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Effect of cleaning procedure and hygienic condition of milking equipment on bacterial count of bulk tank milk

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The aim of the study was to describe the characteristics of cleaning procedures for milking equipment applied in intensive dairy farms in Lombardy (Italy) and to study their relationships with bacterial count of bulk milk and hygienic condition of milking machine components. A group of 22 dairy farms was visited twice (winter and summer) in order to collect bulk tank milk and post-rinse water samples and swabs from liners and milk receiver. Samples were analysed to determine: standard plate count (SPC), laboratory pasteurization count (LPC), psychrotrophic bacteria count (PBC), coliform count (CC) and *Escherichia coli*. Cleaning procedures were monitored using electronic milk flow meters with specific software for the measurement of the duration of each cleaning phase, circulating solution temperature and electrical conductivity, turbulence and water filling percentage of pipelines. The results showed that farms classified as high and low milk total bacteria count significantly differed both in terms of liners and receiver bacterial contamination and in terms of water temperature reached during the detergent phase of cleaning milking equipment. Significant positive correlations were found among total bacteria count in milk and bacterial contamination of the liners. Maximum water temperature reached during the cleaning cycle of milking equipment was very low (34·4 ± 8·9 °C on average); most of the observations (88·6%) corresponded to water temperatures < 45 °C. Cleaning temperature was related to psychrotrophic bacteria count of milk and post-rinse water and coliform count in liners. Routine check and regulation of water temperature during the washing phase of the milking machine can be a simple and effective way to control one of the main risk factors for bacteriological quality of bulk tank milk.

Keywords: Milking equipment, cleaning procedure, milk, bacteria count.

The production of milk with low bacteria count is important to guarantee high safety standards for consumers and to preserve sensory traits and shelf-life of milk and milk derivatives. At a farm level microbial contamination of raw milk can occur from many sources: i.e. dirty udders and animals, improperly cleaned and sanitized milking equipment (Kelly et al. 2009).

Standard plate count (SPC) is used as an indicator of the level of bacteria in milk (Chambers, 2002). SPC does not measure the entire bacterial population, but rather the number of bacteria that grow in the presence of oxygen (aerobically) and at medium range (mesophilic) temperatures. SPC in bulk tank milk is the most common way to evaluate the hygienic quality of raw milk at farm level. A high SPC can affect the quality of pasteurized milk and milk products, shelf-life and organoleptic characteristics (Keefe & Elmoslemany, 2007). The EU maximum limit for SPC was set at 100 000 cfu/ml by Regulation CE No 853/2004 (European Commission, 2004); in Lombardy the legal requirement for raw milk sold directly at farm is 25 000 cfu/ml (Regione Lombardia, 2007). SPC has low diagnostic value in identifying the source of bacterial contamination (Reinemann et al. 1997); determination of laboratory pasteurization count (LPC), psychrotrophic bacteria count (PBC) and coliform count (CC) can help in diagnosing the causes of contamination. LPC assesses the number of thermoduric bacteria in milk; they can multiply in biofilms on milking equipment, but their growth in refrigerated raw milk is low. Consequently a high count of thermoduric bacteria in bulk tank milk suggests extensive proliferation of

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this flora in the dairy equipment (Villar et al. 1996) and can be assumed to indicate poor cleaning efficiency of milking equipment. Psychrotrophic bacteria are able to grow at temperature close to 0 °C (Sørhaug & Stepaniak, 1997). The quality of dairy products can be affected by heat-resistant enzymes that are secreted by psychrotrophs in raw milk before heat treatment or during the cold storage of dairy products; PBC is usually a sign of inadequate refrigeration temperature or long storage time. CC can be an indicator of faecal contamination from dirty udders and teats. High CC and high LPC can also denote improper cleaning of milking equipment. High CC in bulk tank milk is related to low water temperatures during the cleaning cycle of milking equipment, as well as to coliform growth in milk residues in dairy equipment between milkings (Villar et al. 1996). Water used for cleaning the milking equipment is another potential source of coliforms and other undesirable organisms in bulk tank milk (Jayarao et al. 2004; Perkins et al. 2009).

Milk residues or remaining washing water left on the contact surface of milking equipment between milkings support the growth of a variety of microorganisms (Murphy & Boor, 2000; Holm et al. 2004) notably when the cleaning and disinfection process is inadequate (Perkins et al. 2009). Cleaning and sanitation of milking equipment is a combination of chemical, thermal and physical processes which need a minimum reaction time to be effective (Reinemann et al. 2000). The typical automatic cleaning process can be divided into three different main phases: pre-rinse, washing phase and post-rinse. The most important parameters of the washing process are water temperature, duration of each phase, detergent concentration, amount and turbulence of circulating water.

The pre-rinse phase is essential to remove most milk residues. Temperature of pre-rinse water should be between 38 °C and 55 °C (Reinemann et al. 2000). The lower limit is set above the melting point of butterfat; exceeding the upper limit can cause protein deposition on milking equipment. The use of warm water during the initial rinse can help to warm the equipment and reduce temperature drop during the subsequent phases.

During the washing phase, alkaline or acid detergent should be used. Alkaline detergent helps to remove organic deposits, as milk protein and fat. Most detergents work at hot temperature from 43 °C to 77 °C (Reinemann et al. 2000) while others are formulated to be active at room temperature. According to Keown & Kononoff (2006) the optimal range of temperature for washing solution is 60–73 °C. The concentration of detergent used, which can be measured through water solution conductivity, can affect the washing efficacy. Water hardness can also influence detergent effectiveness. Acid detergent is used periodically to remove mineral deposits from water and milk. The frequency of acid washing depends on the hardness of water used in the farm; in Italy acid washing is usually performed once a week.

The amount and turbulence of circulating water guarantees the proper distribution of the detergent solution throughout the pipe system and affects physical removal of residues. Turbulence is created by steady air admission to produce a two-phase air-water mixture that increases flow during cleaning and improves the cleaning action of the water-detergent solution.

The goal of this study was to describe the characteristics of cleaning procedures of milking equipment used in intensive dairy farms in Lombardy (Italy) and to study their relationships with bacteria count of bulk tank milk and hygienic condition of the milking machine.

Materials and Methods

Farm characteristics and classification

A total of 22 farms were chosen from the 250 farms belonging to the same milk cooperative in Lombardy, in the North of Italy. The choice was based on the following criteria: herringbone milking parlour; herds with <200 milking cows; twice a day milking. Moreover all fortnightly analyses of SPC of bulk tank milk were collected from the cooperative database from 2006 to 2008. In these analyses SPC was determined by an opto-fluorimetric method using Bactoscan FC (Foss-Electric). Monthly bulk milk SPC of each herd, obtained as an average of fortnightly analyses, was classified as 'high' if it was greater than the general arithmetic mean of log-transformed counts (4·18 $\log_{10}$ cells/ml equal to 15 136 cells/ml) plus SD (0·4 $\log_{10}$ cells/ml) of the 250 farms; otherwise it was classified as 'low'. Starting from this classification, two groups of farms were chosen: the first group (HSPC herds) collected 14 farms with high variability of SPC results, attested by the large percentage ($\geqslant$ 24%) of monthly bulk milk with SPC classified as 'high', while in the second group (LSPC herds) there were 8 farms with very constant results and very low percentage ($\leqslant$ 6%) of monthly bulk milk SPC classified as 'high'. Each farm was visited twice, during winter and summer of the same year, at the evening milking. During the first visit a questionnaire was filled out to collect information on housing, barn design, milking parlour, milking equipment, milking routine, washing and cooling system.

Sampling and analyses

Bulk milk was sampled from the tank before evening milking, while post-rinse water samples were collected at the end of the cleaning cycle of milking equipment. All the samples were transported to the laboratory under refrigeration (4 °C) no later than 12 h from collection, and subjected to microbiological analysis. SPC, LPC, PBC and CC were determined on each sample according to ISO methods (SPC ISO: 4833:2003; CC: ISO 4832:2006; PBC: ISO 6730: 2005 incubated at 6·5 °C for 10 d; LPC: ISO 4833:2003 incubated at 30 °C for 72 h, after heat treatment at 63 °C for 30 min, sample preparation was made following ISO 8261:2001). *Esch. coli* was counted with Petrifilm *Escherichia coli* count plates (3M, Minneapolis MN, USA) incubated for 24 h at

30 °C and also 24 h at 37 °C. Before milking four different liners (in two different clusters) and milking receiver were swabbed, in order to measure SPC, LPC, PBC, CC and *Esch. coli*. Disposable swabs filled with 1 ml of Liquid Amies (ESwab, Copan, Brescia, Italy) were used; samples were taken by rolling only once the swabs into the internal surface of the mouthpiece chamber of each teatcup and around the drain at the bottom of the receiver. Swabs were homogenized for 1 min then serially diluted in quarter-strength Ringer's solution. Analyses were performed as previously described. Results were expressed in cfu/swab.

Cleaning procedure was monitored using electronic milk flow meters (Lactocorder, WMB, Balgach, Switzerland) with specific software. Two milk flow meters were set in two distant milking units (at the beginning and at the end of one milking line) during the cleaning process. Each parameter of the cleaning cycle was recorded every 0·7 s by inner sensors and saved every 11·2 s. The duration of the cleaning cycle started with the first detection of water motion through the pipe system, and ended when no more moving water was found. Generally three different cleaning phases were identified (pre-rinsing, detergent and post-rinsing phases) but the differentiation was possible only when there was an interval time without water in the pipes between phases. Moreover Lactocorder measured for each phase: duration, circulating solution temperature and electrical conductivity, turbulence and percentage of water filling of pipelines. For each phase Lactocorder provided three different measurements of circulation solution temperature: average, minimum and maximum. Using known and increasing amounts of each kind of detergent, a calibration curve was built for each detergent to link the electrical conductivity to the concentration of detergent solution.

Statistical analysis

All data from the questionnaire were analysed using the Chi-square test (PROC FREQ; SAS, 2001). Data collected during winter and summer tests were analysed by ANOVA using a generalized linear model (GLM; SAS, 2001).

The model used for testing the effect of SPC classification was:

$$Y_{ijkl} = \mu + C_i + T_j + H_k(C_i) + e_{ijkl}$$

where Y = dependent variables; μ = general mean; C_i = effect of SPC classification (i = HSPC, LSPC); T_j = effect of test (j = 1, 2); $H_k(C_i)$ = effect of herd (k = 1–22) nested in SPC classification; e_{ijkl} = residual error.

The model used for testing the effect of maximum temperature used during washing cycle was:

$$Y_{ijkl} = \mu + W_i + T_j + H_k(W_i) + e_{ijkl}$$

where Y = dependent variables; μ = general mean; W_i = effect of maximum water temperature during washing cycle (i = < 35 °C, ⩾ 35 °C); T_j = effect of test (j = 1, 2); $H_k(W_i)$ = effect of herd (k = 1–22) nested in maximum water temperature during washing cycle; e_{ijkl} = residual error.

Pearson correlation analysis was performed by using the CORR procedure (SAS, 2001).

Moreover a canonical discriminant analysis (PROC CANDISC; SAS, 2001) was carried out to find linear combinations of the quantitative variables (SPC, CC and PBC of bulk tank milk; CC and PBC of liner swabs; electrical conductivity and maximum water temperature of detergent phase) that provide the best separation among observations. Based on parameters of the cleaning system, each monitored cleaning process was allotted to one of three classes: if maximum water temperature was higher than 35 °C then the cleaning process was defined as 'good cleaning procedure'; if maximum water temperature was lower than 35 °C and detergent solution conductivity lower than 4 mS/cm then the cleaning process was defined as 'conductivity and temperature failure' and when maximum water temperature was lower than 35 °C and detergent solution conductivity higher than 4 mS/cm then the cleaning process was classified as 'temperature failure'.

Results

Farm characteristics and classification

Mean value of total bacteria count in bulk tank milk was 4·05 $\log_{10}$ cfu/ml with a minimum value of 2·95 $\log_{10}$ cfu/ml and a maximum of 5·43 $\log_{10}$ cfu/ml. Thermoduric bacteria were 2·45 ± 0·56 $\log_{10}$ cfu/ml on average, whereas psychrotrophic and coliform bacteria were, respectively, 3·60 ± 0·82 $\log_{10}$ cfu/ml and 1·90 ± 0·91 $\log_{10}$ cfu/ml.

Based on SPC in bulk tank milk of the previous 3 years, 14 farms were defined as high SPC (HSPC) and 8 farms as low SPC (LSPC). Main farm characteristics are shown in Table 1. Most of the farms, in both categories, had cubicles as the type of housing, while the remaining farms housed cows on straw pack. Average number of milking cows in 22 herds was 71·9 ± 40·8; the frequency distribution was not significantly different between HSPC and LSPC groups, even though there were smaller farms (< 50 cows) in the HSPC class than in the LSPC one. Most of the LSPC farms sold raw milk directly. Regarding udder preparation, forestripping, pre- and post-milking teat dip were carried out more frequently in LSPC farms than in HSPC ones (P < 0·05). Use of gloves by milkers and frequency of milk filter changes were not different between classes.

Milk, liner and receiver swabs and post-rinse water bacteriological analyses for HSPC and LSPC farms are shown in Table 2. Bacteria counts on bulk tank milk samples showed important differences between the two farm classes. The number of thermoduric bacteria was slightly higher in HSPC herds than in LSPC ones, while SPC, PBC and CC were strongly different with the same trend (P < 0·01). Microbiological analyses of receiver swabs always showed higher values in the HSPC farms than in the others; in particular the differences for LPC and CC of receiver swabs were statistically significant (P = 0·05 for LPC and P < 0·05 for

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Table 1 Characteristics of the 22 dairy farms classified on the basis of milk standard plate count (LSPC, low standard plate count; HSPC, high standard plate count) of the previous 3 years

	Percentage of herds		
	LSPC ($n=8$)	HSPC ($n=14$)	P†
Type of housing cubicles (yes *v.* no)	87·5	71·4	0·387
Number of milking cows			
<50	12·5	42·9	0·316
50–100	62·5	35·7	
>100	25·0	21·4	
Selling raw milk directly yes *v.* no	75·0	7·14	0·001
Milk collection frequency every day *v.* every second day	37·5	28·6	0·665
Number of milking units <10 *v.* >10	37·5	64·3	0·225
Milking procedures			
pre-dipping (yes *v.* no)	75·0	28·6	0·035
forestripping (yes *v.* no)	100	35·7	0·003
washing with water (yes *v.* no)	0	28·6	0·095
post-dipping (yes *v.* no)	100	57·1	0·029
Floor cleaning during milking with *v.* without cows inside parlour	62·5	78·6	0·416
Milkers with gloves yes *v.* no	75·0	64·3	0·604
Frequency of filter changes			
every milking	87·5	71·4	0·617
every second milking	12·5	21·4	
no filter	0	7·14	

† *P* value from Chi-square test

CC). Average SPC on receiver was 3·06 ± 1·12 $\log_{10}$ cfu/swab in 22 farms whereas average SPC on liner swabs was 3·72 ± 0·83 $\log_{10}$ cfu/swab. Also SPC, PBC and CC in liner swabs were higher in HSPC farms than in LSPC ones ($P<0·05$). *Esch. coli* in milk, liner and receiver swabs did not differ between HSPC and LSPC farms.

Cleaning procedure of milking equipment

Bacteriological analyses of post-rinse water samples collected at the end of the cleaning cycle of milking equipment did not show any difference between LSPC and HSPC farms (Table 2).

With regard to cleaning procedure of milking equipment, minimum, maximum and average water temperatures during the detergent phase were significantly higher in LSPC than in HSPC ($P<0·05$). In particular maximum water temperature reached in the detergent phase was 39·6 °C and 31·4 °C ($P<0·001$) in LSPC and HSPC farms, respectively.

Maximum water temperature registered during the detergent phase of cleaning cycles on the total sample of 22 farms was on average 34·4 ± 8·9 °C with wide range of variability of 10–50·3 °C. To study the effect of water temperature, bacteriological analyses were classified on that basis: about half of the cleaning tests registered maximum water temperatures lower than 35 °C. Figure 1 shows the frequency distribution of water temperature of the two classes (low temperature <35 °C; medium temperature ⩾35 °C). There was great variability of temperatures in the two classes; in particular in the first class it can be noticed that two observations correspond to very low temperatures (<20 °C)

Table 2 Bacterial counts† of milk and post-rinse water, receiver and liner swabs and characteristics of detergent phase during cleaning cycle of milking equipment of farms classified high (HSPC) or low (LSPC) standard plate count. Values are least squares means

Farms	LSPC ($n=8$)	HSPC ($n=14$)	SE	P
Bulk tank milk				
SPC, $\log_{10}$ cfu/ml	3·68	4·25	0·13	0·003
LPC, $\log_{10}$ cfu /ml	2·30	2·53	0·11	0·097
PBC, $\log_{10}$ cfu ml	3·34	3·95	0·16	0·003
CC, $\log_{10}$ cfu /ml	2·98	3·93	0·20	0·001
Escherichia coli, $\log_{10}$ cfu /ml	0·96	1·08	0·08	0·227
Receiver swabs				
SPC, $\log_{10}$ cfu/swab	2·59	3·21	0·31	0·125
LPC, $\log_{10}$ cfu/swab	1·41	1·86	0·17	0·050
PBC, $\log_{10}$ cfu/swab	2·07	2·86	0·37	0·103
CC, $\log_{10}$ cfu/swab	0·28	0·74	0·16	0·032
Escherichia coli, $\log_{10}$ cfu/swab	0·29	0·43	0·08	0·169
Liner swabs				
SPC, $\log_{10}$ cfu/swab	3·36	3·92	0·18	0·022
LPC, $\log_{10}$ cfu/swab	1·78	2·10	0·14	0·090
PBC, $\log_{10}$ cfu/swab	2·66	3·44	0·24	0·017
CC, $\log_{10}$ cfu/swab	0·36	0·69	0·09	0·007
Escherichia coli, $\log_{10}$ cfu/swab	0·35	0·48	0·07	0·139
Post-rinse water				
SPC, $\log_{10}$ cfu/ml	2·45	2·44	0·20	0·978
LPC, $\log_{10}$ cfu /ml	1·24	1·34	0·16	0·608
PBC, $\log_{10}$ cfu ml	1·44	1·43	0·19	0·966
CC, $\log_{10}$ cfu /ml	0·21	0·20	0·14	0·939
Escherichia coli, $\log_{10}$ cfu /ml	0·12	0·00	0·05	0·069
Detergent phase				
Duration of detergent phase, min	17·3	17·8	1·03	0·734
Minimum water temperature, °C	17·0	14·1	0·90	0·017
Maximum water temperature, °C	39·6	31·4	1·46	0·000
Average water temperature, °C	28·0	24·2	0·90	0·005
Electrical conductivity, mS/cm	3·31	4·06	0·39	0·141
Turbulence, %	25·2	23·1	3·07	0·598
Water filling, %	66·5	64·8	3·04	0·696

† SPC = standard plate count; LPC = laboratory pasteurization count; PBC = psychrotrophic bacteria count; CC = coliform count

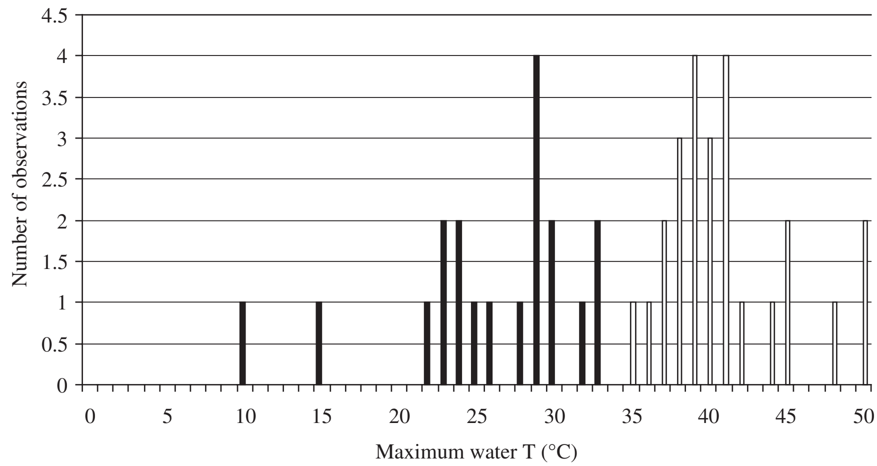

Fig. 1. Frequency distribution of maximum water temperatures (T) during the detergent phase of milking equipment cleaning cycles (■ maximum water T < 35°C; □ maximum water T ⩾ 35°C).

while in the second class there were five observations with temperatures higher than 45 °C.

Effects of maximum water temperature during the cleaning process on bacteria counts of milk and equipment are presented in Table 3. PBC in bulk tank milk and in post-rinse water was significantly lower ($P<0{\cdot}05$) in farms that used medium water temperature (⩾ 35 °C) to wash milking equipment. On the contrary SPC, LPC and CC in milk were not significantly different between classes, even if the mean values were slightly lower in the medium temperature class than in the other one. CC on liner swabs was significantly lower in farms where maximum water temperature was above 35 °C than in the others ($P<0{\cdot}01$). LPC, PBC and *Esch. coli* trends in liner swabs were similar to CC but the differences between classes were not significant. Receiver swab analyses did not show any significant difference between classes, probably because of the high variability of the samples. In addition the other parameters registered by Lactocorder during the cleaning procedure (water filling, turbulence and electrical conductivity of circulating solution) were all more favourable in the medium temperature class than in the other one.

Figure 2 shows coefficients of variation (CV) of mean turbulence (%) and mean water filling (%) between the recordings of the two flow meters simultaneously used on the same milking machine. Wide variability was found for low turbulence values (<20%). High CV for water filling were found for percentages lower than 55%. For both parameters CV decreased with the increase of the mean. On the contrary the CV of maximum water temperature was less than 5% on average, attesting a good stability of water temperature between milking units.

Figure 3 shows the relationship between the electrical conductivity of the circulating solution, measured by Lactocorder, and the detergent concentration estimated by the calibration curves built for each detergent. The same figure displays also the detergent concentrations declared by the farmers (from questionnaire) in relation to expected conductivity. Concentrations calculated from conductivity measurements were always lower than the farmers' reports.

Correlation analysis

Correlation coefficients between bacteria count of bulk tank milk and bacteria count of liner swabs are shown in Table 4. SPC of bulk tank milk was positively correlated with SPC, LPC and PBC of liner swabs. In particular, thermoduric bacteria of liner swabs were the bacterial group with the highest correlation coefficient with milk SPC ($r=0{\cdot}46$). PBC in milk was correlated with bacteria count in liner swabs, in terms of LPC, PBC and CC. The count of *Esch. coli* in milk was low and there was a positive correlation with coliform count ($r=0{\cdot}34$, $P<0{\cdot}05$). There were no significant correlations among SPC of bulk tank milk and receiver swabs and post-rinse water samples.

Canonical discriminant analysis

A canonical discriminant analysis was carried out to underline the relations between cleaning procedure parameters and bacteria count of bulk tank milk and liner swabs (Fig. 4). All quantitative variables were significant ($P<0{\cdot}10$) to provide maximal separation between the three groups ('good cleaning procedures', 'conductivity and temperature failure', 'temperature failure').

Discussion

Average SPC of bulk tank milk of sample herds was very low (geometric mean of 11 266 cfu/ml), below the EU legal limit of 100 000 cfu/ml (European Commission, 2004) and also the requirements of Lombardy region to sell raw milk directly at farm (25 000 cfu/ml; Regione Lombardia, 2007). Thermoduric bacteria count in milk (LPC) should be less than <200 cfu/ml to be considered normal, but less than

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Table 3 Effect of maximum water temperature (°C) during the detergent phase on bacterial count† of milk and post-rinse water, receiver and liner swabs. Values are least squares means

Maximum water temperature	<35° C	⩾35° C	SE	*P*
Number of observations	19	25		
Bulk tank milk				
SPC, $\log_{10}$ cfu/ml	4·20	3·96	0·12	0·142
LPC, $\log_{10}$ cfu /ml	2·44	2·41	0·12	0·815
PBC, $\log_{10}$ cfu ml	4·06	3·32	0·20	0·014
CC, $\log_{10}$ cfu /ml	2·14	1·83	0·18	0·225
Escherichia coli, $\log_{10}$ cfu /ml	1·03	1·05	0·06	0·772
Receiver swabs				
SPC, $\log_{10}$ cfu/swab	3·11	2·86	0·32	0·587
LPC, $\log_{10}$ cfu/swab	1·75	1·53	0·15	0·315
PBC, $\log_{10}$ cfu/swab	2·76	2·32	0·39	0·440
CC, $\log_{10}$ cfu/swab	0·61	0·47	0·18	0·578
Escherichia coli, $\log_{10}$ cfu/swab	0·43	0·34	0·08	0·410
Liner swabs				
SPC, $\log_{10}$ cfu/swab	3·92	3·57	0·16	0·132
LPC, $\log_{10}$ cfu/swab	2·23	1·80	0·18	0·097
PBC, $\log_{10}$ cfu/swab	3·52	3·02	0·21	0·097
CC, $\log_{10}$ cfu/swab	0·84	0·46	0·09	0·010
Escherichia coli, $\log_{10}$ cfu/swab	0·55	0·37	0·07	0·084
Post-rinse water				
SPC, $\log_{10}$ cfu/ml	2·89	2·21	0·16	0·009
LPC, $\log_{10}$ cfu /ml	1·53	1·23	0·13	0·115
PBC, $\log_{10}$ cfu ml	1·96	1·22	0·14	0·002
CC, $\log_{10}$ cfu /ml	0·46	0·15	0·14	0·116
Escherichia coli, $\log_{10}$ cfu /ml	0·07	0·05	0·05	0·741
Detergent phase				
detergent phase, min	17·1	18·4	0·99	0·344
conductivity, mS/cm	3·01	3·97	0·42	0·108
Turbulence, %	18·6	27·9	3·18	0·048
Water filling, %	59·8	69·9	3·93	0·077

†SPC = standard plate count; LPC = laboratory pasteurization count; PBC = psychrotrophic bacteria count; CC = coliform count

Table 4 Correlation coefficients between bacterial count† of bulk tank milk and bacterial count of liner swabs

	Bulk tank milk			
Liner swabs	SPC	LPC	PBC	CC
SPC	0·33*	0·39*	0·24	0·17
LPC	0·46*	0·40*	0·44*	0·32*
PBC	0·38*	0·23	0·41*	0·26
CC	0·19	0·08	0·38*	0·40*

†SPC = standard plate count; LPC = laboratory pasteurization count; PBC = psychrotrophic bacteria count; CC = coliform count
*Statistically significant (*P* < 0·05)

10 cfu/ml when the equipment hygiene is excellent (Ruegg & Reinemann, 2002); in this study LPC was higher than the first threshold suggested (283 cfu/ml). Ruegg & Reinemann

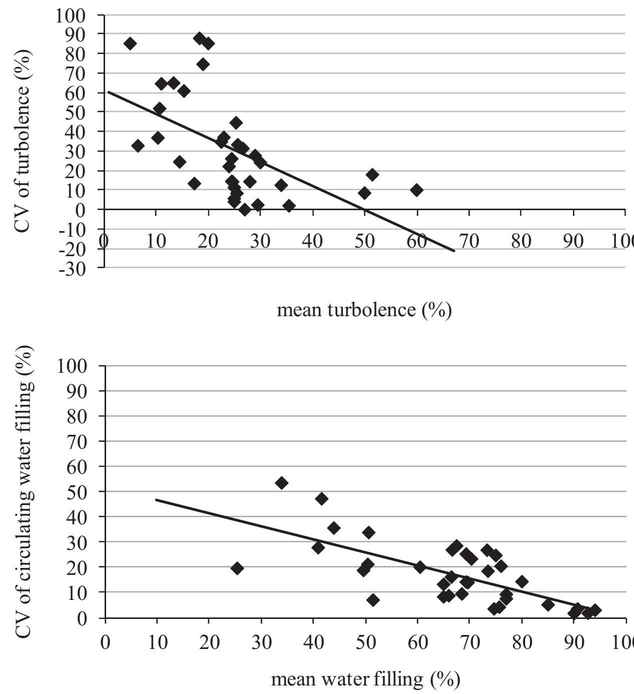

Fig. 2. Relationships between coefficients of variation (CV) and means of all observations for turbulence (y = −0·52x + 52·03; r^2 = 0·476) and percentage of water filling (y = −1·22x + 60·98; r^2 = 0·314).

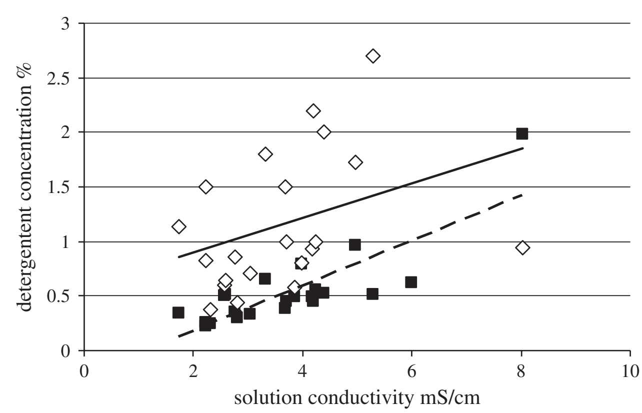

Fig. 3. Relationship between electrical conductivity of the detergent solution (measured by Lactocorder) and detergent concentration ■: concentration of detergent determined by calibration curves; ◊: concentration of detergent declared by farmers; linear ----- (concentration of detergent determined by calibration curves; y = 0·206x − 0·227; r^2 = 0·667); linear — (concentration of detergent declared by farmers; y = 0·159x + 0·577; r^2 = 0·126).

(2002) reported also that high milk LPC could be associated with poor milking hygiene, unclean equipment, improper sanitizing practices and milk stone deposits in the pipes. The highly significant correlation coefficients found between LPC in milk and LPC and SPC in liner swabs suggest that inadequate cleaning system can represent a significant source of contamination of bulk tank milk. Average PBC

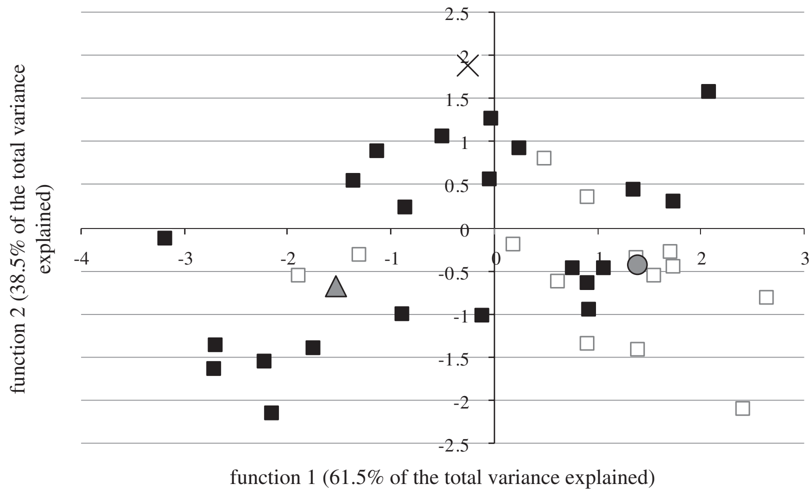

Fig. 4. Canonical discriminant analysis for type of cleaning procedure and problems. ■: HSPC (high standard plate count) farm observations; □: LSPC (low standard plate count) farm observations; ●: mean value for 'good cleaning procedures' group; ▲: mean value for 'conductivity and temperature failure'; **X**: mean value for 'temperature failure'.

(3981 cfu/ml) in milk was equal to 35 % of SPC (11266 cfu/ml); a similar percentage was reported by Jayarao et al. (2004). PBC in milk was correlated with bacteria count in liner swabs, in terms of LPC, PBC and CC.

Bacteriological analyses of bulk tank milk, receiver and liner swabs confirmed the less careful hygiene conditions at all levels in the HSPC herds compared with LSPC ones. These results could be caused by inadequate sanitation of milking equipment in HSPC herds, as suggested by differences in water temperature of cleaning cycle between the two classes of farms, but also by different hygiene procedures at milking (pre-dipping, forestripping and post-dipping).

Total coliform bacteria are used to assess external contamination. CC can be used as an indicator of potential contamination from pathogenic organisms of faecal origin (Yin & Ding, 2009). CC in milk higher than 1000 cfu/ml suggests that bacterial growth is occurring on milking equipment (Reinemann et al. 1997; Murphy & Boor, 2000). In the present study milk coliform contamination was correlated with liner contamination, in terms of LPC and CC, confirming the relations between milk contamination and milking equipment hygiene, as reported by Elmoslemany et al. (2009a).

PBC can be influenced by inadequate refrigeration temperature or long storage time, but it is also related to the total SPC of milk; HSPC herd class had higher PBC than LSPC one ($P<0{\cdot}01$).

SPC of receiver swabs showed higher variability compared with SPC of liner swabs; it was probably due to the high variability in receiver shape, which differed from farm to farm, whereas liner shape was often similar.

Water temperatures during the detergent cycle of milking equipment were significantly lower in the HSPC farms than in LSPC ones. This difference confirms the influence of hygiene operations of milking equipment on milk bacteria count; in particular cleaning temperature seems to be one of the risk factors for bacteriological contamination of milk at farm level. Similarly Elmoslemany et al. (2009b) concluded that high temperature of the wash solution is a protective factor.

For the whole group of monitored farms, maximum water temperature registered during the detergent phase of cleaning cycles was, on average, below 43 °C, which is the minimum temperature suggested for most of the alkaline detergents (Reinemann et al. 2000) and very much lower than the optimal range of 60–73 °C reported by Keown & Kononoff (2006).

All the components of milking machine (claw, liners, long milk tube and long pulse tube) are made of rubber, steel or plastic. All these materials are potential sites for biofilm formation, even when hygiene and sanitation programmes are correctly applied. Biofilm can be a source of milk contamination owing to the persistent release of microorganisms from the surface to the bulk fluid (Teixeira et al. 2005).

Milk samples from farms with medium washing water temperature (⩾ 35 °C) had significantly lower PBC than the other class (<35 °C). This is consistent with the low water temperature reached during the washing cycle: the dominance of thermolabile species such as *Pseudomonas* spp. and coliforms in milk, indicates the use of low (<42 °C) cleaning temperature (Murphy & Boor, 2000). The trend of all liner swab bacteria counts was the same: cleaning milking equipment with higher water temperature reduced bacteria counts, in particular CC. The effect of water temperature was more marked in liner swabs than in milk: liners are the surface where biofilm can easily grow.

Moreover, water filling and turbulence percentages were higher and more favourable in farms with medium washing water temperature (⩾35 °C) than in the other ones; this underlines that in many cases it was not just a temperature

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failure but a more general problem of washing system due to improper setting or old equipment.

Coefficient of variation of cleaning parameters between units in the same milking equipment showed a scarce homogeneity of cleaning process. Sometimes problems of water/air admission in the farthest units could occur, because of old pipes, pipe bends or some kind of obstruction. Turbulence had higher CV in comparison with water filling, in particular with low values (<20%); also water filling percentage showed higher variability with low values. These results suggested that, when the washing cycle does not work correctly (low water filling and low water turbulence), uniformity of washing power between milking units could be negatively affected.

Detergent concentration during the cleaning procedure is another important parameter that can influence cleaning efficiency of milking equipment. The concentration suggested by the producers is 0·5–1% for most of the detergents used by farmers in this research. Lactocorder measurements showed conductivity values ranging from 1·74 to 6 mS/cm, corresponding to adequate detergent concentration. The only outlier value (8·03 mS/cm) presented in Fig. 3 corresponds to a cold water detergent that needed a higher concentration (1–3%) than other detergents. Detergent concentrations declared by farmers in many cases did not correspond to the measurements.

Canonical discriminant analysis suggested that cleaning procedures of milking equipment contribute significantly to discriminate between farms with low and high total bacteria count of bulk tank milk: most of HSPC herds showed water temperature failure or temperature and conductivity failure (due to improper detergent concentration), whereas the majority of LSPC herds used water with temperature >35 °C during the washing phase. A few HSPC herds apparently used correct cleaning procedures of milking equipment; in these cases the high level of contamination of bulk tank milk was probably due to other factors such as poor cow and bedding hygiene, improper milking operations or inadequate milk cooling system.

The results showed that farms classified as high and low total bacteria count in milk significantly differed both in terms of liners and receiver bacterial contamination and in terms of water temperature reached during the detergent phase of cleaning milking equipment. Significant positive correlations were found among total bacteria count in milk and bacterial contamination of the liners. Cleaning procedures of the milking machine, expressed as washing temperature and detergent concentration, contributed significantly to discriminate between farms with low and high total bacteria count of bulk tank milk. In particular water temperature during the washing cycle significantly influenced PBC of milk and post-rinse water and CC in liners. Most of the farms used very low water temperatures during the washing cycle.

Routine check and regulation of water temperature during the washing phase of the milking machine can be a simple and effective way to keep under control one of the main risk factors for bacteriological quality of bulk tank milk. Liner swabs can be useful tools to detect microbiological problems of milking equipment and to evaluate the efficiency of the cleaning procedure.

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