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Araştırma Makalesi ***Research Article***

Discrepancies in Evaluating Farm Management Routines as Risk Factors of Raw Milk and Udder Hygiene in Selected Dairy Farms of Fars Province, Iran

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Abstract

The management practices relevant to bulk tank milk quality were studied in 29 dairy farms of Fars Province, Iran. Farm management practices were obtained by completion of a questionnaire and direct observation. Bulk milk was evaluated by performing standard plate count (SPC), preliminary incubation count (PIC), laboratory pasteurization count (LPC), coliform count (CC), somatic cell count (SCC) and detection of the contagious mastitis agents. The farms were divided into low and high SPC groups (below and above 100,000 CFU/mL) based on Iranian standards. Comparisons of the laboratory results between groups were done using two independent samples *t*-test. The relationships between the laboratory results were studied by Pearson's correlation coefficients, all after logarithmic transformation. Associations of managerial risk factors (obtained by the questionnaire and one time of observation) with laboratory results were investigated using two independent samples *t*-test. P-values <0.05 were considered as significant. Both low and high SPC farms had PIC, LPC, CC and SCC levels above the relevant intervention limits, although the low SPC group had lower PIC and CC levels (P<0.001) and numerically lower LPC and SCC levels (P>0.05). Strong correlations were detected between SPC and PIC, SPC and CC, and PIC and CC, but many of the well explained risk factors of undesired milk quality lacked any relation with high bacterial counts of raw milk. This could be due to the small number of the studied farms, almost similar faults in the farms, wrong answers of the employees to the questions and modification of the milking practices in the presence of an inspector. Infections with *Staphylococcus aureus* and *Mycoplasma bovis* could be potential problems in the studied farms, contributing to the elevation of SCC and/or SPC levels. Veterinary interventions could not be based on the questionnaire results. Direct and frequent observations of farm routines could be recommended.

Introduction

Many Iranian dairy producers are rewarded for producing high quality milk. However, a number of large (Bolourchi et al., 2004; Bolourchi et al., 2008) and small (Hashemi and Shekarforoush, 2007; Hashemi and Shekarforoush, 2008) studies have found that a great many of the farmers do not produce high grade milk based on the current standards (Iran Standard Organization, Procedure No. 164; Jayarao and Wolfgang, 2003) by evaluating standard plate count (SPC) and somatic cell count (SCC) of bulk milk. The Iranian veterinarians who are asked for solving the problems routinely gather some information on farm management practices mostly based on questions (history taking) and recommend some interventional

practices. However, it appears from the personal experiences of the authors that these events will not suffice to localize and to resolve the problems. Bulk tank milk (BTM) analysis including SPC, SCC and more specific tests such as preliminary incubation count (PIC), laboratory pasteurization count (LPC), coliform count (CC) and culture of mastitis agents is known as a time saving, inexpensive tool to determine and to localize the existing and potential problems with milk quality and mastitis (Birtten, 1998; Birtten and Emerson, 1996; Bramley et al., 1984; Bramley and McKinnon, 1990; Emerson, 1989; Fenlon et al., 1995; Jayarao and Wolfgang, 2003; Jeffrey and Wilson, 1987; Keeter, 1997). The present study was conducted to study the accordance of the results of BTM analysis (SPC, SCC, PIC, LPC, CC and contagious mastitis agents) and the relevant management practices obtained by questionnaires and quick observation.

Materials and Methods

Study Design

This study was conducted in summer 2012 (temperature-humidity index >72 during daytimes) in 29 dairy farms with 21-160 milking cows in Fars province, Iran (the cities of Shiraz, Marvdasht, Zarghan, Sarvestan, Beyza, Saadatshahr and Abadeh). A questionnaire, pre-tested in three farms, was completed in all farms by a trained person of the study team who was also responsible for sample collection and farm inspection. The questionnaire comprised of 42 items in 3 categories: a) farm demographics (Table 2, the first 5 items); b) general farm management and cleanliness of cows' bodies; and c) milking practice and milk storage sanitation (Table 5). Only one herd was visited per day for inspecting the farm, interviewing with the farmer and/or milking stuff and attending a whole milking operation. Two hours after milking, the BTM samples of the same milking were collected with a clean dipper from the top of the milk surface after 10 minutes of agitation as described by Jayarao and Wolfgang (2003). A 25 mL sample was poured into a sterile screw-cap tube for microbiological experiments and an additional 50 mL was transferred into a clean vial for SCC determination. The temperature of the BTM was also measured using a digital thermometer. The water of the farm was also sampled (25 mL) from a faucet in the milking parlor into a sterile tube. The water inside the pipe was run for a few seconds, then the tip of the faucet was heated for 30 seconds and the sample was taken after a few seconds of water running. The samples were transferred on ice to the laboratory within 2 to 4 hours after collection.

Microbiological tests and SCC

Microbiological assessments were started within 6 hours of sampling. Milk SPC, PIC, LPC and CC as well as water total count (WTC) and water coliform count (WCC) were determined using routine microbiological tests (Feng, 2002; Maturin, 2001). All milk samples were also subjected to DNA extraction and PCR assays for the detection of causative agents of contagious mastitis. The PCR conditions are provided in Table 1 (*Staphylococcus aureus* (Boss et al., 2011)*, Mycoplasma bovis* (Foddai et al., 2005)*, Streptococcus agalactiae* (Ahmadi et al., 2009) *and Corynebacterium bovis* (Lee et al., 2008). Somatic cell count was also estimated within 6 hours of sampling with an automated cell counter (Fossomatic™ FC, Denmark).

Table 1. Details of PCR assays for further identification of the bacteria.

	Primer pair (5'-3')	Anealing Temp. (°C)	Fragment size (bp)
S. aureus	ATAGAGATGCTGGTACAGG GCTTCCGATTGTTCGATG	57	720
M. bovis	AAGGTACACCAGCTAACCCAG GATCACTTTTTGGAAACTTAT	55	1420
	TATTGGATCAACTGCTGGAT AGATGCTCCACTTATCTTAG		
Step. Agalactiae	TTTGGTGTTTACACTAGACTG TGTGTTAATTACTCTTATGCG	59	207
Coryn. Bovis	CGTGCTTTAGTGTGTGCG GGCACGGAAATCGTGGAAG	60	750

Table 2. Frequencies of the farms with BTM microbial and somatic cell counts above the intervention levels.

	Intervention levels				
	SPC>1×10^5 CFU/mL*	PIC>5×10^4 CFU/mL**	LPC>2×10^2 CFU/mL**	CC>5×10^1 CFU/mL**	SCC>200,000 Cell/ml**
Number (out of 29)	15	17	7	24	19
Percent	52%	59%	24%	93%	66%

*Iran Standard Organization, Procedure No. 164
**Jayarao and Wolfgang, 2003

Table 3. Mean±SD of the quantitative data of the studied farms and low and high SPC groups.

	All farms (n=29)	Low SPC farms (≤1×10^5; n=14)	High SPC farms (>1×10^5; n=15)	P value
Daily milk average [a]	27.55±3.71	28.04±4.49	27.10±2.90	0.507
No. of all cows [a]	55.41±36.68	67.93±39.98	43.73±30.07	0.041
No. of workers [a]	3.17±2.16	4.00±2.69	2.4±1.12	0.027
No. of parlor workers [a]	1.48±0.57	1.71±0.61	1.27±0.46	0.034
Cow: worker ratio [a]	29.70±15.50	33.21±18.51	26.4±11.75	0.336
Tank temperature 2h after milking [a]	9.18±5.92	11.26±7.41	7.24±3.29	0.081
SPC [b]	5.04±1.09	4.09±0.38	5.94±0.68	<0.001
PIC [b]	5.48±1.28	4.52±0.94	6.35±0.86	<0.001
LPC [b]	2.02±0.63	1.81±0.59	2.21±0.62	0.090
CC [b]	3.10±1.15	2.14±0.58	3.99±0.74	<0.001
SCC [b]	5.41±0.42	5.27±0.23	5.54±0.52	0.079
WTC [b]	2.23±0.87	2.34±1.02	2.11±.73	0.487
WCC [b]	1.16±0.79	1.30±0.91	1.06±0.71	0.506

[a] True data are shown ; [b] Log.transformed data are shown
SPC: standard plate count; LPC: laboratory pasteurization count (thermoduric bacteria); PIC: preliminary incubation count (psychotropic bacteria); CC: coliform count; SCC: somatic cell count; WTC: water total count; WCC: water coliform count

Statistical Analysis

Data were analyzed using SPSS statistical software (version 16.0, SPSS, Inc., Chicago, IL, USA). The distribution of the quantitative data was tested by Kolmogorov–Smirnov test; logarithmic transformation was performed in the case of non-normality. The farms were divided into low and high SPC groups (below and above 100,000 CFU/mL) based on the Iranian standard (Iran Standard Organization, Procedure No. 164) to compare the results obtained by questionnaire and laboratory tests between the farms with superior/grade 1 milk (<30,000 and 30,000-100,000 CFU/mL, respectively) and those with poorer quality milk (>100,000 CFU/mL). The comparisons were also done in the farms with low (<200,000 cell/mL) and high (>200,000 cell/mL) SCC groups (Jayarao and Wolfgang, 2003) regards to affection by sub-clinical mastitis. Comparisons between groups were done using two independent samples t-test. The relationships between the SPC, PIC, LPC, CC and SCC levels of BTM were studied by Pearson's correlation coefficients, all after logarithmic transformation. Associations of managerial risk factors (obtained by the questionnaire and one time of observation) with SPC, PIC, LPC, CC and SCC were investigated using two independent samples *t*-test. Factors with P-values less than 0.15 were then evaluated in linear regression models in four subsets of management routines as a) premilking hygiene, b) hygiene during milking, c) equipment and environmental hygiene and d) miscellaneous factors (Table 5). Significant factors at P<0.05 in these models, then introduced in a final model and using stepwise procedure, final significant factors were identified. Separate models were constructed for each of SPC, PIC and SCC. In the final analysis, P-values less than 0.05 were considered as significant.

Table 4. Pearson's correlation coefficients between the microbial components of BTM (after logarithmic transformation) in 29 studied farms and the farms with SPC levels below and above the intervention level.

		LPC	PIC	CC	SCC
All farms (n=29)	SPC	0.22 P=0.24	**0.86** **P<0.001**	**0.88** **P<0.001**	**0.39** **P=0.035**
	LPC		0.26 P=0.17	0.25 P=0.20	-0.16 P=0.40
	PIC			**0.81** **P<0.001**	**0.39** **P=0.038**
	CC				**0.39** **P=0.046**
Low SPC farms <1×10^{5}CFU/mL (n=14)	SPC	0.34 P=0.229	**0.78** **P=0.001**	**0.66** **P=0.015**	-0.28 P=0.326
	LPC		0.23 P=0.43	-0.045 P=0.88	-0.18 P=0.54
	PIC			0.33 P=0.268	-0.15 P=0.617
	CC				-0.03 P=0.926
High SPC farms >1×10^{5}CFU/mL (n=15)	SPC	-0.34 P=0.220	**0.69** **P=0.005**	**0.60** **P=0.025**	0.35 P=0.206
	LPC		-0.14 P=0.629	0.09 P=0.761	-0.37 P=0.177
	PIC			**0.72** **P=0.004**	0.43 P=0.11
	CC				0.35 P=0.224

SPC: Standard plate count; LPC: laboratory pasteurization count (thermoduric bacteria); PIC: preliminary incubation count (psychotropic bacteria); CC: coliform count; SCC: somatic cell count

Results

Microbial and somatic cell count

In a number of the studied farms the microbial and SCC contents of BTM exceeded the relevant intervention levels (Iran Standard Organization, Procedure No. 164; Jayarao and Wolfgang, 2003) as shown in Table 2.

The mean±SD of the quantitative data of the studied farms are presented in Table 3. The means of SPC, PIC, LPC, CC and SCC in the sum of all studied farms were all above the relevant intervention levels. Both low and high SPC farms had PIC, LPC, CC and SCC levels above the intervention limits, although the low SPC group had lower (P<0.001) PIC and the CC levels (Table 3). LPC and SCC levels were numerically higher in the high SPC group (P>0.05). Comparison between high and low SCC groups revealed no difference in the levels of SPC, PIC, LPC and CC (P>0.05).

Correlation analyses between SPC and other bacterial components of BTM of all studied farms (Table 4) revealed that it had high correlations for PIC (r=0.86, P<0.001) and CC (r=0.88, P<0.001). PIC also showed a correlation with CC (r=0.81, P<0.001). LPC, however, did not show any correlation with other bacterial contents. SCC showed low correlations (r<0.4) with SPC, PIC and CC in the sum of all farms. In both low and high SPC groups the correlations between SPC with PIC and CC were repeated (r>0.5) but those of SCC with other components were absent. No correlation was detected between SCC and the microbial components of BTM in the studied farms with low and high SPC.

Some managerial factors showed significant associations with SPC, PIC and SCC at P<0.05 (Table 5). Neither of the managerial factors showed correlations with LPC. In the final regression models (Table 7) some managerial factors showed associations with bulk milk quality (P<0.05). Tank temperature control two hours after milking was inversely associated with SPC and PIC.

Table 5. Distribution of some management factors and their association with microbial components of BTM (after logarithmic transformation) in 29 studied farms in Fars province.

Management routines	SPC Mean±SD	PIC Mean±SD	SCC Mean±SD
Premilking hygiene			
Fore-stripping			
Yes (n=20; 69%)	4.95±1.06	5.36±1.31	5.43±0.42
No (n=9; 31%)	5.25±1.19	5.71±1.26	5.37±0.46
Using strip cups (No: 100%)			
Washing udders before milking			
Yes (n=25; 86%)	4.96±1.04	5.32±1.23	5.40±0.41
No (n=4; 14%)	5.57±1.38	6.39±1.39	5.51±0.55
Drying udders after wash			
Yes (n=21; 84%)	4.86±0.96	5.20±1.17	5.34±0.33
No (n=4; 16%)	5.48±1.48	5.92±1.56	5.72±0.67
Pre-dipping of the teats (No: 100%)			
Individual towels for drying udders			
Yes (n=16; 76%)	4.77±0.90	4.99±1.08	5.34±0.24
No (n=5; 24%)	5.15±1.19	5.90±1.29	5.35±0.59
Warm/hot water for washing the udders/cleaning milking equipment			
Yes (n=19; 76%)	5.03±1.06	5.20±1.18	5.46±0.43
No (n=6; 24%)	4.76±1.05	5.68±1.42	5.21±0.27
Wearing gloves during milking [ab]			
Yes (n=3; 10%)	3.86±0.54	3.90±0.52	5.30±0.15
No (n=26; 90%)	5.18±1.06	5.65±1.22	5.43±0.44

SPC: Standard plate count; LPC: laboratory pasteurization count (thermoduric bacteria); PIC: preliminary incubation count (psychrotrophic bacteria); CC: coliform count; SCC: somatic cell count; P<0.05, a: for SPC, b: for PIC, c: for SCC

Feeding cows immediately after milking showed associations with SPC, PIC and SCC. Cleaning in place (CIP) versus hand washing of the storage tanks was associated with PIC. Milking mastitic cows at the end of milking was significant in the final model for SCC in an inverse manner.

Agents of Contagious Mastitis

Nine farms out of 29 (31%) were contaminated in their BTM with one or two of the agents of contagious mastitis (Table 6). The contamination rate of the examined farms for *Staphylococcus aureus* and *Mycoplasma bovis* was 17.2% each, whereas for *Streptococcus agalactiae* and *Corynebacterium bovis* it was 6.9% and 3.4%, respectively. All farms contaminated with the agents of contagious mastitis had SCC levels above 200,000 cells/mL.

Discussion

Discrepancies between laboratory and questionnaire results

In a great proportion of the studied farms the means of measured components of BTM were above the intervention levels (Table 2). Even in low SPC farms the high levels of PIC, LPC, CC and SCC were concerning (Table 3). These findings could reveal a great need for revision of milking practices in the studied farms. The interventional events, however, could not be based on questionnaire results (yes/no answers) or one time observation of a whole milking operation (Tables 5). Such results were inconclusive in the present study.

The first discrepancy was observed in pre-milking udder preparation and could be concerning as it has deep influences on milk quality (Elmoslemany et al., 2009a, Elmoslemany et al., 2009b; Elmoslemany et al., 2010; Pankey, 1989). Fore-stripping, washing and drying of the udders and using individual towels were ignored in a number of farms, while none of the farms used strip cups or pre-dipped the teats (Table 5). The laboratory results, however, were almost similar between the two types of farms. Dirty udders and teats increase the levels of SPC, PIC (Elmoslemany et al., 2009a, Elmoslemany et al., 2009b; Elmoslemany et al., 2010; Vissers et al., 2007), LPC and CC (Chambers, 2002; Jayarao and Wolfgang, 2003; Murphy, 1997) in raw milk.

Table 5. Distribution of some management factors and their association with microbial components of BTM (after logarithmic transformation) in 29 studied farms in Fars province. Continue.

Management routines	SPC Mean±SD	PIC Mean±SD	SCC Mean±SD
Hygiene during milking			
Contact of the clusters with ground			
Yes (n=27; 93%)	5.05±1.12	5.46±1.29	5.42±0.44
No (n=2; 7%)	4.91±0.63	5.60±1.59	5.34±0.04
Contact of the clusters with cow tail			
Yes (n=23; 79%)	5.23±1.12	5.63±1.28	5.47±0.44
No (n=6; 21%)	4.34±0.58	4.84±1.17	5.19±0.25
Vacuum stop before removal of the clusters			
Yes (n=25; 86%)	5.04±0.99	5.43±1.22	5.38±0.42
No (n=4; 14%)	5.43±1.22	5.68±1.82	5.63±0.44
Automated cluster removal			
Yes (n=7; 28%)	4.36±0.65	5.01±1.42	5.22±0.24
No (n=18; 72%)	5.26±1.12	5.61±1.24	5.47±0.45
Flushing liners between milking two cows			
Yes (n=3; 10%)	5.34±1.08	5.33±1.36	5.12±0.16
No (n=26; 90%)	5.01±1.10	5.48±1.30	5.45±0.43
Alkaline wash of the equipment after all milking times			
Yes (n=7; 24%)	5.00±1.11	5.36±1.33	5.22±0.22
No (n=22; 76%)	5.06±1.11	5.50±1.30	5.48±0.46
Chlorinated alkaline detergent			
Yes (n=1; 3%)	4.79	6.86	5.39
No (n=28; 97%)	5.05±1.11	5.42±1.28	5.42±0.43
Acid wash of the equipment after all milking times [b]			
Yes (n=3; 10%)	3.97±0.52	3.94±0.50	5.29±0.16
No (n=26; 90%)	5.17±1.07	5.64±1.23	5.43±0.44
Cleaning in place (CIP)versus hand washing of the storage tanks [ab]			
Yes (n=4; 14%)	4.14±0.33	4.03±0.32	5.26±0.09
No (n=25; 86%)	5.19±1.10	5.70±1.23	5.44±0.45
Consideration of water hardness			
Yes (n=3; 10%)	5.04±1.48	5.54±1.79	5.23±0.26
No (n=26; 90%)	5.05±1.07	5.46±1.36	5.44±0.444
Rapid drainage of water/manure removal			
Yes (n=15; 52%)	5.08±1.16	5.34±1.33	5.34±0.39
No (n=14; 48%)	5.01±1.04	5.65±1.27	5.50±0.45
Teat dipping			
Yes (n=17; 59%)	4.88±0.99	5.38±1.30	5.30±0.38
No (n=12; 41%)	5.28±1.22	5.59±1.31	5.57±0.45

SPC: Standard plate count; LPC: laboratory pasteurization count (thermoduric bacteria); PIC: preliminary incubation count (psychrotrophic bacteria); CC: coliform count; SCC: somatic cell count; P<0.05, a: for SPC, b: for PIC, c: for SCC

Table 5. Distribution of some management factors and their association with microbial components of BTM (after logarithmic transformation) in 29 studied farms in Fars province. Continue.

Management routines	SPC Mean±SD	PIC Mean±SD	SCC Mean±SD
Equipment and environment hygiene			
Milking mastitic cows at the end of milking[c]			
Yes (n=24; 86%)	5.07±1.12	5.53±1.35	5.49±0.42
No (n=5; 14%)	4.92±1.03	5.18±0.99	5.06±0.23
History of chronic mastitis (recurrence 3 times or more per lactation)			
Yes (n=22; 76%)	5.06±1.07	5.47±1.29	5.43±0.42
No (n=7; 24%)	5.01±1.23	5.45±1.37	5.38±0.46
Replacement of the liners based on recommended milking times (No: 100%)			
Tank temperature control two hours after milking (declaring by the farmer)[ab]			
Yes (n=23; 79%)	5.27±1.05	5.81±1.17	5.47±0.45
No (n=6; 21%)	4.15±0.79	4.16±0.77	5.18±0.14
Free stalls versus open shed housing (milking cows)			
Yes (n=6; 21%)	4.92±0.56	5.57±1.11	5.34±0.41
No (n=23; 79%)	5.08±1.20	5.44±1.35	5.43±0.43
Free stalls versus open shed housing (dry cows)			
Yes (n=2; 7%)	5.34±0.02	6.24±0.69	5.67±0.47
No (n=27; 93%)	5.02±1.13	5.40±1.31	5.40±0.42
Dirt thighs and udders			
Yes (n=26; 90%)	5.38±0.93	5.82±1.19	
No (n=3; 10%)	5.01±1.11	5.43±1.31	
Feeding cows immediately after milking			
Yes (n=22; 76%)	4.85±0.96	5.25±1.16	5.32±0.30
No (n=7; 24%)	5.64±1.33	6.16±1.48	5.73±0.52
Specific cloths and boots for milking parlor (No: 100%)			
Supervision of the milking operation by the owners or managers			
Yes (n=2; 7%)	4.32±0.20	5.05±0.80	5.48±0.19
No (n=27; 93%)	5.10±1.11	5.50±1.32	5.41±0.44
Miscellaneous factors			
Feeding waste/mastitic milk to calves			
Yes (n=24; 86%)	4.97±0.89	5.93±0.99	5.29±0.47
No (n=5; 14%)	5.06±1.14	5.37±1.33	5.44±0.42
Receiving official reports on SPC and SCC (Yes: 100%)			
Bulk milk culture and analysis (No: 100%)			
Familiarity with California Mastitis test			
Yes (n=14; 48%)	4.93±0.95	5.45±1.22	5.30±0.37
No (n=15; 52%)	5.15±1.33	5.48±1.38	5.52±0.45
Performing California Mastitis Test routinely (No: 100%)			

SPC: Standard plate count; LPC: laboratory pasteurization count (thermoduric bacteria); PIC: preliminary incubation count (psychrotrophic bacteria); CC: coliform count; SCC: somatic cell count; P<0.05, a: for SPC, b: for PIC, c: for SCC

Table 6. Frequencies of the farms with bulk tank milk contaminated to agents of contagious mastitis.

	Number of contaminated farms (out of 29)	Percent
Contamination to one or several agents	9	31
Staphylococcus aureus	5	17.2
Mycoplasma bovis	5	17.2
Streptococcus agalactiae	2	6.9
Corynebacteriumbovis	1	3.4
Staphylococcus aureus + Mycoplasma bovis	1	3.4
Staphylococcus aureus + Corynebacteriumbovis	1	3.4
Mycoplasma bovis + Streptococcus agalactiae	2	6.9

Table 7. Results of the final regression models for association of managerial risk factors with standard plate count (SPC), preliminary incubation count (PIC) and somatic cell count (SCC).

	B[a]	SE[b]	T[c]	P-Value
SPC				
Constant	5.370	-	7.561	0.000
Tank temperature control two hours after milking (declaring by the farmer)	-1.212	-0.459	-2.798	0.010
Feeding cows immediately after milking	0.917	0.367	2.236	0.034
PIC				
Constant	3.747	-	2.768	0.010
Tank temperature control two hours after milking (declaring by the farmer)	-1.481	-.475	-3.224	0.004
Feeding cows immediately after milking	1.071	.363	2.574	0.016
Cleaning in place (CIP)versus hand washing of the storage tanks	1.169	.320	2.181	0.039
SCC				
Constant	5.398	-	18.031	0.000
Feeding cows immediately after milking	0.397	0.410	2.531	0.018
Milking mastitic cows at the end of milking	-0.406	-0.370	-2.283	0.031

a: Regression coefficient; b: Standard error; c: Statistical parameter
Adjusted R square for SPC, PIC and SCC were 26%, 45% and 27%, respectively

Drying of the teats is the most important step in udder preparation protocols (Elmoslemany et al., 2010) since the water remained on the teats caries some bacteria to milk (Galton et al., 1982). In the study of Elmoslemany et al. (2010) milking wet teats was associated with elevated SPC and PIC and even application of disinfectant towels without drying was associated with the highest bacterial counts. Also, using shared towels for drying several cows increased the bacterial counts of raw milk. Shared towels also reduce the efficiency of drying of the teats by increasing the risk of transmission of mastitis agents among the cows (Elmoslemany et al., 2010). Many farms in the present study that applied single-use towels used each side of the paper for drying separate cows.

Improper application and sanitation of the clusters during milking could also be areas of concern in the present study. Unclean or contaminated milking equipment is among the reasons for elevated SPC and CC (Jayarao and Wolfgang, 2003). Hashemi and Shekarforoush (2006 and 2008) reported a significant increase in SPC and CC in the milk samples obtained from milking machine compared with those obtained directly from cows' udders. Contact of the cluster with the floor of the parlor and cows' tails was seen in most of the studied farms (Table 5). Flushing and sanitation of the liners greatly reduce bacterial contaminations (Smith et al., 1985). None of the farms in the present study were equipped with automatic back-flushing systems. A few farms (n=3) immersed the clusters in large buckets of water, but their laboratory results were not different from those of other farms. The recommended method (Smith et al., 1985) of rinse-disinfectant-rinse hot solutions was not followed: all cups were dipped simultaneously that causes air lock in the claw. In addition, the buckets were placed so that they could be contaminated by manure spatter. Most of the farms (n=25) stopped the vacuum before detaching of the

clusters, but their laboratory results were not different from those that did not stop the vacuum (n=4). Automated removal of the clusters was not associated with lower microbial counts.

Cleaning and sanitizing of the milking machine and the storage tanks could reveal serious problems in the studied farms (Table 5) leading to elevated microbial counts in their milk (Table 3). Alkaline and acid wash of the equipment after each milking time was practiced only in 3 farms out of 29. Most of the farms only rinsed the equipment and applied alkaline and/or acid wash infrequently. It was surprising that with only rinsing without a chemical wash or hot water sanitization on a daily basis, the bacterial counts were not so different. Nearly all farms used sodium hydroxide instead of chlorinated alkaline detergents. Inadequate washing of the equipment could have allowed the precipitation of organic and inorganic materials inside the equipment leading to formation of milk stones as media for attachment and growth of bacteria. Alkali dissolves fat, protein and lactose while chlorine facilitates detachment of protein deposits (Jayarao and Wolfgang, 2003; Murphy, 1997). Acid prevents deposition of inorganic minerals (Reinemann et al., 2003) and also provides a bacteriostatic pH (Jayarao and Wolfgang, 2003; Murphy, 1997). Inadequate frequency of acid wash has been associated with elevated LPC (Elmoslemany et al., 2010). In the present study acid wash after all milking times was inversely correlated with PIC (Table 5). Most of the farms were not equipped with cleaning in place (CIP) systems and cleaned the storage tanks manually that increases the risk of elevated SPC and PIC (Elmoslemany et al., 2010) probably associated with infrequent use of detergent and acid and low water temperature (Elmoslemany et al., 2009b). Five farms did not have warm and hot water at the time of the visit. In the present study CIP of the storage tanks done in a few farms (n=4) was inversely correlated with SPC and PIC (Table 5).

Correlations

In the sum of all studied farms (n=29) strong correlations (r>0.8) were observed between SPC with PIC and CC as well as PIC and CC (Table 4). In low SPC farms, SPC had relationships with PIC and CC. In high SPC farms, an additional correlation was detected between PIC and CC. SCC showed low correlation coefficients (<0.4) with SPC, PIC and CC. LPC, however, did not show any correlation with other studied components of BTM. No correlation was detected between SCC and the microbial components of BTM in the studied farms with low and high SPC. Correlations between SPC and SCC with other bacterial components of BTM have been explained by others (Chambers et al., 2002; Jayarao, et al., 2004).

The bacterial composition of BTM changes with an increase in SPC (Jayarao, et al., 2004), which is often associated with unclean udders before milking, inefficient teat sanitation, unclean equipment and cooling of milk (Chambers, 2002). About 10 to 50% of the SPC may be the psychrotrophic bacteria, detected as PIC (Chambers, 2002). The most common psychrotrophs in raw milk are the gram-negative bacteria including CC (Cousin, 1982) that gain access to BTM from intramammary infections (environmental mastitis) or nonspecific contaminations from skin, bedding, manure and water (Godkin and Leslie, 1993) regards to stall management, udder hygiene, and milking practices (Jayarao and Wolfgang, 2003). High LPC (thermoduric bacteria) has been associated with poor milking hygiene, unclean equipment, improper sanitizing practices, and milkstone deposits (Murphy, 1997).

In the present study, except a few correlations between managerial factors and SPC and PIC, most of the well explained risk factors lacked any relation with high bacterial counts of raw milk (Table 5). No correlation was detected between LPC and managerial factors and an unexpected positive correlation was seen between CC and milking mastitic cows at the end. These findings could be due to the small number of the studied farms and almost similar faults in the studied farms. Selected wrong answers of the employees to the questions and/or modification of the milking practices in the presence of an inspector could also be the underlying reasons. In fact, a majority of the results obtained by the questionnaire or one time observation were one sided. Close watching of milking routines and facilities of the farms revealed some incoherence between the answers and the actual conditions. For example, a number of farms declared they cleaned the floor of the parlor after milking each sort of cows, but they lacked effective water drainage and manure removal systems. Many farms said that they apply single-use towels but used each side of the paper for drying two separate cows.

A few managerial factors were found in the regression models to influence SPC, PIC and SCC (Table 7). The results confirmed that the questionnaire-based assessment of milking routines alone may not be reliable particularly for the factors that cannot be observed directly. In farms that declared they controlled the tank temperature 2 hours after milking, higher SPC and PIC counts were obtained, shown by negative regression coefficients. Similarly, the declaration of milking mastitis cows at the end of other cows was not in consistent with SCC. Feeding cows immediately after milking and

cleaning in place (CIP) which were observable during farm inspection had positive influences.

The effect of herd size

Herd size has been reported to have a positive correlation with total aerobic count and CC probably due to confinement and greater contamination (Elmoslemany et al., 2010; Goldberg et al., 1992). In the study of Jayarao et al. (2004) herd size did not affect the SPC, PIC and LPC but affected the CC. In the present study no correlation was detected between raw milk bacterial counts and number of cows (data not shown). However, the herds with high SPC levels had less cows (Table 3). In high SPC farms the mean SPC, PIC and CC were 184, 14 and 99 folds of those of low SPC herds, respectively. This could be a result of fewer workers in the farm and milking parlor (Table 3) that had to perform multiple duties. This could lessen the precision of the operation and reduce the level of hygiene in the milking parlor and the whole farm. The employee did not have specific clothing for milking parlor in neither of the farms and there was no noticeable supervision on the quality of their work.

Somatic cell count and contagious mastitis bacteria

Nineteen farms (66%) had SCC levels above 200,000/mL, of which 9 farms (24% of all farms and 47% of high SCC farms) showed contaminations with one or two agents of contagious mastitis (Table 6). Such contaminations were not detected in the farms with SCC<200,000/mL. In both low and high SPC groups the SCC level was above the action level (Table 2). These findings could reveal that all farms, irrespective of their SPC level in bulk milk, needed close attention regards to contagious mastitis. Considering the high levels of CC, the potential influence of environmental mastitis pathogens in elevated bacterial counts and SCC should not be overlooked.

While infections with *Streptococcus agalactiae* and, to some extent, *Corynebacterium bovis* can increase the SPC level in raw milk (Jayarao and Wolfgang, 2003) they appeared not to have major effects in the studied farms. The contamination rates of these organisms were 6.9% (2 farms) and 3.4% (one farm), respectively. As these bacteria are easily shed in milk and are easily isolated from BTM (Jayarao and Wolfgang, 2003), their low infection rate could be due to their responsiveness to a combination of dry cow antibiotic therapy and rapid treatment of clinical mastitis. On the other hand, infections with *Staphylococcus aureus* and *Mycoplasma bovis* appeared to be serious problems in the studied farms, contributing in the elevation of SCC and/or SPC levels. Each organism was detected in 5 farms revealing that 56% of the infected farms and 17.2% of all farms were infected with these unresponsive agents and could not get rid of the infection easily. The infection rate with *Staphylococcus aureus* could still be higher than the detected level since it has an intermittent shedding in milk affecting both SCC and SPC levels. Probable infections with *Mycoplasma bovis* are not reflected on the SPC because isolation and detection of this organism require specific media.

Many of the risk factors of contagious mastitis caused by *Staphylococcus aureus* and *Mycoplasma bovis* such as udder preparation, using individual towels and milking the mastitic cows at the end are the same as described for microbial counts of bulk milk (Ellis et al., 2007; Elmoslemany et al., 2010; Reneau et al., 2005; Schreiner and Ruegg, 2003). There are still a number of other risk factors, related or non-related to milking practices that needed more serious identification and extension advises in the studied farms (Table 5). Post-milking teat dipping as a critical step in controlling mastitis (Erskine, 2001) had problems in nearly half of the farms as they ignored it or did not renew the dip liquid during milking (Table 5). All cows were milked into bulk tank and thus contaminated milk could be fed to calves. Feeding mastitic milk to calves was a part of feeding programs in most of the farms (86%-Table 5). Culling, as the only effective way of controlling nonresponsive contagious mastitis (Erskine, 2001; Jayarao and Wolfgang, 2003), had not been clarified for the farmers and/or had not been accepted by them (data not shown). Diagnostic protocols such as California Mastitis Test (CMT) and evaluation of bulk milk had no places in the farms' routines, although some farms declared that they knew the CMT. Although all farms received official reports on SPC and SCC of their milk, none of them considered any modifications in their routines. Respiratory diseases and joint problems (Jayarao and Wolfgang, 2003) of calves were not concerned as concurrent diseases of Mycoplasma mastitis infections (data not shown). Consequently, many cows with contagious mastitis could remain in the herds for long periods of time without any decision. All of these could be potential factors for elevated microbial and SCC in the studied farms (Jayarao and Wolfgang, 2003).

Conclusion

The laboratory testing of the bulk tank milk revealed undesired milk quality in the studied farms. However, the underlying reasons could not be extracted from the questionnaire (history taking) and one time observation of the milking routines. Accordingly, the veterinary interventions could not be based on the questionnaire

results. Direct and frequent observations of farm routines, instead, could be recommended. Nearly all managerial factors related to the raw milk quality appeared to be inefficient and needed to be revised.

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