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Associations among milk quality indicators in raw bulk milk

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ABSTRACT

The objective of this study was to determine characteristics and associations among bulk milk quality indicators from a cohort of dairies that used modern milk harvest, storage, and shipment systems and participated in an intensive program of milk quality monitoring. Bulk milk somatic cell count (SCC), total bacteria count (TBC), coliform count (CC), and laboratory pasteurization count (LPC) were monitored between July 2006 and July 2007. Bulk milk samples were collected 3 times daily (n = 3 farms), twice daily (n = 6 farms), once daily (n = 4 farms), or once every other day (n = 3 farms). Most farms (n = 11) had direct loading of milk into tankers on trucks, but 5 farms had stationary bulk tanks. The average herd size was 924 cows (range = 200 to 2,700), and daily milk produced per herd was 35,220 kg (range = 7,500 to 105,000 kg). Thresholds for increased bacterial counts were defined according to the 75th percentile and were >8,000 cfu/mL for TBC, >160 cfu/mL for CC, and ≥310 cfu/mL for LPC. Means values were 12,500 (n = 7,241 measurements), 242 (n = 7,275 measurements), and 226 cfu/mL (n = 7,220 measurements) for TBC, CC, and LPC, respectively. Increased TBC was 6.3 times more likely for bulk milk loads with increased CC compared with loads containing fewer coliforms. Increased TBC was 1.3 times more likely for bulk milk with increased LPC. The odds of increased TBC increased by 2.4% for every 10,000-cells/mL increase in SCC in the same milk load. The odds of increased CC increased by 4.3% for every 10,000-cells/mL increase in SCC. The odds of increased CC increased by 1% for every 0.1°C increase in the milk temperature upon arrival at the dairy plant (or at pickup for farms with bulk tank). Laboratory pasteurization count was poorly associated with other milk quality indicators. Seasonal effects on bacterial counts and milk temperature varied substantially among farms. Results of this study can be used to aid the interpretation and analysis of indicators of milk quality intensively produced by dairy processors' laboratories.

Key words: bacterial count, milk quality, dairy, bulk milk

INTRODUCTION

Bacterial and somatic cell counts are reference methods used as indicators of raw milk quality (Costello et al., 2003). When regulatory standards for bacterial counts in raw milk are met, pasteurization is highly effective in destroying pathogenic microorganisms that can present a threat to human health (Boor and Murphy, 2002). In the United States, the Pasteurized Milk Ordinance requires grade A milk produced on individual farms to have a total bacteria count (**TBC**) <100,000 cfu/mL and bulk tank SCC to be <750,000 cells/mL (Pasteurized Milk Ordinance, 2003). Dairy processors perform the tests and provide state regulatory agencies with monthly bulk milk SCC and TBC values from individual dairies to enforce milk quality regulations. In most regulatory programs, milk samples used to determine SCC and TBC are collected 1 to 3 times per month. However, growth in the number of larger herds and changes in testing technology have resulted in an increasing number of dairy processors who intensively monitor microbial quality of bulk milk and provide financial premiums to producers for maintaining minimal microbial counts (Jayarao et al., 2004). Although several researchers have reported characteristics of milk quality indicators collected on a monthly or weekly basis, there are no reports of the characteristics of these indicators when they are sampled one or more times per day. A better understanding of the relationships among intensively monitored milk quality indicators would be helpful for troubleshooting milk quality problems and developing premium payment systems.

Standard plate count, Petrifilm aerobic count (**PAC**), and plate loop count are methods used to estimate TBC in milk (Laird et al., 2004). Increased TBC can be caused by growth of bacteria on unsanitary milking equipment, contamination from soiled cow udders, inadequately cooled milk, and occasionally by milking of mastitic cows (Murphy and Boor, 2000; Hayes et al., 2001; Chambers, 2002; Costello et al., 2003). Some processors perform additional tests on raw milk. Laboratory pasteurization count (**LPC**) estimates the

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Table 1. Bulk milk load sampling frequency, number of milk loads, and number of total bacteria counts (TBC), coliform counts (CC), laboratory pasteurization counts (LPC), and SCC performed from July 2006 to July 2007, ordered by sampling frequency

Farm	Lactating cows, n	Milk l oads/wk	Bulk milk sampling frequency	CC		LPC		TBC		SCC		Total milk loads
				Total counts[1]	% Total loads[2]	Total counts	% Total loads	Total counts	% Total loads	Total counts	% Total loads	
J	370	4.1	Once every other day	165	77.1	163	76.2	168	78.5	204	95.3	214
B	400	4.6	Once every other day	162	68.4	161	67.9	163	68.8	221	93.2	237
C	340	4.9	Once every other day	183	72.3	182	71.9	182	71.9	236	93.3	253
M	375	5.2	Once daily	217	80.1	214	79.0	216	79.7	263	97.0	271
F	347	5.7	Once daily	233	78.2	232	77.9	232	77.9	293	98.3	298
K	580	7.8	Once daily	293	72.2	293	72.2	292	71.9	369	90.9	406
L	200	9.1	Once daily	310	65.4	310	65.4	308	65.0	459	96.8	474
A	1,110	13.2	Twice daily	542	78.8	539	78.3	541	78.6	666	96.8	688
N	1,150	14.6	Twice daily	529	69.9	521	68.8	522	69.0	695	91.8	757
H	1,250	15.0	Twice daily	568	72.7	560	71.7	557	71.3	680	87.1	781
O	1,200	15.2	Twice daily	631	79.7	626	79.0	625	78.9	772	97.5	792
G	690	15.2	Twice daily	618	78.3	614	77.8	617	78.2	769	97.5	789
D	410	16.0	Twice daily (4 d a week)	341	41.1	341	41.1	339	40.8	812	97.8	830
P	1,312	17.7	Three times daily	618	67.3	615	67.0	613	66.8	883	96.2	918
I	2,700	22.8	Three times daily	906	76.5	899	75.9	909	76.7	1,142	96.4	1,185
E	2,350	22.8	Three times daily	959	80.9	950	80.1	957	80.7	1,162	98.0	1,186
Total				7,275	72.2	7,220	71.6	7,241	71.8	9,626	95.5	10,079

[1] Number of tests that were included in the study.

[2] Percentage of all milk loads shipped on which the test was performed during the study period.

number of thermoduric bacteria present in milk after pasteurization (Frank and Yousef, 2004). Thermoduric bacteria can multiply in biofilms present on milking-equipment surfaces; thus, LPC has been used as an indicator of milking-equipment sanitation (Guterbock and Blackmer, 1984; Murphy and Boor, 2000). The enumeration of coliform bacteria in raw milk has been used as an indication of fecal contamination (Davidson et al., 2004). Soiled udders and teats are common sources of fecal contamination and often indicate inadequate premilking cow preparation. Increased numbers of coliforms in bulk milk can also occur when coliforms grow on residual milk left on milk contact surfaces or in poorly sanitized milking equipment (Guterbock and Blackmer, 1984; McKinnon et al., 1990; Chambers, 2002).

Hayes et al. (2001) studied daily variation in bulk milk bacterial counts over 14 d and suggested that analysis of differentiated bacterial counts (in addition to TBC) would aid in the identification of sources of bacterial contamination. However, little research has been conducted using longitudinal monitoring of specific bacterial counts in raw bulk milk. Costello et al. (2003) reported weekly values of SCC, SPC, and coliform count (**CC**) for a single university dairy herd over an 11-yr period but did not report measures of variation. The objective of this study was to determine characteristics and associations among bulk milk quality indicators from a cohort of dairies that used modern milk harvest, storage, and shipment systems and participated in an intensive program of milk quality monitoring.

MATERIALS AND METHODS

Farm Selection and Data Collection

Farms were eligible to participate in the study if they shipped milk to a common dairy processor, had milk quality data (bacterial counts and SCC) determined for most milk loads produced, and used milking parlors equipped with modern milking technology. Enrolled farms (n = 16) had bulk milk samples collected twice daily (n = 6 farms), once daily (n = 4 farms), once every other day (n = 3 farms), or 3 times daily (n = 3 farms) (Table 1).

Farms were visited monthly between July 1, 2006, and June 30, 2007, to assess changes in management practices. Data (SCC, TBC, CC, LPC, and milk temperature at arrival to the dairy plant) from individual bulk milk loads were downloaded from the processor's Web site. Temperature was recorded for milk loads either at arrival to the dairy plant (for farms that stored milk in tankers; n = 11) or at pickup (for farms that had a bulk tank; n = 5).

Table 2. Summary statistics and cumulative frequency distribution of total bacteria count (TBC), coliform count (CC), laboratory pasteurization count (LPC), SCC, and milk temperature (TEMP)

Item	TBC, cfu/mL	CC, cfu/mL	LPC, cfu/mL	SCC, × 10^3 cells/mL	TEMP, °C
Percentile					
10th	0	0	10	126	2.8
20th	1,000	10	20	154	3.3
25th	1,000	20	30	163	3.3
30th	1,000	20	40	172	3.3
40th	2,000	30	70	188	3.3
50th	3,000	50	120	204	3.9
60th	4,000	70	180	220	3.9
70th	6,000	120	250	237	3.9
75th[1]	8,000	160	310	247	3.9
80th	11,000	250	380	258	4.4
90th	26,000	1,140	610	286	4.4
n	7,241	7,275	7,220	9,626	10,079
Mean	12,545.9	242	226.4	206.4	3.7
SD	50,182.8	446.6	332.4	61.5	0.8
CV	400	184.5	146.8	29.8	21.6
Minimum	0	0	0	44	0
Maximum	2,000,000	1,520	11,140	625	8.3
Log$_{10}$ values					
n	7,241	7,275	7,220		
Mean	3.1	1.7	1.9		
SD	1.5	0.9	0.8		
CV	47.3	52.2	42.6		
Minimum	0	0	0		
Maximum	6.3	3.2	4.1		

[1] Thresholds used to define increased bacterial counts.

Bacteriology and SCC

Immediately after arrival of loads at the plant, milk samples were obtained using a standard procedure certified by the Wisconsin regulatory officials. Milk was mechanically agitated for 15 min, and approximately 50 mL was collected by a licensed technician using a sanitized stainless-steel dipper. Milk samples were immediately refrigerated and transported to the dairy-processor laboratory. For farms that stored milk in bulk tanks, milk samples were collected at farm pickup by a licensed milk hauler.

Milk samples used for regulatory purposes (SCC and TBC) were processed using approved methods in an approved laboratory (Wehr and Frank, 2004). Total bacteria count was performed using PAC plates (3M, St. Paul, MN; Laird et al., 2004). In brief, diluted milk samples (1:1,000) were plated on PAC using a plate-loop-count device and incubated for 48 h at 32°C. Coliform bacteria were counted using the Petrifilm coliform count plate (3M) method according to Davidson et al. (2004). Diluted milk samples (1:10) were plated on Petrifilm coliform plates, which were incubated for 24 h at 32°C. Laboratory pasteurization count was performed according to the following procedure: Milk samples (5 mL) were heated for 30 min at 62.8°C, immediately cooled in ice, diluted at 1:10, plated on PAC plates (1 mL), and incubated for 48 h at 32°C. For all described methods, colonies were counted manually by trained laboratory technicians according to Laird et al. (2004). Somatic cell counts in bulk milk were determined using an electronic cell counter (Foss Electric, Hillerød, Denmark), and all milk loads were tested for the presence of antibiotic residues using the SNAP test (IDEXX Laboratories Inc., Westbrook, ME) as described by Bulthaus (2004).

Statistical Analysis

Definitions Used for Analysis. Somatic cell count, TBC, CC, and LPC were used as indicators of milk quality. Increased bacterial counts were defined using thresholds according to the 75th percentile of their frequency distributions (Table 2). The thresholds were TBC $\geq$8,000 cfu/mL, LPC $\geq$310 cfu/mL, and CC $\geq$160 cfu/mL. Distributions of bacterial counts (TBC, LPC, and CC) were examined using both original and log$_{10}$-transformed values. Original values were transformed to log$_{10}$ as follows: log$_{10}$ value = log$_{10}$(original value + 1).

Statistical Procedures. Univariate analysis was performed to determine measures of central tendency, dispersion, and distribution characteristics. Histograms were created to study the distributions of TBC, LPC, and CC. Correlations among indicators of milk quality (using original values) were assessed using Spearman rank correlation coefficient (Pagano and Gauvreau, 2000).

Logistic regression was used to assess the effect of changes in selected milk quality indicators on increased counts of other indicators in the same bulk milk load. Odds ratios (**OR**) were estimated with respective 95% confidence intervals (**CI**). Separate models were constructed for TBC, CC, or LPC as binary outcome variables (increased or normal). Explanatory variables were all other bacterial counts (increased or normal), SCC (cells/mL × 10^3), milk temperature (°C), season (summer, June to August; fall, September to November; winter, December to February; and spring, March to May), and farm. Nonsignificant explanatory variables, interactions, and quadratic terms were excluded from the models according to a stepwise variable-selection procedure and biological significance of variables. A potential clustering effect of individual observations of outcome variables within farm was assessed using logistic regression with generalized estimated equations (Palta, 2003). As a result, farm was included in all models as a fixed effect because individual TBC, CC, or LPC obtained from the same farm were poorly correlated. Two separate ANOVA models were used to assess the effects of season and farm on milk temperature, and the effect of season and bulk milk storage system (bulk tank or tanker) on milk temperature. Goodness of fit for logistic regression and the ANOVA models were assessed using the Hosmer and Lemeshow test and graphical analysis of residuals and predicted values, respectively (Palta, 2003). Descriptive statistics (PROC UNIVARIATE, PROC FREQ, and PROC MEANS), correlation analysis (PROC CORR), logistic regression (PROC GLIMMIX and PROC LOGISTIC) and ANOVA (PROC MIXED) were performed with SAS version 9.1 (SAS Institute, 2008). The level of statistical significance was set at 0.05 for all analyses.

RESULTS

Farm Characteristics

Participating farms used modern technology including parlor efficiency reports (n = 8), electronic milk meters (n = 10), plate milk coolers (n = 16), milk and wash-water temperature-control charts (n = 13), computerized milking-equipment wash controllers (n = 4), and automatic unit removers (n = 16 farms). Farms had parallel (n = 10), herringbone (n = 5), or rotary parlors (n = 1) with an average of 32 (range = 10 to 88) milking units per farm. Most farms (n = 11) had direct loading of milk into tankers, but 5 farms stored milk in bulk tanks before it was picked up by haulers. Except for one farm that milked twice daily, all dairies milked cows 3 times per day. Milking-machine sanitation (postmilking rinse, detergent wash, acid rinse, and premilking sanitation) was performed after each milking. All farms had milking equipment inspected and maintained by manufacturer-authorized dealers at least twice per year. Herd size ranged from 200 to 2,700 lactating cows (Table 1), and daily milk production per cow was 39.2 kg (range = 34.2 to 42.1 kg). Cows were housed in free-stalls containing sand (n = 11), shavings (n = 2), or biosolids (n = 3 farms) as bedding. Although different parlor work routines were used among farms, teats were always disinfected before and after milking (14 farms used iodine and 2 farms used chlorhexidine-based dip solutions) and dried with individual cloth towels before cluster attachment.

Descriptive Statistics

Of enrolled farms (n = 16), complete data were obtained from 15. One farm interrupted its milk supply to the dairy processor 30 d before the end of the study; therefore, data from July 2007 were not available for this farm. Of 10,079 milk loads, bacterial counts were obtained for 72.2% , and SCC was determined for 95.5% of loads (Table 1). Two milk loads were discarded because of the presence of antibiotic residues.

Bacterial Counts. The distributions of bacterial counts are presented in Figures 1, 2, and 3. The mean and median TBC were 12,500 and 3,000 cfu/mL, respectively (Table 2). Total bacteria counts ranged from 0 to 2 × 10^6 cfu/mL, and of the total counts performed during the study period (n = 7,241), 1.6% (n = 142) were >10^5 cfu/mL (Pasteurized Milk Ordinance legal limit). The percentage of increased TBC during the study period varied greatly among farms (5.0 to 72.3%; Table 3).

Coliform counts ranged from 0 to 1,520 cfu/mL, and the CC distribution had great variation (Table 2). The percentage of increased CC (≥160 cfu/mL) varied greatly among farms (6.5 to 62.3%; Table 3). Laboratory pasteurization counts ranged from 0 to 11,140 cfu/mL. Among bacterial counts, the LPC distribution (Figure 3) exhibited the least variation (Table 2). Of LPC (n = 7,220), the proportion of increased counts varied greatly among farms (0.9 to 89.8%; Table 3).

Milk Temperature. Of milk load temperature measurements that were performed during the study period (n = 10,079), 0.06% (n = 6) had temperatures >7.2°C (Pasteurized Milk Ordinance legal limit). The seasonal effect on milk temperature varied significantly among farms (interaction between season and farm, $P < 0.01$). Most farms (n = 10) had milk loads with greater temperature in summer (as compared with winter), but for 6 farms, the mean milk temperature was not different

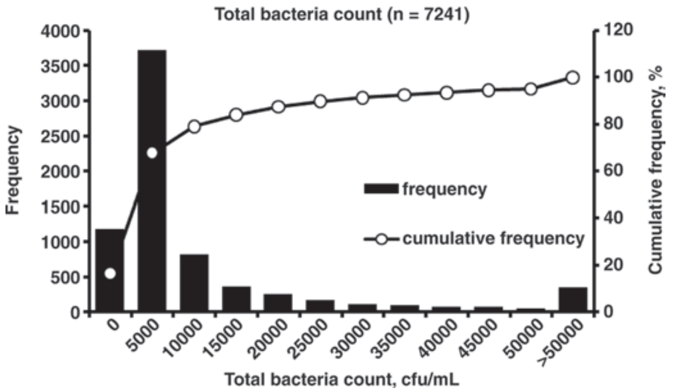

Figure 1. Frequency distribution of total bacteria count for all farms combined, from July 2006 to July 2007.

between summer and winter (Table 4). Except during summer ($P = 0.99$), milk loads stored in bulk tanks had greater temperatures than loads stored in tankers ($P < 0.01$; Figure 4). However, seasonal variation in milk temperature was greater when milk was stored in tankers compared with bulk tanks (Figure 4).

Associations Among Indicators of Milk Quality

The greatest correlation was observed between TBC and CC (r = 0.41; Table 5). Somatic cell count was significantly correlated with TBC (r = 0.25) and CC (r = 0.19), whereas less correlation was found between other pairwise combinations of milk quality indicators (Table 5).

Somatic cell count, LPC, CC, and the interaction between season and farm were associated with TBC ($P < 0.01$). The odds of increased TBC were 6.3 (OR 95% CI: 5.5 to 7.3) times greater for milk loads with increased CC compared with milk loads with CC less than the threshold and 1.3 (OR 95% CI: 1.1 to 1.6) times greater for milk loads with increased LPC compared with milk loads with LPC less than the threshold. Every 10,000-cells/mL increase in bulk milk SCC increased the odds of increased TBC by 2.4% (OR = 1.02, 95% CI: 1.01 to 1.04). The probability of increased TBC was associated with season for 3 farms (Table 6). Two farms had greater odds of increased TBC during summer (relative to winter), and one farm had greater odds of increased TBC during winter (relative to summer).

Somatic cell count, milk temperature, and the interaction between season and farm were associated with CC ($P < 0.05$). For every 0.1°C increase in milk temperature, the odds of increased CC increased by 1% (OR = 1.01, 95% CI: 1.01 to 1.02). For every 10,000-cells/mL increase in SCC, the odds of increased CC increased by 4.3% (OR = 1.04, 95% CI: 1.02 to 1.06). The seasonal effect on the probability of increased CC varied substantially among farms (Table 6). Greater odds of increased CC were observed during summer (compared with winter) for 5 farms, whereas greater odds during winter were observed for the other 5 farms.

The effect of season on LPC varied significantly among farms ($P < 0.01$; Table 6). Greater odds of an increased LPC during summer (compared with winter) were observed for 3 farms, whereas greater odds during winter relative to summer were observed for the other 4 farms.

DISCUSSION

Demographic characteristics of milk production have changed dramatically in the United States. In 2006, 1.9% of US herds contained more than 1,000 cows, but those herds held 34% of US dairy cows and produced 37% of total milk (MacDonald et al., 2007). Although Wisconsin continues to have many smaller herds, the same demographic changes are occurring. In 2007, of 14,400 dairy herds, 1.7% contained more than 500 cows, but those herds held 18% of the cows and produced 22% of total milk produced in Wisconsin (USDA

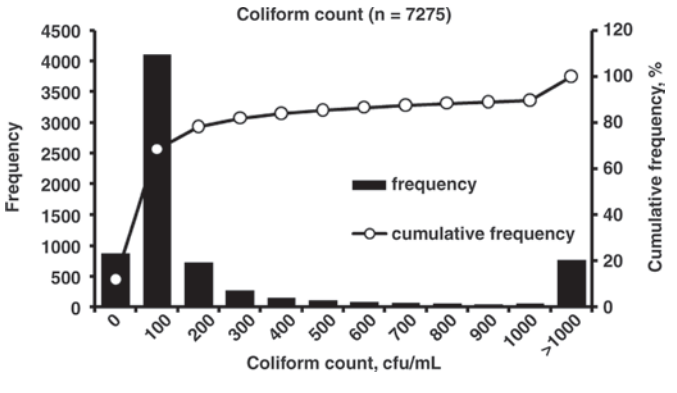

Figure 2. Frequency distribution of coliform count for all farms combined, from July 2006 to July 2007.

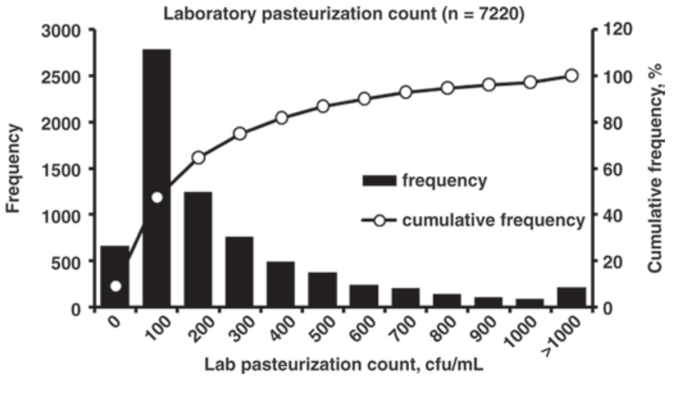

Figure 3. Frequency distribution of laboratory pasteurization count for all farms combined, from July 2006 to July 2007.

Table 3. Number and percentage of increased total bacteria count (TBC), coliform count (CC), and laboratory pasteurization count (LPC) during the entire study period by farm, ordered by percentage of increased TBC[1]

Farm	TBC			CC			LPC		
	Increased counts, n	%	Total counts, n	Increased counts, n	%	Total counts, n	Increased counts, n	%	Total counts, n
A	27	5.0	541	35	6.5	542	12	2.2	539
E	83	8.7	957	198	20.7	959	197	20.7	950
B	20	12.3	163	12	7.4	162	3	1.9	161
F	46	19.8	232	42	18.0	233	20	8.6	232
H	113	20.3	557	153	26.9	568	189	33.8	560
L	67	21.8	308	28	9.0	310	82	26.5	310
I	198	21.8	909	191	21.1	906	152	16.9	899
G	160	25.9	617	124	20.1	618	294	47.9	614
D	92	27.1	339	55	16.1	341	3	0.9	341
J	47	28.0	168	40	24.2	165	74	45.4	163
C	53	29.1	182	24	13.1	183	117	64.3	182
O	186	29.8	625	236	37.4	631	9	1.4	626
M	66	30.6	216	130	59.9	217	31	14.5	214
K	104	35.6	292	81	27.7	293	9	3.1	293
N	201	38.5	522	101	19.1	529	468	89.8	521
P	443	72.3	613	385	62.3	618	148	24.1	615
Total	1,906	26.3	7,241	1,835	25.2	7,275	1,808	25.0	7,220

[1] Thresholds used to define increased bacterial counts were TBC $\geq$8,000, CC $\geq$160, and LPC $\geq$310 cfu/mL.

NASS, 2007). Many of these farms with larger herds have technology and milk-handling practices that differ significantly from those with smaller herds. Many large-herd farms milk directly into milk tankers, and processors often assess milk bacterial counts on each load and use a variety of thresholds to define milk quality payment systems. Bulk milk bacterial-count data are also used to troubleshoot farm-based milk quality problems such as investigation of sources of bacterial contamination in bulk milk, milking hygiene, and milking-machine sanitation. Although characteristics of cross-sectional measurements of bacterial counts have been defined (Boor et al., 1998; Jayarao et al., 2004), measurement of bacterial counts on daily milk loads is a recent phenomenon, and no studies have evaluated the descriptive characteristics of these measurements on modern, large dairy farms that account for an increasing share of milk produced in the United States. The associations among counts and the selection of thresholds for defining increased counts have not been described previously.

Characteristics of Milk Bacterial Counts

The current study highlights important characteristics of bulk milk bacterial-count data that have

Table 4. Milk temperature (°C) by farm and season

Farm	Temperature measurement point	Summer		Winter		Fall		Spring	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
A	Dairy plant	4.36^{d}	0.05	2.48^{c}	0.06	3.54^{a}	0.06	3.38^{a}	0.05
B	Dairy plant	4.95^{a}	0.08	3.13^{b}	0.09	3.75^{c}	0.09	4.18^{c}	0.09
C	Dairy plant	4.43^{b}	0.09	3.40^{a}	0.08	3.20^{a}	0.09	4.27^{b}	0.08
D	Farm bulk tank	3.95^{a}	0.04	4.10^{a}	0.05	3.66^{b}	0.05	3.91^{ab}	0.05
E	Dairy plant	3.61^{a}	0.04	3.74^{ab}	0.04	3.94^{b}	0.04	2.92^{c}	0.04
F	Farm bulk tank	4.84^{a}	0.07	2.80^{b}	0.08	4.29^{c}	0.08	3.45^{d}	0.08
G	Farm bulk tank	3.82^{ab}	0.04	3.62^{ab}	0.05	3.97^{a}	0.05	3.61^{b}	0.05
H	Dairy plant	3.77^{a}	0.05	3.47^{b}	0.05	3.55^{ab}	0.05	3.47^{b}	0.05
I	Dairy plant	4.37^{b}	0.04	3.62^{a}	0.04	3.76^{a}	0.04	4.28^{b}	0.04
J	Dairy plant	4.45^{a}	0.08	2.16^{b}	0.10	2.80^{c}	0.10	4.71^{a}	0.10
K	Dairy plant	4.91^{a}	0.06	2.98^{b}	0.07	4.16^{c}	0.07	4.41^{c}	0.07
L	Farm bulk tank	3.43^{a}	0.06	3.61^{a}	0.06	3.44^{a}	0.06	3.51^{a}	0.07
M	Farm bulk tank	4.69^{a}	0.07	4.59^{a}	0.09	4.44^{a}	0.09	5.30^{b}	0.09
N	Dairy plant	3.83^{a}	0.05	3.52^{b}	0.05	3.64^{ab}	0.05	3.60^{ab}	0.05
O	Dairy plant	4.22^{b}	0.05	3.47^{a}	0.05	3.62^{a}	0.05	3.57^{a}	0.05
P	Dairy plant	3.27^{ab}	0.04	3.40^{a}	0.05	3.13^{b}	0.05	3.25^{ab}	0.05

$^{a–d}$Means with different superscripts in the same row differ (Tukey-adjusted $P < 0.05$).

Table 5. Spearman correlation coefficients between SCC, total bacteria count (TBC), coliform count (CC), laboratory pasteurization count (LPC), and milk temperature (TEMP)

Item	SCC	TBC	CC	LPC	TEMP
SCC		0.25*	0.19*	−0.15*	0.02
TBC			0.41*	0.17*	0.02
CC				0.08*	0.04*
LPC					−0.04*

*Statistically significant ($P < 0.05$).

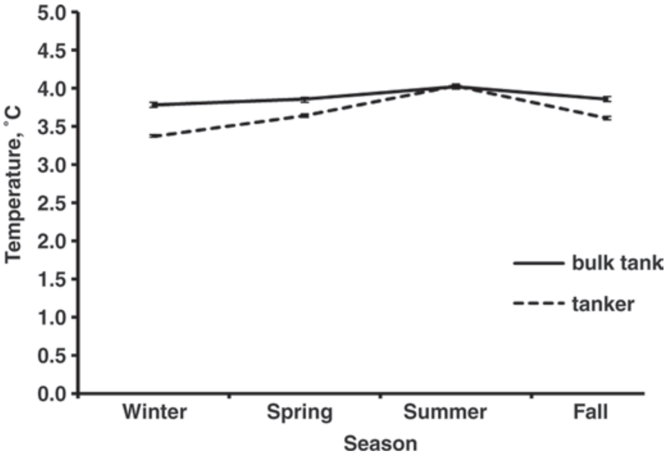

Figure 4. Effect of season of the year and on-farm milk storage system (bulk tank or tanker) on the mean milk temperature. Seasons were defined as summer (June to August), fall (September to November), winter (December to February), and spring (March to May).

been produced by many processors' laboratories using approved methods. Bacteria grow in an exponential manner, which normally results in skewed distributions. The distribution characteristics of TBC, CC, and LPC observed in this study are partly determined by laboratory procedures used to enumerate bacteria (e.g., dilution of milk to count plates with specified numbers of colonies). Tests indicating no growth of bacteria greatly contributed to the clustering at the left side of the TBC, CC, and LPC distributions. Some of these results are likely false-negatives that are a result of growth below the detection limit of the test procedure, but regardless, these apparent negative samples have counts that fall far below thresholds of regulatory or processor interest. In addition, the maximum count reported for coliform bacteria was 1,500 cfu/mL per plate, which truncated the CC distribution. From an analytical standpoint, these distribution characteristics violate the assumption of normality and require the use of categorical data-analysis techniques, which can limit the use and development of analytical tools based on data normally distributed to monitor daily bacterial counts in bulk milk. Thresholds used in this study to define increased SCC and bacterial counts were based on the 75th percentile of observed data from participating dairy farms. Additional studies are needed to identify biologically meaningful thresholds that affect the finished quality of dairy products.

Table 6. Odds of increased total bacteria count (TBC), coliform count (CC), and laboratory pasteurization count (LPC) in summer compared with winter, by farm[1]

	TBC			CC			LPC		
Farm	OR[2]	LCL[3]	UCL[3]	OR	LCL	UCL	OR	LCL	UCL
A	3.43	0.74	15.86	4.45*	0.99	20.02	1.01	0.28	3.65
B	9.23*	1.09	78.06	0.66	0.11	3.82	0.57	0.03	9.54
C	1.10	0.43	2.81	—[4]	—	—	2.13	0.83	5.45
D	1.99	0.97	4.08	0.24*	0.11	0.54	—	—	—
E	1.79	0.82	3.87	0.84	0.52	1.37	2.39*	1.32	4.32
F	0.33*	0.30	0.36	4.52*	1.48	13.84	—	—	—
G	0.99	0.55	1.77	0.25*	0.13	0.47	0.01*	0.01	0.03
H	0.64	0.33	1.25	0.52*	0.30	0.90	0.12*	0.07	0.21
I	0.92	0.59	1.45	1.89*	1.17	3.05	3.11*	1.87	5.15
J	1.28	0.42	3.83	3.52	0.97	12.75	2.60*	1.06	6.38
K	1.86	0.82	4.19	2.01	0.78	5.14	—	—	—
L	1.25	0.57	2.75	0.50	0.16	1.64	0.28*	0.14	0.59
M	1.24	0.49	3.11	5.48*	2.19	13.67	—	—	—
N	0.55*	0.33	0.93	0.28*	0.14	0.55	0.39*	0.17	0.90
O	0.91	0.54	1.55	1.73*	1.03	2.91	0.37	0.07	2.06
P	0.88	0.47	1.63	0.24*	0.13	0.46	0.88	0.53	1.46

[1] Thresholds for increased bacterial counts were TBC ≥8,000, CC ≥160, and LPC ≥310 cfu/mL.

[2] Odds ratio of increased bacterial count during summer compared with winter.

[3] Lower (LCL) and upper (UCL) 95% confidence limits for odds ratio.

[4] Inestimable odds ratio and confidence limits because of lack of events.

*$P < 0.05$.

Associations Among Indicators of Milk Quality

Correlations among indicators of milk quality found in the current study ranged from weak to moderate and were similar to those reported by Jayarao et al. (2004). Boor et al. (1998) reported a greater correlation between LPC and TBC or CC than those observed in this study. For this group of herds, milk loads with increased LPC were more likely to have increased TBC. Increased LPC are often used to estimate the presence of biofilms on milking equipment, and it is possible that these biofilms could support growth of coliforms, *Bacillus* spp., and other gram-positive bacteria (Wong, 1998; Sharma and Anand, 2002; Teixeira et al., 2005). It is interesting to note that within farm, the variation was least for LPC, but among farms, LPC had the greatest variation of any milk quality indicator. This suggests that although farms varied in LPC, these values did not vary as much over time as compared with TBC and CC. This finding suggests that the underlying fault was of a more chronic nature (such as the presence of biofilms) rather than a sporadic event (such as incubation of milk in the milk line). Of enrolled farms, the 2 farms that had the greatest proportion of increased LPC (farms C and N) had relatively less variation and may be representative of farms with chronic LPC problems. Nonetheless, the relationship between LPC and TBC seems to be of moderate magnitude, and further studies are necessary to investigate the role of biofilms as a risk factor for increased TBC and other bacterial counts in raw milk.

Bulk milk loads with increased CC were much more likely to have increased TBC compared with milk loads with CC <160 cfu/mL. This association might be explained by considering potential sources of bacterial species found in milk. For example, coliforms and streptococci are commonly found in fecal matter and in the environment of dairy cows. The identification of on-farm risk factors for increased CC in bulk milk might also identify sources of increased TBC. Coliform count was the only milk quality indicator associated with milk load temperature, but the effect was of small magnitude. Psychrotrophic strains of coliform bacteria can rapidly multiply in milk loads held at low temperatures (5°C; Thomas and Druce, 1972). During milk transport in cooled tankers, greater increases in coliform and psychrotrophic bacteria have been reported compared with total bacteria and thermoduric bacteria (Thomas, 1974). Possible risk factors for increased CC during transport, such as dirty milk hoses, meters, or pumps, have not been reported. Although tankers held milk at lower temperatures than bulk tanks in most seasons of the year, milk temperature when stored in tankers was more susceptible to the effect of season. However, these differences were of small magnitude, and data from this study suggest that both milk storage systems efficiently hold milk temperature within regulatory limits.

Mastitis-causing bacteria have been suggested to be potential contaminants of raw bulk milk (Gonzalez et al., 1986; Hayes et al., 2001; Zadoks et al., 2004), and it might be hypothesized that IMI could result in increased TBC and CC in bulk milk. Hayes et al. (2001) reported that 70% (n = 14) of bacterial spikes (sudden increase in bacterial counts) measured from daily bulk tank milk samples were caused by *Streptococcus uberis* and suggested that IMI could be a likely source of this organism because these spikes occurred with no increase in other environmental bacteria in the same milk sample. However, bacterial spikes in bulk milk due to IMI would be more likely to occur in small herds because shedding of mastitis pathogens from individual cows would have a bigger effect on smaller milk volumes (Hayes et al., 2001). In this study, every 10,000-cells/mL increase in SCC within a milk load increased the odds of increased TBC and CC by 2.4 and 4.3%, respectively. The biological effect of these associations is in agreement with the fact that mastitis organisms can be associated with bulk milk bacterial contamination, but for the large dairy herds used in this study, the strength of these associations did not seem to be of great magnitude. Other researchers (Costello et al., 2003; Jayarao et al., 2004) have reported small to moderate correlations between SCC and TBC and SCC and CC from the same milk load. It is important to note that the associations between milk quality indicators assessed in this study might not represent causal associations. The magnitude of the coefficients of correlation relative to pairwise associations between bacterial counts suggests that most of the variation in TBC, CC, or LPC is explained by unmeasured factors (e.g., farm risk factors). However, it should be taken into account that when these associations were measured using odds ratio, they represented the probability of reaching extreme values greater than the 75th percentile of the bacterial counts' distributions.

Studies have shown different seasonal effects on bulk milk bacterial counts. Costello et al. (2003) studied an 11-yr series of weekly TBC from a single herd and reported greatest SPC during winter, whereas van Shaik et al. (2002) reported greatest plate loop counts during summer from a 22-mo series of monthly counts obtained from a large sample of farms in New York. In our study, the seasonal effect on TBC, CC, LPC, and milk temperature varied substantially among farms, which suggests that in some instances, seasonal effects may be overwhelmed by larger, farm-specific risk factors.

Participating farms were a convenience sample of Wisconsin dairies, and thresholds used to define increased bacterial counts in bulk milk were based on bacterial counts from this particular cohort. Consequently, the target population to which results of this study can be extrapolated is limited to large, high-producing herds on farms that use state-of-the-art technology to harvest and store milk. One important characteristic of most such farms is the production and shipment of multiple milk loads per day. Direct loading of milk into tankers has been increasingly used in Wisconsin in response to the expansion and development of farms and dairy processors and will probably be the main farm milk storage system in the near future. Although results of this study contribute to a better understanding of the relationships among selected indicators of milk quality, observational studies are limited in assessing causes of bacterial contamination in bulk milk. Additional cross-sectional studies with more herds would be valuable in determining associations between management practices and specific bacterial counts. Future research is also needed to define biologically meaningful thresholds that affect product quality.

CONCLUSIONS

Frequently monitored bulk milk bacterial counts demonstrated great variation. Milk loads with increased CC and LPC were more likely to have increased TBC compared with milk loads with CC and LPC less than the threshold used in this study. The odds of increased TBC increased as SCC increased in the same milk load. These findings estimate the effect of specific groups of bacteria on TBC. The odds of increased CC increased as SCC and milk temperature increased in the same milk load. Laboratory pasteurization count was poorly associated with other milk quality indicators. The seasonal effect on TBC, CC, LPC, and milk temperature varied substantially among farms. Results of this study can be extrapolated to a specific population of large, modern dairy farms similar to those described in this study. Further studies are necessary to identify biologically meaningful thresholds to define increased bacterial counts in raw milk and investigate on-farm risk factors associated with bacterial counts in bulk milk.

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