Effect of Different Premilking Manual Teat-Cleaning Methods on Bacterial Spores in Milk

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ABSTRACT

Different teat-cleaning methods were evaluated to determine their effect on the presence of spores from anaerobic bacterial spore-formers in the milk. Artificial contamination was used to achieve uniform contamination of teats to reduce the number of cows and samples needed in the experiments and still obtain adequate power to detect differences among tested methods. Teats were contaminated experimentally with a large amount of *Clostridium tyrobutyricum* spores in a manure–water slurry. Various types of dry and moistened towels and different combinations of methods using soap or 2 types of towels, together with cleaning times of 10 or 20 s, were compared in 2 Latin square-designed experiments with 7 cows, 7 treatments, and 4 replications in each experiment. In comparison with control (no cleaning and no forestripping), cleaning teats with dry paper towels for 10 s reduced concentration of spores in milk by 45 to 50%. A 50 to 74% reduction was achieved using different types of moist towels for 10 s. Methods using 2 towels, soap, or a longer cleaning time reduced bacterial contamination by 85 to 91%. The most effective methods in reducing milk spore content (96% reduction) were use of a moist washable towel with or without soap followed by drying with a dry paper towel, for a total time of 20 s per cow. One of the best cleaning methods was studied in an additional experiment to determine the effect of different teat contamination mixtures. The Latin square-designed experiment with 8 cows, 8 treatments, and 2 replications showed that cleaning was independent of the tested contamination matrix (manure, soil, or sawdust), type of spores (*Cl. tyrobutyricum* and *Bacillus cereus*), or degree of contamination (manure or extra manure).

Key words: premilking teat cleaning, *Clostridium tyrobutyricum* spore, *Bacillus cereus* spore, milk quality

INTRODUCTION

Bacterial spore contamination of milk can cause processing problems for the dairy industry because spores can survive pasteurization. Presence of *Bacillus cereus* is a limiting factor for the shelf life of pasteurized milk (Griffiths, 1992) and a potential food poisoning agent (Granum and Lund, 1997). It is a spore-forming bacterium commonly found in soil and is most frequently found in milk during the grazing season when the risk of teat contamination with the soil is greatest (Slaghuis et al., 1997; Christiansson et al., 1999). In addition, spore-forming bacteria of the *Clostridium* species cause late blowing in some types of cheese (Stadhouders et al., 1985). The major source of the clostridia found in the milk is feeding silage, especially in the poor-quality portions of the silage (Stadhouders and Spoelstra, 1990). Spores can then be found in feces of animals consuming the silage, and manure contamination on the teats results in the spores being transferred to the milk (Stadhouders and Jørgensen, 1990; Herlin and Christiansson, 1993).

Worldwide, several different premilking manual cleaning methods are practiced, but comparative information about their effect on milk quality is scarce. Previous systematic investigations generally considered the effect of cleaning on bacterial contamination. Cleaning teats reduced milk bacterial counts more than when no cleaning was done, and wet cleaning was better than dry cleaning (Galton et al., 1982, 1986; Pankey, 1989). Washing teats with water directly from a hose should be followed by careful drying of the teats (Galton et al., 1984). Use of different sanitizers or disinfectants in teat preparations improved cleaning better than cleaning with water from a hose followed by drying with a paper towel (Adkinson et al., 1991; Ingwa et al., 1992), or using a dry or wet towel, but was not more effective than using a wet towel followed by drying with a paper towel (Galton et al., 1986). Using a disinfectant entails a risk of adding residues to milk (Galton et al., 1984; Rasmussen et al., 1991).

Few investigations of the effect of teat cleaning on the presence of spores in the milk have been conducted. McKinnon et al. (1983) found that the total bacterial

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Table 1. Description of cleaning methods using different commercially available teat-cleaning paper towels, a washable cotton towel, and a synthetic towel (Experiment 1)

Treatment	Description of towel ^{1,2}	Size of towel, cm
1A (control)	No cleaning of udder and teats, no forestripping	—
1B	Dry paper towel a, smooth surface	25.5 × 33
1C	Dry thin paper towel b, rough surface	25 × 28
1D	Moist thin paper towel c, premoistened with 10% isopropanol	25 × 29
1E	Moist paper towel d, premoistened with <0.5% ethanol and 1.5 to 3.0% alkyl benzene sulfonate	23 × 28
1F	Moist washable cotton towel, dipped in water and firmly wrung	35 × 36
1G	Moist washable towel of synthetic fiber, dipped in water and firmly wrung	36 × 51

¹Four kinds of paper towels (a, b, c, and d) were used.

²Cleaning time was fixed to 10 s.

contamination and aerobic spore counts in milk decreased when teats were washed and dried before milking in comparison with no cleaning or washing without drying. Washing teats with hypochlorite before drying did not further affect the aerobic spore counts in milk. Rasmussen et al. (1991) showed that cleaning teats with a dry paper towel produced fewer bacterial and anaerobic spore counts in milk than no cleaning. Longer cleaning time together with use of a wet paper towel, followed by drying with a paper towel, was even more effective. Long cleaning time and more active scrubbing of the teat ending with a moist cotton towel further decreased bacterial counts in milk. Their results indicated that the physical structure of the towel and the physical action were important. Similarly, Stadhouders and Jørgensen (1990) concluded that the more intensive the cleaning and drying of the teats, the smaller the concentration of clostridial spores in the milk.

The objectives of this study were to 1) compare effectiveness of using different methods for premilking manual teat cleaning under controlled conditions with teats experimentally contaminated with spores of *Clostridium tyrobutyricum*, and 2) evaluate the efficiency of the best cleaning methods according to the type of spores, matrix (contamination carrier), and degree of contamination by using different matrices mixed with spores of *Cl. tyrobutyricum* or *B. cereus* or both.

MATERIALS AND METHODS

Animals and Treatments

In each of 3 different experiments, the teats of 7 or 8 Swedish Friesian cows producing 17 to 35 kg of milk daily were experimentally contaminated with spores of *Cl. tyrobutyricum* or *B. cereus* mixed with different matrices and various premilking cleaning methods were tested. Cows were housed in tie stalls on rubber mats bedded with sawdust. All cows in each experiment were fed similar rations (including the same batch of silage) and were fed at least 30 min before milking.

Experiment 1. Effect of using 4 commercially available teat-cleaning paper towels (a, b, c and d; Table 1)

and towels of cotton or synthetic fiber was studied. In this experiment, cleaning time was fixed at 10 s. Teats were contaminated before milking with a sterilized manure–water slurry containing *Cl. tyrobutyricum* spores. Treatments for Experiment 1 are listed in Table 1.

Experiment 2. Effect of teat-cleaning time in combination with different teat-cleaning methods was studied. Teats were contaminated before milking with a sterilized manure–water slurry containing *Cl. tyrobutyricum* spores. Treatments for Experiment 2 are shown in Table 2.

Experiment 3. Cleaning efficiency as affected by the contamination matrix, type of spores, and degree of contamination was studied. Influence of the matrix was studied by using manure, soil, or sawdust contaminated with the most common bacterial spores for each matrix (manure: *Cl. tyrobutyricum*; soil and sawdust: *B. cereus*). Influence of degree of contamination was studied by using 2 contamination mixtures having different concentrations of manure (manure and extra manure). Influence of type of spores (*B. cereus* and *Cl. tyrobutyricum*) was studied by using 2 contamination mixtures (manure and soil) containing both types of spores. Teats were purposely contaminated before milking with 4 different combinations of contamination sources and spores, 1 per cow. Cleaning method 2F described in Table 2 and a control was used for all contamination mixtures. Treatments for Experiment 3 are shown in Table 3.

Experimental Design

In Experiment 1 and 2, each of 7 cows was assigned to 1 of 7 different cleaning methods (Tables 1 and 2, respectively) at 7 milking occasions in a Latin square design. In each experiment, 4 replications (2 milkers and morning vs. evening milking) were conducted for a total of 28 milking occasions. Each experiment was carried out for 2 wk. In Experiment 3, each of 8 cows at 8 milking occasions was assigned to 1 of 4 different combinations of contamination mixtures, and with or without teat cleaning before milking, in a Latin square

Table 2. Description of treatments using different teat-cleaning times in combination with different teat-cleaning methods (Experiment 2)

Treatment	Cleaning method ¹	Duration, s
2A (control)	No cleaning of udder and teats, no forestripping	—
2B	Soap solution (nonionic surface-active agent) applied on teats by spraying, and left for 30 s before drying with a dry paper towel b	Application + 10
2C	Soap solution applied on teats by dipping in foam (1 to 5% lactic acid, 1 to 5% fatty acid derivative, <1% hydrogen peroxide, <1% fatty alcohol ether sulfate), and left for 30 s before drying with a dry paper towel b	Application + 10
2D	One moist paper towel d, premoistened with <0.5% ethanol and 1.5 to 3.0 % alkyl benzene sulfonate	20
2E	Two moist paper towels d, premoistened with <0.5% ethanol and 1.5 to 3.0 % alkyl benzene sulfonate	10 + 10
2F	Towel of synthetic fiber dipped in water and firmly wrung + dry paper towel b	10 + 10
2G	Towel of synthetic fiber dipped in soap solution (nonionic surface-active agent) and firmly wrung + dry paper towel b	10 + 10

¹Two kinds of paper towels (b and d) were used (see Table 1).

design. Two replications with 1 milker (morning vs. evening milking) were conducted for a total of 16 milking occasions during 8 consecutive days. The study of cleaning effects on different types of spores was made as an incomplete Latin Square design because only 2 of the 4 different contamination mixtures contained both types of spores.

Teat Contamination

Artificial contamination to achieve a high and uniform contamination of the teats was used to reduce numbers of cows and samples needed to increase the power of resolution between cleaning methods tested. Spore concentration in the mixtures was chosen to leave detectable amount of spores (>10 spores/L) in the milk after teat cleaning. Based on previous experience to achieve a suitable degree of contamination in the milk, more *B. cereus* spores than *Cl. tyrobutyricum* spores were added to the contamination mixtures. Teats were experimentally contaminated before milking by dipping the entire teat, but not the udder, in a contamination mixture containing spores. To prevent bacterial growth, portions of contamination mixtures sufficient for one milking occasion were stored in a refrigerator (soil or sawdust) or in a freezer (manure). Before each milking, a portion was thawed and spores were added together with some water. The mixture was well shaken, poured into a 250-mL plastic jar, and teats were dipped into the mixture and allowed to dry for 20 min before milking. Teats were not cleaned before contamination, but if larger flakes of manure or sawdust were present, they were removed. For manure mixtures, each teat was dipped twice; for soil mixtures, each teat was dipped thrice; and for sawdust mixtures, each teat was dipped in the jar while the jar was shaken for 10 s to achieve a realistic degree of dirtiness of the teats. Cows were not allowed to lie down between teat contamination and milking.

Experiment 1. A contamination mixture consisting of 1 part sterilized cow manure and 3 parts water (wt/wt) was used. Manure was sterilized for hygienic reasons. A dose of spores was added so that the prepared mixture contained 155,000 *Cl. tyrobutyricum* spores/g. The DM content was 3.0%. Slight visible dirtiness of the teats was evident upon contamination.

Experiment 2. A contamination mixture of 1 part sterilized cow manure and 3 parts water (wt/wt) was used and spores were added to a concentration of 260,000 *Cl. tyrobutyricum* spores/g. The DM content was 3.5%. Slight visible dirtiness of the teats was evident upon contamination.

Experiment 3. Four different contamination mixtures were used: Manure (A): a mixture of 1 part sterilized cow manure and 3 parts water (wt/wt) was used, and spores were added to a concentration of 210,000 *Cl. tyrobutyricum* spores/g and 1,260,000 *B. cereus* spores/g. The DM content was 3.9%. Slight visible dirtiness of the teats was evident upon contamination. Extra manure (B): a mixture of 1 part sterilized cow manure and 1.5 parts water (wt/wt) was used and spores were added to a concentration of 240,000 *Cl. tyrobutyricum* spores/g. The DM content was 5.8%. Somewhat more visible dirtiness of the teats was evident upon contamination than observed for mixture A. Soil (C): a mixture of 1 part soil (not sterilized) and 1 part water (wt/wt) was used and spores were added to a concentration of 230,000 *Cl. tyrobutyricum* spores/g and 740,000 *B. cereus* spores/g. The DM content was 44.2%. More visible dirtiness of the teats was evident upon contamination than observed for mixture A. Sawdust (D): before each milking occasion, sawdust (not sterilized) was mixed with water and spores so DM content of 37.6% was achieved. The mixture contained 3,100,000 *B. cereus* spores/g. After contamination, a moderate amount of visible sawdust adhered to the teats.

Table 3. Description of treatments using different contamination matrices, type of spores (*Clostridium tyrobutyricum* and *Bacillus cereus*), and degree of contamination on cleaning efficiency (Experiment 3)

Treatment	Matrix	Type of spores	Cleaning
3Aa	Manure	<i>Cl. tyrobutyricum</i> and <i>B. cereus</i>	Control ¹
3Ab	Manure	<i>Cl. tyrobutyricum</i> and <i>B. cereus</i>	Method 2F ²
3Ba	Extra manure	<i>Cl. tyrobutyricum</i>	Control
3Bb	Extra manure	<i>Cl. tyrobutyricum</i>	Method 2F
3Ca	Soil	<i>Cl. tyrobutyricum</i> and <i>B. cereus</i>	Control
3Cb	Soil	<i>Cl. tyrobutyricum</i> and <i>B. cereus</i>	Method 2F
3Da	Sawdust	<i>B. cereus</i>	Control
3Db	Sawdust	<i>B. cereus</i>	Method 2F

¹Control = no cleaning, no forestripping.

²Method 2F = cleaning with a moist washable synthetic towel for 10 s followed by drying using a dry paper towel for 10 s (see Table 2).

Preparation of Spores

Spores of *B. cereus* strain A205, isolated from spent bedding material (sawdust), were prepared by surface-spreading 0.1-mL aliquots of a fully grown culture on sporulation agar plates. The sporulation agar had the following composition: 8 g of nutrient broth (Difco, Boule Nordic, Huddinge, Sweden), 1 g of KCl, and 30 g of Bacto-Agar (Difco) per L, with addition of 10 mM $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 10 μM $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 1 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 10 μM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. Plates were incubated aerobically at 30°C for approximately 1 wk. Proportion of spores was checked regularly by phase-contrast microscopy. Spores were harvested by washing the plates with sterile physiological saline and centrifuging the washings at $7,500 \times g$ for 10 min. Spores were washed twice with physiological saline and finally resuspended in saline.

The *Cl. tyrobutyricum* strain SMR 226, isolated from raw milk, was grown on the surface of Reinforced Clostridial Agar (RCA; Oxoid Ltd., Basingstoke, UK) during 1 to 2 wk at 37°C under anaerobic incubation. Spores were harvested and washed as previously described for *B. cereus* spores. Spore count was determined after heating an aliquot of the spore preparation at 72°C for 5 min. Serial 10-fold dilutions of *B. cereus* were surface-plated on blood agar plates and incubated under aerobic conditions for 24 h at 30°C. The *Cl. tyrobutyricum* spores were analyzed on the surface of RCA agar plates following anaerobic incubation at 37°C for 72 h. Spore suspensions were diluted and frozen at -20°C in aliquots suitable for the experiments.

Milking Conditions

Rear parts of the stalls were carefully cleaned of manure and sawdust 30 min before milking. Cows were milked at 0630 and 1600 h while in tie stalls, using 7 or 8 milking units with claw size of 360 mL, short milk tube diameter (14 mm), dual vacuum system, and auto-

matic claw removal (Alfa Laval Agri, Tumba, Sweden). Each unit was only used for 1 cow per milking and 1 unit was used consistently for the same treatment during each experiment. Cows were milked in the same order at every milking occasion. The milkers washed their hands carefully with soap between cows and new teat-cleaning towels were used, one for each cow. Only experienced milkers were used and they were told to wipe the udder and teats as usual, but to do it carefully, actively cleaning the teats during the entire cleaning time and especially clean the teat ends. Cleaning times for udders and teats were fixed at 10 or 20 s depending on the experimental treatment. Forestripping of 2 squirts of milk was performed after udder and teat cleaning in all treatments, except for that of the control. Milking machines were attached immediately after forestripping. After milking, the udder and teats were carefully wiped with moist paper towels and postmilking teat dip containing iodine was used (Proactive; Hygenius, Drongen, Belgium).

Cleaning of Milking Equipment

Before each experimental series, milking machines were dismantled and carefully cleaned by hand. After each milking, milking equipment was cleaned automatically, alternately with a chlorine-free alkaline detergent (Eco alkali; Juvelit, Söderhamn, Sweden) and with an acid detergent (Eco cid; Juvelit), followed by circulation with chloramine. Before every milking occasion, milking equipment was rinsed with water.

Sampling Procedures

Milk yields were recorded and a milk sample was collected from each cow at each milking occasion with a milk meter (Tru-test Ltd., Auckland, New Zealand). To evaluate whether natural contamination with cow manure or contamination from milking equipment might have contributed to additional *Cl. tyrobutyricum*

spores during the experiments, samples of cow manure and rinse water from the milking equipment were collected and analyzed. Manure was taken from the stalls every morning as a composite sample from 3 cows. Rinse water from milking equipment was collected at 2 occasions: at the beginning and at the end of each experiment. Samples were collected following rinse water circulation before milking from 2 samplings points: the releaser and at the outlet in the milk storage room. To evaluate natural contamination with *B. cereus* spores in Experiment 3, 3 composite samples of used bedding material from 3 different stalls were collected on the last experimental day, including a sample of unused sawdust. All fluid samples of 200 mL and solid samples of 100 to 200 g were frozen until analyses were carried out within 14 d.

Hygiene Scores

To determine whether natural contamination of udder and teats affected results, visual observations of udder and teat hygiene (manure and sawdust) were made before the experimental contamination of the teats. Dirtiness of udder, teats, and teat ends of all cows were graded on a 5-point scale (Christiansson et al., 1999): 1 = completely clean; 2 = some visual dirt; 3 = less than 25% of the area dirty; 4 = 25 to 50% of the area dirty; and 5 = more than 50% of the area dirty.

Microbiological Analyses

Milk and water samples were analyzed by filtration. Water samples of 100 mL in duplicate were heat-treated at 72°C for 5 min and filtered through a Sartorius membrane filter (LKB-Sartorius AB, Sundbyberg, Sweden) with 0.8- μ m pore size (11404-47-CAN) using a filtration apparatus equipped with sterilizable filter support and funnels (Sartorius SM16831). Milk samples were heat-treated as described above and treated with trypsin and Triton X-100 (Christiansson et al., 1997). From each sample, duplicates of 10 to 100 mL of milk were filtered depending on the expected degree of contamination. Following filtration, filters were rinsed with 100 mL of sterile water at a temperature of 55°C. Filters for *B. cereus* counts were placed on the surface of blood agar plates [blood agar base No.2 (Oxoid Ltd.); 10 ppm of polymixin B sulfate (Sigma, St Louis, MO); and 5% bovine defibrinated blood]. Plates were incubated aerobically at 20°C for 48 h, and typical colonies of *B. cereus* with a zone of hemolysis were counted. For *Cl. tyrobutyricum* spore counts, filters were placed on the surface of RCA agar plates with 200 ppm D-cycloserine (Sigma) and anaerobically incubated at 37°C for 72 h before counting.

Solid 25-g samples of manure or sawdust were added to 225 mL of sterile peptone-water (2 g of peptone/L and 0.1 g of Tween 80/L) in a Colworth Stomacher bag (Seward Ltd., London, UK) and then homogenized twice in a Colworth Stomacher for 30 s each. From the Stomacher bag, 100 mL of liquid was transferred to a sterile 100-mL cylinder. Following 2 min of sedimentation, 10 mL were collected at the 50-mL mark. The liquid was transferred to a test tube and was heat-treated at 72°C for 5 min before analysis of *B. cereus* spores, and at 80°C for 10 min before analysis of *Cl. tyrobutyricum* spores. Serial 10-fold dilutions were surface-plated in duplicate for the determination of *B. cereus* spores on blood agar and for *Cl. tyrobutyricum* spores on RCA agar plates. Blood agar plates were incubated aerobically at 20°C for 48 h and RCA agar plates anaerobically at 37°C for 72 h. To determine concentrations of spores in the contamination mixtures, samples were heat-treated, surface-plated, and incubated as previously described before the colonies were counted.

Statistical Analyses

Statistical calculations were performed using Minitab version 14 for Windows (Minitab, 2003). Logarithms of total numbers of spores in the milk for each cow at each milking occasion were calculated as $[\log_{10} (\text{spores/mL} \times \text{mL of milk/milking occasion})]$ and used for statistical analyses by ANOVA. Pairwise comparisons were made by the Tukey's test. Possible effects on the presence of spores in the milk in response to dirtiness of the udder and teats before experimental contamination were analyzed. Pearson correlations were calculated between the hygiene score and the logarithm of the total number of spores in the milk. Differences in hygiene scoring among treatments were analyzed by ANOVA.

Experiments 1 and 2. The model used to analyze effects of the different cleaning methods was as follows:

$$Y_{ijklm} = \mu + \alpha_i + b_j + c_k + \delta_l + (c\delta)_{kl} + t_{m(kl)} + e_{ijklm},$$

where Y_{ijklm} = $\log_{10}$ spores in milk at each milking, μ = overall mean, α_i = effect of cleaning method ($i = 1$ to 7), b_j = random effect of cow ($j = 1$ to 7), c_k = random effect of milker ($k = 1$ to 2), δ_l = effect of time of milking ($l = 1$ to 2, morning or evening), $(c\delta)_{kl}$ = effect of interactions with milker and time of milking ($k = 1$ to 2, $l = 1$ to 2, morning or evening), $t_{m(kl)}$ = the random effect of milking occasion within Latin square ($m = 1$ to 7), and e_{ijklm} = residual error.

Experiment 3. Cleaning efficiency as affected by the contamination matrix and degree of contamination was analyzed using the most common spore for each matrix. The model was as follows:

Table 4. Effect of different type of towels on the reduction of spores in milk (Experiment 1)

Teat-cleaning method ¹	Mean log ₁₀ spores per milking ²	Reduction compared with control, %
1A) Control: no cleaning, no forestripping	5.00 ^a	—
1B) Dry paper towel a, 10 s	4.74 ^b	45
1C) Dry paper towel b, 10 s	4.70 ^b	50
1D) Moist paper towel c, 10 s	4.70 ^b	50
1E) Moist paper towel d, 10 s	4.42 ^c	74
1F) Moist washable cotton towel, 10 s	4.41 ^c	74
1G) Moist washable synthetic towel, 10 s	4.42 ^c	74

^{a-c}Means having different superscript letters differ ($P \leq 0.05$).

¹Four kinds of paper towels (a, b, c, and d) were used (see Table 1).

²SD = 0.18, df = 156.

$$Y_{ijkl} = \mu + \alpha_i + b_j + \delta_k + t_{l(k)} + e_{ijkl}$$

$$\text{where } \alpha_i = \tau_m + \gamma_n + (\tau\gamma)_{mn},$$

where Y_{ijkl} = log₁₀ spores in milk at each milking, μ = overall mean, α_i = effect of treatment ($i = 1$ to 8, contamination mixture and cleaning or no cleaning), τ_m = effect of contamination mixture ($m = 1$ to 4), γ_n = effect of cleaning ($n = 1$ to 2, cleaning or no cleaning), $(\tau\gamma)_{mn}$ = effect of interaction with contamination mixture and cleaning ($m = 1$ to 4, $n = 1$ to 2, cleaning or no cleaning), b_j = random effect of cow ($j = 1$ to 8), δ_k = effect of time of milking ($k = 1$ to 2, morning or evening), $t_{l(k)}$ = the random effect of milking occasion within Latin square ($l = 1$ to 8), and e_{ijkl} = residual error.

Cleaning efficiency as affected by the type of spores was analyzed by the ratio of the concentration of *Cl. tyrobutyricum* spores vs. the concentration of *B. cereus* spores in the milk. The model was as follows:

$$Y_{ijkl} = \mu + \alpha_i + b_j + \delta_k + t_{l(k)} + e_{ijkl},$$

where Y_{ijkl} = (log₁₀ *Cl. tyrobutyricum* spores – log₁₀ *B. cereus* spores) in milk at each milking, μ = overall mean, α_i = effect of treatment ($i = 1$ to 4, contamination mixture and cleaning or no cleaning), b_j = random effect of cow ($j = 1$ to 8), δ_k = effect of time of milking ($k = 1$ to 2, morning or evening), $t_{l(k)}$ = the random effect of milking occasion within Latin square ($l = 1$ to 8), and e_{ijkl} = residual error.

RESULTS

Experiment 1

Treatments differed ($P < 0.001$) for logarithms of total number of spores in milk (Table 4). Compared with control (no cleaning and no forestripping), cleaning routines using different types of dry paper towels for a

period of 10 s reduced ($P < 0.001$) content of spores in milk by 45 to 50%. A 50 to 74% reduction was achieved by using different types of moist towels for 10 s. No significant differences were detected between the observations for the 2 milkers or between the morning and evening milking occasions.

Milking equipment did not seem to contribute to any spore contamination of the milk because rinse water contained <10 *Cl. tyrobutyricum* spores/L. Samples of cow manure contained between 87,000 and 620,000 *Cl. tyrobutyricum* spores/g. Greater hygiene scores reflecting teat dirtiness prior to contamination (much dirt on udder, teat, and teat ends) contributed to some extent to the milk spore contents ($r = 0.30$; $P < 0.001$). No significant differences in hygiene scoring were detected among treatments.

Experiment 2

Treatments differed ($P < 0.001$) for logarithms of total number of spores in milk (Table 5). The most effective treatments ($P < 0.001$) for cleaning teats were use of a moist synthetic towel with or without soap followed by drying with a dry paper towel for 10 + 10 s, which reduced the spore content in the milk by about 96%. Treatments with 2 towels, soap, or a longer cleaning time had greater effects than did the treatments in Experiment 1 (Figure 1). No significant differences were detected between the 2 milkers or between the morning and evening milkings.

Rinse water in the milking equipment contained <10 *Cl. tyrobutyricum* spores/L. Samples of cow manure contained between 6,100 and 194,000 *Cl. tyrobutyricum* spores/g. No significant relationship was found between hygiene score and numbers of spores in the milk. No significant differences in hygiene scoring were detected among treatments.

Table 5. Effect of cleaning time and different cleaning procedures on the reduction of spores in milk (Experiment 2)

Teat-cleaning method ¹	Mean log ₁₀ spores per milking ²	Reduction compared with control, %
2A) Control: no cleaning, no forestripping	5.33 ^a	—
2B) Soap solution with sprayer + dry paper b, 10 s	4.52 ^b	85
2C) Soap solution by dip in foam + dry paper b, 10 s	4.26 ^c	91
2D) One moist paper towel d, 20 s	4.47 ^b	86
2E) Two moist paper towels d, 10+10 s	4.30 ^c	91
2F) Moist washable synthetic towel + dry paper b, 10+10 s	3.94 ^d	96
2G) Moist washable synthetic towel with soap + dry paper b, 10+10 s	3.93 ^d	96

^{a-d}Means having different superscript letters differ ($P \leq 0.05$).

¹Two kinds of paper towels (b and d) were used (see Tables 1 and 2).

²SD = 0.18, df = 156.

Experiment 3

Because no differences between milkers were found in Experiments 1 and 2, only 1 milker was used in Experiment 3. When teats were contaminated with mixtures containing different matrices and the most common type of spores for each matrix (Table 6), no interactions were detected between type of matrix and cleaning method (control or cleaning). Lack of an interaction indicated that neither type of matrix (manure, soil, or sawdust) nor degree of contamination (manure or extra manure) affected cleaning efficiency. The logarithm of the ratio of concentration of spores of *Cl. tyrobutyricum* over *B. cereus* equal to ($\log_{10} Cl. tyrobutyricum$ spores – $\log_{10} B. cereus$ spores) was calculated for control milk and milk from cows following cleaning. No significant differences were detected between the ratios (Table 7) for either manure or soil. The cleaning method used had similar effects on both types of spores (*Cl. tyrobutyricum* and *B. cereus*). The results indicated that when cleaning by using a moist washable synthetic towel for 10 s followed by drying with a dry paper towel for 10 s, efficiency of cleaning was independent of the contamination matrix, type of spores, or degree of contamination. No significant differences were detected between morning and evening milking.

Rinse water in the milking equipment contained <10 *Cl. tyrobutyricum* spores and <21 *B. cereus* spores/L. Between 12,700 and 89,000 *Cl. tyrobutyricum* spores/g were found in samples of cow manure. Unused sawdust contained <100 *B. cereus* spores/g, and used sawdust from the stalls contained between 100 and 1,750 *B. cereus* spores/g. No positive correlation was found between hygiene score and number of spores in milk for either *B. cereus* or for *Cl. tyrobutyricum*. No significant differences in hygiene scoring were detected between treatments.

DISCUSSION

The method used to experimentally contaminate teats with *Cl. tyrobutyricum* spores was a practical way to evaluate effectiveness of different premilking teat-cleaning methods. Uniform teat contamination made it possible to reduce the numbers of cows and samples in the experiments and provides more power of resolution between cleaning methods. It was also a cost-effective approach to compare new teat-cleaning methods. Anaerobe spores are excellent microbiological cleaning indicators because they do not grow aerobically and are not killed by the cleaning agents. It was important to use contamination mixtures containing a large concentration of spores and to minimize influence of natural

Figure 1. Percentage of spores in milk after different methods of cleaning contaminated teats compared with no cleaning and no forestripping (control; Experiment 1 and 2).

Table 6. Effect of contamination matrix and degree of contamination on the reduction of spores (*Clostridium tyrobutyricum* and *Bacillus cereus*) in milk (Experiment 3)

Matrix	Type of spores	Mean log ₁₀ spores per milking ¹		Reduction, cleaning compared with control, %
		Control	Cleaning	
Manure	<i>Cl. tyrobutyricum</i>	5.01 ^a	3.23 ^b	98.3
Extra manure	<i>Cl. tyrobutyricum</i>	5.01 ^a	3.40 ^b	97.5
Soil	<i>B. cereus</i>	5.23 ^a	3.48 ^b	98.2
Sawdust	<i>B. cereus</i>	5.18 ^a	3.24 ^b	98.8

^{a,b}Means having different superscript letters differ ($P \leq 0.05$).

¹SD = 0.31, df = 98. Control = no cleaning, no forestripping; Cleaning = cleaning with a moist washable synthetic towel for 10 s followed by a dry paper towel for 10 s.

teat contamination from manure and bedding. In addition, using silage of good quality is recommended to maintain a low spore content in the manure.

During these experiments, a large concentration of *Cl. tyrobutyricum* spores was found in manure in Experiment 1, probably because of poor silage quality. Elevated teat and udder hygiene scores before spore contamination produced slightly elevated concentrations of spores in milk, but no differences in hygiene scoring were detected among treatments. Therefore, it was most likely that natural manure contamination had not affected the final observations. In Experiments 2 and 3, contamination mixtures having elevated concentrations of spores were used and feeding a new batch of silage produced smaller concentrations of spores in the manure. Under these conditions, positive correlations between hygiene scores and presence of spores in the milk were not found. The limited spore content observed in rinse water showed that milking equipment did not contribute to the spore contamination during these experiments.

Different cleaning methods gave clear differences in the results and confirmed observations from previous studies. For example, spore reductions of 45 to 50% found with dry paper toweling was comparable with findings by Rasmussen et al. (1991) when a dry paper towel was used for 6 s. Use of moist towels often gave better results than did the use of dry paper towels as previously noted for bacterial counts by others (Galton et al., 1986; Pankey, 1989). Type of paper influenced the cleaning effect; 1 type of moist paper towel had a similar effect as the dry ones (50% reduction), but the other type of moist paper towel gave a better result (74% reduction). The least efficient moist paper towel was thinner and often ripped apart when used. Effect of using washable towels of synthetic material or cotton was as good as that of the best moist paper towel (74% reduction). Using a moist washable synthetic towel with or without soap to clean followed by drying with a dry paper towel produced the best results (96% reduc-

tion). Adding soap to the moist synthetic towel did not affect the results. Because similar results were obtained with one of the moist paper towels and with both of the moist washable towels, it could be concluded that using any moist towel (washable or not) that had a strong mechanical effect, followed by drying with a dry paper towel should produce a good cleaning effect. Rasmussen et al. (1991) found that use of a moist cotton towel for 20 s was more effective than cleaning with a wet and a dry paper towel for 20 s, but their results were related not only to type of towel but also to more intense cleaning with the cotton towel.

When the teats were sprayed or dipped with soap followed by drying with a paper towel, cleaning effect was much better than that obtained just using a dry paper towel, as found for bacterial counts by Galton et al. (1986). The differences obtained when soap was applied to the teats by spray or by foam, that of 85 and 91% reduction in the milk spore content, respectively, could be because of the application method or might have been caused by the different compounds in the soap solutions. When dipping teats in soap foam, they were more easily completely covered with soap, whereas this was more difficult to achieve using a spray. Best results were obtained when soap was applied by a moist synthetic towel followed by drying with a paper towel (96% reduction). This method produced similar results with or without soap. With longer manual cleaning times, soap seemed to be of less importance. Many disinfectants affect bacterial counts in milk (Adkinson et al., 1991; Ingwa et al., 1992). Soap and disinfectants, however, can have less influence on milk spore content because spores are not killed by these agents because of their short contact time.

Duration of cleaning time of teats was also of importance. In accordance with the present results, Rasmussen et al. (1991) found that teat cleaning with firmly wrung cotton towels for 6 s was less efficient than cleaning for 20 s. When the same moist paper towel was used for 10 or 20 s, 74 and 86% reductions in milk

Table 7. Effect of cleaning on different type of spores (*Clostridium tyrobutyricum* and *Bacillus cereus* (Experiment 3)

Matrix	Type of spores	Mean log ₁₀ spores per milking ¹		Reduction, cleaning compared with control, %
		Control	Cleaning	
Manure	<i>Cl. tyrobutyricum</i>	5.01	3.23	98.3
	<i>B. cereus</i>	4.88	3.10	98.3
	Ratio of <i>Cl. tyrobutyricum</i> to <i>B. cereus</i>	0.13 ²	0.13 ²	—
Soil	<i>Cl. tyrobutyricum</i>	5.21	3.62	97.4
	<i>B. cereus</i>	5.23	3.48	98.2
	Ratio of <i>Cl. tyrobutyricum</i> to <i>B. cereus</i>	-0.02 ²	0.14 ²	—

¹Control = no cleaning, no forestripping; Cleaning = cleaning with a moist washable synthetic towel for 10 s followed by a dry paper towel for 10 s.

²The 4 means of the ratios do not differ ($P > 0.05$); SD = 0.26, df = 38.

spores, respectively, were achieved. With the same cleaning time, using 2 moist paper towels for 10 s each was better than using one moist paper towel for 20 s (91 and 86% reduction, respectively). If only 1 towel is used for a longer time on severely contaminated teats, contamination is likely spread about the teat, but not removed.

Results of Experiment 3 showed that it was possible to remove *B. cereus* spores from the teats with an efficiency similar to that obtained for the *Cl. tyrobutyricum* spores. Cleaning efficiency was not affected by the type of matrix (manure, soil, or sawdust). Problems with *B. cereus* spores in milk by soil contamination of teats during pasture season or bedding contamination of teats during winter can be reduced by good cleaning routines. Degree of contamination, with respect to more manure on teats, did not affect cleaning results. Similar percentages of manure were removed from more or less manure-contaminated teats. In practice, the more manure and spore contamination of teats there is, the more intense teat cleaning is needed to produce milk with low spore concentration.

Spores are hydrophobic and are considered to be harder to remove from surfaces than vegetative bacterial cells (Rönner et al., 1990). It might be expected that any cleaning method should have as good or even better effect on bacterial contamination than it has on spore contamination. An appropriate premilking cleaning method is also important to reduce risk of infection by mastitis pathogens (Pankey, 1989). To avoid transferring pathogens from cow to cow, each towel should only be used for 1 cow, and washable towels should be washed at 90°C before reuse.

Applying longer cleaning times, such as 20 s, in large dairy herds might extend milking time. Duration of teat cleaning, vigorousness of forestripping, and time between stimulation and unit attachment will affect the milk flow and milking efficiency (Rasmussen et al., 1992). Optimal duration of prestimulation to receive an

immediate and continuous milk flow has been shown to be 20 to 90 s, depending on how full the udder is at the time. Longer prestimulation times for udders that were not full also lead to reduced total vacuum load on the teats when milking (Weiss and Bruckmaier, 2005). Presumably, total milking time will not be prolonged when using a cleaning time of 20 s plus forestripping. On the other hand, it will contribute to an increased physical load on the milker.

Teat cleaning is also of great importance when using automatic milking systems. Cleaning effectiveness when tested with the same methods as those used in these experiments with experimental contamination of teats with *Cl. tyrobutyricum* spores, showed that a good cleaning effect with a 98% spore reduction can be achieved in the automatic milking systems (Melin et al., 2002). Field studies testing different methods for determining cleanliness have shown that cleaning efficiency between various brands of automatic milkers also differ and can be improved (Knappstein et al., 2004).

The present studies demonstrated that it is possible to reduce spore content in milk even when manure spore content is elevated. Even the best cleaning routine, however, does not eliminate all spores. When feeding poor quality silage it is not uncommon to find very large concentrations of *Clostridium* spores in the manure, which can be more than 10 times greater than those used in contamination mixtures applied in the present series of experiments (Stadhouders and Jørgensen, 1990). Under those circumstances it is very important to keep the udders clean by having clean free stalls and alleys. The best strategy of all is, of course, to feed good quality silage.

CONCLUSIONS

Cleaning teats and udders with a moist towel is often better than cleaning with a dry paper towel. Using dip

or spray with a soap solution before drying with a paper towel also will improve cleaning results. Longer manual cleaning times are important, and use of 2 moist towels produced better results than use of only 1 moist towel. Using a moist towel with or without soap followed by drying with a dry paper towel significantly improved cleaning results. In herds with spore problems, this teat-cleaning strategy is recommended.

The nature of the contamination matrix, such as manure, soil or sawdust, type of spores, or degree of contamination on the teats does not influence the effect of the cleaning method. Not even the best cleaning method removes all bacteria and spores; consequently, it is of great importance to take steps to maintain good hygiene in all parts of dairy production.

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