The evaluation of udder health status in Holstein dairy farms located in Qom province

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Key words:
bulk tank milk, dairy cow, mastitis

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Received: 15 April 2014
Accepted: 23 June 2014

Abstract:

BACKGROUND: Mastitis is the most economically important disease in dairy industry worldwide. Bulk tank milk (BTM) analysis has been suggested for monitoring the udder health status at herd level. **OBJECTIVES:** A cross-sectional study was conducted to evaluate udder health status in 15 Holstein dairy farms located in Qom province. **METHODS:** Bulk tank somatic cell count (BTMSCC) was analyzed using opto-fluoro-electronic counter. Standard plate count, preliminary incubation count, laboratory pasteurized count, and the number of environmental streptococci, *S. agalactiae*, *S. dysgalactiae*, *S. uberis*, *E. coli*, *K. pneumoniae*, coagulase negative streptococci, *S. aureus* and *C. bovis* were determined using specific culture media. Moreover, clinical and subclinical mastitis were diagnosed using physical examination and California mastitis test, respectively. **RESULTS:** Most herds had moderate to high BTMSCC and high bacterial counts. The prevalence of clinical and subclinical mastitis was 1.3% and 24.7%, respectively. **CONCLUSIONS:** The present study indicated inefficient stall management, udder hygiene, and milking practices in the herds investigated.

Introduction

Mastitis is the most economically important disease in dairy industry worldwide (Blosser, 1979, Harmon, 1994, Sargeant et al., 1998), which not only decreases milk production but also diminishes milk quality (Philpot and Stephen, 2000, Radostits et al., 2007). Mastitis might not be eradicated but can be controlled (Philpot and Stephen, 2000, Radostits et al., 2007). Control of mastitis necessitates the evaluation of udder health status leading to knowledge of the prevalence of mastitis and mastitis-associated pathogens. In this context, bulk tank milk (BTM) analysis has been applied to monitor udder health status at herd level, to troubleshoot herds with mastitis and to determine milk quality (Jayarao and Wolfgang, 2003, Radostits et al., 2007).

Qom province is located in the centre of Iran and has dry climate, with low humidity and scanty rainfall. In total, approximately 10,000 Holstein dairy cattle are raised in Qom province with daily milk production of 200,000 kg. Dairy farms located in Qom province provide milk for dairy industries not only in Qom province but also in adjacent provinces. A cross-sectional study was conducted to evaluate udder health status in 15 farms located in Qom province.

Materials and Methods

Dairy herds: The study was implemented from June to November 2012 in 15 dairy farms with 1,740 Holstein dairy cows. All herds were milked three times a day, had open shed housing, and utilized dried manure solids as bedding. All herds implemented some of the principles of mastitis control program

(Radostits et al., 2007) such as proper milking hygiene, use of functionally adequate milking machine, and post-milking teat dipping; however, none of the herds practiced pre-milking teat dipping.

BTM sample collection and analysis: BTM sample collection was performed as described by National Mastitis Council (Jayarao and Wolfgang, 2003). All BTM samples were transported on ice within 24 hours to the laboratory. BTM somatic cell count (SCC) was determined using an opto-fluoro-electronic counter (Fossomatic 90®; Foss Electric, Denmark). Standard plate count (SPC), preliminary incubation count (PIC), and laboratory pasteurized count (LPC) were determined using plate count agar (Merck, Germany). The number of environmental streptococci (ES) and *Streptococcus agalactiae* (*S. agalactiae*), *Streptococcus dysgalactiae* (*S. dysgalactiae*) and *Streptococcus uberis* (*S. uberis*) were determined using Modified Edward's agar (HiMedia, India). EMB and MacConkey agar (Merck, Germany) were used to count *E. coli* and *Klebsiella pneumoniae* (*K. pneumoniae*), respectively. Baird Parker's agar (Merck, Germany) was used to enumerate coagulase negative streptococci (CNS) and *Staphylococcus aureus* (*S. aureus*). Corynebacterium selective agar (Merck, Germany) was used to determine the number of *Corynebacterium bovis* (*C. bovis*). Plates for enumeration of SPC, PIC, and LPC were incubated at 32°C for 48 h. Plates for enumeration of CNS, ES, *S. aureus*, *Streptococci* spp., *E. coli*, *K. pneumonia*, and *C. bovis* were incubated at 37°C for 48h.

Clinical examination and california mastitis test (CMT): Initially, the quarters (n=6,960) were physically examined for the presence of the signs of clinical mastitis including warm, hard, and swollen quarter and/or abnormal milk appearance. Quarters that were not blind and were apparently healthy were then subjected to CMT to determine quarters with subclinical mastitis. In brief, 2 mL of milk were mixed with 2 mL of CMT reagent (DeLaval, Poland). Reactions were graded as 0, trace, 1+, 2+, and 3+ (Kasravi et al., 2010).

Statistical analysis: Initially, data were tested for normality using Kolmogorov-Smirnov test. Given that the assumptions of parametric tests were not achieved, spearman's rank correlation was used to analyze the relationship between data resulted from BTM analysis. All analyses were conducted in SAS Version 9.2 (SAS Institute Inc., USA). Correlations were considered significant at the $p<0.01$ level.

Results

BTM sample collection and analysis: Values for SCC and bacterial counts in BTM are summarised in Table 1 and 2. In terms of BTMSCC, SPC, PIC, LPC, CNS, and ES, 13%, 0%, 0%, 13%, 13%, and 0% of herds had low values, 47%, 0%, 0%, 67%, 0%, and 0% had moderate values, and 40%, 100% 100%, 20%, 87%, and 100% had high values, respectively (Table 3). *S. aureus*, *S. agalactiae*, and *K. pneumoniae*, which ideally must not be observed in BTM, were detected in 100% (15.15), 33% (5/15) and 87% (13/15) of herds, respectively. With regard to the count of *S. dysgalactiae*, *S. uberis* and *C. bovis*, 100% (15/15), 93% (14/15), and 60% (9/15) of herds had >500 CFU (the goal was BTM bacterial count of the mentioned bacteria). The goal for *E. coli* count in BTM was considered <50 CFU; however, all herds (100%; 15/15) had >50 CFU *E.coli* in BTM.

BTMSCC was correlated with SPC, PIC, *S. aureus*, *S. agalactiae* and *C. bovis* ($p< 0.01$; $r = 0.79$, 0.79, 0.76, 0.74 and 0.84, respectively; Table 4). SPC was correlated with PIC, ES, *S. aureus*, *S. dysgalactiae* and *C. bovis* ($p<0.01$; $r = 0.97$, 0.68, 0.78, 0.67 and 0.86, respectively). PIC was correlated with ES, *S. aureus*, *S. dysgalactiae* and *C. bovis* ($p<0.01$; $r = 0.68$, 0.79, 0.70 and 0.84, respectively). There was correlation between LPC and ES ($p<0.01$; $r=0.66$). CNS was correlated with ES, *S. dysgalactiae*, and *E. coli* ($p<0.01$; $r = 0.68$, 0.67 and 0.80, respectively). ES was correlated with *S. dysgalactiae* and *E. coli* ($p<0.01$; $r = 0.95$ and 0.75, respectively). *S. aureus* was correlated with *S. dysgalactiae* and *C. bovis* ($p< 0.01$; $r = 0.70$ and 0.73, respectively). *E. coli* was correlated with *S. dysgalactiae* and *K. pneumoniae* ($p< 0.01$; $r = 0.68$ and 0.71, respectively; Table 4).

Prevalence of clinical and subclinical mastitis: Values for prevalence of clinical and subclinical mastitis in each individual farm are presented in Table 5. In total, the proportion of blind quarters was 1.3% (89/6960). The prevalence of clinical and subclinical mastitis was 1.3% (90/6960) and 24.7% (1,721/ 6960), respectively. The proportion of trace, 1+, 2+,

Table 1. Somatic cell count (SCC), standard plate count (SPC), preliminary incubation count (PIC), laboratory pasteurized count (LPC), coagulase negative staphylococci (CNS), environmental streptococci (ES), *E. coli*, *S. aureus*, *S. agalactiae*, *S. dysgalactiae*, *S. uberis*, *C. bovis* and *K. pneumoniae* in bulk tank milk (BTM) in each individual farm.

Parameter	Farm 01	Farm 02	Farm 03	Farm 04	Farm 05	Farm 06	Farm 07	Farm 08	Farm 09	Farm 10	Farm 11	Farm 12	Farm 13	Farm 14	Farm 15
Number of cows	166	140	34	57	102	50	295	157	24	250	66	163	30	71	135
SCC (Cells/ml)	423000	481000	338000	963000	1273000	216000	211000	289000	142000	336000	218000	787000	173000	333000	437000
SPC (CFU/ml)	82000	84000	93000	116000	419000	41000	74000	49000	40000	74000	49000	115000	78000	122000	88000
PIC (CFU/ml)	176000	169000	216000	227000	963000	77000	128000	81000	5000	122000	72000	548000	149000	359000	159000
LPC (CFU/ml)	110	130	285	90	1250	100	125	100	75	105	125	350	130	100	145
CNS (CFU/ml)	1100	1000	2400	400	2200	1900	1000	1100	450	1000	2700	6000	1100	3400	3900
ES (CFU/ml)	15900	14500	19300	1400	21300	8100	11900	10900	4000	10900	8700	21000	11400	26500	24500
S. aureus (CFU/ml)	40	20	100	70	75	20	15	40	10	50	15	210	10	75	70
S. agalactiae (CFU/ml)	6200	5200	0	28000	17500	0	0	0	0	0	0	0	0	0	6200
S. dysgalactiae (CFU/ml)	7600	7200	8100	700	11200	2100	6800	2100	600	1800	1200	8100	1600	11600	10900
S. uberis (CFU/ml)	2100	1700	4900	150	4900	4500	1300	4600	2500	4500	4500	2500	5900	7400	5200
C. bovis (CFU/ml)	375	900	750	875	1500	350	200	400	100	500	300	1000	500	800	850
E. coli (CFU/ml)	481	322	482	121	349	325	209	316	60	428	371	1120	387	1388	491
K. pneumoniae (CFU/ml)	50	15	50	0	60	30	0	30	40	85	20	125	30	50	60

Table 2. Descriptive statistics of somatic cell count (SCC), standard plate count (SPC), preliminary incubation count (PIC), laboratory pasteurized count (LPC), coagulase negative staphylococci (CNS), environmental streptococci (ES), *E. coli*, *S. aureus*, *S. agalactiae*, *S. dysgalactiae*, *S. uberis*, *C. bovis* and *K. pneumoniae* in bulk tank milk (BTM).

Parameter	Minimum	Median	Maximum
Number of cows	24	102	295
SCC (Cells/ml)	142,000	336,000	1,273,000
SPC (CFU/ml)	40,000	82,000	419,000
PIC (CFU/ml)	50,000	159,000	963,000
LPC (CFU/ml)	75	125	1,250
CNS (CFU/ml)	400	1,100	6,000
ES (CFU/ml)	1,400	11,900	26,500
S. aureus (CFU/ml)	10	40	210
S. agalactiae (CFU/ml)	0	0	28,000
S. dysgalactiae (CFU/ml)	600	6,800	11,600
S. uberis (CFU/ml)	150	4,500	7,400
C. bovis (CFU/ml)	100	500	1,500
E. coli (CFU/ml)	60	371	1,388
K. pneumoniae (CFU/ml)	0	40	125

and 3+ grades of CMT was 38.0% (654/1721), 20.0% (344/1721), 21.2% (365/1721), and 20.8% (358/1721), respectively.

Discussion

Bulk tank milk analysis revealed moderate to high values of BTMSCC in the majority of the herds (87%), which shows the presence of intramammary infection within the herds (Philpot and Stephen, 2000, Bradley and Green, 2005). Major contagious pathogens of mastitis including *S. aureus* and *S.*

Table 3. Number and proportion of farms in different categories of bulk tank milk SCC and bacterial counts. [(a)]Categories and values were suggested by Jayarao et al. (10).

Parameter	Category (count)[a]	Number of farms (%)
BTMSCC	Low (<200,000)	2/15 (13)
	Moderate (200,000-400,000)	7/15 (47)
	High (>400,000)	6/15 (40)
SPC	Low (<5,000)	0/15 (0)
	Moderate (5,000-10,000)	0/15 (0)
	High (>10,000)	15/15 (100)
PIC	Low (<10,000)	0/15 (0)
	Moderate (10,000-20,000)	0/15 (0)
	High (>20,000)	15/15 (100)
LPC	Low (<100)	2/15 (13)
	Moderate (100-200)	10/15 (67)
	High (>200)	3/15 (20)
CNS	Low (<500)	2/15 (13)
	Moderate (500-1,000)	0/15 (0)
	High (>1,000)	13/15 (87)
ES	Low (<500)	0/15 (0)
	Moderate (500-1,000)	0/15 (0)
	High (>1,000)	15/15 (100)

Table 4. Spearman correlation coefficients. (*) p<0.01. (**) p<0.001. (***) p<0.0001.

	BTM SCC	SPC	PIC	LPC	CNS	ES	*S. aureus*	*S. agalactiae*	*S. dysgalactiae*	*S. uberis*	*C. bovis*	*E. coli*	*K. pneumoniae*
BTMSCC	1	0.79**	0.79**	0.45	0.21	0.43	0.76*	0.74*	0.50	-0.14	0.87***	0.28	0.34
SPC		1	0.97***	0.48	0.35	0.68*	0.78**	0.54	0.67*	0.20	0.86***	0.48	0.33
PIC			1	0.51	0.34	0.68*	0.79**	0.50	0.70*	0.11	0.84***	0.48	0.37
LPC				1	0.56	0.66*	0.40	0.16	0.59	0.27	0.55	0.49	0.40
CNS					1	0.68*	0.53	-0.16	0.67*	0.61	0.32	0.80**	0.55
ES						1	0.61	0.16	0.95***	0.49	0.55	0.75*	0.58
S. aureus							1	0.27	0.70*	0.23	0.73*	0.60	0.61
S. agalactiae								1	0.21	-0.25	0.53	-0.11	-0.02
S. dysgalactiae									1	0.42	0.56	0.68*	0.54
S. uberis										1	0.17	0.57	0.46
C. bovis											1	0.38	0.38
E. coli												1	0.71*
K. pneumoniae													1

Table 5. Prevalence of clinical and subclinical mastitis. Numbers in parentheses are actual numbers.

Parameter	Farm 01	Farm 02	Farm 03	Farm 04	Farm 05	Farm 06	Farm 07	Farm 08
Number of quarters	664	560	136	228	408	200	1180	628
Proportion of blind quarters (%)	2.0 (13/664)	1.6 (9/560)	1.5 (2/136)	0.9 (2/228)	1.0 (4/408)	0.5 (1/200)	0.3 (4/1180)	2.4 (15/628)
Prevalence of clinical mastitis (%)	0.6 (4/664)	0.0 (0/560)	0.0 (0/136)	4.4 (10/228)	3.4 (14/408)	0.0 (0/200)	0.3 (4/1180)	0.2 (1/628)
Prevalence of subclinical mastitis (%)	19.4 (129/664)	16.4 (92/560)	33.1 (45/136)	34.6 (79/228)	38.5 (157/408)	18.0 (36/200)	13.6 (161/1180)	32.6 (205/628)
Proportion of grade trace quarters (%)	10.0 (13/129)	20.7 (19/92)	35.6 (16/45)	24.1 (19/79)	26.1 (41/157)	50.0 (18/36)	42.9 (69/161)	57.6 (118/205)
Proportion of grade 1+ quarters (%)	20.2 (26/129)	10.9 (10/92)	8.9 (4/45)	15.2 (12/79)	15.9 (25/157)	11.1 (4/36)	19.9 (32/161)	11.2 (23/205)
Proportion of grade 2+ quarters (%)	31.0 (40/129)	29.3 (27/92)	24.4 (11/45)	32.9 (26/79)	27.4 (43/157)	22.2 (8/36)	25.4 (41/161)	13.6 (28/205)
Proportion of grade 3+ quarters (%)	38.8 (50/129)	39.1 (36/92)	31.1 (14/45)	27.8 (22/79)	30.6 (48/157)	16.7 (6/36)	11.8 (19/161)	17.6 (36/205)

Parameter	Farm 09	Farm 10	Farm 11	Farm 12	Farm 13	Farm 14	Farm 15
Number of quarters	96	1000	264	652	120	284	540
Proportion of blind quarters (%)	1.0 (1/96)	2.1 (21/1000)	0.8 (2/264)	0.8 (5/652)	0.8 (1/120)	0.4 (1/284)	1.5 (8/540)
Prevalence of clinical mastitis (%)	0.0 (0/96)	2.7 (27/1000)	1.1 (3/264)	3.5 (23/652)	0.0 (0/120)	1.4 (4/284)	0.0 (0/540)
Prevalence of subclinical mastitis (%)	14.6 (14/96)	35.6 (356/1000)	35.6 (94/264)	27.0 (176/652)	13.3 (16/120)	22.9 (65/284)	17.8 (96/540)
Proportion of grade trace quarters (%)	50.0 (7/14)	50.0 (178/356)	45.7 (43/94)	31.8 (56/176)	31.3 (5/16)	40.0 (26/65)	27.1 (26/96)
Proportion of grade 1+ quarters (%)	28.6 (4/14)	27.5 (98/356)	30.9 (29/94)	24.4 (43/176)	25.0 (4/16)	24.6 (16/65)	14.6 (14/96)
Proportion of grade 2+ quarters (%)	14.3 (2/14)	12.9 (46/356)	16.0 (15/94)	20.5 (36/176)	31.2 (5/16)	21.5 (14/65)	23.9 (23/96)
Proportion of grade 3+ quarters (%)	7.1 (1/14)	9.6 (34/356)	7.4 (7/94)	23.3 (41/176)	12.5 (2/16)	13.9 (9/65)	34.4 (33/96)

agalactiae have the most contribution to the elevation of BTMSCC (Wilson et al., 1997, Olde Riekerink et al., 2006), which is probably the reason for correlation of BTMSCC with *S. aureus* and *S.*

agalactiae in the present study.

Moreover, the majority of BTM bacterial counts in the herds were substantially higher than goals. High SPC values could originate from milking cows with mastitis or contaminated teats, defective sanitation of milking equipment, or the delayed cooling of BTM (Jayarao and Wolfgang, 2003, Radostits et al., 2007). High PIC, affecting the keeping quality of milk, reflects substandard hygiene practices (Jayarao and Wolfgang, 2003). Correlation of *S. aureus* and environmental streptococci, particularly *S. dysgalactiae*, with SPC and PIC implies the contribution of these organisms to the rise of SPC and PIC. Intramammary infection with *S. aureus* increases bacterial count of individual cows, which could subsequently lead to elevation of SPC in BTM (Radostits et al., 2007). Jayarao et al., (2004) have also reported correlation of ES with SPC, and PIC. CNS, ES, *E. coli* and *K. pneumoniae* originates from not only intramammary infections, but also nonspecific contamination of cow skin, bedding, and water (Jayarao and Wolfgang, 2003). Elevation of the number of these organisms in BTM suggests defective stall management, udder hygiene, and milking practices (Godkin and leslie, 1993, Hayes et al., 2001, Jayarao et al., 2003, 2004). *C. bovis* is highly susceptible to teat disinfection and is, in turn, suggested as an indicator of teat dipping efficiency (Radostits et al., 2007). In this context, high number of *C. bovis* (which was strongly correlated with BTMSCC, SPC and PIC) in majority of herds indeed indicates inefficient teat-dipping. The considerably high count of environmental streptococci, particularly *S. dysgalactiae*, in all herds and its correlation with SPC and PIC implicates the need for improvement of stall management as well as proper implementation of pre-milking teat dipping in herds investigated (Radostits et al., 2007).

As it was previously indicated, the prevalence of subclinical mastitis (24.7%) was considerably higher than that of clinical mastitis (1.3%) in the present study (Philpot and Stephen, 2000, Radostits et al., 2007). The prevalence of clinical and subclinical mastitis in the present study is approximately comparable with that previously reported in Fars province (0.7% and 21.6%, respectively) (Hashemi et al., 2011).

In conclusion, the high BTM bacterial counts in the present study suggest defective stall management, udder hygiene, and milking practices. Although all herds practiced post-milking teat dipping, high values of *C. bovis* in the majority of the herds indicates substandard execution of teat disinfection.

Acknowledgements

The authors thank Dr. F. Moosakhani for his kind assistance in BTM sample analysis.

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مجله طب دامی ایران، ۱۳۹۳، دوره ۸، شماره ۴، ۲۶۰-۲۵۵

ارزیابی وضعیت سلامت پستان در مزارع گاو شیری هلشتاین واقع در استان قم

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(دریافت مقاله: ۲۶ فروردین ماه ۱۳۹۳، پذیرش نهایی: ۲ تیر ماه ۱۳۹۳)

چکیده

زمینه مطالعه: ورم پستان زیان بار ترین بیماری در صنعت گاوشیری به لحاظ اقتصادی می باشد. ارزیابی شیر تانک جمع آوری شیر به عنوان روشی جهت مانیتورکردن وضعیت سلامت پستان درسطح گله پیشنهادشده است. **هدف:** مطالعه ای به روش مقطعی به منظور ارزیابی وضعیت سلامت پستان در ۱۵ گله گاو شیری هلشتاین واقع در استان قم به انجام رسید. **روش کار:** شمارش سلول های سوماتیک شیر تانک جمع آوری شیر با استفاده از دستگاه فوزوماتیک صورت پذیرفت. شمارش پلیت استاندارد، شمارش انکوباسیون اولیه، شمارش پاستوریزاسیون آزمایشگاهی و تعداد استرپتوکوکوس های محیطی، استرپتوکوکوس آگالاکتیه، استرپتوکوکوس دیس گالاکتیه، استرپتوکوکوس یوبریس، ای کولای، کلبسیلا نومونیه، استافیلوکوکوس های کوآگولاز منفی، استافیلوکوکوس اورئوس و کورینه باکتریوم بوویس با استفاده از محیط های کشت اختصاصی انجام شد. بعلاوه، تشخیص ورم پستان بالینی از طریق معاینه هرکارتیه و تشخیص ورم پستان تحت بالینی توسط تست ورم پستان کالیفرنیایی صورت پذیرفت. **نتایج:** غالب گله های مورد بررسی مقادیر متوسط تا بالای شمارش سلول های سوماتیک شیر تانک جمع آوری شیر و مقادیر بالای شمارش باکتریایی شیر تانک جمع آوری شیر را دارا بودند. شیوع ورم پستان بالینی و تحت بالینی به ترتیب ۱/۳٪ و ۲۴/۷٪ بود. **نتیجه گیری نهایی:** مطالعه حاضر بیانگر وضعیت نامناسب مدیریت جایگاه، بهداشت پستان و شیردوشی در گله های مورد بررسی می باشد.

واژه های کلیدی: شیر تانک جمع آوری شیر، گاوشیری، ورم پستان

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