

Using Quick Test, California Mastitis Test, and Somatic Cell Count for Diagnosis of Subclinical Mastitis Related with Human Health Risk

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BACKGROUND/AIMS

The aim of the present study was to compare Quick Test (QT) in the diagnosis of subclinical mastitis related with human health risk with California Mastitis Test (CMT) and to determine the compatibility of QT with Somatic Cell Count (SCC) using reference data from Fossomatic.

MATERIAL and METHODS

The study was performed using milk samples collected from 160 individual udder quarters of 40 Holstein cows at three different farms. Milk samples were initially checked by CMT and QT. Then, the samples were sent for SCC analyses.

RESULTS

The SCC results (0-100,000 cell/mL) of 101 (101/160) samples belonging to ≤ 100 standards of QT category were found to be compatible in the ratio of 60.39%. When QT standards of ≤ 100 and 250 were compared with the SCC results, the negative subclinical mastitis values (0-250,000 cell/mL) were compatible in the ratio of 84%. When CMT was compared with SCC, the negative subclinical mastitis values of CMT were found to be compatible with the SCC results (0-250,000 cell/mL) in the ratio of 85.9%. For the determination of all cases with subclinical mastitis, SCC data were found to be compatible with QT and CMT in the ratios of 97.6% and 63.4%, respectively. When QT and SCC were compared with each other, the specificity, sensitivity, and false ratio of QT were detected as 84.0%, 97.5%, and 16.7%, respectively.

CONCLUSION

Quick Test is a supporting method to other tests or alternative method to CMT in the diagnosis of subclinical mastitis related with human health risk.

Keywords: California mastitis test, quick test, somatic cell count, subclinical mastitis

INTRODUCTION

Generally, mastitis is the inflammation of the mammary glands with no involvement of the skin tissue (1). The use of bulk milk Somatic Cell Count (SCC) as an indicator of farm hygiene has been related to the potential human health risk. Viable pathogens and their toxins can be transferred from the milk of infected quarters directly to humans. A large and diverse group of human pathogens reside in the cow's environment, such as *Salmonella dublin*, *Campylobacter jejuni*, and *Listeria monocytogenes*. These bacteria are often pathogens or normal flora of dairy cows. Evidence has shown that *Mycobacterium avium* subsp. *paratuberculosis*, associated with Johne's disease in cattle and isolated from human patients with Crohn's disease, may survive some accepted milk pasteurization procedures. Although the possible association between shedding of *M. avium* subsp. *paratuberculosis* in milk and subsequent survival after pasteurization is compelling, the rate of shedding is low in infected cows and not related to an increase in SCC. Evidence suggests that contamination of milk with most of these pathogens occurs during or after harvest of milk and is not due to intramammary infections. However, herds with high bulk milk SCC are more likely to have these pathogens infecting cows and are present in elevated populations in the farm environment. The tempting inference is that farms ineffective in implementing hygiene practices to reduce bulk milk SCC are also ineffective in other farm hygiene measures aimed at reducing exposure of milk to human pathogens via routes other than intramammary infections (2, 3). Mastitis is a

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disease that causes important economic losses (4-6). In cows, it is generally accepted as the most complicated and high-cost disease, causing the highest economic losses to the milk producer (7-9). Subclinical mastitis is an overemphasized form of mastitis in economic terms due to its deleterious effect on both milk production and quality (10). It is a kind of mastitis in which factors causing infection are found in the mammary glands; however, there is no visible disturbance on the mammary or in milk. Among the mastitis originated losses, 70% of the loss is related to subclinical mastitis (11). The development of subclinical mastitis increases during the early lactation period, and a great percentage of intramammary infections appear in the postpartum period (12-14). There have been many studies that aim to develop treatments for clinical mastitis and to obtain high milk by controlling subclinical mastitis (15, 16). An increase in SCC is observed following inflammation of the mammary glands (17). The SCC value of milk from healthy mammary glands is <200,000 cell/mL, involving epithelial cells and leukocytes (18). Direct and indirect methods are required for SCC. Electronic devices are used in the laboratory for the direct determination of SCC (19). The SCC values of milk samples are the indicator of inflammation level of the mammary glands and form the basis of various indirect tests for mastitis. High values of SCC are generally an indicator for inflammation in the mammary glands (17). In 1957, the California Mastitis Test (CMT), which is a quick and reliable method, was developed to identify unhealthy milk (20). It is a cheap, fast and easy test and is one of the indirect methods used in the determination of SCC in milk (19, 21). Under field conditions, although microbiological analysis from each mammary lobe and analysis of active substance are the most confidential methods in the diagnosis of mastitis, they are time-consuming and expensive. Therefore, CMT and SCC provide economic, quick, and reliable results in the diagnosis of infected mammary lobes (22).

MATERIAL and METHODS

Milk samples were collected from 160 individual udder quarters of 40 Holstein cows at three different farms. Foremilk was obtained from cows without mastitis, udder lobes and cow numbers were recorded, and samples were placed in tubes. First, CMT analysis was performed on all samples at the farm, followed by SCC and QT. Finally, all samples were sent to the Cyprus-Turkish Dairy Industry Institution, Quality Control Laboratory for the determination of the SCC values.

The tests were evaluated as follows:

California Mastitis Test (CMT): CMT was performed according to the Schalm and Noorlander principles (20). For this purpose, the CMT solution was obtained from DeLaval (Cardiff, UK). In test chambers, milk samples were evaluated in accordance with the structure and color change of the reaction after the addition of the CMT solution. The condition without any reaction was evaluated as negative (-); whereas positive reactions were categorized as weak positive (+), positive (++), and strong positive (+++).

Quick Test (QT): The PortaSCC Quick Test (PortaCheck, Moorestown, USA) was used for the practical determination of the SCC. QT standards were categorized as ≤ 100 , 250, 500, 750, 1500, and ≥ 3000 . The results were evaluated based on color change according to different shades of blue.

Somatic Cell Count (SCC): The SCC was performed in milk samples by using the Fossomatic™ FC 5000 (Foss, Hillerod, Denmark) device, and the results were evaluated as SCC.

In the present study, ranges including ≤ 100 : 0 to $\leq 100 \times 10^3$ cell/mL, 250: 101-250 $\times 10^3$ cell/mL, 500: 251-500 $\times 10^3$ cell/mL, 750: 501-750 $\times 10^3$ cell/mL, 1500: 751-1500 $\times 10^3$ cell/mL, and ≥ 3000 : 1501 to $\geq 3000 \times 10^3$ cell/mL were selected as the baseline for the QT standards, and the results were compared with those from the CMT and SCC analyses.

According to the -, +, ++, and +++ classification of CMT, SCC was determined as -: 0-250 $\times 10^3$, +: 251-500 $\times 10^3$, ++: 501-1500 $\times 10^3$, and +++: 1501 to $\geq 3000 \times 10^3$ cell/mL, respectively.

The SCC values were accepted as the reference comparison values during laboratory evaluations.

Statistical Analysis

The method by Roelofs et al. (23) was modified for the calculation of sensitivity, specificity, and false ratio and applied as follows:

Diagnosis	QT or CMT	SCC
Mastitis +	a	b
Mastitis -	c	d

CMT: California Mastitis Test; QT: Quick Test; SCC: Somatic Cell Count.

In the diagnosis of mastitis, the sensitivity (efficiency, detection rate, and treatment ratio), specificity (negative diagnosis), and false ratio were calculated according to the formulas: $(a/(a+c) \times 100)$, $(d/(b+d) \times 100)$, and $(b/(a+b) \times 100)$, respectively (23). Sensitivity calculates the positive diagnosis and the detection reliability of mastitis. Specificity calculates the negative diagnosis and the detection reliability of mastitis. False ratio calculates the mistakes in the deviations. Descriptive tests were performed in the calculation of mean values and standard deviations. The PASW Statistics (IBM, SPSS Corp.; Armonk, NY, USA) version 18.0 software for Windows was used for all statistical analyses. The aim of the study was to determine the use of QT in comparison with CMT and SCC in the diagnosis of subclinical mastitis.

The present study was laboratory based and did not use any human materials. Therefore, ethical approval and informed consent are not necessary. The study was performed in accordance with the principles of the Declaration of Helsinki.

RESULTS

Among 160 samples, 101 had a standard value ≤ 100 with QT. When the SCC results (0-100,000 cell/mL) of 101 samples were compared, the results were found to be compatible in the ratio of 60.4% (n=61). Of 101 samples with ≤ 100 standard value, 28.7% (n=29) were evaluated as negative in terms of subclinical mastitis according to the SCC (100-250,000 cell/mL) interval. When QT ≤ 100 standards were compared with the SCC results that were accepted as negative subclinical mastitis (0-250,000 cell/mL), the results were found to be

TABLE 1. The compatibility ratios between QT and SCC

QT/SCC		SCC (x1000/cell/mL)					
		0-100	101-250	251-500	501-750	751-1500	1501-3000 ↑
QT	≤100	61 60.39%	29 28.71%	8 7.92%	-	2 1.98%	1 0.99%
	250	1 5.55%	9 50%	3 16.66%	4 22.22%	1 5.55%	-
	500	-	1 7.14%	2 14.28%	4 28.57%	5 35.71%	2 14.28%
	750	-	-	1 16.66%	3 50%	1 16.66%	1 16.66%
	1500	-	-	-	-	4 44.44%	5 55.55%
	3000	-	-	-	-	-	12 100%

QT: Quick Test; SCC: Somatic Cell Count

TABLE 2. The compatibility ratios between CMT and SCC

CMT/SCC		SCC (x1000/cell/mL)					
		0-100	101-250	251-500	501-750	751-1500	1501-3000 ↑
QT	Negative (0-250)	53 67.94%	14 17.94%	7 8.97%	2 2.56%	1 1.28%	1 1.28%
	+ (251-500)	7 16.67%	15 35.72%	10 23.81%	3 7.14%	6 14.28%	1 2.38%
	++ (501-1500)	2 9.09%	6 27.27%	3 13.63%	3 13.63%	4 18.18%	4 18.18%
	+++ (1501-≥3000)	-	-	-	1 5.55%	3 16.66%	14 77.77%

QT: Quick Test; SCC: Somatic Cell Count

TABLE 3. The compatibility ratios between CMT and QT

CMT/SCC		SCC (x1000/cell/mL)					
		0-100	101-250	251-500	501-750	751-1500	1501-3000 ↑
CMT	Negative (0-250)	67 84.81%	6 7.59%	6 7.59%	-	-	-
	+ (251-500)	25 60.97%	10 24.39%	2 4.87%	1 2.43%	3 7.31%	-
	++ (501-1500)	7 31.81%	2 9.09%	5 22.72%	4 18.18%	3 13.63%	1 4.54%
	+++ (1501-≥3000)	2 11.11%	1 5.55%	1 5.55%	1 5.55%	3 16.66%	10 55.55%

CMT: California Mastitis Test; QT: Quick Test

TABLE 4. The manifestation of specificity, sensitivity, and false ratio results in comparison with half-quantitative test number (QT and CMT) with SCC

Parameters	Comparisons		
	QT/SCC	CMT/SCC	CMT/QT
Subclinical mastitis negative 0-250(x1000/cell/mL) (specificity)	84.0% (100/119)	85.9% (67/78)	92.40% (73/79)
Subclinical mastitis positive ≥250 (x1000/cell/mL) (sensitivity)	97.5% (40/41)	63.4% (52/82)	41.97% (34/81)
False ratio	16.7% (20/120)	27.5% (44/160)	39.1% (47/120)

CMT: California Mastitis Test; QT: Quick Test; SCC: Somatic Cell Count

highly compatible with ratios of 89.1% and 10.9%, respectively. When the results of QT ≤100 and 250 standards were compared with SCC, the negative subclinical mastitis values (0-250,000 cell/mL) were found to have a compatibility ratio of 84.0% (Table 1).

When CMT was compared with SCC, the negative subclinical mastitis values (0-250,000 cell/mL) of CMT were found to be compatible in the ratio of 85.9% (Table 2).

When the results of QT and CMT were compared, the negative subclinical mastitis values (0-250,000 cell/mL) of CMT were found to be compatible in the ratio of 93.3% (Table 3).

Proportional compatibility was determined between QT and SCC and CMT and SCC with the ratios of 97.57% and 63.42%, respectively, in all positive subclinical mastitis cases (p<0.001).

When QT was compared with the SCC results, the specificity, sensitivity, and false ratio of QT were 84.0% (negative), 97.5% (positive), and 16.7%, respectively. In other CMT/SCC and CMT/QT comparisons, the specificity ratios of CMT were determined as 85.9% and 92.4%, respectively (Table 4).

DISCUSSION

In cows, mastitis has been generally accepted as the most complicated and costly disease, resulting in the highest economic losses in the milk industry (8-10, 24, 25). Subclinical mastitis is the overemphasized form of mastitis in terms of economical aspect due to its effects on milk production and milk quality. The losses caused by mastitis involve deteriorating milk quality, lack of quality premium milk for buyers, treatment expenses for clinical mastitis, risks of antibiotic residuals, slaughtering, and death (10). Most of the farmers considered the losses for clinical cas-

es, animals that had to be killed, veterinary services, and drug costs (24, 25). The high losses of milk production are unclear or non-relevant reasons of subclinical mastitis cases (25).

Dohoo and Meek (26) evaluated the SCC limit of subclinical infected mammary lobes and mixed milk as 300,000 cell/mL and 250,000 cell/mL, respectively. Direct (e.g., Fossomatic) and indirect (CMT) methods are used for the determination of SCC in subclinical mastitis. PortaSCC® QT is an important indirect and simple test, showing the categories of <100,000 cell/mL and 250,000 cell/mL, separately. Our study was performed to show the compatibility levels and practicability of QT with CMT and SCC (Fossomatic Test). Sanford et al. (27) have stated that CMT is the gold standard and has wide ranges. In the present study, the SCC results were considered as the standard value, and the accuracy of both tests (QT and CMT) was emphasized in accordance with the SCC results.

When healthy values below subclinical mastitis levels (<250,000 cell/mL) were considered, the compatibility ratios between QT and SCC and CMT and SCC were found as 81% and 85.9% (CMT negative values), respectively. Casura et al. (28) stated that CMT does not provide an adequate safety level in comparison with SCC for the diagnosis of subclinical mastitis, and that SCC is more reliable than CMT. The relationship between CMT and bacteriological results was between 70% and 86% (27, 29), and these results are supported by the CMT results obtained in the present study. The same result ratios were obtained from QT and SCC in this value category. The comparison ratio of 93.3% between CMT and QT revealed that both tests were reliable in the diagnosis of negative subclinical mastitis (0-250,000 cell/mL).

For the determination of all positive cases of subclinical mastitis, SCC data were found to be compatible with QT and CMT with ratios of 97.57% and 63.42%, respectively. The results of + subclinical mastitis were found to be compatible with SCC (0-250,000 cell/mL) in the ratio of 92.4%, particularly for CMT controls. There were statistically significant differences between both tests in terms of determination ratios of positive results ($p < 0.001$). The sensitivity (63.4%) and specificity (85.9%) ratios between CMT and SCC demonstrated the possibility of mistakes in positive values using CMT. In studies comparing CMT with bacteriological results according to all CMT factors, Baştan et al. (29) detected a compatibility ratio of 85% between CMT and bacteriological results, whereas Varatanovic et al. (30) found a ratio of 55.7%. Obviously, compatibility ratios are dependent on bacterial types. In the present study, the SCC results were similar to those of Varatanovic et al. (30) although their comparison was performed using bacteriological results. Data obtained from SCC showed that CMT tests were less compatible than QT in cases of positive mastitis results with SCC. Studies were also performed on sheep and goat (31-33). The CMT control results showed that negative scores were more sensitive than positive scores. In the present study, the same result was also found in cows.

Sensitivity shows the true positive ratio (34). The higher sensitivity ratio of QT obtained by SCC revealed a more reliable determination of positive cases with QT in comparison with CMT. Specificity is the determination of true negative cases (34). The close and high specificity ratios in CMT and QT suggested that

negative cases had the same reliability in both tests. Mastitis was not detected when CMT and QT results were compared, and the compatibility ratio of 93.33% (0-250,000 mL/milk) between both tests supports these findings.

Most studies indicate that decreasing the limits of SCC will positively influence acceptability and suitability of milk as measured by improved safety, milk quality, and value-added products. The relationship of high SCC milk with poor farm hygiene, antibiotic residues, and presence of pathogenic organisms and toxins offers an insight into the potential increase in safety risk factors to consumers when high SCC milk is marketed. However, consuming milk with high SCC does not appear to pose direct, specific health risks to humans. In conclusion, scientific studies have not shown that the ingestion of large numbers of bovine leukocytes is harmful to humans (35).

When comparing QT and CMT with both SCC and between themselves (CMT/QT), the specificity ratios were calculated as 84.0% and 92.4%, respectively. This result revealed the high sensitivity ratio of both tests in the determination of healthy cases (negative mastitis). When this result was compared with SCC, the sensitivity of QT was found as 97.5%. It is concluded that QT can be used easily in the field, and the ratios of "mastitis is present" and "mastitis is absent" are detected in higher ratios with QT in the diagnosis of subclinical mastitis related with human health risk.

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