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The Effect of Parity, Age and Season on Somatic Cell Count of Dairy Cows with Subclinical Mastitis

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Abstract: This study aimed to determine the effect of parity, age and season on somatic cell count of dairy cows. For this purpose, 310 milk samples were collected from 9 different dairy farms located around Tekirdao. The milk samples were examined in terms of California Mastitis Test (CMT), Somatic Cell Count (SCC) and Total Viable Bacteria Counts (TVBC). According to CMT examination, 256 milk samples were evaluated as CMT + and the average age of the cows was 3.84 years, 46 samples were evaluated as CMT ++, the average age of the cows was 5.61 years and 8 samples were evaluated as CMT +++ while their average age of the cows was 6 years. In conclusion, it is seen that when the age and the parity of dairy cows increase, CMT and SCC increase thus mastitic milk incidence may be rise up. This affects the quality of milk thus the dairy industries should be more sensitive about this issue and they should take extra cautions for mastitis control in order to obtain healthy milk and milk products.

Key words: Parity, age, season, somatic cell count, dairy cows, subclinical mastitis

INTRODUCTION

Mastitis is one of the major problems in dairy industries that cause economic losses. It is defined as the inflammatory changes of the udder that is caused by bacteria and its toxins which manifests itself by the physical, chemical and bacteriological changes in the milk (Sharma, 2007). Subclinical mastitis leads to reduction in milk production, therapy costs, poor milk hygiene and variations in the milk composition (Atasever and Erdem, 2009; Brightling *et al*., 1998; Chagunda *et al*., 2006; Halasa *et al*., 2009; Seegers *et al*., 2003). Clinical mastitis can be noticed by the farmer with its visible changes in the udder quarter but subclinical mastitis is sly so it can not be determined unless evaluating of the pathogens and inflammatory components in the milk (Nielen *et al*., 1993; Pillai *et al*., 2001).

Various methods have been used to determine of subclinical mastitis such as California Mastitis test, Somatic Cell Count (SCC), some biochemical and microbiological methods in the milk and electrical conducivity (Bastan *et al*., 1997; Lafi, 2006).

When there is an infection in the mammary gland, leucocytes and epithelial cells (somatic cell) increase in the milk (Coban *et al*., 2007). Somatic cell count is a good parameter that indicate the milk quality. Increase of somatic cells in the milk can be considered as the main factor for the infection (Coban *et al*., 20007; Souza *et al*., 2005). Even many chemical and microbiological tests being used to diagnose subclinical mastitis, the tests based on the identification of somatic cell count in milk become more important in recent years (Risvanli and Kalkan, 2002; Arda *et al*., 1982). In the determination of the subclinical mastitis SCC in the milk is an important criteria but there are lots of factor that effect SCC such as animal's age, breed, parity, season, stress, milking interval, lactation period, sexual cycle, nutrition, other infections in the body of the animal, mastitis pathogens (Arda *et al*., 1982; De Haas, 2003; Deveci *et al*., 1994; Eyduran, 2002; Goncu and Ozkutuk, 1998; Harmon, 1994; Sederevicius *et al*., 2006). Increase in SCC can be detected with the increase in age have been reported earlier by so many researchers, this increase was linked to the increased incidence of infection in elderly cows (Beckley and Johnson, 1966; Blackburn, 1966; Reichmuth, 1975).

There are some reports about the seasonal effect on SCC that the incidence of mastitis can be increase in Spring and Summer (Busato *et al*., 2000; Miller *et al*., 1975; Risvanli and Kalkan, 2002), some others report that in Winter and Autumn incidence of mastitis can increase (Alrawi *et al*., 1979; Batra *et al*., 1977; Kennedy *et al*.,

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1982). SCC is between 50.000 and 100.000 cells mL^{-1} in a healthy udder's milk whereas in a diseased udder's milk SCC is >200.000 cells mL^{-1} (Harmon, 2001; Skrzypek *et al*., 2004).

The aim of this study is to determine the effect of parity, age and season on somatic cell count of the dairy cows with subclinical mastitis.

MATERIALS AND METHODS

A total of 310 milk samples were collected from quarters of 225 cows aged between 2-8 years at 9 different small holder dairy farms around Tekirdao and were examined using the CMT, SCC, TVBC. Clinical examination of the udder and the CMT evaluations were carried out on farms in order to determine subclinical mastitisc in dairy cows. CMT positive milk samples (CMT +, ++, +++) are accepted as subclinical mastitis suspected samples and these milk samples were collected from the related quarters for the analysis.

Examination of the udder: Udders of the cows were checked for redness, pain, heat and swelling by inspection. Samples from each quarter were collected and controlled for any variation in milk colour and consistency.

Collection of samples: Milk samples were collected during hot-humid and cold season periods while the cows were strained in standing position after all the quarters were cleaned, they were washed with tap water. The teat end was dried and cleaned lastly with alcohol. Approximately, 100 mL of milk samples were collected into the sterile bottles aseptically from subclinical mastitic quarters and transported for analysis of SCC within 4 h to the laboratory of Faculty of Veterinary Medicine, Istanbul University.

CMT evaluation and CMT scoring: California Mastitis test solution consisted of 3% sodium lauryl sulphate and bromocresol (DeLaval, Cardif, UK) was mixed the same amount with the milk, CMTs were scored due to no reaction; as 0 for a weak positive (+), for a distinct positive (++) for a strong positive (+++).

SCC analyse: Somatic cell counts were measured using a Fossomatic 90 instrument (Foss Electric, Hillerod, Denmark) after 40°C heat treatment at duration of 15 min (32). SCC of the samples were measured separately for hot-humid and for cold season.

TVBC analyse: A 1 mL milk sample was diluted using serial decimal dilutions up to 10^{-7}. Then, 0.1 mL of the diluents was transferred to the plate count agar (Oxoid CM463, UK) and was incubated at 30°C for 48 h.

Statistical analysis: A one way Analysis of Variance (ANOVA) was performed using SPSS 13.0 statistical package program (SPSS, 2001) in order to determine the significant differences among means of effects. Duncan's multiple range tests was used to evaluate the significant difference ($p<0.05$) between characteristics. Moreover, Pearson correlations among defined characteristics were also estimated and 2-paired t-test was used to identify SCC in hot-humid and cold season.

RESULTS

Detected milk samples results are summarized in Table 1-3. Animals' ages were between 2-8, the mostly

Table 1: Age, parity, cow no, hot-humid and cold season SCC, TVBC results of subclinical suspected milk samples depending on CMT levels

Levels	n	Age	Parity	Cow No.	Hot-humid SCC (cells mL^{-1})	Cold SCC (cells mL^{-1})	TVBC (cfu mL^{-1})
CMT+	256	3.84^{b}	1.71^{b}	115.26	58309^{c}	113289^{b}	489010^{c}
CMT++	46	5.61^{a}	2.78^{a}	101.63	508587^{b}	175218^{a}	1690848^{b}
CMT+++	8	6.00^{a}	2.75^{a}	130.38	1200000^{a}	-	3641250^{a}
p	310	0.00	0.00	NS	0.000	0.006	0.000

Table 2: CMT, SCC (hot-humid and cold season), TVBC, cow number and parity results of subclinical suspected milk samples depending on animal's ages

Age (years old)	n	Parity	Cow No.	CMT	Hot-humid SCC (cells mL^{-1})	Cold SCC (cells mL^{-1})	TVBC (cfu mL^{-1})
2	40	1.00^{e}	108.70	1.00^{c}	22125^{c}	70850^{ab}	108845^{c}
3	89	1.02^{e}	110.94	1.04^{c}	78506^{c}	95708^{ab}	500203^{bc}
4	54	1.98^{d}	115.61	1.13^{c}	104778^{c}	161667^{a}	494834^{bc}
5	65	2.31^{c}	119.28	1.17^{c}	142923^{c}	145154^{a}	1245730^{ab}
6	43	3.05^{b}	113.65	1.65^{ab}	362372^{b}	150233^{a}	1091981^{abc}
7	10	3.60^{a}	103.30	1.50^{b}	425000^{b}	108500^{a}	1091000^{abc}
8	9	3.78^{a}	120.22	1.78^{a}	585556^{a}	-	1962722^{a}
p	310	0.00	NS	0.00	0.000	0.008	0.000

Mean values within the same row with different superscript small letters are different ($p<0.05$); NS = Not Significant ($p>0.05$)

Table 3: The results of age, cow number, CMT, TVBC, SCC (hot-humid and cold season) of subclinical suspected milk samples depending on parities

Parity	n	Age	Cow No.	CMT	Hot-humid SCC (cells mL^{-1})	Cold SCC (cells mL^{-1})	TVBC (cfu mL^{-1})
1st parity	136	2.79^{d}	111.210^{ab}	1.05^{b}	68728^{c}	90309^{c}	455989^{b}
2nd parity	89	4.51^{c}	110.380^{ab}	1.11^{b}	99978^{c}	163652^{ab}	684335^{ab}
3rd parity	65	5.72^{b}	131.080^{a}	1.54^{a}	381723^{a}	101077^{bc}	1328166^{a}
4th parity	20	6.80^{a}	87.550^{b}	1.50^{a}	243250^{b}	182250^{a}	1142225^{a}
p	310	0.00	0.042	0.00	0.000	0.002	0.002

Table 4: The correlation between age, parity, cow number, CMT, hot-humid and cold SCC, TVBC of the milk samples considered as subclinical mastitis

Samples	Significance	Age	Parity	Cow No.	CMT	Hot-humid SCC (cells mL^{-1})	Cold SCC (cells mL^{-1})	TVBC (cfu mL^{-1})
Age	p	1	0.885^{**}	0.030	0.446^{**}	0.429^{**}	0.075	0.256^{**}
		-	0.000	0.597	0.000	0.000	0.190	0.000
Parity	p	-	1	0.022	0.401^{**}	0.355^{**}	0.111	0.202^{**}
		-	-	0.704	0.000	0.000	0.051	0.000
Cow No.	P	-	-	1	-0.030	0.243^{**}	-0.466^{**}	0.078
		-	-	-	0.595	0.000	0.000	0.174
CMT	p	-	-	-	1	0.804^{**}	0.028	0.399^{**}
		-	-	-	-	0.000	0.623	0.000
Hot-humid SCC	p	-	-	-	-	1	-0.396^{**}	0.407^{**}
		-	-	-	-	-	0.000	0.000
Cold SCC	p	-	-	-	-	-	1	-0.060
		-	-	-	-	-	-	0.292
TVBC (cfu mL^{-1})	p	-	-	-	-	-	-	1

**Correlation is significant at the $p<0.01$ level (2-tailed)

affected animals with subclinical mastitis were found in 3-5 years of age. According to 310 milk sample examinations from 9 different dairy farms, 256 milk samples were evaluated as CMT +, their average age was 3.84 years, 46 of them were evaluated as CMT ++, their average age was 5.61 years and 8 of them were evaluated as CMT +++ while their average age was 6 years. The correlation between age, parity, cow number, CMT, TVBC, hot-humid and cold SCC of the milk samples considered as subclinical mastitis is given in Table 4.

According to the CMT evaluation, age, parity, TVBC ($p<0.001$), SCC (hot-humid and cold season) ($p<0.01$) were showed significant differences whereas no statistically differences were observed in cow number of the samples (Table 1).

According to the age, statistically significance was found between parity, CMT, TVBC, hot-humid SCC ($p<0.001$) and cold season SCC ($p<0.01$) scores while cow number did not show any significant differences. Additionally, the 2-paired t-test between hot-humid and cold somatic cell count showed no significant difference ($p>0.05$). However, significant correlations were obtained between analyzed parameters (Table 2). According to the parity, age, CMT, hot-humid SCC ($p<0.001$), cow number ($p<0.05$), TVBC and cold season SCC ($p<0.01$) were determined significantly different (Table 3).

DISCUSSION

Subclinical mastitis is one of the major problem that causes high economic losses in dairy industries. Microbial infections of the udder change milk composition and alter milk less suitable for human consumption. Also, it leads the reduction of milk yield per animal. Milk is a valuable product for human beings. The nutritional requirement of the human body obtained from milk is vitamin A, ascorbic acid and thiamine. SCC is an important criteria to identify mastitis and milk quality in dairy cows.

Randy *et al*. (1990) reported high SCC results during hot-humid season can be obtained in order to extra exposure of teat ends to microorganisms so the incidence of the mastitis increase (up to 40%). The high somatic cell count found in this study when compared to cold season might be due to the high humidity and high temperature as these conditions lead stress on the animals and can cause the increase in the exposure of the microorganisms that lead to mastitis. According to the result of this study, none of the milk samples were evaluated as CMT +++ in cold weather. Low somatic cell counts during cold season were earlier reported in buffaloes (Singh and Ludri, 2000), in this study according to the CMT scores from (+, ++, +++), the results were detected as 113289, 175218, none for cold season whereas the results were detected as 58309, 508587, 1200000 for hot-humid season, respectively.

In this study, CMT +, ++, +++ cows mean age was found 3.84; 5.61 and 6 years, respectively (Table 1). According to this study, there is no connection between the parity and SCC related to season of the milk samples when the parity of the animal's increases in the cold season SCC found to be increased at first then decrease and increased again, in hot-humid season SCC firstly increased and then decreased (Table 3). Singh and Ludri (2001) compared SCC and parity in different cow breeds and they reported that in Tharparkar breed in the Winter season, there was a fluctuation like this study. But in this study the breed classification have not been recorded.

In present study, it is found that when the age increases the mastitis incidence found to be increased (Table 4). Risvanli and Kalkan (2002) reported in their study that due to the age of the cows did not reflect any statistical difference between the groups while Matthews *et al*. (1992) reported in their study that SCC of the milk increased with increasing age.

In this study, when the age of the animals increase the hot-humid SCC of the animals were increased too but the same can not be said for the cold season SCC (Table 4).

Karimuribo *et al*. (2006) reported the best diagnosis aid for subclinical mastitis is California Mastitis test, in this study subclinical mastitis of the mammary quarters were first evaluated with CMT and then confirmed with SCC.

The rapid result of subclinical mammary infection can be diagnosed also with somatic cell count as in this study. If there is an increase above 200.000 cells mL^{-1} it is considered as unhealthy milk for consumers. At the end of SCC evaluation out of 310 milk samples ≤500.000 SCC is accepted as CMT +, 500.000-100.000 SCC is accepted as CMT ++ ≥1000.000 SCC is accepted as CMT +++.

In a study conducted by Khate and Yadav (2010), it was reported that somatic cell count are detected low in cold weather while they were high in Summer season similarly with the present study.

Kasikci *et al*. (2012) reported in their study that SCC was 249.453, 1.167.058 and 2.108.139, respectively according to the CMT results. They also added that total viable bacteria counts ranged between 3.4771-6.9395 from 3.4771-7.3617 and from 4.7782-7.5315 log CFU mL^{-1}, respectively. In this study according to the CMT results CMT + total bacteria value was found as 489010 CFU mL^{-1}, CMT ++ was found as 169084 CFU mL^{-1} and CMT +++ was found as 3641250 CFU mL^{-1}. It is seen a positive correlation between SCC and TVBC similarly with the present study. SCC increase due to mammary gland infections thus increase TVBC.

Control of subclinical mastitis can be achieved by routine screening tests in order to prevent economical losses caused by subclinical mastitis and acquisition of good quality milk. In mastitis, treatment with antibiotics remains only moderately effective for a dairy industry, control systems such as proper milking techniques, improved sanitation, effective use of teat-dipping and dry period therapy and improvement in management are more important initiatives for animals. Optimum production, maximum daily milk yield and healthy milk production can be achieved by adapting the mastitis control programme in farm conditions and herd.

CONCLUSION

It can be stated that when the age and the parity of dairy cows increase, SCC and CMT increase and this increase might lead to the mastitic milks. This result affects the quality of the milk so the dairy industries should be more careful on that issue and they should take extra cautions for mastitis control.

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