

ORIGINAL ARTICLE

Effectiveness of selected premilking teat-cleaning regimes in reducing teat microbial load on commercial dairy farms

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Keywords

dairy hygiene, milk quality, premilking, teat bacterial load, teat cleaning.

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2007/1213 received 19 September 2007,
revised 18 October 2007 and accepted
7 November 2007

doi:10.1111/j.1472-765X.2007.02308.x

Abstract

Aims: To determine the effectiveness of premilking teat-cleaning regimes in reducing the teat microbial load and effect on milk quality.

Methods and Results: The effectiveness of several premilking teat-cleaning regimes in reducing teat microbial load was assessed using 40 cows on each of the four commercial UK dairy farms with herringbone parlours during two sampling periods. In the first experiment, all the treatments reduced teat total viable count (TVC), but there was no significant difference between the hypochlorite wash and dry wipe, iodine dip and dry and alcohol-medicated wipe or dry wipe alone. In the second experiment, the chlorine wash and dry wipe was significantly more effective in reducing teat TVC than a water wash and dry, chlorine dip and dry or a dry wipe. There was no relationship between cleaning regime and milk TVC, Enterobacteriaceae or *Escherichia coli* levels.

Conclusions: All of the cleaning techniques studied reduced teat microbial load, however, the chlorine wash and dry was the most effective.

Significance and Impact of the study: The premilking teat-cleaning techniques studied reduced the teat microbial load and therefore reduced the potential for milk contamination; however, a wash including an effective disinfectant followed by a dry wipe was the most effective.

Introduction

The safety and quality of pasteurized milk is dependent on its bacterial levels throughout the food production chain. Microbiologically, the processes involved on farms were the major contributors to potential dairy hazards (Valeeva *et al.* 2005) with three main sources of milk contaminants from within the udder, the external udder surface and the equipment surfaces (Holm *et al.* 2004). Good hygiene and husbandry practices are therefore essential in minimizing the bacterial contamination of milk from the environment and infected udders. The level of contamination is influenced by animal health and nutrition, housing and feeding facilities, parlour design, milking procedures, herd management techniques, herd size and milk yield (Bramley *et al.* 1992; Sanaa *et al.* 1993; Köster *et al.* 2006). A hazard analysis approach advocated by the dairy hygiene regulations (Komorowski

2006) leads to the identification of microbial load on teats on dairy cows as a key factor in the reduction of microbial contamination in raw milk.

The objective of teat cleaning and udder hygiene is to enhance milk quality and aid in the control of mastitis (Galton *et al.* 1984; Pankey 1989). Various studies have shown that premilking teat disinfection is beneficial (Galton *et al.* 1986; Rasmussen *et al.* 1991; Oliver *et al.* 1993; Skrzypek *et al.* 2004; Magnusson *et al.* 2006), whilst other work has shown limited effect (Grindal and Bramley 1989). Different cleaning regimes vary in their effectiveness in reducing teat microbial levels. In previous work (Gibson *et al.* 2005), only 10% of the premilking teat-cleaning regimes sampled on commercial UK dairy farms achieved high levels of cleaning (>76% removal of teat bacterial load), while 28% of premilking regimes increased teat microbial level and may therefore increase the microbial load in raw milk. Cleaning regime factors

considered to contribute to poor parlour hygiene included: sharing paper towels, cloths or medicated wipes between cows, weak disinfectant, milking wet teats, contaminated dip-cups or cleaning solutions. The most important step in an effective teat-cleaning regime is the drying of the teats prior to milking (National Mastitis Council 2004) to remove bacteria and minimize bacterial growth (Galton *et al.* 1986; Ruegg 2004). In a direct comparison between cleaning regimes, washing teats with a sanitizer compared with no premilking treatment reduced microbial load by 44%, whilst washing with a sanitizer and drying decreased microbial load by 85% (Galton *et al.* 1984).

Premilking teat-cleaning regimes have been studied previously (Galton *et al.* 1984, 1986; Pankey 1989); however, there is no one recommended method and the efficacy of common premilking treatments in reducing teat microbial load is still unclear. The objective of this study was to investigate the effectiveness of specific commercially available teat-cleaning regimes under controlled conditions on four commercial dairy herds milked through herringbone parlours during two sampling periods. The effectiveness of treatments was assessed by teat total viable count (TVC) before and after cleaning, TVC in the milk (experiment 1) and Enterobacteriaceae and *Escherichia coli* levels in the milk (experiment 2).

Materials and methods

Farm and cow selection

From 37 commercial dairy farms previously involved in a microbiological survey (Gibson *et al.* 2005), four herringbone parlours were selected because of their locality and because the existing premilking teat-cleaning regime on the farms was a dry wipe. The regimes investigated were based on current commercial practices determined from a previous survey (Gibson *et al.* 2005).

At the start of each study, 40 cows that were between 50 and 200 days in milk, in their second, third or fourth lactation cycle and with both their last recorded somatic cell count (SCC) and cumulative SCC below 150 000 ml⁻¹ were selected and 10 cows randomly allocated to one of the four treatments.

Premilking teat-cleaning regimes

Between December 2003 to April 2004 (experiment 1) and May to August 2004 (experiment 2), the 40 cows from each farm were swabbed and composite milk samples collected during the morning milking on four separate occasions on a three to five weekly cycle. In

experiment 1, the four premilking teat-cleaning regimes were: a dry wipe with two-ply blue roll (Deosan, JohnsonDiversey UK Ltd, Northampton, UK); an individual alcohol-based medicated wipe [50% (v/v) ethanol, 0.1% (v/v) cetrinide, 0.1% (v/v) chlorhexidine gluconate; Teisen Teat Wipes, Teisen Products Ltd, Worcestershire, UK]; an iodine-based dip for 15 s and dry wipe (1000 ppm available iodine; Downland Pre-dip, Downland Marketing Ltd, Carlisle, UK using a nonreturn dip-cup; Ambic Equipment Ltd, Oxfordshire, UK); a hypochlorite and glycerine wash and dry wipe 500 ml of 10% (v/v) hypochlorite (Red Label Hypochlorite, Emprasan Chemicals Ltd, Merseyside, UK) was added to 500 ml glycerine (Battle, Hayward and Bower Ltd, Lincoln, UK) for skin condition and made up to 5 l with water (to give 125 ppm available chlorine) and applied using an udder cloth (Honeycomb Udder Cloths, Dairy Spares Ltd, Whitchurch, UK). In experiment 2, the four premilking teat-cleaning regimes were: a dry wipe; a chlorine-based dip for 15 s and dry wipe (150 ppm available chlorine; Chlorisept Tablets, Davidson Agricultural Solutions, Hampshire, UK); a water wash and dry wipe; a chlorine-based wash and dry wipe (150 ppm available chlorine; Chlorisept Tablets, Davidson Agricultural Solutions). Each farm was supplied with the products, measuring equipment, instructions and demonstrations to ensure adequate training. The cleaning regimes were applied six milkings prior to the sampling date to the same 10 cows per cleaning regime.

Sample collection

Two rear teat swab samples per cow were collected before and after cleaning (alternating between left and right) during the morning milking using sterile swabs (Bibby Sterilin Ltd, Staffordshire, UK) according to the method of Ingawa *et al.* (1992). Before sampling the swab was moistened and postsampling the swab was placed in 5 ml of sterile recovery medium 1.0 g l⁻¹ protease-peptone A (Lab M, Lancashire, UK), 8.5 g l⁻¹ sodium chloride (VWR International Ltd, Dorset, UK) and 2.0 g l⁻¹ sodium thiosulfate (Fischer Scientific Ltd, Loughborough, UK).

One composite milk sample of the minimum practical volume, approx. 20 ml, was collected into a sterile plastic universal from each cow on each sampling occasion (using the on-farm milk collection apparatus available). Samples were transported from the farm to the laboratory in an insulated box with ice blocks and stored at 4°C for a maximum of 4 h prior to analysis.

At the end of each sampling period, the length of time for each premilking cleaning regime and the time to clean 17 cows were recorded in one parlour.

Sample analysis

To assess TVC, swab samples were vortexed twice for 5 s and 50 μ l subsamples inoculated onto Tryptone Soya Agar (Lab M) in duplicate using a Whitley Automatic Spiral Plater (Don Whitley Scientific Ltd, West Yorkshire, UK). Following incubation at 30°C for 2 days, viable counts were performed using an ACOLyte (Don Whitley Scientific Ltd).

During experiment 1, milk samples were assessed for TVC by pour plating with milk agar (Oxoid Ltd, Basingstoke, UK). Following incubation at 30°C for 2 days, viable counts were performed with the aid of an ACOLyte. In experiment 2, milk samples were spread plated (100 μ l) in duplicate onto Violet Red Bile Glucose Agar (Lab M) for Enterobacteriaceae and Chromocult TBX (Merck, Darmstadt, Germany) for *Escherichia coli*. Following incubation at 37°C for 24 h, colonies were counted manually.

Statistical analysis

The before and after cleaning teat TVCs were transformed to \log_{10} and the difference calculated, with negative values indicating a reduction in microbial load. The milk data were also transformed to \log_{10} . Data were subjected to analysis of variance using SPSS for Windows, version 10.0.7 (SPSS Inc., Chicago, IL, USA). Mean values were also compared using the Student–Newman–Keuls *post hoc* test. Correlations were calculated to assess the relationships between TVC and cleaning regime.

Results

In experiment 1, the mean TVC levels on teats before cleaning ranged from \log_{10} 4.57 to 5.71 and after cleaning from \log_{10} 4.04 to 5.22 (Table 1), showing that the pre-milking cleaning techniques reduced teat TVC. There was a large variation in milk TVC levels between farms, with

Table 2 Mean \log_{10} difference in teat total viable count as a result of premilking cleaning during experiment 1

Cleaning regime	Difference (mean \log_{10})
Dry wipe	-0.32
Alcohol-based medi-wipe	-0.46
Iodine-based dip and dry	-0.50
Hypochlorite wash and dry	-0.52
SEM	0.060

the mean \log_{10} milk TVC ranging from 3.66 to 5.33. There was no difference ($P > 0.05$) in milk TVC as a result of the different cleaning regimes. Similarly, there was no correlation between milk and teat TVC levels before cleaning ($r = 0.113$, $P > 0.05$, $n = 8$), after cleaning ($r = 0.077$, $P > 0.05$, $n = 8$) or the difference because of cleaning ($r = -0.153$, $P > 0.05$, $n = 8$). The removal of microbial load by each premilking teat-cleaning regime (Table 2) showed no significant difference between the dry wipe, medi-wipe, iodine dip and dry and hypochlorite wash and dry in the reduction of teat TVC ($P < 0.05$).

During the second experiment, the four farms had similar teat TVC levels prior to cleaning with a mean \log_{10} ranging between 4.79 and 5.06 (Table 3). Because of the lack of correlation between milk and teat TVC in the first experiment, Enterobacteriaceae and *E. coli* levels were assessed as alternative indicators of microbial milk quality and hygiene. There was a large variation between farms in Enterobacteriaceae levels, which ranged from mean \log_{10} 0.72 to 2.01 (Table 3). As may be as expected as a subset of the Enterobacteriaceae, the *E. coli* levels were considerably less variable. There was a weak correlation between teat TVC before cleaning and milk Enterobacteriaceae ($r = 0.285$, $P < 0.01$, $n = 128$) and *E. coli* ($r = 0.278$, $P < 0.01$, $n = 116$), but there was no overall relationship ($P > 0.05$) between the cleaning regimes and Enterobacteriaceae and *E. coli* levels in milk.

Table 1 Mean \log_{10} teat total viable count (TVC) levels before and after premilking cleaning* and mean \log_{10} milk TVC levels during experiment 1

Herringbone parlour no.	No. of samples†	Teat TVC (mean \log_{10})			No. of samples	Milk TVC (mean \log_{10})
		Before	After	Difference		
1	118	5.18	4.79	-0.40	99	5.33
2	169	4.93	4.55	-0.38	160	4.94
3	158	4.57	4.04	-0.53	160	3.66
4	138	5.71	5.22	-0.49	132	5.25
Mean		5.10	4.65	-0.45		4.37
SEM		0.050	0.030	0.050		0.045

*For each parlour, approx. 100 cows were cleaned with one of the four pre-milking cleaning treatments (dry wipe, alcohol-based medi-wipe, iodine dip) or hypochlorite wash) on four occasions.

†Practicalities during sampling resulted in variation in sample numbers between farms.

Herringbone parlour no.	No. of samples†	Teat TVC (mean log ₁₀)			No. of samples	Milk (mean log ₁₀)	
		Before	After	Difference		Enterobacteriaceae	<i>E. coli</i>
1	160	4.88	4.21	-0.66	134	2.01	0.30
2	154	4.79	4.13	-0.67	138	1.28	0.24
3	166	4.87	4.48	-0.39	165	0.72	0.15
4	119	5.06	4.64	-0.42	115	1.78	0.42
Mean		4.90	4.37	-0.54		1.45	0.28
SEM		0.005	0.010	0.006		0.019	0.009

*For each of parlour, approx. 10 cows were cleaned with one of the four premilking cleaning treatments (dry wipe, chlorine dip, water wash or chlorine-based wash) on four occasions

†Practicalities during sampling resulted in variation in sample numbers between farms.

Table 3 Mean log₁₀ teat total viable count (TVC) levels before and after premilking cleaning* and mean log₁₀ milk Enterobacteriaceae and *E. coli* levels during experiment 2

Table 4 Mean log₁₀ difference in teat total viable count as a result of premilking cleaning during experiment 2

Cleaning regime	Difference (mean log ₁₀)
Dry wipe	-0.39a
Chlorine-based dip and dry	-0.39a
Water wash and dry	-0.59a
Chlorine-based wash and dry	-0.81b
SEM	0.060

Mean values with different letters differ ($P < 0.05$).

In the second experiment, the effectiveness of three additional premilking cleaning regimes on the removal of microbial of teat microbial load was determined (Table 4). The chlorine wash and dry regime was the most effective ($P < 0.05$) in reducing teat microbial load compared with any of the other three treatments, which did not differ ($P > 0.05$).

The time taken to apply the premilking teat-cleaning regimes may influence the ability of farms to incorporate it into their regular routine. Analysis of the time taken to perform the cleaning techniques shows that the dry wipe and medicated wipe treatments were approx. one- to two-fold quicker to apply than any of the other treatments (Table 5).

Discussion

The variation in individual farm mean teat and milk TVC may be due to factors that influence milk microbial load such as herd size, milk yield, field conditions, general management techniques and housing (Sanaa *et al.* 1993; Hogan *et al.* 1999; Zdanowicz *et al.* 2004; Köster *et al.* 2006). The variation in milk TVC levels between farms was consistent with the log₁₀ 3.96 reported by McKinnon *et al.* (1990) and log₁₀ 3.45 (SD 3.69) reported by Silk *et al.* (2003). There was also a large variation between farms in milk Enterobacteriaceae levels; however, *E. coli*

Table 5 Mean time taken in seconds to clean four teats per cow with each premilking cleaning regime

Cleaning regime	Time (s) taken to clean four teats on one cow	
	Experiment 1	Experiment 2
Dry wipe	7	8
Alcohol-based medi-wipe	9	
Iodine-based dip and dry	18	
Hypochlorite wash and dry	20	
Chlorine-based dip and dry		16
Water wash and dry		19
Chlorine-based wash and dry		19

levels were considerably less variable and, although low, were consistent with other studies (Desmasures *et al.* 1997).

There was no correlation between teat TVC levels (before cleaning, after cleaning or the difference) and milk TVC, which may be due to the inter-farm variation and/or other contributors to milk contamination. There was a weak correlation between teat TVC before cleaning and milk Enterobacteriaceae or *E. coli* level, suggesting that these may be better indicators of teat hygiene. However, studies by Kagkli *et al.* (2007) concluded that bovine faecal organisms were not the principle source of coliforms in raw milk and that other sources such as the environment, milking equipment and water were contributors and may explain the weak relationship observed in this study. Similarly, Hutchison *et al.* (2005), investigating indicators of farm and milk hygiene including coliforms and *E. coli*, concluded that there were low correlations with milk levels. In this study, there was no difference in milk TVC, Enterobacteriaceae or *E. coli* levels as a result of the different cleaning regimes. A lack of correlation between premilking teat cleaning and bulk tank milk contamination with environmental bacteria was also reported by Feldmann *et al.* (2006). Milk TVC levels may be influenced

by contamination from other external sources such as teat liners, milking clusters or pipe work. McKinnon *et al.* (1990) reported that milk TVC and coliform levels increased as the milk passed through the milking equipment and therefore contamination from the equipment may mask any beneficial effects of the premilking teat-cleaning techniques on milk microbial load. By contrast, however, other workers have reported a positive effect of premilking teat-cleaning regime on reducing milk microbial levels (Galton *et al.* 1984; Rasmussen *et al.* 1991).

In the first experiment, all of the premilking cleaning regimes reduced teat TVC; however, there was no significant difference between the regimes. This is in contrast to Galton *et al.* (1986) who reported that a wet wipe with sanitizer resulted in a significant reduction in teat microbial levels compared with a dry wipe alone. In studies using teats artificially contaminated with clostridial spores, Magnusson *et al.* (2006) also found that a dry wipe was the least effective, with similar levels of removal as reported in this study; the most effective method was a moist towel used with or without soap, followed by a dry wipe.

As there was no difference between treatments in the first experiment, additional treatments were assessed in the second experimental period. The chlorine wash and dry regime was the most effective in reducing teat microbial load compared with any of the other three treatments, which did not differ. The same strength chlorine solution was used in both the wash and dry and dip and dry treatments, along with the same dry wiping routine. This difference between regimes suggests that the effectiveness of cleaning is influenced by the application method, with mechanical action of the wash enhancing removal, a finding in agreement with previous studies (Galton *et al.* 1984; Magnusson *et al.* 2006). The water wash and dry and chlorine wash and dry employed the same application method and as the chlorine wash and dry was more effective at reducing microbial load, the use of a disinfectant in the regime clearly enhanced the cleaning effectiveness. Other studies have also highlighted the importance of chemical formulations in premilking teat cleaning effectiveness (Pankey 1989; Rasmussen *et al.* 1991; Ingawa *et al.* 1992; Magnusson *et al.* 2006).

The most effective premilking cleaning technique in this study, the chlorine wash and dry, took two to three times longer to apply than the dry wipe or medicated wipes. Farmers may be concerned about the impact of longer premilking teat-cleaning regimes on the overall herd milking time. Although not evaluated in this study, work by Sandrucci *et al.* (2007) concluded that proper udder preparation (fore-stripping, teat cleaning and pre-dip) resulted in greater milk yield and shorter total milk-

ing time. The current general practice is to apply premilking cleaning only to visibly dirty cows; however, Magnusson *et al.* (2007) concluded that teats can appear clean but still be contaminated with high levels of bacteria and spores and therefore having a standardized premilking routine applied to the whole herd may be the most effective means to reduce microbial contamination from the teats with no overall increase in milking time and possible increase in milk yield (Rasmussen *et al.* 1991; Sandrucci *et al.* 2007).

Premilking teat-cleaning regimes reduce the microbial load on teats and therefore reduce the potential contamination of milk destined for human consumption and also contribute to the control of mastitis. Although this study did not find a significant relationship between cleaning regime and milk microbial load, there was variation in the microbial cleaning capabilities and consequently the ability to reduce teat microbial levels, including potential food-borne pathogens that can enter milk. A premilking teat-cleaning regime involving the washing of teats with an effective disinfectant and then drying was the most effective.

Acknowledgements

We would like to express our sincere thanks to the farmers who participated in this study. This study was funded by the UK Food Standards Agency in partial fulfilment of project B12003.

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