Risk factors for bacteriological quality of bulk tank milk in Prince Edward Island dairy herds. Part 1: Overall risk factors

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ABSTRACT

The objective of this study was to determine on-farm risk factors for bacteriological quality of bulk tank milk. Bulk tank raw milk quality was evaluated on all Prince Edward Island dairy herds ($n = 235$) over a 2-yr period (March 2005 to March 2007). Biweekly total bacterial, preliminary incubation, laboratory pasteurization, and coliform counts were conducted using a Petrifilm culture system. For the assessment of risk factors, a case-control study was conducted from January 2006 to May 2007. Case and control herds were defined based on the last 6 analyses of bulk tank bacterial counts before on-farm evaluation. Cases were herds that had multiple elevated counts for any of the parameters measured. A total of 69 herds (39 cases and 30 control herds) were evaluated. Data collection included 1) observation and questionnaire on basic hygiene and farm management practices; 2) complete wash analysis of the milking equipment, monitoring the presence of bacterial films on equipment and evaluation of cooling system function; and 3) environmental and cow hygiene scoring. Data were analyzed using multivariable logistic regression. The results of the final model indicated that high alkalinity in the wash water and poor teat-end cleanliness were associated with high bacterial counts in bulk tank milk (odds ratios = 12 and 5.3, respectively). It was also observed that high water temperature of detergent wash and the use of a water softener were associated with low bacterial counts in bulk tank milk (odds ratios = 0.87 and 0.11, respectively). A significant association between udder hair clipping and teat-end cleanliness was also observed. In conclusion, this study highlights the importance of udder hygiene and milking system washing factors on hygienic quality of bulk tank milk.

Key words: bulk tank milk, bacteria quality testing, risk factor

INTRODUCTION

Periodic examination of bulk tank milk (BTM) is useful for monitoring and evaluating raw milk quality produced on the dairy farm. High bacterial counts in raw milk can affect the quality of pasteurized milk and milk products, resulting in lowered shelf life and reduced consumer acceptance of milk and milk products (Barbano et al., 2006; Jayarao et al., 2006; Keefe and Elmoslemany, 2007). Estimation of the type and number of bacteria in BTM is valuable in understanding and troubleshooting issues related to udder health, milk harvest hygiene, cleaning practices, and milk storage conditions. Tests for milk quality include total aerobic count (TAC), which is a recognized alternative and is considered equivalent to the standard plate count (Wehr and Frank, 2004), preliminary incubation count (PIC), laboratory pasteurization count (LPC), and coliform count (CC) (Murphy, 1997). Among these tests, TAC is the most frequently used in regulatory programs and reflects the general hygienic condition during milk production. Each of the other tests identifies contamination sources of potential concern for milk quality (Murphy and Boor, 2000).

Microbial contamination of raw milk may occur from 3 main sources: from within the udder (mastitis-associated organisms), from environmental organism transfer via dirty udder and teat surfaces, and from improperly cleaned and sanitized milking equipment. Additionally, improper cooling and prolonged storage of milk can also influence bacterial count by increasing the rate of bacterial growth during storage of milk. Because different types of bacteria can contaminate BTM through various and multiple sources, it is not always straightforward to determine the cause of high bacterial count in milk (Murphy and Boor, 2000). Accordingly, several guidelines have been developed during the last 2 decades to facilitate the interpretation of BTM bacterial counts and to relate these counts to different sources of contamination on the farm (Murphy, 1997; Jayarao and Wolfgang, 2003; Jayarao et al., 2004).

Received October 17, 2008.

Accepted January 21, 2009.

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Although it is well recognized that good quality raw milk is essential for producing quality milk and milk products, limited information is available on the influence of management factors on bulk tank bacterial counts. Furthermore, no observational studies have been conducted to evaluate multiple risk factors and the interactions that might exist between these factors that could affect the quality of raw milk. The focus of this study was to determine on-farm risk factors that could be associated with elevated BTM bacterial count using a case-control study design.

MATERIALS AND METHODS

Herd Demographic Data

The average herd size and production of Prince Edward Island dairy herds was recently reported to be 60 cows and 8,894 kg/cow per year, respectively (Olde Riekerink et al., 2006). Herds in this study were similar in size (58 cows/herd). In this study, lactating cows were housed in tie stalls (60%), free stalls (27%), or straw pack (13%). Additionally, 66% of the farms had a pipeline milking system, 29% had a milking parlor, and 5% used bucket milking systems. On 74% of farms, cows had access to pasture during summer, whereas 13% of herds provided cows only outdoor access to an exercise yard and in 13% of herds cows were confined year round.

Study Design

Bulk tank raw milk was collected from all Prince Edward Island dairy herds ($n = 235$) every other week by licensed milk haulers over a 2-yr period (March 2005 to March 2007). For each sample, TAC, PIC, LPC, and CC counts were conducted using Petrifilms (3M Canada, London Ontario) according to *Standard Methods for Examination of Dairy Products* (Richardson, 1985; Wehr and Frank, 2004). Petrifilms were read electronically using the Petrifilm Plate Reader (3M Canada, London, Ontario, Canada), and data were stored in Excel spreadsheets (Microsoft, Seattle, WA) before transfer into statistical analysis software.

For the assessment of risk factors, a case-control study was conducted from January 2006 through May 2007. Case and control herds were defined based on the last 6 analyses of bulk tank bacterial counts before on-farm evaluation (approximately 3 mo). To be classified as a case, the herd was required to have at least 4 high TAC, PIC, or CC measurements or 3 high LPC measurements out of the last 6 analyses. Case definition was based on the distribution of the number of high counts for each

test for all Prince Edward Island dairy herds in the year preceding the study. Thresholds for high counts were $>20,000$ cfu/mL for TAC, $>50,000$ cfu/mL for PIC, >200 cfu/mL for LPC, and >50 cfu/mL for CC (Murphy, 1997). Control herds had low counts in all of the last 6 analyses for all the parameters (TAC, PIC, CC, and LPC) of interest. Every 3 mo throughout the study period, a list of case and control herds was created and herds were selected from these lists using a formal random procedure. Once a farm was selected, it became ineligible for reselection even if its status changed later on in the study period. Herd was the study unit and the target population included all dairy herds in Prince Edward Island, Canada.

On-Farm Data Collection

There were 3 main aspects in the data collection process. The first aspect was observation and recording of data on basic hygiene and management practices in 4 main areas: 1) general farm demographics and management, 2) cow cleanliness and hygiene, 3) milking procedures and mastitis control, and 4) equipment maintenance and cleaning.

The second aspect was a full evaluation of equipment hygiene and cooling function. This evaluation was carried out by a single experienced technologist trained in equipment analysis on all farms. Evaluations of the equipment wash and sanitization cycle were conducted for both the milking system and the bulk tank. This analysis consisted of recording the start and end water temperature ($^{\circ}\text{C}$) of each cycle (water rinse, alkaline wash, acid rinse). For the milking system, wash solution temperature was recorded on samples taken from the wash sink immediately before the solution went into the system and after draining start using a digital thermometer. For bulk tank, temperature was recorded during the filling and draining of each cycle. For the alkaline wash cycle, the alkalinity (ppm), chlorine content (ppm), and pH were also recorded. For the acid rinse cycle, the pH was recorded. Finally, a water hardness score in grains per gallon (**gpg**) was conducted on the water source used for wash procedures. All chemical testing was conducted using LaMotte water quality testing kits (LaMotte Co., Chestertown, MD).

For evaluation of physical cleaning, the cleaning-ball function of the bulk tank was scored by observing the velocity and spray distribution during the wash cycle and scored on a scale from 1 (poor) to 5 (excellent). To assess the physical cleaning of the pipeline, the same experienced technician on each farm used a variety of methods including graphing the vacuum using Bou-Matic system analyzer SA II (BouMatic, Madison, WI),

comparing firing time to the pipeline length, viewing the slug at the receiver jar, and manual palpation of slug consistency at various sites in the system. These criteria were used to score the slug on a scale from 1 (poor) to 5 (excellent). The presence of organic films on equipment was measured using the ATP bioluminescence technique (Charm LUMinator T and PocketSwab Plus, Charm Science Inc., Lawrence, MA). Eight sites were evaluated: 5 in the milking system (pipeline near and far inlets, receiver jar, diverter valve, and cross-over pipe) and 3 in the bulk tank (outlet, agitator paddle, and back wall). Pocket swabs (Ecolab Inc., St. Paul, MN) were used to swab the surface of each site according to the instruction of the manufacturer. Additionally, pipeline near and far inlets and the bulk tank were visually inspected after drying, using an approximately 10 million lx light, and were given a score from 1 to 6. The scale for milk surface visual scoring was thus: 1 = clean and shiny; 2 = <1% film covering; 3 = 1 to 5% film covering; 4 = >5 and <15% film; 5 = 15 to 40% film; and 6 = >40% film covering.

For evaluation of milk cooling system efficiency, milk holding temperatures were recorded electronically every minute for one on-farm storage cycle (approximately 2 d) using a Hobo U12 temperature data logger (Onset Computer Corp., Bourne, MA).

The third aspect consisted of environmental and cow hygiene scoring. Based on the number of rows in the stables, 4 to 6 milking-cow stalls were selected at random for hygiene scoring of the stalls. Additionally, 2 dry-cow stalls and 1 calving pen were also scored whenever available. If more than 2 dry-cow stalls and 1 calving pen were available on a farm, a formal random selection procedure was followed. Stalls were scored for cleanliness using a 4-point scale: 1 = stall clean with dry bedding; 2 = <20% of the stall soiled with urine or manure; 3 = 20 to 40% soiling of the stall; and 4 = >40% soiling of the stall. At the same time, bedding samples from scored stalls were collected and evaluated for total aerobic and coliform counts. Five bites of bedding from the back of the stall were gathered by hand with sterile gloves into a plastic zip bag and kept in a cooler until submission to the laboratory.

Cows in the selected stalls were also evaluated for hygiene status using the hygiene scoring card from Pharmacia Animal Health (adapted from hygiene scoring card, School of Veterinary Medicine, University of Wisconsin, Madison). Three areas were scored on a scale from 1 (clean) to 4 (dirty): the udder, the lower leg, and the flank. Additionally, 4 milking cows were evaluated for teat-end cleanliness, before attaching the milking unit, using a cotton swab to check the effectiveness of teat sanitation and drying using the 4-point scoring system described by Cook and Reinemann (2007).

Each farm assessment took 2 trained technicians approximately 4 h to complete: one investigator was responsible for evaluation of cow and environment hygiene and the other for evaluation of the milking system. Both investigators and the farmers were blind to the case-control status of the farms. Copies of the materials used in the on-farm data collection are available from the corresponding author upon request.

Statistical Analyses

Data Manipulation. Data from questionnaire and on-farm evaluation forms were coded and entered twice with data-entry software (EpiData Entry; Lauritsen and Bruus, 2006) and both entries were compared with check for errors. Both laboratory and on-farm data were merged into a single database using Stata version 10 (Stata Corp., College Station, TX).

Predictors that were measured at the cow level (udder, leg, flank, teat-end cleanliness, and stall hygiene) were converted to herd-level variables by taking the average score of each category. An average for each of udder, leg, and flank hygiene scores was calculated for milking cows and for combined dry cows and calving pen cows. In the same way, an average stall hygiene score was calculated for each of the milking cow stalls and for combined dry cow stalls and calving pens.

Variables measuring similar management procedures were grouped together by taking their average, and their internal consistency was assessed using Cronbach's α (Cronbach, 1951). Cronbach's α measures the level of correlation among sets of variables recorded at the same time. For a set of variables to be grouped into a block, α should be at least 0.70. Two additional measurements were also calculated during evaluation of α : item rest correlation (IRT), the correlation between an item and the scale that is formed by all other items, and average inter-item correlation (AIIC) of all items.

Unconditional Associations. Unconditional association between the outcome of interest (being a case with high BTM bacterial counts versus control with low BTM bacterial counts) and each of the predictors was examined using simple logistic regression. Only predictors showing association with the outcome of interest at $P \leq 0.15$ were considered for subsequent multivariable analyses.

Multivariable Analyses. Variables that were significant in the unconditional analyses were offered into multivariable logistic regression model. Nonsignificant variables were removed sequentially using backward elimination at $P < 0.05$. Two-way interactions among all predictors that were significant in the final main effect model were evaluated. The fit of the final model was assessed using the Hosmer-Lemeshow test. Obser-

Table 1. Characteristics of 69 dairy herds that participated in a case-control study of on-farm risks for raw milk quality in Prince Edward Island, Canada

Parameter	Percentage of herds ¹		<i>P</i> -value ²
	Cases (n = 39)	Controls (n = 30)	
Type of housing			0.478
Tie-stall	56	70	
Free-stall	31	23	
Manure/straw pack	13	7	
Milking system			0.756
Pipeline	62	70	
Parlor	33	27	
Buckets	5	3	
Bedding material			0.179
Straw	74	63	
Wood products	21	37	
Sand	5	0	
Frequency of bedding change			0.551
Twice a day or more vs. lower freq	67	73	
Udder clipping (yes vs. no)	56	83	0.015
Milking procedures			
Strip = yes	36	40	0.727
Prewipe ³ teats = yes	26	37	0.324
Wash = yes	41	23	0.122
Pre-dip = yes	51	67	0.199
Udder drying = yes	87	90	0.717
Frequency of pipeline cleaner use			
Detergent: 2/d vs. lower	82	96	0.091
Acid: 2/d vs. lower	88	96	0.236
Sanitizer: 2/d vs. lower	85	94	0.325
Frequency of bulk tank cleaner use			
Detergent: after each pickup vs. other	92	96	0.261
Acid: after each pickup vs. other	94	96	0.733
Sanitizer: after each pickup vs. other	89	93	0.694
Bulk tank cleaning			
Automatic vs. manual	79	93	0.105
Milk house water check			
Bacteria: 1/yr vs. longer than 1/yr	31	70	0.001
Hardness: 1/yr vs. longer than 1/yr	31	63	0.007
Water softener: yes vs. no	10	37	0.008

¹Percentage of herds that apply specific management procedure.²*P*-value was calculated based on Pearson chi square.³Udder wiping before disinfectant application.

variations that did not fit or had substantial effect on the model were also examined using standardized residuals, leverage values, and delta-betas (Dohoo et al., 2003).

RESULTS

Data Description

A total of 69 herds (39 cases and 30 controls) were evaluated for on-farm risk factors for high bacterial count in bulk tank milk. Table 1 shows the main characteristics of dairy herds that participated in the study according to their case-control status. Both case and control herds had similar herd size with a mean of 69 cows per herd including milking and dry cows. Compared with control herds, case herds were less likely to clip udder hair, use pipeline detergent twice a day, have

automatic cleaning of bulk tank milk, do a yearly check of milk house water for bacteria and hardness, or use a water softener ($P < 0.10$). On the other hand, a higher percentage of case herds used water to wash the teats for premilking udder preparation ($P \leq 0.15$).

A comparison of the factors related to wash analysis of the milking system between case-control herds is shown in Tables 2 and 3. For wash solution temperature, the main differences between cases and controls were the bulk tank alkaline wash start and ending temperatures, where the proportion of herds that had acceptable or higher water temperature was at least 15% greater in the control group. However, the majority of both cases and controls failed to achieve the recommended temperature. Chemical analyses of the wash solution (Table 3) showed that most of case herds had hard water (>6 gpg) and a high concentration of pipeline

Table 2. Bulk tank and pipeline wash analyses (wash solution temperature) of the 69 herds that participated in a case-control study of on-farm risks for raw milk quality

Parameter	Threshold, ¹ °C	Percentage of herds ²		<i>P</i> -value ³
		Cases (n = 39)	Controls (n = 30)	
Bulk tank wash analyses				
Pre-wash rinse				
Prewash fill temperature	55	27	41	0.140
Prewash drain temperature	38	22	21	0.927
Chlorinated alkaline wash				
Starting temperature	77	0	17	0.008
Ending temperature	43	31	47	0.177
Pipeline wash analyses				
Pre-wash rinse				
Starting temperature	55	0	7	0.102
Ending temperature	38	6	16	0.234
Chlorinated alkaline wash				
Starting temperature	77	0	0	—
Ending temperature	43	31	38	0.537

¹According to IDF Bulletin (Reinemann et al., 2003).

²Percentage of herds that had water temperature $\geq$ recommended value.

³*P*-value was calculated based on Pearson chi square.

alkaline detergent (>500 ppm). Visual inspection of the bulk tank after cleaning and drying indicated that only 11% of case herds and 14% of control herds had a clean and shiny tank, whereas, for pipeline inlets, 21% of cases and 40% of controls were clean and shiny.

There were no differences between cases and controls with regard to different factors related to milk cooling temperature. The overall mean holding temperature was 2.8 versus 3.1°C, and the mean peak temperature after addition of the second milking was 8.5 versus 9°C in case and control herds, respectively. The mean number of minutes above 6°C during the 2-d pickup cycle was 133 versus 151, and the mean number of minutes above 8°C was 33 versus 54 in case and control herds, respectively.

Cow and stall hygiene scores are presented in Table 4. Control herds had a lower (cleaner) hygiene score for each of milking cow stalls, udder, leg, flank, and teat-end cleanliness. The mean natural logarithm of TAC for bedding from milking cow stalls was 18.92 (16.4×10^7 cfu/g) and 18.72 (13.4×10^7 cfu/g) for case and control herds, respectively, and the mean CC was 9.49 (13.2×10^3 cfu/g) and 9.21 (10×10^3 cfu/g) for case and control herds, respectively; however, these differences were not significant.

Unconditional Associations

Table 5 shows predictors that were unconditionally associated ($P \leq 0.15$) with high or low bacterial counts in BTM. These variables can be divided into 4 main groups: 1) hygiene-related factors; 2) variables related

to water temperature during the start and end of different washing cycles of pipeline and bulk tank; 3) predictors related to chemistry of the wash solution; and 4) other equipment-related factors.

The variables udder, leg, and flank hygiene were all significant in the unconditional association and were also highly correlated. Cronbach's α for these variables was 0.84 (Table 6). Therefore, an index (cow hygiene score) was created by taking the average of these variables: cow hygiene score = [(udder + leg + flank scores)/3]. Similarly, the variables describing the start and end temperatures of bulk tank and pipeline alkaline detergent wash cycles were significant and highly correlated; their Cronbach's α was 0.83 (Table 6). As such, an index (detergent wash temperature score) was created. Detergent wash temperature score = [(bulk tank alkaline wash fill + bulk tank alkaline wash drain temperature + pipeline alkaline wash start + pipeline alkaline wash end temperature)/4].

Multivariable Association

Multivariable statistical analyses produced a model with 4 significant ($P < 0.05$) predictors (Table 7). Two-way interaction terms between these variables were not significant. There was no evidence of lack of fit of the model as indicated by the Hosmer-Lemeshow goodness-of-fit test ($P = 0.75$). According to the final model, the risk of being a case (high bacterial counts in BTM) was minimized when detergent wash temperature was high, a water softener was used, pipeline alkaline wash alkalinity was moderate, and teat ends were clean.

Table 3. Bulk tank and pipeline wash analyses (chemistry and physical action) of the 69 herds that participated in a case-control study of on-farm risks for raw milk quality

Parameter	Percentage of herds ¹		<i>P</i> -value ²
	Cases (n = 39)	Controls (n = 30)	
Bulk tank wash analyses ³			
Chlorinated alkaline wash			
Active chlorine level ≥ 100 ppm	44	59	0.244
Active alkalinity level (ppm)			0.946
<400	31	33	
400–800	46	47	
>800	23	20	
Alkaline wash pH ≥ 11	69	73	0.710
Acid rinse pH <3.5	97	96	0.881
Pipeline wash analyses ³			
Chlorinated alkaline wash			
Active chlorine level ≥ 75 ppm	64	72	0.566
Active alkalinity level			0.021
<250	05	13	
250–500	44	67	
>500	51	20	
Alkaline wash pH ≥ 11	92	97	0.442
Acid rinse pH <3.5	91	89	0.773
Water hardness score (gpg) ⁴			0.047
≤ 6	29	57	
7–9	37	29	
≥ 10	34	14	
Physical action			
Cleaning ball function score ≥ 4	77	78	0.974
Slug score ≥ 4			
Beginning	21	31	0.322
Middle	26	28	0.857
End	31	38	0.537

¹Percentage of herds that were $\geq$ recommended value.²*P*-value was calculated based on Pearson chi square.³Thresholds for chlorine, alkalinity, and pH were selected according to IDF Bulletin (Reinemann et al., 2003).⁴gpg = grains per gallon.

DISCUSSION

The production of high-quality milk with low bacteriological counts begins at the farm and involves multiple factors related to cow, environment, and equipment hygiene. The aim of this study was to identify on-farm management factors that influence microbial contamination of BTM, so that the producers can use these results to improve their milk quality.

Data Quality and Study Power

This study involved evaluation of a large number of on-farm factors that may influence bacterial quality of BTM. These factors were related to 3 major areas—environment, cow, and equipment hygiene. All efforts have been made to ensure high quality of the collected data. All evaluations were done by well-trained and experienced technicians who were blind to the case-

control status of the farm. The dairy producers were also blind to the case-control status of their farms and were advised during the on-farm visit to do the procedures as they routinely performed them. If some of the case herds were able to guess their case status, this might have led to underestimation of the effect of some predictors because producers may have used procedures consistent with industry standards during the farm visit, not their routine methods. The majority of risk factors were recorded using objective rather than subjective methods, which ensured correct classification of exposure status of the farms. Even factors that were recorded on a subjective scale were evaluated by the same investigator. Consequently, any source of misclassification would be expected to be equal among cases and controls and if this resulted in any bias, it would be toward the null (i.e., underestimation of the effects). Cases were defined as having 3 to 4 (or more) high counts out of the last 6 analyses to represent vari-

Table 4. Median and mean stall hygiene, cow hygiene, and teat-end cleanliness scores for case and control herds in the study of on-farm risks for raw milk quality

Parameter	Cases (n = 39)		Controls (n = 30)	
	Median	Mean (SD)	Median	Mean (SD)
Milking stall	1.75	1.80 (0.69)	1.75	1.67 (0.55)
Dry stall and calving pen	2.00	2.15 (0.82)	1.42	1.84 (0.61)
Milking cow udder	1.50	1.51 (0.44)	1.33	1.36 (0.30)
Milking cow leg	2.00	2.05 (0.62)	1.75	1.80 (0.51)
Milking cow flank	1.50	1.75 (0.65)	1.29	1.52 (0.50)
Dry and calving cow udder	1.00	1.30 (0.55)	1.00	1.24 (0.39)
Dry and calving cow leg	1.50	1.73 (0.63)	1.83	1.87 (0.66)
Dry and calving cow flank	1.50	1.57 (0.83)	1.58	1.81 (0.81)
Average teat cleanliness	1.44	1.60 (0.61)	1.12	1.27 (0.36)

ous degrees of severity of cases and to avoid evaluation of very bad herds only. On the other hand, controls were limited to herds with low bacterial count in all tests in the last 6 analyses to avoid misclassification of cases as controls.

Although data were extensive and of good quality, we were able to evaluate only 69 herds because of budget and labor limitations. Because of the relatively small number of herds included in the study, only variables

that were strongly associated with bulk tank bacterial count could be evaluated in the final model. The absence of a particular variable from the final model may be due to the limited sample size. Additionally, the effect of some variables could not be evaluated because of an absence of variation between cases and controls. For example, bulk tank milk cooling temperature was relatively consistent between cases and controls for all herds.

Table 5. Unconditional associations ($P \leq 0.15$) between case-control status and on-farm risks for raw milk quality in Prince Edward Island, Canada

Variable	Odds ratio	P-value
Hygiene-related factors		
Teat wash (no vs. yes)	2.28	0.122
Udder hair removal (no vs. yes)	0.26	0.015
Teat-end cleanliness score	4.50	0.007
Cow hygiene score ¹	2.70	0.078
Average udder hygiene	2.52	0.150
Average leg hygiene	2.09	0.095
Average flank hygiene	1.88	0.140
Wash analyses: Temperature		
Bulk tank prewash fill temperature	0.98	0.060
Bulk tank prewash drain temperature	0.96	0.036
Pipeline rinse start temperature	0.96	0.103
Detergent wash temperature score ²	0.90	0.005
Bulk tank alkaline wash fill temperature	0.93	0.014
Bulk tank alkaline wash drain temperature	0.95	0.046
Pipeline alkaline wash start temperature	0.92	0.021
Pipeline alkaline wash end temperature	0.93	0.059
Wash analyses: Chemistry		
Pipeline alkaline wash alkalinity	1.003	0.002
Water hardness ≤ 6 (baseline)		0.047
7–9	2.54	0.114
≥ 10	4.73	0.025
Water softener (no vs. yes)	0.20	0.008
Pipeline detergent frequency (twice vs. once/day)	5.55	0.073
Other milking equipment-related factors		
Bulk tank outlet bioluminescence score >0	2.25	0.104
Pipeline far inlet bioluminescence score >0	2.62	0.053
Tank cleaning (manual vs. automatic)	0.28	0.092
Milk house water bacterial check: yearly vs. longer	5.25	0.001
Milk house water hardness check: yearly vs. longer	3.90	0.007

¹ Average of udder, leg, and flank scores.

² Average of alkaline wash start and end temperatures of bulk tank and pipeline.

Table 6. Evaluation of Cronbach's α for the predictors describing the same management procedure¹

Within-block factor	Observations, n	IRC	AIIC	α
Block 1: cow hygiene score				
Udder	69	0.67	0.70	0.82
Leg	69	0.71	0.64	0.78
Flank	69	0.75	0.60	0.75
Test scale	—	—	0.64	0.84
Block 2: detergent wash temperature				
Bulk tank alkaline wash fill	69	0.63	0.58	0.81
Bulk tank alkaline wash drain	69	0.66	0.56	0.79
Pipeline alkaline wash start	69	0.75	0.50	0.75
Pipeline alkaline wash end	68	0.63	0.58	0.81
Test scale	—	—	0.56	0.83

¹IRC = item rest correlation; AIIC = average inter-item correlation.

Unconditional Association

Four groups of predictors were identified as having unconditional associations. The first group included factors related to cow management: cow hygiene score (combination of udder, leg and flank), teat-end cleanliness score, and udder hair removal. Several studies have identified relationships between cow cleanliness and milk quality as measured by SCC (Schreiner and Ruegg, 2003; Reneau et al., 2005; Ellis et al., 2007). For example, using a 4-point udder hygiene score (clean = 1 or 2) and (dirty = 3 or 4), Schreiner and Ruegg (2003) have shown that SCC and rate of intramammary infection are higher for dirty animals. Our study further identified the relationship between cow hygiene or teat-end cleanliness score and bacterial count in BTM, with high hygiene score (dirty) being associated with increased risk of having a high bacterial count in BTM.

Cow cleanliness can also affect the efficiency of cow preparation before milking, where dirty cows can double cow preparation time (Reneau and Bey, 2007). Cleanliness of the udder and teats can be influenced by several factors, including transition from summer grazing to winter housing (with housed cows being dirtier than grazing cows; Ellis et al., 2007), fecal consistency (where increasingly fluid fecal consistency correlated with dirtier cows), frequency of bedding change and quality of bedding (Ward et al., 2002), and stage of lactation (Reneau et al., 2005).

There are few studies on the effect of udder hair clipping on milk quality. Barkema et al. (1998) reported that herds with low SCC were practicing udder hair clipping more frequently (84 vs. 62%) than herds with high SCC. This study showed that clipping udder hair is associated with having lower bacterial count in BTM (odds ratio = 0.26). Additionally, there was a significant association between udder hair clipping and teat-end cleanliness score ($P = 0.01$), where ud-

der hair clipping was associated with a reduction of 0.35 units on the cleanliness scale (indicating cleaner teats). These results confirm the importance of udder hair clipping in decreasing bacterial contamination of raw milk. A study by Bartlett et al. (1992) reported a weak association between clipping udders and decreased intramammary coliform infection. On the other hand, Silk et al. (2003) evaluated the effect of removing udder hair surrounding the teats on milk quality, and found that udder hair removal had no effect on milk quality as measured by bacterial count of milk and on the teat surface. They suggested that the lack of effect of udder hair removal might be related to the use of predipping for udder preparation in the study herds, which may be sufficient to remove teat skin bacteria regardless of udder hair removal.

The other 3 groups of predictors are all related to cleaning of the milking system. Proper cleaning and sanitation of the milking system is one of the most important factors in producing high-quality raw milk. It involves a combination of thermal, chemical, and physical processes. A deficiency in any of these parameters could result in soil build-up, which provides nutrients for growth and multiplication of bacteria between milking times (Reinemann et al., 2003). In this study, high water temperature of the rinse and alkaline detergent wash solution were always associated with low bacterial count in BTM. Hot water is important for emulsifying milk fat and dispersing milk protein, as well as increasing the cleaning efficiency of chemicals (Reinemann et al., 2003). Therefore, checking the hot water supply for adequate temperature is necessary to ensure proper cleaning temperature.

Water is the most important ingredient in cleaning and sanitizing solutions. The properties of water on the farm can affect the cleaning process and milk quality. Our results showed that herds with medium or high water hardness scores were 2.5 and 4.7 times more likely, respectively, to have high bacterial count in BTM than

Table 7. Final multivariable logistic regression model for on-farm risks for raw milk quality in Prince Edward Island, Canada

Case-control	Odds ratio	P-value	95% confidence interval
Teat-end cleanliness score	5.27	0.027	1.20–23.09
Detergent wash temperature score ¹	0.87	0.014	0.77–0.97
Water softener (yes vs. no)	0.11	0.013	0.02–0.62
Pipeline alkaline wash alkalinity >500 ppm	11.88	0.001	2.66–53.18

¹Average of alkaline wash start and end temperatures of bulk tank and pipeline.

herds with lower hardness score. Hard water can reduce the effectiveness of cleaning chemicals and may lead to formation of films or deposits on the milking system (Cords et al., 2001). Additionally, less-frequent checks of milk house water for bacterial contamination and hardness was associated with higher risk of having high bacterial count in BTM. Untreated water could be a source of contamination of milking equipments with *Pseudomonas* spp., coliforms, and other gram-negative bacteria (Chambers, 2002). Consequently, regular monitoring of microbiological and chemical properties of water will help in identifying water quality problems and implementing the necessary treatment in the proper time.

The efficiency of cleaning and sanitation of the milking equipment was assessed using an ATP bioluminescence method. The ATP method is fast and simple and can monitor both microbial contamination and milk residues (Reinemann and Ruegg, 2000).

In this study, bioluminescence readings of bulk tank outlet and far inlet of the pipeline were associated with BTM bacterial count, with high score being associated with higher bacteria count; however, there was no association for the other 6 sites. Reinemann and Ruegg (2000) indicated that there is considerable variation in the ATP data, and the method must be used carefully and with sufficient number of ATP swabs to obtain meaningful results. Our data support this conclusion and indicate that although ATP testing may be helpful in hygiene investigations, it must be used as part of thorough system evaluation.

Multivariable Association

The final model had 4 significant predictors ($P < 0.05$), with high temperature of the detergent wash and the use of a water softener being protective, and high alkalinity and high (dirty) teat-end cleanliness score being risk factors. Adjusted for other variables in the model, for every 1°C decrease in detergent wash temperature below the average temperature score (52°C), the likelihood of having high bacterial counts in BTM increased by a factor of 1.15. Consequently, a 10°C decrease will increase the risk by a factor of 4.

These results confirm the importance of water temperatures as indicated above. It also supports the report of Guterbock et al. (1984) who noted that cleaning efficiency doubles with every 10°C increase in temperature.

Similarly, the risk of having high bacterial count in BTM will increase by 5.3 for each unit increase in teat-end cleanliness score. Contrary to our expectation, high alkalinity of detergent wash was associated with an increased risk of high bacteria count (odds ratio = 12). Alkaline detergent wash is important for dissolving milk fats, proteins, and carbohydrates and for dispersion and suspension of other soil particles so that they can be removed by mechanical action (Reinemann et al., 2003) and prevented from deposition on the milking system. A possible explanation of this finding is that higher alkalinity was the result of increased detergent use by producers in response to high bacterial count problems. Finally, the use of a water softener was associated with decreased risk of having high bacterial count (odds ratio = 0.11). The effect of water softener use could be explained through the prevention of water hardness problem and its influence on milk quality.

CONCLUSIONS

The results of this study highlight the importance of udder hygiene, temperature of the wash solution used for cleaning the milking system, and water quality-related factors for hygienic quality of BTM. Although only 4 predictors remained in the final models, all factors in the univariable association should be considered for improvement of bacteriological quality of BTM.

ACKNOWLEDGMENTS

The authors acknowledge the technical support of Ron Sampson (Dairy Farmers of Prince Edward Island, Charlottetown, PE), Ricky Milton, Theresa Andrews, and Lloyd Dalziel (Atlantic Veterinary College, Charlottetown, PE). This research was funded by Dairy Farmers of Prince Edward Island, Agricultural Research Investment Fund (PEI Department of Agriculture, Charlottetown, PE), Purity Dairy (Charlottetown,

PE), and Amalgamated Dairies Limited (Summerside, PE). Personal funding for A. M. Elmoslemany was provided by Mission Office, Ministry of Higher Education, Egypt.

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