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Guidelines for Monitoring Bulk Tank Milk Somatic Cell and Bacterial Counts

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ABSTRACT

This study was conducted to establish guidelines for monitoring bulk tank milk somatic cell count and bacterial counts, and to understand the relationship between different bacterial groups that occur in bulk tank milk. One hundred twenty-six dairy farms in 14 counties of Pennsylvania participated, each providing one bulk tank milk sample every 15 d for 2 mo. The 4 bulk tank milk samples from each farm were examined for bulk tank somatic cell count and bacterial counts including standard plate count, preliminary incubation count, laboratory pasteurization count, coagulase-negative staphylococcal count, environmental streptococcal count, coliform count, and gram-negative noncoliform count. The milk samples were also examined for presence of *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma*. The bacterial counts of 4 bulk tank milk samples examined over an 8-wk period were averaged and expressed as mean bacterial count per milliliter. The study revealed that an increase in the frequency of isolation of *Staphylococcus aureus* and *Streptococcus agalactiae* was significantly associated with an increased bulk tank somatic cell count. Paired correlation analysis showed that there was low correlation between different bacterial counts. Bulk tank milk with low (<5000 cfu/mL) standard plate count also had a significantly low level of mean bulk tank somatic cell count (<200,000 cells/mL), preliminary incubation count (<10,000 cfu/mL), laboratory pasteurization count (<100 cfu/mL), coagulase-negative staphylococci and environmental streptococcal counts (<500 cfu/mL), and noncoliform count (<200 cfu/mL). Coliform count was less likely to be associated with somatic cell or other bacterial counts. Herd size and farm management practices had considerable influence on somatic cell and bacterial counts in bulk tank milk. Dairy herds that used automatic milking detachers, sand as bedding material, dip cups for teat dipping instead of spraying, and practiced pre- and postdipping had significantly lower bulk tank somatic cell and/or bacterial counts. In conclusion, categorized bulk tank somatic cell and bacterial counts could serve as indicators and facilitate monitoring of herd udder health and milk quality.
(**Key words:** bulk tank milk, somatic cell, bacterial count, milk quality)

Abbreviation key: BTM = bulk tank milk, **BTSCC** = bulk tank somatic cell count, **CC** = coliform count, **ES** = environmental streptococci, **LPC** = laboratory pasteurization count, **NC** = noncoliform count, **PIC** = preliminary incubation count, **SA** = *Staphylococcus aureus*, **SAG** = *Streptococcus agalactiae*, **SPC** = standard plate count.

INTRODUCTION

Since the early 1990s, researchers have used bulk tank milk (**BTM**) to diagnose multiple problems (current and potential) that might exist in a dairy herd related to milk quality and mastitis pathogens. Progressive dairy producers, veterinarians, and dairy health consultants are interested in BTM analysis as a tool to determine milk quality and troubleshoot herds with mastitis. Many quality-conscious milk cooperatives have implemented BTM analysis to reward dairy producers who excel at producing high quality milk and have a low incidence of mastitis. In addition, milk producers and cooperatives view BTM analysis as an important part of their quality assurance program (Emerson, 1989; Farnsworth, 1993; Bray and Shearer, 1996; Britten and Emerson, 1996; Keeter, 1997; Mickelson et al., 1998; Jayarao et al., 2001).

Successful milk quality assurance programs start with farm BTM free of antibiotic residues and with low somatic cell and bacterial counts, resulting in better quality products with longer shelflife (Boor et al., 1998; Ma et al., 2000; Reugg and Tabone, 2000). Many dairy producers also receive premiums from their milk cooperative for producing milk with low somatic cell and bacterial counts. Several guidelines have been proposed

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to interpret BTM milk bacterial counts (Bray and Shearer, 1996; Britt et al., 1997; Murphy, 1997; Jones and Sumner, 1999; Edmondson, 2000; Jayarao et al., 2001; Jayarao and Wolfgang, 2003). However, many of the guidelines are based on individual or collective experience, or extrapolations from other scientific studies. Further, many of the interpretive guidelines lack validation and provide little insight into the interrelationship between different groups of bacteria found in BTM. An extension and research study, conducted in Pennsylvania from April 2000 through March 2001, focused on BTM analysis. The findings of the milk quality survey were used to establish guidelines for interpreting BTM counts and also to understand the relationship between different bacterial groups that occur in BTM.

MATERIALS AND METHODS

Dairy Herds

The veterinary extension group at Pennsylvania State University with the support of the county extension agents implemented the study. A total of 12 county extension agents and 1 milk cooperative participated in the study. Each participating county extension agent/milk cooperative enrolled 7 to 11 dairy producers from its county or region. Dairy producers who participated in the study were solicited by county extension agents through their extension newsletter or announcements about the study during a monthly dairy extension meeting. For a given county, participation in the study was open to all dairy producers, and the first 12 dairy producers who responded to the invitation were included in the study.

Dairy producers who opted to participate in the program answered a self-administered questionnaire. The questionnaire sought information on the following aspects of the dairy herd: 1) herd size, 2) milk production, 3) milking frequency, 4) milkings per tank pickup, 5) type of milking facility, 6) change in milking facility, 7) use of automatic milking detachers, 8) type(s) of bedding, 9) animals purchased, 10) residue violations in the past 6 mo, 11) milk quality premiums in the past 6 mo, 12) type of milk equipment cleaning system, 13) mastitis prevention and control practices, and 14) milking procedures. The questionnaire used in this study has been successfully used previously (Jayarao and Cassel, 1999). The responses to the questions were analyzed to determine if any of these practices were associated with bulk tank somatic cell count (**BTSCC**) or bacterial counts.

Collection and Processing of BTM

The county extension agent provided on-farm instruction on BTM collection and handling procedures as described by National Mastitis Council BTM sample collection and handling guidelines (NMC, 1999). Dairy producers collected the sample in the first and third week of each month for 2 mo (4 samples total). Sampling kits containing gloves, racks, tubes (50 mL sterile screw cap tubes), and labels were provided. Bulk tank milk samples were collected in sterile 50-mL screw-cap centrifuge tubes. Within 24 hr of collection, all milk samples were shipped on ice overnight to the laboratory. On receipt of the sample in the laboratory, only those samples that recorded a temperature of <7°C were processed. The BTM in the 50-mL centrifuge tube was mixed thoroughly several times, and 20 mL of the milk was transferred to a snap-cap vial containing a preservative and sent to the Dairy One Laboratory in State College, PA, for determination of BTSCC. The remainder of the milk sample was used for bacteriological analysis.

Bacteriological Analysis of BTM

The BTM samples were examined for standard plate count (**SPC**), preliminary incubation count (**PIC**), laboratory pasteurization count (**LPC**), CNS count, environmental streptococci (**ES**) count, coliform count, and gram-negative noncoliform (**NC**) count. Bacteriological tests for milk quality were done as described by the American Public Health Association (Marshall, 1992). The milk samples were mixed thoroughly by gently inverting the milk vial 20 to 25 times. One milliliter of milk was transferred to a sterile tube containing 9 mL of quarter-strength Ringer's solution (Oxoid, Unipath Ltd., UK). The 10-fold diluted sample was vortexed at high speed for 15 s, and 50 μL was plated on selective and nonselective media using a spiroplater (Autoplate 4000, Spiral Biotech, Bethesda, MD). Plate count agar was used for enumeration of SPC, PIC, and LPC. The numbers of ES and *Streptococcus agalactiae* (**SAG**) in BTM samples were estimated using modified Edward's agar supplemented with colistin sulfate and oxolinic acid (Sawant et al., 2002). MacConkey's agar no. 3 (Oxoid) was used to determine coliform and noncoliform counts. Baird Parker's agar (Difco) was used to determine the number of CNS and presence of *Staphylococcus aureus* (**SA**). Plates for enumeration of SPC, PIC, and LPC were incubated at 32°C for 48 h. Plates for enumeration of CNS, ES, coliform count (**CC**), and NC were incubated at 37°C for 48 h. The Autoplate 4000 user guide (Spiral Biotech) was used to enumerate bacterial counts.

Colonies suggestive of SAG from modified Edward's agar supplemented with colistin sulfate and oxolinic acid were randomly selected and streaked on 5% sheep blood agar and incubated for 48 h at 37°C. All isolates were examined for gram's reaction and catalase production, serotyped (Streptex, Oxoid), and identified using API 20 STREP (BioMérieux, Hazelwood, MO) (Sawant et al., 2002). Colonies suggestive of SA from Baird Parker agar were randomly selected, streaked on 5% sheep blood agar, and incubated for 48 h at 37°C. The isolates were examined for hemolysis, catalase production, and coagulase production, and identified using API-STAPH (BioMerieux) (NMC, 1999).

Isolation of *Mycoplasma* was done as described by Gonzalez et al. (1995), with modifications. Briefly, 500 μL of BTM was pre-enriched in modified Hayflick's broth and incubated for 48 h at 37°C in a moist 10% CO_2 incubator. One hundred microliters of the pre-enriched broth was streaked on modified Hayflick's agar and incubated for 7 d at 37°C in a moist 10% CO_2 incubator. *Mycoplasma* colonies were viewed under a low-power microscope. *Mycoplasma* was differentiated from *Acholesplasma laidlawdii* using the digitonin inhibition test as described by Thurmond et al. (1989).

Data Analysis

A total of 149 dairy herds elected to participate in the study of which 4 herds opted not to participate during the course of the study. Of the 145 herds, 7 dairy herds were unable to provide information on farm management practices, and 12 dairy producers, on 2 consecutive occasions, supplied contaminated bulk tank milk, or the milk that was received for analysis had a temperature in excess of 7°C. A total of 126 dairy herds with complete data sets were used for data analysis.

Answers to the questionnaire were transferred to Microsoft Excel and grouped by their categorical response (e.g., *yes*, *no*). To estimate if a response had an influence on the mean BTSCC, SPC, PIC, LPC, SA, CNS, ES, CC, and NC counts for each group within a response were compared with the 3 categories (low, medium, high) within each bacterial count using one-way ANOVA. A P-value of < 0.05 was considered a significant association between the response and a category of the count. All statistical analyses were performed using JMP software version 4.0 (SAS Inst., Inc., Cary, NC). BTSCC and bacterial counts from the 4 BTM samples from each farm were transformed to log10 values. The log10 transformed BTSCC and bacterial counts (SPC, PIC, LPC, SA, CNS, ES, CC, and NC) from the 4 bulk tank samples from each farm were averaged and subjected to correlation coefficient analysis (SAS Inst. Inc.).

The BTSCC, SPC, PIC, LPC, SA, CNS, ES, CC, and NC counts were each classified as low, medium, or high. These 3 categories are the suggested interpretive criteria for monitoring BTM (see Table 2). The average counts for each of the 3 groups were compared using the Tukey-Kramer (equal variance) or Dunnett's T3 (unequal variance) procedures. These 2 procedures were used due to unequal sample sizes observed in the 3 categories of a given count. The Tukey-Kramer procedure performs all pair-wise comparisons, testing whether the 3 means are significantly different. The Dunnett's T3 procedure performs all comparisons with a control category. In our study, the second category (medium) was used as the control because the sample mean falls in this category. $P < 0.05$ was considered significant. Epi-info-2002 (Centers for Disease Control and Prevention, Atlanta, GA), a database and statistics system for epidemiology on microcomputers, was used for performing χ^2-square tests and odds ratio analysis.

RESULTS

Dairy Herds

The responses to the 14 questions on the questionnaire were grouped based on herd size (Table 1). Nearly 71% of the farms had fewer than 100 lactating cattle, typical of farm families engaged in milking cows in Pennsylvania. Farm management practices changed as the herd size increased. This observation can be supported by change in the management practices such as 1) number of milkings per day, 2) type of milking facility, 3) use of automatic milking detachers, 4) type of cow bedding, 5) number of animals purchased, 6) milk equipment cleaning system, 7) mastitis prevention and control, and 8) milking practices. For the majority of the dairy herds, cows were milked twice a day (88%) in stanchion barns (61%) and/or parlors (39%). About 45% of dairy herds had automatic milking detachers. As the herd size increased, so did the use of automatic milking detachers. Sawdust was used as bedding on 44% of the farms surveyed. Nearly 5% of the respondents to the questionnaire indicated antibiotic residues in the last 6 mo. A majority of the dairy producers practiced dry cow treatment (88%), whereas 73% of the dairy producers who teat dipped their cows practiced both pre- and postdipping. Milking practices varied considerably within a given herd size and between the 4 herd size categories (Table 1). Significant differences were observed with respect to the type of bedding used ($P \leq 0.000$), antibiotic residues in bulk tank milk ($P \leq 0.043$), type of milking equipment cleaning system ($P \leq 0.022$), dry cow treatment ($P \leq 0.043$), teat-dipping practices ($P \leq 0.031$), stripping practices before milking ($P \leq 0.028$), and towel type ($P \leq 0.011$) (Table 1).

Table 1. Characteristics of the dairy herds that participated in the study.

Query	Herd size					χ^2 ($P \leq 0.005$)
	< 50 (n = 35)	50 to 99 (n = 55)	100 to 199 (n = 30)	>200 (n = 6)	Total (n = 126)	
No. of cows in milk (average)	38	67	136	294	87	
Milk produced per cow (lb)	33	34	33	30	32.5	
Times milked (%)						
Two	97	91	83	17	88	4.99 (0.111)
Three	3	9	17	83	12	
No. of milkings in bulk tank (%)						
Two	0	10	24	17	11	
Three	0	4	10	0	4	6.63 (0.011)*
Four	97	82	62	50	80	
>Four	3	4	3	13	5	
Milking facility						
Stanchion	94	75	10	0	61	0.28 (0.632)
Parlor	6	25	90	100	39	
Change in milking facility in last 6 mo (%)	14	20	30	33	21	6.89 (0.078)
Automatic milking detachers (%)	26	52	47	83	45	4.54 (0.122)
Cow bedding (%)						
Combination (>1 type bedding, c–i)	11	9	3	0	8	
Corn fodder	3	4	0	0	1	
Hay	0	4	0	0	2	
Mats	3	7	0	0	4	11.86 (0.000)*
Newspaper	6	11	7	0	8	
Sand	3	6	17	0	7	
Sawdust	37	33	63	87	44	
Shavings	14	11	3	13	10	
Straw	23	19	7	0	16	
Animals purchased (%)						
Dry cows	9	9	30	33	15	
Milking cows	11	16	40	50	22	2.70 (0.145)
Spring heifers	20	11	33	50	21	
Antibiotic residues in last 6 mo	3	9	4	0	5	11.3 (0.043)*
Milk premiums in last 6 months	74	47	55	60	58	1.04 (0.382)
Milk equipment cleaning system (%)						
Automatic	74	96	93	100	88	
Manual	3	2	0	0	2	7.64 (0.022)*
Semi-automatic	23	2	7	0	10	
Mastitis prevention and control (%)						
Dry cow therapy (always)	86	88	93	100	88	11.37 (0.043)*
Teat dipping	74	67	87	83	73	14.54 (0.031)*
Predipping only	3	7	0	17	6	12.01 (0.007)*
Postdipping only	18	33	10	0	22	
Pre- and postdipping	79	60	90	83	72	
Milking practices						
Written protocols	3	9	7	17	7	9.73 (0.052)
Check for mastitis	38	36	58	80	42	0.62 (0.486)
Wear gloves	18	12	27	50	19	4.96 (0.112)
Strip before milking	63	63	77	83	67	15.63 (0.028)*
Type of towel						
Common wash cloth	3	4	7	0	4	
Individual wash cloth	11	18	30	67	21	6.69 (0.011)*
Paper towel	71	62	53	33	61	
Medicated towel	15	16	10	0	14	
Milkers						
Employees	3	2	17	17	7	
Family members	49	31	20	0	31	1.92 (0.195)
Self and employees	9	20	43	67	25	
Self	40	37	20	17	37	

*$P \leq 0.05$.

Bulk Tank Somatic Cell Counts

The mean BTSCC (315,190 cells/mL) varied significantly with respect to the herd size. Fifty percent of the BTM samples had a BTSCC <348,000 cells/mL. Paired correlation analysis showed that there was low correlation between BTSCC and different bacterial counts (Table 2). Bulk tank somatic cell counts were categorized

Table 2. Descriptive statistics and correlation coefficients of counts of bulk tank milk (BTM) samples from 126 dairy producers in Pennsylvania.[1]

	Mean count							
Herd size	BTSCC[2]	SPC[3]	PIC[3]	LPC[3]	CNS[3]	ES[3]	CC[3]	NC[3]
<50	320,440	3260	9140	150	760	630	30	170
50–99	375,169	4760	13,950	150	820	900	60	210
100–199	289,175	5500	12,220	110	540	780	80	310
>200	283,895	3100	3740	100	519	1010	200	130
Range[4]	95,250–737,500	180–62,820	500–139,750	5–6,400	60–15,180	15–1,1040	5–4,130	0–15,460
Mean count (all herds)[4]	315,190	4,320	8740	125	650	820	70	200
χ^2 ($P < 0.05$)	2.87 (0.038)	1.41 (0.242)	2.44 (0.067)	0.39 (0.759)	1.18 (0.319)	0.78 (0.506)	5.97 (0.003)	1.12 (0.341)
Cumulative frequency								
<10%	187,250	1,140	2,200	30	190	210	10	30
<50%	348,000	4,210	12,500	133	700	900	60	230
<90%	553,250	19,370	62,000	1,240	2,650	3,100	230	1,240
Correlation coefficients								
BTSCC	1	0.32	0.198	0.148	0.322	0.362	0.18	0.108
SPC	—	1	0.619	0.51	0.571	0.648	0.385	0.415
PIC	—		1	0.502	0.435	0.533	0.239	0.435
LPC	—	—	—	1	0.377	0.405	0.166	0.264
CNS	—	—	—	—	1	0.503	0.121	0.344
ES	—	—	—	—	—	1	0.193	0.221
CC	—	—	—	—	—	—	1	0.279
NC	—	—	—	—	—	—	—	1

[1]BTSCC = bulk tank SCC, SPC = standard cell plate, PIC = preliminary incubation count, LPC = laboratory pasteurization count, CNS = coagulase-negative staphylococci, ES = environmental streptococci, CC = coliform count, NC = noncoliform count.

[2]cells/mL (log transformed values).

[3]cfu/mL (log transformed values).

[4]126 dairy herds.

Table 3. Categorization of mean bulk tank somatic cell and bacterial counts.[1]

	Proposed interpretive criteria										
Bulk tank	Category	Count (cfu/mL)	N[2]	BTSCC	SPC	PIC	LPC	CNS	ES	CC	NC
	Low	<200	19	179,390	2290	6540	90	360*	390	30	120
BTSCC	Medium	200,000–400,000	55	283,320*	4140	10440	130	680	760	60	290
	High	≥400,000	52	497,310	5970	14960	160	940	1080	70	220
	Low	<5,000	70	310,900	1950	6170*	80*	440*	490*	40	130*
SPC	Medium	5,000–10,000	24	303,601	7470*	16870	280	1170	1220	70	350
	High	≥10,000	32	415,040	17680	31290	280	1410	1690	90	470
	Low	<10,000	60	313,610	2370*	3660	80	470*	460*	40	120
PIC	Medium	10,000–20,000	20	337,640	5100	13290*	130	1030	1170	60	200
	High	≥20,000	26	358,110	9280	44230	290*	1040	1350	70	440
	Low	<100	52	307,860	2720	6130*	32	470	530	50	140
LPC	Medium	100–200	21	313,700	2900	11870	140*	630	780	40	270
	High	≥200	53	368,430	8340*	20140	540	1120*	1190*	80	280
	Low	<500	46	277,520*	2490	5810	80	260	450	50	120
CNS	Medium	500–1000	40	350,140	3690	10370*	110	720*	820	50	180
	High	≥1000	40	390,760	10140*	26400	290*	2160	1470*	80	460
	Low	<500	33	284,960	1940	5090	60*	380	190	41	140
ES	Medium	500–1000	35	311,490	3480*	10110*	150	540	690*	60	170
	High	≥1000	58	378,970	8090	18970	200	1190*	1970	70	310
	Low	<50	57	307,990	3130	8520	110	600	620	20	140
CC	Medium	50–100	28	356,160	4980	11630	160	1030	950	70*	290
	High	≥100	41	354,710	6510	16330	170	700	980	220	290
NC	Low	<200	53	303,570	2980*	6790	100	560	650	40	60
	Medium	200–400	44	359,240	4770	12640	140	660	790	60	270*
	High	≥400	29	351,750	7970	24030*	240	1220	1140	90	1300

[1]See Table 2 for abbreviation definitions.

[2]N, number of bulk tanks

*$P \leq 0.05$.

into 3 groups (low, <200,000; medium, 200,000 to 400,000; and high, >400,000 cells/mL) (Table 3). Mean CNS count was significantly associated with mean BTSCC (Table 3). A BTM with a mean BTSCC > 200,000 cells/mL was 5 times more likely to have high CNS (>500 cfu/mL) counts compared with BTM with BTSCC < 200,000 cells/mL (Table 4). Dairy producers who received milk premiums had significantly lower BTSCC (291,300 cfu/mL) compared with the BTSCC (378,090 cells/mL) in BTM of those dairy producers who did not receive premiums [χ^2 (p) = 3.27(0.0014)]. BTSCC was significantly lower when cows were milked using automatic milk detachers as compared with BTM from herds that milked cows without automatic milk detachers. The same observation was made with herds that teat dipped the cows with a dip cup instead of using a spray. Interestingly, BTSCC was significantly higher in herds that practiced fore-stripping before milking compared with BTM from herds that did not. Dairy farms that used sand as bedding had significantly lower BTSCC in their BTM compared with dairy producers who used organic bedding such as shavings, newspaper, and straw (Table 5).

Standard Plate Count

For the 126 dairy herds in the study, the mean SPC for an 8-wk period was 4320 cfu/mL. The herd size did not influence the mean SPC of BTM. Fifty percent of BTM samples had a SPC < 4120 cfu/mL. Paired correla-

Table 4. Odds ratio (confidence interval) estimates for somatic cell and bacterial counts.[1]

Counts	BTSCC >200,000 cells/mL	SPC >5,000 cfu/mL	PIC >10,000 cfu/mL	LPC >100 cfu/mL	CNS >500 cfu/mL
BTSCC >200,000 cells/mL	—	—	—	—	—
SPC >5,000 cfu/mL	—	—	—	—	—
PIC >10,000 cfu/mL	—	9.55 (3.86–24.15)	—	—	—
LPC >100 cfu/mL	—	4.89 (2.07–11.75)	3.02 (1.36–6.77)	—	—
CNS >500 cfu/mL	5.04 (2.11–12.97)	5.86 (2.32–15.12)	3.13 (1.37–7.17)	3.71 (1.56–8.94)	—
ES >500 cfu/mL	—	6.80 (2.23–22.12)	4.22 (1.64–11.12)	2.93 (1.20–7.23)	5.75 (2.25–14.94)
NC >200 cfu/mL	—	6.14 (2.54–15.12)	3.73 (1.66–8.47)	—	—

[1]See Table 2 for abbreviation definitions.

Table 5. Effect of herd size and management practices on somatic cell and bacterial counts.[1]

Count	Practice		Herd size <50	50–99	100–199	>200	Total
SCC	Automatic detachers	Yes	354,580	289,430*	230,890*	473,250	298,560*
		No	363,780	356,000	421,720	—	352,650
	Strip	Yes	409,030*	327,510	341,070	390,020	357,060
		No	293,050	295,570	253,470	313,750	295,760*
	Spray	Yes	515,070	295,580	399,480	361,370	396,600
		No	335,840*	327,510	284,050	459,500	321,550*
	Dip cup	Yes	317,480*	296,950	300,710	361,700	306,360*
		No	429,510	359,450	336,810	459,500	370,090
	Bedding	Combination	295,450	286,420	306,250	—	291,940
		Corn fodder	449,330	—	—	—	449,330
		Hay	—	242,510	—	—	242,510
		Mats	587,000	342,760	—	—	381,700
		Newspaper	479,300	343,490	382,090	—	325,480
		Sand	316,750	206,290*	179,550*	—	241,750*
		Sawdust	350,320	299,020	343,550	—	317,990
		Shavings	315,420	339,270	274,670	370,150	353,580
		Straw	397,140	413,200	588,180	407,500	360,700
SPC	Pre and post	Yes	358,030	330,680	282,800*	408,340	312,290*
		No	368,820	284,310	441,290	319,190	380,680
	Spray	Yes	9450	5100	6330	6200	6710
		No	4690	4330	2430*	2120	3910*
PIC	Spray	Yes	26,030	23,520	15,900	12,990	18,300
		No	14,210	10,100	6200*	16,200	10,170*
CNS	Automatic detachers	Yes	660	610	400*	1880	510*
		No	1110	610	870	—	860
ES	Pre and post	Yes	900	420*	540	520	670*
		No	1390	930	830	1330	1120

[1]See Table 2 for abbreviation definitions.

*$P \leq 0.05$.

tion analyses between SPC and other bacterial counts showed that SPC had correlation coefficients >0.5 for ES (0.648), PIC (0.618), CNS (0.571), and LPC (0.510) (Table 1). SPC were categorized into 3 groups (low, <5000; medium, 5000 to 10,000; and high, >10,000 cells/mL) (Table 3). The mean PIC, LPC, CNS, ES, and NC counts were significantly different for the 3 SPC categories (low, medium, and high) (Table 3). BTM with a mean SPC >5000 cfu/mL were 9.5, 5, 6, 7, and 6 times more likely to have medium or high PIC, LPC, CNS, LPC, ES, and NC, respectively, compared with BTM with SPC <5,000 cfu/mL (Table 4). The SPC was significantly lower in BTM when cows were subjected to both pre- and postdipping. In contrast, BTM samples had significantly higher SPC when cows were sprayed with a teat dip instead of using a dip cup (Table 5).

Preliminary Incubation Count

The mean PIC for an 8-wk period ranged from 500–139,750 cfu/mL with a mean PIC of 8740 cfu/mL. As observed with SPC, herd size did not influence the mean PIC of BTM. Nearly 50% of the BTM milk samples had a PIC of <12,500 cfu/mL. In addition to SPC, PIC had correlation coefficients >0.5 for LPC (0.501) and ES (0.533) (Table 2). The mean SPC, LPC, CNS, and ES counts were significantly different for the 3 PIC categories (low, medium, and high) (Table 3). The BTM with a mean PIC >10,000 cfu/mL were 3, 3, 4, and 4 times more likely to have medium or high LPC, CNS, ES, and NC, respectively, compared with BTM with PIC < 10,000 cfu/mL (Table 4). As observed with SPC, BTM had significantly higher PIC when cows were sprayed with a teat dip instead of using a dip cup (Table 5).

Laboratory Pasteurization Count

The mean LPC for an 8-wk period was 125 cfu/mL. The herd size did not influence the LPC of BTM. Approximately 10% of BTM samples had a LPC <30 cfu/mL, whereas 90% of the BTM samples had a LPC of <1240 cfu/mL (Table 2). The mean SPC, PIC, CNS, and ES counts were significantly different for the 3 LPC categories (low, medium, and high) (Table 3). The BTM with a mean LPC >100 cfu/mL were 4 and 3 times more likely to have medium or high CNS and ES, respectively, compared with BTM with LPC <100 cfu/mL (Table 4). None of the management practices had any significant effect on LPC in BTM (Table 5).

Coagulase-Negative Staphylococci

The mean CNS for an 8-wk period ranged from 60 to 15,180 cfu/mL with a mean CNS of 650 cfu/mL. As

observed with other bacterial counts, herd size did not have any significant effect on CNS count of BTM. Fifty percent of BTM samples had CNS counts of <700 cfu/mL (Table 2). The mean BTSCC, SPC, PIC, LPC, and ES counts were significantly different for the 3 CNS categories (Table 3). The BTM samples with a mean CNS >500 cfu/mL were 6 times more likely to have medium or high ES compared with BTM with CNS <500 cfu/mL (Table 4). The CNS counts were significantly lower in BTM when cows were milked using automatic milk detachers compared with BTM from cows that were milked without the use of automatic milk detachers (Table 5).

Environmental Streptococci

The mean ES for an 8-wk period was 820 cfu/mL. The herd size did not have a significant effect on ES count of BTM. Fifty percent of BTM samples had ES counts of <900 cfu/mL. Ten and 90% of the BTM samples had ES counts of <210 and <3100 cfu/mL, respectively. Paired correlation analyses between ES and CNS had a correlation coefficient of 0.503 (Table 2). The mean SPC, PIC, LPC, and CNS counts were significantly different for the 3 ES categories (Table 3). The ES count was significantly lower in BTM when cows were pre- and postdipped (Table 5).

Coliform Count

The mean CC for an 8-wk period was 70 cfu/mL. A significant association was observed between CC and herd size. As the herd size increased, so did the CC of BTM. About 50% of BTM samples had CC <60 cfu/mL (Table 2). There was no significant difference in the mean of all of the bacterial counts for the 3 CC categories (low, medium, and high) (Table 3).

Gram-Negative Noncoliform Bacteria

For the 126 dairy herds, the mean NC count was 200 cfu/mL. As observed with other bacterial counts, herd size did not have any significant effect on an NC count of BTM. About 50% of the BTM samples had an NC count of <230 cfu/mL (Table 2). The mean SPC and PIC were significantly different for the 3 NC categories (low, medium, and high) (Table 3). The BTM samples with a mean NC >200 cfu/mL were 6 and 4 times more likely to have medium or high SPC and PIC counts compared with BTM with NC <200 cfu/mL (Table 4).

Contagious Mastitis Pathogens

The bulk tank milk that tested positive for contagious mastitis pathogens was categorized as low frequency (1 of 4 samples positive), medium frequency (2 of 4 samples positive), or high frequency (3 or 4 samples positive) (Table 6). Based on the analysis of 4 milk samples from each bulk tank, SA was detected in 39 of 126 (31%) bulk tanks. It was observed that 17, 8, and 6% of the BTM samples had low, medium, and high isolation rates of SA, respectively. As the frequency of sampling increased from 2 to 4 samples, the number of bulk tanks with SA also increased. The mean BTSCC count was significantly associated with the frequency of isolation of SA (Table 6). *Streptococcus agalactiae* was detected at least once in 13 of 126 (10%) bulk tanks (Table 6). Of the BTM samples, 3, 5, and 2% had low, medium, and high isolation rates of SAG, respectively. As seen with SA, with increased frequency of sampling, the number of BTM samples with SAG was also observed (Table 6). *Mycoplasma* was isolated from 3 of 39 (7.5%) BTM samples examined.

DISCUSSION

Farnsworth (1993) presented the first set of guidelines for interpreting BTM counts. This was followed by Bray and Shearer (1996), who developed comprehensive interpretive criteria for BTM counts. Murphy (1997) suggested interpretive guidelines for monitoring BTM counts, focusing on milk and milking system hygiene. Other researchers have also provided guidelines for monitoring bulk tank bacterial counts as they relate to herd udder health and milk quality, with an emphasis on troubleshooting herds with high bacterial counts (Britt et al., 1997; Jones and Sumner, 1999; Edmondson, 2000; Jayarao et al., 2001; Jayarao and Wolfgang, 2003). These recommended guidelines served as the foundation for developing interpretive guidelines for monitoring BTM (Table 3).

Based on the 1996 to 1997 BTSCC data collected from dairy herds from 49 states, Pennsylvania ranked 20th, with a state average of 331,000 cells/mL of milk (Norman et al., 2000). Paired correlation analyses between BTSCC and bacterial counts showed low correlations (Table 1). Van Schaik et al. (2002), studying the trends of somatic cell counts in New York State during 1999 to 2000, observed that the average BTSCC was 363,000 cells/mL. The findings of their study suggest that larger farms had lower BTSCC and plate loop count, but had more antibiotic residue violations.

An individual cow somatic cell count of <200,000 cells/mL is typical of an uninfected udder (Laevens et al., 1997). A similar guideline was used by Van Schaik et al. (2002) for evaluating trends in somatic cell counts in New York. In our study, 15% of the BTM had SCC <200,000 cells/mL. This suggests that lowering BTSCC is still a challenge for many dairy producers in Pennsyl-

Table 6. Relationship between frequency of isolation of *Staphylococcus aureus* and *Streptococcus agalactiae* on mean bulk tank SCC (BTSCC) counts.

Sampling	Cumulative frequency of isolation (category)					Total positive bulk tanks (n = 126)
	Not detected (0/4)	Low (1/4)	Medium (2/4)	High (3/4)	High (4/4)	
Staphylococcus aureus						
1st	111 (88.0)	6 (4.7)	4 (3.2)	3 (2.4)	2 (1.6)	15 (11.9)
2nd	101 (80.1)	13 (10.3)	4 (3.2)	6 (4.8)	2 (1.6)	25 (19.8)
3rd	91 (72.2)	17 (13.4)	10 (7.9)	6 (4.8)	2 (1.6)	35 (27.8)
4th	87 (69.0)	21 (16.7)	10 (7.9)	6 (4.8)	2 (1.6)	39 (30.9)
Mean BTSCC	253,440[1]	244,300	326,810	361,290	458,440	—
Streptococcus agalactiae						
1st	122 (96.8)	1 (0.8)	2 (1.6)	1 (0.8)	0	4 (3.2)
2nd	119 (94.5)	2 (1.6)	3 (2.4)	2 (1.6)	0	7 (5.5)
3rd	117 (92.8)	0	4 (3.2)	3 (2.4)	0	9 (7.1)
4th	113 (89.7)	4 (3.2)	6 (4.8)	3 (2.4)	0	13 (10.3)
Mean BTSCC	243,760[1]	318,940	379,130	519,350	—	—

[1]$P \leq 0.05$.

vania. It was observed that the mean CNS count was significantly associated with mean BTSCC. Coagulase-negative staphylococci are frequently isolated from milk samples and are a significant cause of mild inflammation and elevated cell counts. The CNS generally produce a mild elevation of milk SCC, but if the cows have chronic mastitis, the SCC can elevate to millions (Sears and McCarthy, 2003).

The SPC provides an estimate of the total number of aerobic bacteria present in raw milk. This test is required by the FDA and state regulatory agencies. In our study, 50% of BTM samples had SPC <4120 cfu/mL. Boor et al. (1998) found that 50% of dairy producers in New York routinely produced milk with SPC <10,000 cfu/mL. The Virginia Department of Agriculture and Consumer Services reported that 59% of BTM samples had SPC <5000 cfu/mL, and 76% had <10,000 for the period of December 1997 to November 1998 (Jones and Sumner, 1999).

These observations suggest that SPC is strongly influenced by specific groups of organisms. If SPC increases, counts of specific groups of bacteria (thermoduric, psychrotrophic, and environmental mastitis pathogens) should be examined. With an increase in the SPC, the composition of the bacterial microflora changes considerably. In most cases, an increase in the SPC correlates with unsanitary conditions associated with unclean udders before milking, poor teat and teat-end sanitation, cleaning and sanitation milking equipment, and cooling of milk (Chambers, 2002).

Panes et al. (1979) reported a correlation of 0.65 between thermoduric and SPC when the geometric means were compared for 12 monthly BTM samples from about 350 individual farms. When the numerical means for individual samples were compared, the correlation coefficient between psychrotrophic counts and SPC was 0.66. Boor et al. (1998) observed a correlation of 0.66 between SPC and PIC, whereas Peeler et al. (1989) observed a correlation of 0.71 between SPC and PIC. Based on the findings in our study and those reported by Boor et al. (1998) and Peeler et al. (1989), it can be inferred that correlation coefficients between counts lack predictive value.

The PIC is used as an indicator of the number of psychrotrophic bacteria in raw milk. Milk with high PIC can influence the keeping quality of raw milk and reflect sanitation practices (Jones and Sumner, 1999; Jayarao et al., 2001). The association between PIC and SPC, CNS, LPC, NC, and ES counts can be explained based on the observations of Cousin (1982) and Chambers (2002). The most commonly occurring psychrotrophs in raw milk are the gram-negative bacteria (CC and NC), of which *Pseudomonas* spp. account for nearly 50% (Cousin, 1982). Gram-positive species belonging to the genera *Enterococcus* and *Streptococcus* have been reported to occur with psychrotrophic bacterial flora of raw milk. The psychrotrophic bacteria may account for 10 to 50% of the SPC (Chambers, 2002).

Laboratory pasteurization count determines the number of thermoduric bacteria present in raw milk. Bulk tank milk with an LPC count <200 cfu/mL is considered normal, while a count of <10 cfu/mL indicates excellent equipment hygiene (Ruegg and Reinemann, 2002). In our study, 50% of the dairy producers had a LPC count of <130 cfu/mL. High counts of thermoduric bacteria (>200 cfu/mL) have been associated with herds with poor milking hygiene, unclean equipment, improper sanitizing practices, and milkstone deposits (Murphy, 1997).

Some of the bacterial species that belong to the genera *Micrococci* and *Bacilli* have survived heating at 63°C for 30 min. *Enterococcus faecalis*, lactobacilli, and

some corynebacteria are heat resistant, surviving at 60°C for about 20 min. A very small percentage (<1%) can survive heating at 63°C for 30 min (Chambers, 2002). The association between LPC and gram-positive cocci (CNS and ES) suggests that CNS and ES could contribute to the LPC count in BTM.

The CNS, ES, CC, and NC are collectively termed *environmental mastitis pathogens*. These organisms gain access to bulk tank milk, not only from intramammary infections, but also from nonspecific contamination from cow skin surface, bedding, manure, and water. The presence of these organisms in BTM may relate to the general level of environmental and milking hygiene in the herd (Godkin and Leslie, 1993). An increase in their numbers in BTM is suggestive of problems related to stall management, udder hygiene, and milking practices (Jayarao and Wolfgang, 2003). Coagulase-negative staphylococci are opportunistic pathogens and form a part of the resident bacterial flora on teat skin. When provided with a favorable opportunity to colonize the teat end or teat canal, they grow to considerable numbers and enter the gland to produce mastitis (Sears and McCarthy, 2003).

Catalase-negative, gram-positive cocci belong to a large heterogeneous group of organisms. Members of the genera *Streptococcus*, *Enterococcus*, *Lactococcus*, and *Aerococcus* have been isolated from BTM (Jayarao et al., 2001). With the exception of *Streptococcus agalactiae*, these organisms isolated from milk are collectively termed *streptococci or streptococci-like organisms* (Jayarao et al., 2001) or *environmental streptococci* (Hillerton and Berry, 2003). Environmental streptococci are widely distributed in the cow's environment, including on teat ends, teat skin, bedding, and feces. Bedding materials with high moisture and organic content can serve as reservoirs for ES (Hillerton and Berry, 2003). ES can gain access to the mammary gland through the teat canal and induce changes in the mammary tissue. Amongst the environmental pathogens, *S. uberis* have been shown to increase SPC in BTM (Hayes et al., 2001). In our study, dairy producers who practiced pre- and postmilking teat dipping had significantly lower ES in their BTM compared with dairy producers who did not practice teat dipping. The use of pre- and postmilking teat dipping has been widely advocated for prevention of ES intramammary infections (NMC, 1996).

Presence of coliform bacteria in BTM milk is suggestive of fecal contamination. Coliforms include *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp. These environmental organisms are frequently isolated from BTM. *Escherichia coli* in particular has been shown to elevate bacterial numbers in BTM (Hayes et al., 2001). In our study, we observed a lack of a relationship between CC and other counts. Chambers (2002) reported that although coliforms are the predominant bacteria in cow bedding, CC on teat skin do not exceed 100 cfu/mL. Another potential source of coliforms and other problem organisms in bulk tank milk is the water used for cleaning the milking equipment. Potable water within the dairy production environment can be contaminated by the farm storage tank, rodent and bird droppings, insects, dust, and dirty buckets and hoses (Chambers, 2002).

Gram-negative noncoliform bacteria belonging to 15 different genera have been isolated from BTM. In particular, organisms belonging to the genus *Pseudomonas* are most frequently isolated from raw milk (Jayarao and Wang, 1999). With the exception of *Serratia* spp. and *Pseudomonas aeruginosa*, other noncoliforms bacteria are less frequently isolated from cases of subclinical and clinical mastitis. Noncoliforms in general can serve as indicators of bacterial milk quality but are poor indicators of herd udder health. Gram-negative NC bacteria, such as *Pseudomonas* and *Serratia,* can cause mastitis. They can also grow at low temperatures (4 to 22°C) and colonize the stainless steel surface of the milking system (Cousin, 1982; Jayarao et al., 2001). Failure to cool milk to 4°C or improper sanitation can cause the NC count in bulk tank milk to increase dramatically and result in poor quality milk (Cousin, 1982). The association between NC, SPC, and PIC suggests that NC can influence SPC and, in particular, PIC in BTM. The fact that a large part of the PIC microflora consists of NC sustains this observation.

Responses to the 14 questions on the questionnaire were analyzed to determine if any responses/farm management practices were associated with BTSCC or bacterial counts. Fenlon et al. (1995) showed that herds with high BTSCC had significantly lower milk yield and were less likely to use postmilking teat dip, periodically perform maintenance of the milking system, and use automatic cluster removal. A study conducted in Washington State showed that the dairy farms that produced milk with low SCC milked high-producing cows first and clinical cows last, had automatic milking detachers, attended to cow bedding, and disinfected teat ends prior to intramammary antibiotic treatment (Hutton et al., 1990). The results of our study corroborate the findings reported by Hutton et al. (1990) and Fenlon et al. (1995). The CNS counts were significantly lower in the BTM of those dairy producers who used automatic milking detachers. This observation may not relate directly to the use of automatic milking detachers but to fewer cows with subclinical mastitis.

Hogan et al. (1989) conducted an extensive study that monitored for 1 yr bedding materials on 9 commercial dairies. Their findings revealed that inorganic bedding, such as sand and crushed limestone, had significantly

lower moisture content, coliforms, gram-negative bacterial count, *Klebsiella* spp., and streptococcal counts compared with organic bedding materials such as chopped straw and sawdust. In a subsequent study, Hogan et al. (1990) showed that bacterial counts in bedding did not differ considerably, and using chopped newspaper over pelleted corn cobs or wood shavings did not reduce exposure to teat environmental mastitis pathogens. In our study, dairy herds that used sand as bedding had significantly lower BTSCC compared with dairy herds that used organic bedding. Based on the reports of Hogan et al. (1989, 1990), it can be inferred that the low BTSCC observed in our study could be due to the fact that sand as a bedding material may not be conducive to mastitis pathogens growth and intramammary infections resulting in elevated BTSCC.

The practice of pre- and postmilking teat dipping is one of the critical components of a mastitis prevention and control program in a dairy herd. Teat dipping, or disinfecting of the teat, is now a universally accepted practice for reducing the bacterial population around the teat end, thus decreasing the risk of intramammary infection. Thorough cleaning and drying of teats immediately before milking lowers bacterial numbers as well as coliform and *Staphylococcus* spp. counts and decreases milk sediment (Galton et al., 1984; Pankey, 1989).

Bacterial contamination of the teat and teat end occurs between milkings, when the teat comes in contact with bedding, soil, water, and dung. The number and type of bacteria present on the teat end and teat skin can vary considerably and is affected by season, grazing, and bedding type. Milk from cows with teats soiled with dung have coliform counts as high as 10^6 cfu/mL (Chambers et al., 2002). Milk samples from mastitis-free cows obtained without washing the teats had an average 7000 cfu/mL, whereas milk from cows with teats dipped and dried with paper towels had an average SPC of 1500 cfu/mL (McKinnon et al., 1988). Hogan et al. (1987) observed that application of germicidal teat dips reduced the incidence of CNS infection and selectively altered both the prevalence and distribution of *Staphylococcus* spp. intramammary infections. Based on the findings of our study, it can be inferred that pre- and postmilking teat dipping has the positive effect of reducing not only the number of environmental mastitis pathogens (CNS, ES), but also the number of thermoduric (LPC) and psychrotrophic (PIC) bacteria in the BTM.

In our study we observed that dairy herds that applied teat dip using a dipcup had significantly lower BTSCC, SPC, and PIC in their BTM compared with dairy herds who sprayed the teats with a teat dip. Effective application of a teat dip is achieved when all areas touched by the milking machine are covered in the dip. Applying the dip using a spray is less likely to achieve maximum teat end and teat skin coverage compared with using a dipcup (NMC, 1996). Incomplete coverage of the teat skin could result in areas of teat skin where bacteria could survive and grow between milkings and could result in intramammary infections or contaminate bulk tank milk (NMC, 1996; Edmondson, 2002).

The frequency of isolation (number of positive samples out of 4) of SA, SAG, and *Mycoplasma* was used to monitor contagious mastitis pathogens (Table 6). This approach to interpreting SA, SAG, and *Mycoplasma* in bulk tank milk comes from several reports that suggest that the number of contagious organisms in bulk tank milk provides little or no clear evidence of the severity of the contagious mastitis problem present in the herd. This could be due to several factors; for example, latently infected cows may not be shedding or shedding intermittently, the dilution effect of milk in the bulk tank may cause the organisms to go undetected, and culture techniques may not work for that particular organism (Farnsworth, 1993; Godkin and Leslie, 1993; Kirk and Lauerman, 1994; Fenlon et al., 1995; Ruegg and Reinemann, 2002).

The isolation rates of SA and SAG were significantly associated with BTSCC in BTM (Table 5). Fenlon et al. (1995) showed a significant correlation between number of *S. agalactiae*, *S. dysgalactiae*, and *S. uberis* in BTM. *Staphylococcus aureus* was less significantly correlated to BTSCC. However of herds that had cows with SA infection, BTSCC between 250,000 and 400,000 (borderline) was a good indicator of SA infection. Greer and Pearson (1973) observed that herds with a higher BTSCC had a higher frequency of isolation of SAG.

In our study, *Mycoplasma* was detected in 7.5% of the BTM samples. Kirk et al. (1997) conducted a study to determine the prevalence of *Mycoplasma* spp. in herds that were members of a milk cooperative. They reported that *Mycoplasma*-positive samples ranged from 1.8 to 5.8% for all species of *Mycoplasma*, and 1.2 to 3.1% for *Mycoplasma* spp. known to be mastitis pathogens. In their study, *M. bovis* was the most commonly isolated species, and that the distribution of *Mycoplasma* spp. varied by year, season, and herd. They recommended that BTM samples should be routinely examined for *Mycoplasma*, and all isolates should be speciated.

With an increase in the frequency of sampling, an increase in the number of bulk tanks with SA and SAG was observed. Detection of SA and SAG in successive BTM samples taken from the same herd over a period of time is a good indicator that cows with SA and SAG infection are present in the herd. Based on the findings of our study, it can be inferred that several BTM sam-

ples must be examined before interpreting the findings of BTM analysis. These observations clearly provide evidence to support the recommendations of earlier reports that suggest that frequent sampling of BTM is needed to determine the true estimate of contagious mastitis pathogens (Farnsworth, 1993; Godkin and Leslie, 1993; Ruegg and Reinemann, 2002).

In conclusion, this study found that changes in BTSCC do reflect on the CNS count and frequency of isolation of SA and SAG from BTM. There was a low correlation between bacterial counts. Use of automatic milking detachers, teat dipping practices, pre- and postmilking teat disinfecting, and bedding type, influenced BTSCC and bacterial counts in BTM. Categorization of counts (low, medium, and high) can be used as guidelines for monitoring BTSCC and bacterial counts. The categorization of BTSCC and bacterial counts proposed in this study can be used for monitoring herd udder health and milk quality.

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