 24, 48 and 72 h and analysed for TBC, thermoduric bacteria count, SCC, *B. cereus s. l.* and SRC. Each test for TBC was repeated three times and the average was used in data analysis.

TBC and thermoduric analysis procedure

One millilitre of each milk sample was placed on three Petrifilm plates and incubated at 32 °C for 48 h for TBC. Following incubation, colonies were counted, and the number of micro-organisms/mL of the milk sample was automatically calculated using a 3M Petrifilm Plate Reader (Technopath, Ballina, County Tipperary, Ireland). Thermoduric organisms were defined as those that survived, but did not grow, at pasteurisation temperatures (63.5 ± 0.5 °C for 35 min).

B. cereus s. l. and SRC analysis procedure

To enumerate *B. cereus,* milk samples were serially diluted and surface-plated on mannitol–egg yolk–polymyxin (MYP) agar (Merck, Darmstadt, Germany). The plates were incubated at 32 °C for 48 h. Typical pink colonies surrounded by a zone of precipitation were counted. Presumptive colonies were confirmed by the presence of b haemolysis on blood agar (blood agar base No. 2; Oxoid, Basingstoke, UK) plates after 24 h at 32 °C.

The SRC count of milk samples was determined in accordance with the International Organization for Standardization (ISO)

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standard 15213 (ISO, 2003). Briefly, 1 mL of each milk sample was plated on iron sulphite agar and incubated under anaerobic conditions for 72 h at 37 °C. Black colonies were counted as SRC.

Teat swabs for B. cereus s. l.

All four teats from 10 random cows within each treatment group were swabbed using one sterile swab per cow (Cultiplast; LP Italiana SPA, Milano, Italy) on five occasions (June–October) to measure *B. cereus*. Swabs were dipped in a solution of peptone (0.5%) prior to swabbing to improve recovery. Swabs were drawn across the teat orifice and up the side of each teat, avoiding contact with the udder hair. Each swab was then placed in a sterile Universal bottle containing 2 mL of sterile 0.1% peptone and vortexed for 20 s. A portion (0.5 mL) of the peptone solution was plated, undiluted, on MYP agar for the prepared teats and at a dilution of 10^1 on the MYP agar for untreated teats.

Individual cow measurements – SCC

Milk samples were analysed for SCC using a Somacount 300 (Bentley Instruments, Inc., Chaska, MN, U.S.A.) according to the International Dairy Federation (IDF) guidelines (IDF, 1981). The culture media used on plates to identify whether pathogens were present in the milk sample were CM0271 blood agar base no. 2 (Oxoid, Wade Road, Basingstoke, Hampshire, United Kingdom). Individual quarter foremilk samples, including a pre-trial sample, were collected by hand on four occasions. Quarters with an initial SCC >500,000 cells/mL were excluded from quarter analysis, and cows with a bulk SCC >400,000 cells/mL were not included in the study. All individual quarter incidences of clinical mastitis were recorded and were detected by clinical examination of the udder quarters and determination of abnormalities in milk such as clotting and discolouration. Quarters were considered sub-clinical when the SCC was >500,000 cells/mL, with and without the recovery of pathogens from milk samples, and quarters were considered to a have a latent infection when the SCC was >500,000 cells/mL and pathogens were cultured from the milk samples.

Milk yield and milk composition

Individual cow milk yield was recorded daily, and milk samples for analysis of gross milk composition were taken weekly using the Weighall milk meter (Dairymaster, Causeway, County Kerry, Ireland) and analysed using a Foss instrument (Foss Analytical A/S, Slangerupgade, Hillerod, Denmark).

Statistical analysis

The analysis of the data was carried out with linear models (SAS Institute, 2011) with log transformation as appropriate. Treatment, time and period were the factors in the analyses. For the repeated measures in stored bulk tank milk samples, a covariance model was fitted across observation times to account for the correlations involved. The mixed procedure was used throughout. Residual checks were made to ensure the validity of the analyses. Some variables were recorded for descriptive purposes, at lower sample numbers than those of primary interest, but were statistically tested for completeness. This is highlighted in the Results and Discussion sections. Results are presented as back-transformed median values.

Results

TBCs in bulk tank milk were numerically higher when teat preparation was omitted (3,152 cfu/mL), compared to the TBCs when teats were prepared (1,678 cfu/mL) prior to cluster application for milking ($P < 0.10$; Table 1). Period of sampling had no effect on TBC.

Thermoduric colonies in bulk tank milk were significantly higher where teat preparation prior to milking was omitted, compared with results obtained from teats prepared prior to milking ($P < 0.01$), and differed depending on the period of sampling ($P < 0.001$; Table 1). Higher thermoduric counts were recorded in milk from unprepared teats in August, September

Table 1. Median bacteriological counts (cfu/mL) of bulk tank milk samples from cows with and without teat preparation prior to milking, taken on seven monthly occasions, including sample analysis of stored milk samples (24, 48 and 72 h) (n=42)[1]

	No teat preparation	With teat preparation	S.e.	Significance
TBC (cfu/mL)	3,152	1,678	0.23	0.10
Thermoduric count (cfu/mL)	11	5	0.15	***
SCC (cells/mL)	156,000	102,000	19,197	0.09
Bacillus cereus (cfu/mL)	2.5	1.1	0.36	0.38
SRC (cfu/mL)	0.38	0.25	0.08	0.45

[1]In total, 21 milk samples from each teat preparation treatment were analysed for each bacteriological parameter.
***= $P < 0.001$.
Cfu = colony-forming units; TBC = total bacterial count; S.e. = standard error of mean; SRC = sulphite-reducing *Clostridia;* SCC = somatic cell count.

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and October, compared with the counts in milk from prepared teats ($P < 0.05$) (data not shown). The enumeration of *B. cereus* and SRC in bulk milk samples did not differ between treatments, but levels of *B. cereus* increased with the sampling period ($P < 0.05$; Table 1). Individual quarter SCC tended to be higher for unprepared teats (159,000 cells/mL) compared to that for prepared teats (133,000 cells/mL; $P < 0.09$). A similar trend was observed for bulk tank SCC with milk from unprepared teats tending to have a higher SCC (156,000 cells/mL) compared to the prepared teat treatment (102,000 cells/mL; $P < 0.09$; Table 1). There was a significant effect of storage time on TBC ($P < 0.01$) but no treatment × storage time interaction ($P > 0.05$). TBCs were significantly higher after 72 h storage compared to those after 0, 24 and 48 h ($P < 0.01$) and higher after 48 h compared to 0 h ($P < 0.05$) regardless of treatment (Table 2). There were also significant differences in thermoduric counts depending on the time of storage ($P < 0.05$), with a significant treatment × storage time interaction ($P < 0.05$). There was no effect of storage time on the levels of *B. cereus* or SRC in milk ($P > 0.05$; Table 2)

During lactation, when teats were swabbed for the presence of *B. cereus*, unprepared teats had significantly higher prevalence (50 cfu/mL versus 5 cfu/mL) of *B. cereus* ($P < 0.001$; Table 3) and higher numbers were present on teats for both treatment groups in late lactation (September and October; $P < 0.001$).

The number of clinical mastitis cases, sub-clinical cases and latent infections did not differ between treatments (Table 3). *Staphylococcus aureus* was the single most common contagious pathogen isolated from both clinical and sub-clinical cases.

There were no significant differences in the weekly milk yield or milk composition between treatments or any treatment × date of sampling interactions. However, there were significant effects of sampling period ($P < 0.001$), with weekly milk yield and lactose percentage decreasing and fat and protein percentage increasing as lactation progressed.

Discussion

TBCs in bulk tank milk were numerically higher when teat preparation was omitted compared to when teats were prepared. This agrees with a previous study, which showed that teat preparation reduces TBC levels in bulk milk

Table 2. Median bacteriological counts (cfu/mL) of milk samples taken on seven monthly occasions and stored for 0, 24, 48 and 72 h at 4 °C[1]

Storage time (h)	0	24	48	72	Significance	
					Time	Time × Treatment
TBC (cfu/mL)	1,576[2]	1,721[2,3]	2,174[3]	4,744[4]	**	0.70
Thermoduric (cfu/mL)	112	73	73	93	*	*
Bacillus cereus (cfu/mL)	2.6	1.3	1.3	2.0	0.79	0.52
SRC (cfu/mL)	0.2	0.4	0.5	0.2	0.25	0.79

[1]Totally, 42 milk samples were analysed for each bacteriological parameter.
[2–4]Means within a row with different superscripts differ significantly.
*= $P < 0.05$, **= $P < 0.01$.
TBC = total bacterial count; SRC = sulphite-reducing *Clostridia*.

Table 3. Individual animal measurements from cows with and without teat preparation prior to milking

	No teat preparation	With teat preparation	S.e.	Significance
Milk yield – weekly (kg per cow)	118	113	6.08	0.41
Fat (%)	4.6	4.6	0.11	0.49
Protein (%)	3.7	3.7	0.05	0.54
Lactose (%)	4.5	4.5	0.02	0.36
Bacillus cereus (cfu/mL)[1]	50	5	0.15	***
		Intramammary infections		
Clinical infections	2	1		NS
Sub-clinical infections	24	24		NS
Latent infections	5	4		NS

[1]Teat swab data.
***= $P < 0.001$.
NS = Non significant; S.e. = standard error of mean.

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(Murphy *et al.*, 2005). Although the initial TBC of milk at 0 h (2,263 versus 1,097 cfu/mL) differed numerically between unprepared and prepared teats, respectively, levels for both teat preparation treatments were considered low and this may explain why it had no significant effect on TBC levels when subsequently stored for 72 h. TBCs doubled from 48 to 72 h and were three times higher compared to the initial counts regardless of treatment. This indicates that extending the storage time by an additional day over the processor's normal 48 h collection period can result in increased milk TBC levels, and this increase could be dependent on the initial TBC of the milk. While TBC levels differed numerically between treatments and were significantly higher at 72 h, levels were well within the EU regulatory limit of 100,000 cfu/mL (EEC, 1992) as well as the processor limit of 30,000 cfu/mL required in Ireland, and differences observed between treatments could not be considered biologically important. A high standard of farm roadway, parlour/milking equipment hygiene and tail clipping were implemented on this farm and some of these factors may also influence the TBC levels reported. Similar farm management factors have been shown to affect farm bulk tank milk TBC (Kelly *et al.*, 2009).

Teat skin is considered the major source of thermoduric bacteria in raw milk and subsequent attachment to equipment surfaces and growth are responsible for the majority of contamination in bulk-tank milk (McKinnon *et al.*, 1983; McKinnon *et al.*, 1990; Christiansson *et al.*, 1999). Thermoduric bacteria counts in bulk tank milk were higher where teat preparation prior to milking was omitted compared to when teats were prepared. This is in agreement with a number of studies that showed that teat preparation reduced thermoduric counts in bulk milk (Galton *et al.*, 1982; Magnusson *et al.*, 2006). Thermoduric counts did not increase with storage time, and this would indicate that the thermoduric population present probably did not contain psychrotrophic strains that grow in the temperature range of 0–20 °C (Gleeson *et al.*, 2013). In this study, milk was stored at a controlled temperature of 4 °C for 72 h. A high thermoduric reading (> 1,000 cfu/mL) can indicate a chronic cleaning failure (Pantoja *et al.*, 2009). While thermoduric counts were significantly different between teat preparation treatments and different storage times, counts were not biologically important as the thermoduric levels reported in this study were well within the limits considered satisfactory for good-quality milk. A thermoduric count <200 cfu/mL is considered normal, while a count <10 cfu/mL indicates excellent equipment hygiene (Ruegg and Reinemann, 2002). Milking equipment cleaning was carried out according to the recommended cleaning guidelines (Gleeson, 2015), and therefore, equipment surfaces had little impact on either thermoduric counts or TBC levels. In this study, a sample from the 1 d milk from each treatment group was analysed and stored at 4 °C (in refrigerator) for further testing at 24, 48 and 72 h. Larger bacterial differences may be apparent in stored milk if the initial milk was of less inferior quality.

A larger difference in infection rates and SCC between treatments, over the trial period, may have been observed if the initial cow SCC was higher at the trial start date. The prevalence of intramammary infection is highly correlated with the number of mastitis pathogens on the teat end at milking (Galton *et al.*, 1988). Advantages in terms of reduced bacterial levels in milk have been highlighted when teats are dry prior to cluster application (McKinnon *et al.*, 1983; Galton *et al.*, 1986). During this study, unprepared teats were considered dry as were teats that were prepared prior to cluster application. The washing of teats followed by drying with paper towels or indeed the daily drawing of foremilk may have contributed to the numerically lower SCC observed with prepared teats in this study. The differences for both quarter and group milk SCC observed between treatments were considered important; however, the current study was underpowered to detect significant differences for these variables.

In cases where teats were swabbed prior to milking, unprepared teats had higher colony counts of *B. cereus* present, in particular, during late lactation. Contamination of teats with soil is the main route of *B. cereus* contamination of bulk tank milk during the grazing season (Christiansson *et al.*, 1999). Therefore, higher numbers would be expected on teats where teat preparation was omitted. The EU (Anon., 2007; EC, 1441/2007) has set a threshold for *B. cereus* in dried infant milk formulae, and in order to achieve this target level, the specifications for *B. cereus* in raw milk are frequently set at <10 cfu/mL. The current study had insufficient sample size to detect levels of difference for both *B. cereus* and SRC in fresh and stored bulk milk between treatments; however, *B. cereus* counts observed in bulk milk from prepared and unprepared teats were within industry specifications. The lack of growth during storage may be related to the storage temperature and the optimum temperature necessary for these bacteria to grow. The optimum temperature for growth of the *B. cereus* group is 30–37 °C (Claus and Berkeley, 1986), and the ideal temperatures for SRC germination can vary from 3.3 to 80 °C (Aureli and Franciosa, 2002). Storing milk at a stable temperature of 4 °C for the duration of the study may have limited any possible growth of SRC. This highlights the importance of storage temperature in preventing the growth of these spores. Reduced cow milk yield and changes in milk composition in late lactation were as expected from a seasonal calving herd, with peak milk production occurring in May and yields decreasing in November, when cows were approaching involution (McCarthy *et al.*, 2013). Milk composition and weekly milk yields did not differ between treatments, as would be expected, as the management of all animals was similar except for the teat preparation procedure.

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Conclusion

This is the first study in Ireland that has investigated the effect of pre-milking teat preparation on the microbial quality of milk during the outdoor grazing period and on the subsequent storage of that milk for 72 h. The raw milk used for this study was of excellent quality, reflecting good practices on the research farm. The results indicate that under good hygienic conditions, in an outdoor grazing situation, where teats with a high hygiene teat score are cleaned and where good equipment hygiene is implemented, the omission of pre-milking teat preparation has a minimal effect on TBC as the levels observed for both teat preparation treatments were low. Individual quarter milk samples and bulk tank milk tended to have higher SCC when teat preparation was omitted. However, these results may not extrapolate to the early spring period where cows spend a greater proportion of time in a housed environment. Further studies are required to investigate the effect of omitting teat preparation during this period on milk quality and the effect that it may have on the storage capacity of that milk.

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