Manageable risk factors associated with bacterial and coliform counts in unpasteurized bulk milk in Flemish dairy herds

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ABSTRACT

Associations between herd management practices and both bacterial counts (BC) and coliform counts (CC) from 254 and 242 dairy herds in Flanders (Belgium), respectively, were studied. Data were analyzed using multivariable, multilevel linear regression analysis, allowing variance components analyses. Both BC and CC fluctuated throughout the year, although the milk quality parameters followed an opposite pattern. Bacterial count values decreased with each increase of the cleaning frequency of the cubicles (once per week, once per day, twice per day, or more than twice per day) between January and March. Herds with a conventional milking parlor had substantially lower BC than herds where the cows were milked using an automatic milking system. Lower BC were observed when the milking parlor was equipped with an automatic cluster removal system, when premilking teat disinfection was applied, when the dry cows were supplemented with a mix of minerals and vitamins, and when the teats were prepared either first wet and dried or via an automatic milking system. Milking cows with a high-pipeline milking parlor setup or with an automatic milking system was associated with substantially higher CC values. Herds where prepartum heifers were often treated with antimicrobials before calving had a lower CC than farms where heifers were either not or only rarely treated. Most variation in BC and CC resided at the herd level rather than at the observation level, indicating that management is important in the control of both BC and CC. Still, only a small proportion of the total variance was explained by factors capturing information related to the milking, herd health, and dry cow management, which suggests that the bacteriological milk quality and, in particular, CC is primarily driven by other factors than the ones included in this study.

Key words: bacterial count, bulk milk, coliform count, dairy herd, management practice

INTRODUCTION

High bacterial levels in milk, whether originating from the cow or the environment, substantially affect the quality, safety, and consumer acceptance of milk and dairy-derived products. Some bacteria found in unpasteurized milk such as *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Mycobacterium tuberculosis*, and *Salmonella* spp. pose a potential risk for human health (Gilmour and Rowe, 1990; Murphy and Boor, 2000). Bacteria that are not known to be pathogenic can cause flavor changes, rancidity, and thus reduced shelf life (Boor, 2001; Barbano et al., 2006). Bacterial quality of milk can be determined using several parameters including bacterial counts (BC), preliminary incubation counts, laboratory pasteurization counts, and coliform counts (CC; Murphy, 1997). Among these, BC is the most commonly used one in regulatory programs (Murphy and Boor, 2000) and estimate the number of colony-forming units or individual BC (IBC) present in unpasteurized bulk tank milk.

In Flanders (Belgium), the official mandatory milk-quality regulations follow European legislation and require a geometric mean BC over the last 2 mo (based on 2 recordings per month) $<100 \times 10^3$ IBC/mL of milk. In contrast, testing of CC is nonobligatory for milk quality in Flanders, yet implemented as part of an incentive program. Similar to other countries, such as Ireland (Berry et al., 2006) and different regions in the United States (Jayarao et al., 2004), the majority of milk processors in Flanders pay incentives of up to €0.75/100 L of milk to farmers that meet higher quality requirements, including geometric mean bacterial counts $<50 \times 10^3$ IBC/mL and geometric mean CC <50 cfu/mL over the last 2 mo (4 recordings) in combination with a geometric mean SCC $<350 \times 10^3$ cells/mL, in the absence of antibiotic residues in any milk delivery, all to ensure the image of milk as a high-quality and safe product. Although bulk tank BC and CC in Flanders decreased by 19.2% between 2005 and 2008, an increase of almost 10% was observed between 2008 and 2009 (K. Lommelen, Milk Control Centre, Lier,

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Belgium, personal communication), warranting the need to understand the reasons behind this tendency.

Most studies have identified factors holding information on milking and equipment hygiene, sanitizing procedures (Elmoslemany et al., 2009a,b; Pantoja et al., 2011), and milk storage conditions (Murphy and Boor, 2000), explaining variability in BC and CC. Apart from 2 studies, of which one was conducted in Chile (van Schaik et al., 2005) and the other in Canada (Elmoslemany et al., 2010), factors related to either herd health management, transition and feeding management, or housing, which are known to affect udder health, have not been studied as potential risk factors. Still, mastitis-causing streptococci such as *Streptococcus uberis* and *Streptococcus agalactiae* can be important contributors to bacterial levels of unpasteurized bulk tank milk (Zadoks et al., 2004). Given this information, one could anticipate that the latter management practices are also relevant for milk quality and BC in particular, and could explain the increase in BC and CC in Flanders, coincident with the increase in the average bulk milk SCC (BMSCC) during the same period.

The main objective of this study was to evaluate to what extent differences in management practices different from those related to milking and equipment hygiene are associated with BC and CC in unpasteurized bulk milk on Flemish dairy herds. A secondary objective was to assess whether the variation in BC and CC resided mostly at the herd or at the observation level.

MATERIALS AND METHODS

Herd Demographic Data

In 2009, Flanders had 6,971 dairy herds, with an average herd size and milk production of 40.9 cows and 8,059 milk kg/cow per year. The average milk quota size was 310,708 kg. Herds included in this study were, on average, larger (65.8 cows/herd) in both size and milk

production (8,503 kg of milk/cow per year). The average of the geometric mean of BC and CC in Flanders was 11.3×10^3 IBC/mL and 10 cfu/mL, respectively. In 2009, 97% of the herds met the requirements for BC according to the European legislation (geometric mean BC $<100 \times 10^3$ IBC/mL), whereas approximately 85% of the herds met the specific requirements for higher-quality milk for CC (geometric mean CC <50 cfu/mL). The average of the geometric mean BMSCC was 230,000 cells/mL. The milk quality of the herds included in this study was comparable.

Herd Selection and Data Collection

A written web-based questionnaire was conducted between January 2010 and July 2010. The questionnaire was pretested and fine-tuned in close cooperation with 4 dairy farmers before the start of the study.

In total, 254 farmers completed the online questionnaire that consisted of 39 questions concerning general management (n = 8), herd health management (n = 5), milking management (n = 11), calving (n = 3) and dry cow management (n = 6), and nutrition (n = 6) in place on farm during 2009 (Table 1).

From all farms that completed the online questionnaire, the bulk tank milk BC and CC records measured at 2-wk intervals from January 2009 to December 2010 were retrieved from the Milk Control Centre Flanders that executes the (regulatory) farm screening program in Flanders, Belgium. Bacterial counts and CC were examined on unpasteurized bulk milk samples collected in 30-mL sterile screw-cap tubes by trained milk haulers. The samples were kept cooled ($\pm 4^{\circ}\text{C}$) until arrival at the laboratory.

Total BC and CC

All microbiological analyses were performed within 24 h after pick up at the farm. The milk samples were

Table 1. Overview of all herd management practices collected via a web-based questionnaire on 254 dairy herds in Belgium

Management type	Description
General management	Type of livestock farming, expected time the farm will still exist, herd size, number of lactating cows, milk quota size, duration of access to pasture during summer, barn type, cleaning frequency of the housing
Herd health management	Registration of animal diseases, herd health monitoring by veterinarian, monthly incidence of clinical mastitis, antimicrobial treatment during lactation of subclinical mastitis, treatment of prepartum heifers with antimicrobials
Milking management	Milking machine type, milking parlor type, cows kept in headlock after milking, use of automatic cluster removal, providing a preparation lag time of 60 s, teat-preparation method, application of premilking teat disinfection, application of postmilking teat disinfection, machine unit liner, rinsing of machine unit liners, replacement of machine unit liners
Calving management	Calving on pasture, presence of calving pen, use of calving pen for sick cows
Nutrition management	Concentrate provided during milking, concentrate provided on top of forage, concentrate provided via TMR, concentrate provided via automatic feeder, forage provided, type of forage feeding system
Dry cow management	Drying-off procedure, use of long-acting antimicrobials, adapted diet provided, mineral/vitamin mix provided, use of external teat sealer, use of internal teat sealer

vortexed before the start of the analyses. For BC, undiluted milk samples were used and automatically analyzed by means of a BactoScan FC (Foss Electric A/S, Hillerød, Denmark). Bacterial counts were expressed as the number of IBC/mL of milk. For CC, milk samples were first diluted using tryptone salt broth at 1:10. One milliliter of diluted milk was plated on 3M Petrifilm CC plates (3M, St. Paul, MN) and incubated at 30°C for 24 h. Colony-forming units were counted electronically using an automated colony counter (protoCOL; Synbiosis, Cambridge, UK). Coliform counts were expressed as the number of colony-forming units per milliliter of milk. Per month, a geometric mean BC and CC was calculated based on the last 4 recordings (last 2 mo). A logarithmic transformation of the geometric mean BC and CC was performed to normalize the data before statistical analyses.

Statistical Analysis

All data were entered in an electronic spreadsheet program (Excel 2010; Microsoft Corp., Redmond, WA) and were checked for unlikely values. Monthly geometric means for BC ($n = 3046$) and CC ($n = 2895$) were available for 254 and 242 of the farms, respectively.

The regression model-building process to identify management practices associated with BC and CC involved several steps as described previously (De Vliegher et al., 2004) and is presented in a flowchart (Figure 1). Linear mixed regression models with BC and CC as dependent variables were fit using SPSS software (version 17.0; SPSS Inc., Chicago, IL). In all models, herd was included as random effect to model the repeated measurements within herds using a first-order autoregressive correlation structure.

Initially, unconditional associations were tested between the continuous dependent variables at the herd level (logarithm of BC and logarithm of CC, respectively) and all management practices ($n = 39$; Table 1) and month of observation as fixed effects, the latter to take into account the longitudinal nature of the data. Statistical significance at this step was assessed at $P \leq 0.20$ (Maldonado and Greenland, 1993). Some of the categorical variables were recoded based on biological reasons because of low frequencies in 1 or more categories in this step. Second, Pearson and Spearman rank correlation coefficients were calculated among the significant independent variables to avoid multicollinearity in the next steps. If 2 management practices had a correlation coefficient ≥ 0.6 , only 1 was withheld for further analysis. In the third step, multivariable models were built with the remaining management variables as independent variables, with logarithm of BC and logarithm of CC, respectively, as dependent variables

(Tables 2, 3, and 4). Nonsignificant variables were removed using backward elimination at $P \leq 0.05$. In both models, all first-order interactions among month of observation and the remaining variables in the multivariable model were tested and removed when nonsignificant (Wald test, $P > 0.05$). The adequacy of the final model was tested by examining normal probability plots of residuals and plots of residuals versus predicted values to check whether the assumptions of normality and homogeneity of variance had been fulfilled. No patterns indicating heteroscedasticity were revealed.

To evaluate the proportion of variance occurring at the different levels of the data hierarchy, 2-level null models (intercept and month of observation as fixed effects only, to take into account the longitudinal nature

Figure 1. Flowchart of variable reduction through different steps in the statistical analysis. All univariable models were corrected for month of observation to take into account the longitudinal nature of the data. BC = bacterial count; CC = coliform count; $n = 7 + 1$ indicates interaction between month of observation and 1 other variable.

Table 2. Unconditional associations between general and herd health management factors and logarithm of bacterial count (BC) and logarithm of coliform count (CC) on 254 and 242 Flemish dairy farms, respectively ($P \leq 0.20$)

Independent variable	BC (IBC $\times 10^3$ /mL) ¹			CC (cfu/mL)		
	Herds [no. (%)]	LSM ² /β ³	P-value	Herds [no. (%)]	LSM ²	P-value
General management						
Number of lactating cows	254 ³	−0.000691 ³	0.02			NS
Expected time the farm will still exist			0.01			NS
<5 yr	30 (11.8)	8.4				
5–10 yr	18 (7.1)	9.0				
≥10 yr	204 (80.3)	7.2				
No idea	2 (0.8)	5.6				
Cows on pasture during summer			0.23			NS
No	30 (11.8)	6.9				
<4 h	24 (9.4)	6.8				
4–12 h	136 (53.5)	7.7				
>12 h	64 (25.2)	7.5				
Barn type			<0.001			NS
Freestall with slatted floor	202 (79.5)	7.2				
Freestall with nonslatted floor	12 (4.7)	7.7				
Straw box with slatted or nonslatted floor ⁴	24 (9.4)	8.1				
Tie-stall with slatted or nonslatted floor	16 (6.3)	10.2				
Cleaning frequency of cubicles			0.01			NS
Once per week	9 (3.5)	10.8				
Once per day	13 (5.1)	8.6				
Twice per day	174 (68.5)	7.5				
>2 times per day	58 (22.8)	6.7				
Herd health management						
Herd health monitoring performed by veterinarian			0.02			0.10
No	108 (42.5)	7.9		104 (43.0)	8.5	
Yes	146 (57.5)	7.1		138 (57.0)	7.3	
Treatment of prepartum heifers with antimicrobials			NS			0.06
Never/rarely/sometimes				233 (96.3)	7.9	
Usually/always				9 (3.7)	4.8	
Monthly incidence of clinical mastitis in 2009			0.01			0.10
≤3%	122 (48.0)	6.9		116 (47.9)	7.1	
>3%	132 (52.0)	7.9		126 (52.1)	8.4	

¹IBC = individual BC.²Back-transformed LSM of logarithm of BC ($\times 10^3$ IBC/mL) or logarithm of CC (cfu/mL), corrected for month of observation.³Regression coefficient of the model.⁴On 5 farms, the barn consisted of a straw box and some cubicles.

of the data), with herd as random effect were fit for both BC and CC.

RESULTS

Descriptive Statistics

The average herd size of the 254 herds included in the study was 65.8 [interquartile range (IQR) from 45 to 80] lactating cows per herd with an average milk quota size of 551,937 kg of milk (IQR from 375,750 to 676,500) per year. The average of the geometric mean BC was 9.1×10^3 IBC/mL (IQR from 5×10^3 to 11×10^3 cfu/mL) and the average of the geometric mean CC was 21.3 cfu/mL (IQR from 2 to 16 cfu/mL). Approximately 94% of the herds met the specific requirements for higher-quality milk in Belgium for BC (geometric mean BC $< 50 \times 10^3$ IBC/mL), whereas 98.4% of the herds met the requirements according to the European

legislation (geometric mean BC $< 100 \times 10^3$ IBC/mL) for the whole year. However, 38.5% of the herds exceeded at least once in the studied years the specific requirements for higher-quality milk for CC (geometric mean CC < 50 cfu/mL). The average BMSCC was 223,621 cells/mL, with an IQR from 173,000 to 271,000 cells/mL. Similar figures were obtained using the subset of 242 farms of which monthly geometric mean CC values were available for further analyses.

Of the 254 herds, 60.2% ($n = 153$) only housed dairy cows, 22.9% ($n = 58$) also kept double-purpose or beef cows, and 16.9% ($n = 43$) also farmed other animals such as pigs. In almost 60% of the herds ($n = 146$), some herd health aspects such as fertility, and (or) udder health, and (or) heifer rearing, and (or) claw health were monitored by a veterinarian on a regular basis. Disease registration was systematically done on approximately half of the herds. Almost 80% of the farms ($n = 202$) housed their cows in a freestall with slatted floor.

Table 3. Unconditional associations between milking routine and logarithm of bacterial count (BC) and logarithm of coliform count (CC) on 254 and 242 Flemish dairy farms, respectively ($P \leq 0.20$)

Milking management	BC (IBC $\times 10^3$ /mL) ¹			CC (cfu/mL)		
	Herds [no. (%)]	LSM ²	P-value	Herds [no. (%)]	LSM ²	P-value
Milking machine type			<0.001			0.10
Low line	189 (74.4)	7.2		179 (73.9)	6.3	
High line	24 (9.4)	10.0		22 (9.1)	9.3	
With glass jars	28 (11.0)	6.9		28 (11.6)	5.6	
Automatic milking system	13 (5.1)	7.7		13 (5.4)	16.1	
Milking parlor type			<0.001			0.01
Fishbone	145 (57.3)	6.9		139 (57.6)	7.3	
Tandem	53 (20.9)	8.1		51 (21.2)	7.5	
Rotary	19 (7.5)	6.8		17 (7.1)	6.6	
Side-by-side	6 (2.4)	7.2		5 (2.1)	10.7	
Automatic milking system	13 (5.2)	8.6		13 (5.4)	17.0	
Tie-stall	17 (6.7)	10.4		16 (6.6)	7.9	
Cows kept in headlocks after milking			0.17			NS
No, although possible	150 (59.1)	7.5				
Yes, but not all animals	32 (12.6)	8.1				
Yes	72 (28.3)	7.0				
Use of automatic cluster removal			<0.001			0.06
No	36 (14.2)	10.7		35 (14.5)	9.7	
Yes	218 (85.8)	7.0		207 (85.5)	7.5	
Method of teat preparation			0.01			NS
Wet and dry after/automatic	42 (16.5)	7.0				
Dry with paper towel	155 (61.0)	7.9				
Disinfection towels	10 (3.9)	6.2				
Dry with cloths	47 (18.5)	7.8				
Providing a preparation lag time of 60 s			<0.001			0.03
Never/rarely/sometimes	94 (37.0)	8.2		88 (36.3)	8.2	
Not applicable (rotary milking parlor)	20 (7.9)	7.5		19 (7.9)	12.2	
Always/usually/sometimes	140 (55.1)	7.0		135 (55.8)	7.1	
Application of premilking teat disinfection			0.01			NS
Never/rarely/sometimes	229 (90.2)	7.6				
Always/usually	25 (9.8)	6.0				
Application of postmilking teat disinfection			0.13			NS
Never/rarely/sometimes	27 (10.6)	8.2				
Always/usually	227 (89.4)	7.3				
Type of milking machine unit liner			0.06			NS
Rubber	229 (90.2)	7.5				
Silicone	25 (9.8)	6.5				

¹IBC = individual BC.²Back-transformed LSM of logarithm of BC ($\times 10^3$ IBC/mL) or logarithm of CC (cfu/mL), corrected for month of observation.

Unconditional Associations

A first reduction based on unconditional associations and highly correlated variables revealed 22 and 9 herd management practices to be associated with logarithm of IBC and logarithm of CC, respectively (Tables 2, 3, and 4). A strong correlation was found between the number of cows, number of lactating cows, and milk quota size. The number of lactating cows was selected for further analysis.

Final Multivariable, Multilevel Models

The final multivariable, multilevel models for logarithm of BC and logarithm of CC are presented in Tables 5 and 6, respectively. Although the correlation coefficient between type of milking machine and type

of milking parlor did not exceed the cut-off value of 0.6 ($r = 0.27$), a multivariable model for logarithm of BC in which both variables were included did not reach convergence, as all tie-stall milking parlors were obviously equipped with a high-pipeline setup (Table 7). Therefore, it was decided to exclude the variable type of milking machine from the multivariable model for logarithm of BC.

Factors Associated with BC. Individual BC varied throughout the year, with the highest values being observed from January to March and the lowest values from June to November ($P < 0.001$). Individual BC values decreased with each increase of the cleaning frequency of the cubicles (once per week, once per day, twice per day, or more than twice per day) between January and March (interaction between month of observation $\times$ cleaning frequency of cubicles, $P < 0.05$;

Table 4. Unconditional associations between nutrition, calving, and dry cow management and logarithm of bacterial count (BC) and logarithm of coliform count (CC) on 254 and 242 Flemish dairy farms, respectively ($P \leq 0.20$)

Independent variable	BC (IBC $\times 10^3$ /mL) ¹			CC (cfu/mL)		
	Herds [no. (%)]	LSM ²	P-value	Herds [no. (%)]	LSM ²	P-value
Nutrition management						
Type of forage feeding system			<0.001			NS
Layers	56 (22.0)	7.4				
Partial mixed ration	56 (22.0)	7.4				
TMR	131 (51.6)	7.1				
Completely separated	11 (4.3)	12.2				NS
Concentrate provided on top of forage			<0.001			
No	222 (87.4)	7.1				
Yes	32 (12.6)	9.9				
Concentrate provided via automatic feeder			<0.001			NS
No	81 (31.9)	8.4				
Yes	173 (68.1)	7.0				
Dry cow management						
Drying-off procedure			0.11			NS
Intermittent milking	20 (7.9)	7.9				
Abrupt	234 (92.1)	7.3				
Adapted diet provided			0.01			0.08
No	40 (15.7)	8.8		40 (16.3)	9.3	
Yes	214 (84.3)	7.2		202 (83.7)	7.5	
Mineral/vitamin mix provided			<0.001			NS
No	67 (26.4)	9.1				
Yes	187 (73.6)	6.9				
Calving management						
Presence of calving pen			0.01			NS
No (cows calve in tie-stall/cubicles)	45 (17.7)	8.5				
Yes	209 (82.3)	7.2				
Calving pen used for sick cows			0.01			NS
No	76 (29.9)	7.4				
No, as there is no calving pen	39 (15.4)	8.9				
Yes	139 (54.7)	7.1				

¹IBC = individual BC.²Back-transformed LSM of logarithm of BC ($\times 10^3$ IBC/mL) or logarithm of CC (cfu/mL), corrected for month of observation.

Figure 2). When cows were milked using an automated milking system, BC were higher than in other herds using different systems ($P < 0.001$). A lower BC was observed when the milking parlor was equipped with an automatic cluster removal system as opposed to when the milking clusters were removed manually ($P < 0.001$). Lower BC was observed in herds where the teats were prepared either first wet and then dried or via an automatic milking system ($P < 0.05$). Premilking teat disinfection via dipping or spraying as well as supplementation of dry cows with minerals were associated with lower BC as well ($P < 0.05$).

Factors Associated with CC. Coliform counts varied throughout the year ($P < 0.001$; Figure 3). Herds with a high-line milking parlor setup or automatic milking system had substantially higher CC compared with herds using a low-line milking parlor setup or reservoir ($P < 0.001$). Farms that treated prepartum heifers with antimicrobials before calving had lower CC than farms where heifers were either not or only rarely treated before calving ($P < 0.05$).

Variance Components

When fitting null models, 93.6% of the variation in BC occurred at the herd level and 6.4% at the observation level, whereas for CC, 68.1 and 31.9% of the variation occurred at the herd and observation level, respectively, (Table 8). These proportions only changed slightly in the final multivariable models. Of the total variance in BC and CC, only 4.6 and 2.1% of the variation, respectively, was explained by the fixed effects in the final models.

DISCUSSION

Several studies have identified risk factors associated with milk-quality parameters such as BC and CC (Elmoslemany et al., 2009a,b; Pantoja et al., 2011). Apart from 2 studies of which one was conducted in the 10th region of Chile (van Schaik et al., 2005) and another on Prince Edward Island in Canada (Elmoslemany et al., 2010), most studies have focused on factors associated

Table 5. Final multivariable, multilevel model for logarithm of bacterial count (BC) of 254 Flemish dairy farms

Independent variable	β^1	SE	LSM ²	<i>P</i> -value ³
Intercept	1.20	0.079	—	<0.001
Month of observation				<0.001
January	Ref. ⁴	—	10.0	
February	0.100	0.049	11.6	0.04
March	0.016	0.065	11.3	0.80
April	−0.148	0.075	10.2	0.05
May	−0.197	0.082	9.2	0.02
June	−0.280	0.087	7.6	0.01
July	−0.367	0.090	6.8	<0.001
August	−0.274	0.093	7.3	0.01
September	−0.216	0.094	7.4	0.02
October	−0.227	0.096	7.3	0.02
November	−0.200	0.097	7.8	0.04
December	−0.105	0.064	8.9	0.29
General management				
Cleaning frequency of the housing				0.22
Once per week	Ref.	—	10.1	—
Once per day	−0.114	0.094	8.2	0.22
Twice per day	−0.212	0.074	8.5	0.01
>2 times per day	−0.312	0.078	7.9	0.01
Milking management				
Milking parlor type				<0.001
Fishbone	Ref.	—	7.6	—
Tandem	0.056	0.022	8.6	0.01
Rotary	0.003	0.034	7.6	0.92
Side-by-side	0.063	0.059	8.7	0.28
Automatic milking system	0.179	0.045	11.4	<0.001
Tie-stall	0.048	0.041	8.4	0.24
Use of automatic cluster removal				<0.001
No	Ref.	—	10.3	—
Yes	−0.148	0.029	7.3	—
Teat preparation method				0.01
Wet and dry after/automatic	Ref.	—	7.9	—
Dry with paper towel	0.082	0.028	9.6	0.01
Disinfection towels	0.037	0.052	8.7	0.47
Dry with cloths	0.025	0.033	8.4	0.43
Application of premilking teat disinfection				0.01
Never/rarely/sometimes	Ref.	—	9.3	—
Always/usually	−0.063	0.030	8.0	0.04
Dry cow management				
Mineral/vitamin mix provided				0.01
No	Ref.	—	9.2	—
Yes	−0.057	0.021	8.1	—
Month of observation × cleaning frequency of barn ⁵				0.01

¹Estimate.²Back-transformed LSM of logarithm of BC [$\times 10^3$ individual BC (IBC)/mL].³*P*-value of the fixed effects.⁴Ref. = reference.⁵See Figure 2 for more detailed information.

with milking equipment and cow hygiene rather than on factors related to herd health in general. In the current study, several manageable risk factors associated with BC and CC that can be readily implemented by the Flemish dairy farmer to control BC and CC at acceptable levels were identified.

Web-based questionnaires are commonly used tools, as they allow for relatively easy and cheap data collection. This approach allowed us to include data of farms that were distributed over the 5 different Flemish prov-

inces. The need to have internet access, however, might have selected the input of more contemporary farmers that are also better managers, potentially diminishing the external validity of the data (Dohoo et al., 2009). However, the high variation in BMSCC between the respondents suggests a wide diversity of management styles (Barkema et al., 1998). Also, both the average BMSCC and BC of the selected herds approached the average BMSCC and BC in 2009 for the whole of Flanders, being 230,000 cells/mL and 11.3×10^3 IBC/mL,

Table 6. Final multivariable, multilevel model for logarithm of coliform count (CC) of 242 Flemish dairy farms

Independent variable	β^1	SE	LSM ²	P-value
Intercept	0.694	0.039	—	<0.001
Month				<0.001
January	Ref. ³	—	4.9	—
February	−0.020	0.028	4.7	0.47
March	−0.043	0.036	4.5	0.01
April	−0.007	0.042	4.9	0.24
May	0.026	0.045	5.3	0.86
June	0.073	0.047	5.9	0.11
July	0.232	0.048	8.4	<0.001
August	0.309	0.049	10.1	<0.001
September	0.260	0.050	9.0	<0.001
October	0.213	0.051	8.1	<0.001
November	0.201	0.051	7.9	<0.001
December	0.137	0.051	6.8	0.01
Milking management				
Milking machine type				<0.001
Low line	Ref.	—	5.7	—
High line	0.171	0.076	9.6	0.03
With glass jars	−0.064	0.069	4.9	0.35
Automatic milking system	0.419	0.098	13.8	<0.001
Herd health management				
Treatment of prepartum heifers with antimicrobials				0.02
Never/rarely/sometimes	Ref.	—	8.7	—
Usually/always	−0.261	0.115	4.9	—

¹Estimate.²Back-transformed LSM of logarithm of CC (cfu/mL) obtained from the final model.³Ref. = reference.

respectively (Annual Report 2009; Milk Control Centre Flanders, 2009). Altogether, the results are believed to reflect the BC and CC status of typical dairy herds in Flanders and are likely valid for the Flemish dairy situation and herds of other regions and countries with similar management conditions.

The average BC in our study was slightly lower than the 11.4×10^3 IBC/mL found in New York State (van Schaik et al., 2002). The average CC, on the other hand, was almost half of the average CC of 44 cfu/mL across several studies (e.g., Elmoslemany et al., 2009b; Pantoja et al., 2011), suggesting better conditions on the Flemish dairy farms compared with the farms included in the other studies (Jayarao and Wolfgang, 2003). As was expected, the average CC and BC strongly varied

among herds, which are comparable with other studies (Elmoslemany et al., 2009a,b; Pantoja et al., 2011; Mallet et al., 2012).

Several variables reflecting milking, herd health, and dry cow management were significantly associated with either BC or CC, but explained only a small proportion of their total variance. The latter suggests that the bacteriological milk quality and, in particular, the CC is primarily driven by other management practices than those studied, such as factors related to milking equipment and milk storage, although unknown factors should be suspected as well. Obviously, the fact that several associations were statistically significant in the analyses not necessarily indicates that a causal relation exists (Dohoo et al., 1997). Rather, some associations

Table 7. Cross tabulation of the 2 independent variables milking machine type and milking parlor type

Variable	Milking machine type				Total
	Low line	High line	With glass jar	Automatic milking system	
Milking parlor type					
Fishbone	116	4	25	0	145
Tandem	48	3	2	0	53
Rotary	18	0	1	0	19
Side-by-side	6	0	0	0	6
Automatic milking system	0	0	0	13	13
Tie-stall	0	17	0	0	17
Total	188	24	28	13	253

Figure 2. Back-transformed LSM of logarithm of bacterial count [BC; $\times 10^3$ individual BC (IBC)/mL] throughout the year (---) for herds where the cubicles were cleaned once per week (■), once per day (▲), twice per day (◆), or more than 2 times per day (○).

Figure 3. Back-transformed LSM of logarithm of coliform count (CC; cfu/mL) throughout the year.

need confirmation, whereas others, such as the association between prepartum antibiotic treatment in heifers and CC as well as the negative association between feeding diets supplemented with minerals to the dry cows and BC, generate new hypotheses on controlling BC and CC that should be tested.

Bacterial counts and CC changed over time. Bacterial counts were highest during the winter and spring months, whereas CC followed the opposite pattern. In other studies conducted in the Balearic Islands, Chile, and Prince Edward Island, both BC and CC were highest in summer (Soler et al., 1995; van Schaik et al., 2002; Elmoslemany et al., 2010). The discrepancy between our results and those reported by others can yet not be fully explained, but might be related to the temperate maritime climate in Belgium with ample rainfall

all year round. The fact that cows on Flemish dairy farms are in confined housing from October to May might expose the udder and teats under the rather mild and humid weather conditions to high bacterial levels, increasing the risk for contamination of the udder and teats in the winter and spring months. The opposite pattern of CC compared with BC indicates that both are partly determined by other factors. Coliforms only start to grow and multiply when the ambient temperature exceeds 10°C (Naemura and Seidler, 1978). Accumulation of soil from the teats and udders somewhere in the milking equipment will, therefore, have a larger effect on CC in the summer than in the winter.

The dose-response relationship of frequency of cleaning of the housing on BC between January and March corresponded well with the data published by Kelly et al. (2009). Frequency of bedding change has been asso-

Table 8. Variance components at each level of the null and final multivariable models for logarithm of bacterial count (BC) and logarithm of coliform count (CC), respectively

Data hierarchy	Null model ¹			Final model ²		
	Estimate	SE ³	% ⁴	Estimate	SE ³	% ⁴
BC						
Herd level	0.798	0.010	93.6	0.767	0.011	94.3
Observation level	0.054	0.003	6.4	0.046	0.002	5.7
Total variance	0.852	—	100	0.813	—	100
CC						
Herd level	0.719	0.012	68.1	0.709	0.013	68.6
Observation level	0.337	0.014	31.9	0.325	0.014	31.4
Total variance	1.056	—	100.0	1.034	—	100.0

¹Model containing only month of observation as fixed effect to take the longitudinal nature of the data set into account.

²Model containing all fixed effects as presented in Tables 5 and 6.

³Standard error of the variance estimate of the parameter.

⁴Variance proportion explained at the level of the data hierarchy.

ciated with the cleanliness of the udder and teats and, thus, the exposure of teats to bacteria (Ward et al., 2002). Also, dirty teats increase the risk of elevated BC (Elmoslemany et al., 2009a). Keeping in mind the confinement housing and the mild and wet Belgian weather conditions in wintertime, farmers should, in particular during those months, put more effort in keeping the housing of the lactating cows clean.

The higher BC and CC levels on farms equipped with an automatic milking system are complementary to the increasing bulk milk BC levels observed on farms switching from conventional to automatic milking (Rasmussen et al., 2002; de Koning et al., 2003). The increase in BC shortly after introduction of the automatic milking system has been suggested to partly originate from contamination of milk from the teat surfaces and partly from the inappropriate cleaning of the milking equipment and cooling of the milk (Rasmussen et al., 2002). The CC was also affected by the type of milking machine. Higher CC were observed in milking parlors with a high-pipeline setup compared with those with a low-pipeline setup or reservoir (Reinemann et al., 2003). A common denominator among the studies that have already been performed is the overriding importance of proper cleaning and sanitation of the milking system in producing high-quality milk (Elmoslemany et al., 2009a; Pantoja et al., 2011). The cleaning and sanitation of the pipelines in stalls with a high-pipeline setup are more often lacking than in other milking systems, likely due to the higher risk for accumulation of milk fat and drying of milk in the high-pipeline setup and, in particular, in the cool line at the high end of the system (Reinemann et al., 2003).

Individual BC was affected by premilking teat cleaning practices, which was not that unexpected, as Elmoslemany et al. (2009a) reported that the risk of having high BC increased by 5.3 for each unit increase in the teat-end cleanliness before the milking unit was attached. In our study, a wet teat preparation followed by dry wiping was more strongly associated with lower BC than dry wiping with a paper towel alone, corresponding well with the results of Kelly et al. (2009). Still, in almost 25% of the cases, the wet teat preparation consisted of premilking teat disinfection, which was associated with lower BC as well, which is obviously positive. The latter finding corresponds well to the numerous studies that showed a strong reducing effect of premilking teat disinfection on the teat microbial load (e.g., Galton et al., 1986; Magnusson et al., 2006; Gibson et al., 2008).

A negative relationship was found between the presence of automatic removal and BC, which is in accordance with the results reported by Kelly et al. (2009). This finding might be related to the increased

risk for teat end callosity and hyperkeratosis in the case of manual cluster removal. Teats with a highly caloused teat end have an increased load of environmental pathogens (Paduch et al., 2012). Also, teats with a distinct callosity ring are most likely less effectively cleaned during premilking teat preparation, leaving more bacteria on the teat end when the milking cluster is attached (Hovinen et al., 2005). Although few peer-reviewed publications are available on the risk factors associated with teat-end callosity, it is reasonable to accept that manual cluster removal might more often lead to overmilking compared with automatic cluster removal, thereby compromising the teat-end quality (Rasmussen et al., 1991; Gleeson et al., 2003).

CONCLUSIONS

Multivariable, multilevel regression analysis revealed several management practices associated with either BC or CC. Increasing the cleaning frequency of the housing during wintertime under the Belgian weather conditions and implementing premilking teat disinfection by either dipping or spraying the teats before attaching the milking unit will likely result in lower BC values. The variation in both BC and CC seems to be mainly determined by differences in management between the herds, as most variation in both parameters resided at the herd rather than at the observation level. As only a small proportion of the total variance was explained by the management practices included in our study, bacteriological milk quality seems to be primarily driven by other factors than the ones included in this study.

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