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Effects of season, milking routine and cow cleanliness on bacterial and somatic cell counts of bulk tank milk

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The aim of the study was to investigate the effects of season, cow cleanliness and milking routine on bacterial and somatic cell counts of bulk tank milk. A total of 22 dairy farms in Lombardy (Italy) were visited three times in a year in different seasons. During each visit, samples of bulk tank milk were taken for bacterial and somatic cell counts; swabs from the teat surface of a group of cows were collected after teat cleaning and before milking. Cow cleanliness was assessed by scoring udder, flanks and legs of all milking cows using a 4-point scale system. Season affected cow cleanliness with a significantly higher percentage of non-clean (NC) cows during Cold compared with Mild season. Standard plate count (SPC), laboratory pasteurization count (LPC), coliform count (CC) and somatic cell count, expressed as linear score (LS), in milk significantly increased in Hot compared with Cold season. Coagulase-positive staphylococci on teat swabs showed higher counts in Cold season in comparison with the other ones. The effect of cow cleanliness was significant for SPC, psychrotrophic bacterial count (PBC), CC and *Escherichia coli* in bulk tank milk. Somatic cell count showed a relationship with udder hygiene score. Milking operation routine strongly affected bacterial counts and LS of bulk tank milk: farms that accomplished a comprehensive milking scheme including two or more operations among forestripping, pre-dipping and post-dipping had lower teat contamination and lower milk SPC, PBC, LPC, CC and LS than farms that did not carry out any operation.

Keywords: Milk, bacteria count, somatic cell count, hygiene score, milking routine.

Raw milk quality at farm level is an important component influencing the performance of the whole dairy chain. A key parameter of raw milk quality is its hygienic profile, which is characterized by contamination levels and specific distributions of microorganisms. Hygienic quality of milk is influenced by many factors, several of which depend on farm management. Season effect is also important; temperature and humidity variations can have strong effects on bacterial counts in milk. Season affects total aerobic count with a positive trend during summer and an opposite one in winter, as shown by IZSLER (2010) for cow bulk tank milk in Lombardy. Similar results were reported by Elmoslemany et al. (2010) in a study on dairy herds of Prince Edward Island.

Hygienic conditions of the cows are essential for the production of high quality milk. In particular a strong association between udder hygiene and bacterial counts in bulk tank milk was found by Elmoslemany et al. (2009). Different methods were developed to assess cow cleanliness and farm environment hygiene (Bartlett et al. 1992; Barkema et al. 1998; Ward et al. 2002; Schreiner & Ruegg, 2003). Assessment of cow cleanliness can be referred to the whole body of the animal, or alternatively to specific parts of the body such as legs, flanks and udders. A positive correlation was also found between frequency of dirty udders and new intramammary infections (Schreiner & Ruegg, 2003). In fact, many factors influencing bacterial count of milk also affect udder safety and milk somatic cell count.

Pre-milking udder preparation is essential to produce high quality milk; it includes teat end sanitation and pre-milking stimulation (Wagner & Ruegg, 2002). A proper prestimulation is needed to obtain continuous and rapid milk removal. In contrast, without prestimulation, the milk ejection reflex is often delayed (Sandrucci et al. 2007), milking time is prolonged and udder health can be compromised. Pre-milking teat sanitation can be performed in different ways using wet

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or dry towels, water, spray or foamy solutions. According to Reinemann et al. (2008) pre-dipping can reduce teat surface bacteria by 75%. Forestripping consists of the removal of the first streams of milk to check for clinical mastitis. It determines a strong stimulus for milk letdown, ensures that all abnormal milk is diverted from the human food chain, and should be a standard food safety practice in all farms (Wagner & Ruegg, 2002; Ruegg, 2003). Disinfection of the teat after the end of the milking (post-dipping) can reduce the numbers of bacteria on the teat skin and, at the same time, can help to protect the stressed teat orifice after milking and to prevent bacteria entering the teat canal (Murphy & Boor, 2000).

A limited number of field studies have analysed the effects of different environmental and management factors on microbiological quality and somatic cell count of milk. The goal of the present study was to investigate the effects of season, cow cleanliness and milking routine on bacteria and somatic cell count of bulk tank milk in intensive dairy farms in Lombardy (Italy).

Material and Methods

Farm questionnaire and environmental data

A group of 22 dairy farms situated in the north of Italy (Lombardy) were involved in the study. Sample farms had on average 71·9 (± 40·8) milking cows milked twice a day. Each farm was visited three times: during the Cold season (December, January and February), the Hot season (June and July) and the Mild season (8 farms in April; 14 farms in October) of the same year, at evening milking. During the first visit a questionnaire was completed to collect information on housing, barn design, milking parlour, milking equipment, milking routine and milk cooling system. Environmental temperature data were obtained from the database of Regional Weather Bureau (ARPA, 2009).

Milk and teat swab analyses

Bulk milk was sampled from the tank after evening milking. All samples were transported to the laboratory under refrigeration (4 °C) no later than 12 h after collection, and submitted for microbiological analyses. Standard plate count (SPC), coliform count (CC), psychrotrophic bacteria count (PBC), laboratory pasteurization count (LPC) and *Escherichia coli* 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). Coliforms and *Esch. coli* were counted with Petrifilm *Esch. coli* Count Plates (3M, Minneapolis MN, USA) incubated for 24 h at 30 °C (for coliforms) and additional 24 h at 37 °C (for *Esch. coli*).

Somatic cell count per ml (SCC) was detected by Fossomatic (TM 400, Foss, Hillerød, Denmark); SCC values were converted to Linear Scores (LS) by the following equation: $LS = \log_2 (SCC/12\,500)$ (Wiggans & Shook, 1987).

Swabs were performed on a single teat of 6–8 cows randomly selected from the herd during each farm visit at the evening milking. Moistened commercial paper towels, without perfume or other additives, were used as swabs to wipe the whole surface of the teat after pre-milking cleaning (if performed) and before claw attachment. Care was taken to touch only the teat surface (not the base of the udder) and to remove as much as possible of the debris on the teat barrel and end surface, as described by Bade et al. (2008). Teat swabs were placed immediately in sterile plastic 'zip-loc' bags, transported to the laboratory under refrigeration (4 °C) and analysed the morning after the collection day. A peptone solution (90 ml; 0·1% w/v) was added to every bag and samples were homogenized for 30 s in a stomacher blender (Interscience; St. Nom, France) at high speed. Decimal dilutions of the homogenates were prepared with sterile Ringer solution (Scharlau Microbiology, Spain). Aliquots of the dilutions were plated onto the following media: Standard Plate Counts Petrifilm Aerobic Count Plates-3M, Minneapolis, USA incubated at 30 °C for 72 h; coagulase-positive staphylococci Baird-Parker RPF agar, Biolife, Italy (ISO 6888-2/IDF 145:1999) at 37 °C for 48 h. Results were expressed in cfu/swab.

Cow cleanliness

Hygiene scores were assessed through direct observation in the milking parlour at each farm visit according to Schreiner & Ruegg (2003): udder, flanks and legs of each milked cow (4216 cows) were scored in the same way based on a 4-point scale system, where score 1 indicates very clean skin while score 4 indicates skin completely covered with dirt. In order to classify the whole-cow cleanliness, the scores given to udders, flanks and legs were used for a combined classification: when a cow had udder, flank and leg scores always different from 3 and 4 the cow was defined as 'clean' (C), otherwise it was classified as 'non-clean' (NC). For each visit, farms were divided into two groups on the basis of the percentage of cows defined as NC (< 50% or ⩾ 50% in the total herd).

Statistical analysis

Data were analysed by ANOVA using a generalized linear model (proc GLM; SAS, 2001).

The model used for testing the effects of cow cleanliness (C) and season (S) was:

$$Y_{ijkl} = \mu + C_i + S_j + CS_{ij} + H_k(C_i) + e_{ijkl}$$

where Y_{ijkl} = dependent variables; μ = general mean; C_i = effect of cow cleanliness class (i = 1–2; < 50% of NC cows in the herd, ⩾ 50% of NC cows in the herd); S_j = effect of season (j = 1–3); CS_{ij} = interaction effect between cow cleanliness and season; $H_k(C_i)$ = effect of herd (k = 1–22) nested in cow cleanliness classification; e_{ijkl} = residual error.

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The model used for testing the effects of milking routine (R) classification was:

$$Y_{ijkl} = \mu + R_i + S_j + RS_{ij} + H_k(R_i) + e_{ijkl}$$

where Y_{ijkl} = dependent variables; μ = general mean; R_i = effect of milking routine type (i = 1–3; no operation, one operation, two or more operations); S_j = effect of season (j = 1–3); RS_{ij} = interaction between milking routine type and season; $H_k(R_i)$ = effect of herd (k = 1–22) nested in milking routine type ; e_{ijkl} = residual error.

The dependent variables considered were: SPC, PBC, LPC, CC, *Esch. coli* and LS of bulk tank milk; SPC and coagulase-positive staphylococci of teat swabs; cow cleanliness (% of NC cows).

The percentage of variance explained by each fixed effect was estimated from an univariate analysis using the VARCOMP procedure of SAS (SAS, 2001).

Relationships among hygiene score data on flanks, legs and udders were studied using proc REG procedure (SAS, 2001).

Results

The 22 farms belonged to the same milk cooperative in Lombardy (northern Italy). Most of the farms had cubicle sheds while only 5 of them housed cows on straw pack. Other characteristics of the sample farms were previously described in a companion paper (Bava et al. 2011).

As expected, large differences of mean outside temperatures were observed among the three periods of farm visits: 3·8 °C (with a minimum of − 6·4 °C) in Cold season; 12·1 °C during Mild season and 23·5 °C (with a maximum of 28 °C) in the Hot season (June and July).

Season affected cow cleanliness with a significantly higher percentage of NC cows during Cold (66%) compared with Mild season (52%) (Table 1). Milk SPC, LPC, CC and LS increased in Hot season compared with Cold season ($P < 0·01$) and intermediate values were observed in Mild season. On the contrary coagulase-positive staphylococci on teat swabs showed higher count in Cold season in comparison with other seasons ($P < 0·01$).

Results from the VARCOMP statistics revealed that season effect explained 4·5–9·9% of the total variance, and herd-effect explained 18–61% of the total variance.

In Fig. 1 two regression lines are represented: one shows the relationship between flank and udder hygiene scores (% cows in each herd scored 3 or 4), the other relates, with the same approach, leg and udder scores. The high regression coefficients ($r^2 = 0·73$, $P < 0·001$ and $r^2 = 0·70$, $P < 0·001$, respectively) underline the strong association among hygienic conditions of udder, legs and flanks.

Classification of whole-cow cleanliness revealed great variability among farms in terms of percentage of NC cows (Fig. 2), with high number of farms (14) with more than half the animals classified as NC. Moreover in 16 farms, where days in milk (DIM) of each cow was obtained, the percentage of NC cows was higher in early lactating cows (64%, <100 DIM) in comparison with cows in late lactation (40%, >200 DIM).

Table 1. Effect of season on cow cleanliness, milk and teat swab bacterial counts and linear score. Values are least squares means. Means in rows followed by different letters are significantly different ($P < 0·01$)

	Seasons			
	Hot	Mild	Cold	SEM
Observations, n	22	22	22	
Cow cleanliness				
NC† cows, %	55·7ab	52·3b	65·7a	3·774
Bulk tank milk (log$_{10}$cfu/ml)				
SPC	4·20a	3·96b	3·88b	0·070
PBC	3·84	3·54	3·59	0·119
LPC	2·55a	2·47ab	2·26b	0·092
CC	2·41a	1·92b	1·66b	0·136
Escherichia coli	1·01	1·10	1·05	0·060
Bulk tank milk				
LS	4·55a	4·29ab	4·07b	0·120
Teat swabs (log$_{10}$ cfu/swab)				
SPC	5·37	5·34	5·28	0·095
Coagulase-positive staphylococci	2·96b	2·95b	3·23a	0·042

†NC cows = non-clean cows; SPC = standard plate count; PBC = psychrotrophic bacterial count; LPC = laboratory pasteurization count; CC = coliform count; LS = linear score

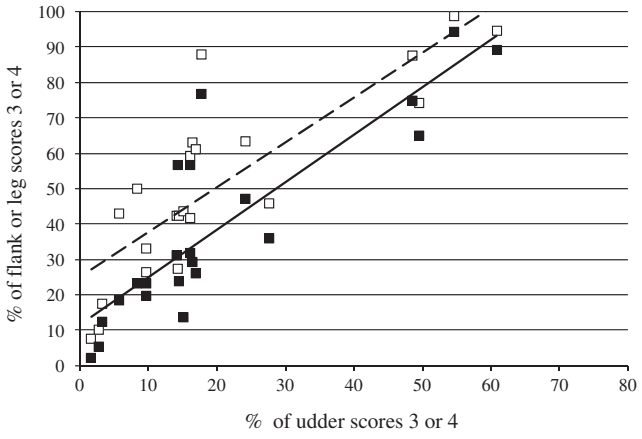

Fig. 1. Regression analysis: ■ regression between udder scores and flank scores (percentage of 3 or 4 scores) linear – (y = 1·34x + 11·6 $r^2 = 0·73$); □ regression between udder scores and leg scores (percentage of 3 or 4 scores) linear --- (y = 1·26x + 25·2 $r^2 = 0·70$).

Effects of cow cleanliness on bacterial counts of bulk tank milk and teat skin bacterial contamination are shown in Table 2. In particular, SPC, PBC and CC in bulk tank milk were significantly higher in the group of observations with more than 50% of NC animals in comparison with the other group.

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Table 2. Effect of cow cleanliness on milk and teat swab bacterial counts and linear score. Values are least squares means

	< 50% NC† cows	⩾ 50% NC cows	SEM	*P*
Observations, n	29	36		
Bulk tank milk (log_{10}cfu/ml)				
SPC	3·88	4·06	0·070	0·05
PBC	3·40	3·87	0·112	0·00
LPC	2·46	2·44	0·069	0·79
CC	1·78	2·19	0·140	0·03
Escherichia coli	0·97	1·12	0·058	0·06
Bulk tank milk				
LS	4·07	4·32	0·122	0·14
Teat swabs (log_{10}cfu/swab)				
SPC	5·19	5·41	0·103	0·12
Coagulase-positive staphylococci	3·01	3·05	0·042	0·52

†NC cows = non-clean cows; SPC = standard plate count; PBC = psychrotrophic bacterial count; LPC = laboratory pasteurization count; CC = coliform count; LS = linear score

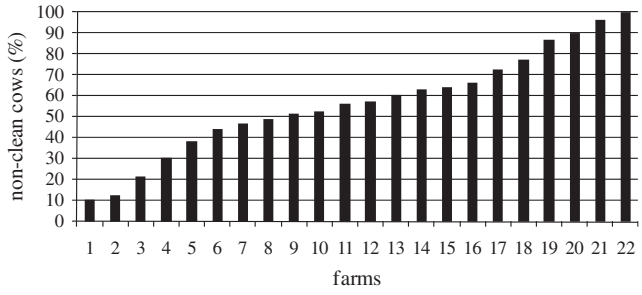

Fig. 2. Percentage of non-clean cows in the monitored farms (means of three visits).

The interaction between cow cleanliness and season was not significant for all variables. Results from the VARCOMP statistics revealed that the cow cleanliness effect explained 4·9–24·2% of the total variance.

When only udder scores were considered, a significant association with somatic cell counts was found: in the herds where < 15% of udders were scored 3 or 4, milk LS was 4·01 while in the herds with ⩾15% of udders scored 3 or 4 milk LS was 4·34 ($P < 0·05$).

To study the effects of milking procedures on cow cleanliness, bacterial counts of bulk tank milk and teat swabs, and LS, the 22 farms were classified into three groups based on the combination of the three most common milking operations: forestripping, pre-dipping and post-dipping. Three farms did not carry out any of these milking procedures (Table 3); 7 farms performed only one operation (forestripping or pre-dipping or post-dipping using dipcup) and 12 farms accomplished a more comprehensive scheme with two or more operations; in particular all farms of the third group carried out forestripping. The most common milking procedure was post-dipping (16 farms).

Table 3. Effect of milking operations on milk and teat swab bacterial counts and linear score. Values are least squares means. Means in rows followed by different letters are significantly different ($P < 0·001$)

	Pre and post milking operations			
	No operation	One operation	Two or more operations	SEM
Observations, n	9	21	36	
Milking operations				
Forestripping	0	3	36	
Pre-dipping	0	3	27	
Post-dipping	0	15	33	
Cow cleanliness				
NC† cows, %	82·4a	72·8a	43·2b	5·730
Bulk tank milk (log_{10}cfu/ml)				
SPC	4·36a	4·34a	3·73b	0·097
PBC	4·21a	3·97a	3·34b	0·160
LPC	2·50a	2·72a	2·23b	0·141
CC	2·46a	2·30a	1·71b	0·191
Escherichia coli	1·16	1·10	1·00	0·087
Bulk tank milk				
LS	4·59a	5·05a	3·77b	0·175
Teat swabs (log_{10}cfu/swab)				
SPC	5·89a	5·60a	5·04b	0·149
Coagulase-positive staphylococci	3·14	3·02	3·04	0·063

†NC cows = non-clean cows; SPC = standard plate count; PBC = psychrotrophic bacterial count; LPC = laboratory pasteurization count; CC = coliform count; LS = linear score

Bacterial counts and somatic cell count of bulk tank milk were significantly lower in the group of farms that implemented two or more operations at milking compared with the other two groups; the only exception was *Esch. coli*. Carrying out two or more milking operations also significantly reduced SPC of teat swabs. On the contrary coagulase-positive staphylococci on teat swabs were not affected by milking operations. The percentage of NC cows was significantly lower in the group of farms that performed two or more operations at milking in comparison with the other farms ($P < 0·001$).

Results from the VARCOMP statistics revealed that herd-effect explained 17·1–44% of the total variance.

Discussion

Cows were significantly dirtier in the Cold season in comparison with the Mild season; this was probably due to the difficulty in keeping cow bedding and alleys dry and

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clean during the rainy and snowy season, and the consequent increasing amounts of manure on legs, flanks and udders.

High values of milk SPC, LPC, CC and LS in Hot season agree with the results reported by Elmoslemany et al. (2010), who concluded that high milk bacterial counts during summer and spring were related to warmer environmental temperature, allowing bacteria to grow faster than in the other seasons. Seasonal variation of LS, with high values during the Hot season, is consistent with the study of Olde Riekerink et al. (2007). On the contrary coagulase-positive staphylococci on teat swabs increased during the Cold season. Similar results for *Staphylococcus aureus* were obtained by Olde Riekerink et al. (2007) and Makovec & Ruegg (2003), who observed a peak of these bacteria in milk during December and January. The same strain of bacteria is able to produce a biofilm on teat skin but also on milking unit liners (Fox et al. 2005); this type of biofilm is resistant to antimicrobial agents (Costerton et al. 1999) and, during winter, the temperature drop of the water in the liners, during the washing cycle of milking equipment, can facilitate its survival.

The high regression coefficients among udder, flank and leg hygiene scores suggest the importance of maintaining high level of cleanliness of all parts of the cow body. Positive correlation coefficients among udder, flank and leg hygiene scores were also obtained in a previous study on ten herds (Bava et al. 2008).

In the 16 farms cows at the beginning of lactation were dirtier than late lactation cows; this result could be due to the generally loose consistency of faeces in fresh cows. This is in agreement with the results of Ward et al. (2002) who reported worse hygienic condition of early lactation cows in comparison with cows in mid to late lactation and dry cows. The study showed that early-lactation cows had generally loose faeces and the cleanliness scores of the udders, flanks and legs of early-lactation cows were significantly related to faecal consistency. These results suggest that farmers have to take a special care with fresh cow cleanliness, i.e. by better cleaning of cubicles and better milking care.

The relation between cow cleanliness and bacterial counts of bulk tank milk agrees in part with the conclusions of Elmoslemany et al. (2010) who demonstrated that the amount of dirt on teats before pre-milking udder preparation was positively associated with SPC and psychrotrophic bacterial count in milk. In our study, LPC was not affected by cow cleanliness; this was probably due to the extensive proliferation of thermoduric bacteria in tank milk, mainly associated with poor cleaning of milking equipment (Villar et al. 1996).

In a companion paper (Bava et al. 2011) CC in milk showed a relation with hygienic conditions of milking equipment, in particular with liner bacterial contamination. The present study suggests also a relationship between milk CC and cow hygiene: CC was higher in the group of herds with higher percentage of NC cows than in the other one. According to Reinemann et al. (2000) CC is linked to cow cleanliness, cow environment and efficacy of milking equipment sanitization.

In the present study somatic cell count, expressed as LS, was lower in the group with a lower percentage of NC cows but the difference between the groups was not statistically significant. On the other hand LS showed a significant association with hygiene score of the udders. Barkema et al. (1998) demonstrated that SCC in bulk tank milk was lower (< 150 000 cell/ml) in herds with clean udders in comparison with herds with dirty udders (> 251 000 cell/ml). Similar results were obtained by Ellis et al. (2007).

A considerable percentage of farms did not carry out any procedure to clean and/or sanitize teats before or after milking; this practice, besides the effect on milk bacterial count, could expose the herd to an increase of bulk somatic cell count (Barkema et al. 1998).

The association of two or more milking operations (forestripping, pre-dipping, post-dipping) showed a positive effect on microbiological quality of milk; these results are in agreement with Jayarao et al. (2004) who found that bulk milk SPC was significantly lower when cows were treated with both pre- and post-dipping. Moreover Galton et al. (1986) found a relation among udder preparation care before teat cup attachment and reduction of SPC and CC in bulk tank milk. On the contrary *Esch. coli* was not affected by milking operations, in agreement with the results of Gibson et al. (2008). The presence of coliforms in milk is generally regarded as indicating direct contamination with faecal material but this assumption might not be completely true because the resident flora of the milking system might contain coliforms (Reinemann et al. 2000). This could explain the inefficacy of premilking operations.

Somatic cell count showed the lowest value in the farms that carried out two or more milking operations. This is in agreement with Barkema et al. (1998) who showed that post-milking teat disinfection had important effects in decreasing bulk tank milk somatic cell count. While Köster et al. (2006) did not find any association between bulk milk somatic cell count and forestripping, this was probably due to the different quality in performing these procedures. A similar conclusion was reported by Rodrigues et al. (2005).

Milking routine influenced also SPC on teat swabs. Similar results were reported by Gibson et al. (2008) who studied the effectiveness of selected pre-milking teat cleaning regimes on teat microbial load; the authors noticed a significant reduction of bacterial counts on teat swabs after teat cleaning operations.

Low percentages of NC cows were observed in the farms that accomplished a comprehensive milking routine consisting of two or more operations. This result suggests a special attention by these farmers both to milking routine and to the cleanliness of cow environment (bedding materials, alleys).

In conclusion, season affected cow cleanliness, milk bacterial counts, LS and coagulase-positive staphylococci count on teats. Microbiological quality of milk was

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influenced by cow cleanliness while LS showed an association with udder hygiene score. Milking operation routine showed one of the most important effects on both bacterial counts and LS of bulk tank milk: farms that accomplished a comprehensive milking scheme including two or more operations among forestripping, pre-dipping and post-dipping, had lower contamination of teats before cluster attachment, lower milk bacterial counts and lower LS than farms that carried out one operation or none.

This study suggests that implementing and maintaining few and simple hygienic practices in terms of barn cleaning and milking procedures (forestripping, pre-dipping and post-dipping) can significantly improve microbiological quality of cow milk and reduce somatic cell count also in the intensive farming conditions of northern Italy where animals are kept in the barn all year. In this context the highly confined animal density involves a number of hygienic hazards that require proper management interventions.

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