Research Article

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Relations between electrical conductivity, somatic cell count, California mastitis test and some quality parameters in the diagnosis of subclinical mastitis in dairy cows

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Abstract: This study was conducted to determine the effectiveness of the electrical conductivity (EC) method on the diagnosis of subclinical mastitis in dairy cows comparing with somatic cell count (SCC) and California mastitis test (CMT), and also to investigate the effect of these values on the amount of total viable bacteria, density, freezing point, and mineral substances. A total of 386 milk samples collected from quarters of 188 cows at 10 different farms were used as materials. Of the samples, 258 (66.85%) were CMT (+), 85 (22.02%) were CMT (++), and 43 (11.13%) were CMT (+++). The mean EC and SCC were 25.71, 28.02, and 29.63 mS and 249,453, 1,167,058, and 2,108,139, respectively, according to the CMT results. The total viable bacteria counts ranged from 3.4771 to 6.9395, from 3.4771 to 7.3617, and from 4.7782 to 7.5315 log CFU/mL, respectively. The average amounts of mineral substances were 0.65%, 0.70%, and 0.76%; the density values were 1.031, 1.0297, and 1.029; and the freezing points were –0.5281, –0.5285, and –0.5282, respectively. As a result, it has been concluded that EC showed similarity with the CMT and the SCC in the detection of subclinical mastitis; furthermore, its reliability would further increase when used together with the other diagnostic methods.

Key words: Milk, electrical conductivity, somatic cell count, California mastitis test, subclinical mastitis

İneklerde subklinik mastitis tanısında elektrik iletkenliği, somatik hücre sayısı, Kaliforniya mastitis testi ve bazı kalite parametreleri arasındaki ilişkiler

Özet: Bu araştırmada ineklerde subklinik mastitis tanısında kullanılan elektriksel iletkenlik (EC) yönteminin etkinliğinin, somatik hücre sayısı (SCC) ve Kaliforniya Mastitis Testi (CMT) ile karşılaştırarak araştırılması; aynı zamanda bu değerlerin toplam canlı mikroorganizma sayısı, yoğunluk, donma noktası ve mineral madde miktarı üzerine etkilerinin belirtilmesi amaçlanmıştır. Çalışmada 10 farklı çiftlikte bulunan 188 ineğin toplam 386 meme lobundan alınan süt örnekleri materyal olarak kullanılmıştır. Bu örneklerin 258'i (% 66,85) CMT (+), 85'i (% 22,02) CMT (++) ve 43'ü (% 11,13) CMT (+++) olarak değerlendirilmiştir. CMT sonuçlarına göre ortalama elektriksel iletkenlik değerleri sırasıyla 25,71, 28,02 ve 29,63 mS ve ortalama somatik hücre sayıları ise sırasıyla 249.453, 1.167.058 ve 2.108.139 olarak belirlenmiştir. Toplam canlı mikroorganizma sayısı sırasıyla 3,4771-6,9395, 3,4771-7,3617 ve 4,7782-7,5315 Log CFU/mL olarak bulunmuştur. Mineral madde miktarı ortalama olarak sırasıyla % 0,65, % 0,70 ve % 0,76; yoğunluk 1,031, 1,0297 ve 1,029 ve donma noktası ise –0,5281, –0.5285 ve –0,5282 olarak saptanmıştır. Sonuç olarak, subklinik mastitisli sütlerde elektriksel iletkenliğin saptanmasının CMT ve SCC ile benzerlik gösterdiği; dolayısıyla subklinik mastitislerin teşhisi için kullanılabilir bir yöntem olabileceği; ayrıca diğer tanı yöntemleri ile kullanıldığında güvenilirliğinin daha da artacağı kanısına varılmıştır.

Anahtar sözcükler: Süt, elektrik iletkenliği, somatik hücre sayısı, Kaliforniya mastitis testi, subklinik mastitis

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Introduction

Mastitis has been recognized as a major disease affecting the dairy industry, especially in its subclinical form (1,2). Subclinical mastitis causes economic losses by decreasing milk production, costs of therapy, and unused milk during the withdrawal period (3). Clinical mastitis can be detected by the farmer, but subclinical mastitis can only be detected by the measurement of inflammatory components and pathogens in the milk (2). Since somatic cell count (SCC) in milk has been shown to be an excellent marker for subclinical mastitis (1), the use of SCC is inevitable for specifying the reduction of the milk yield.

The contents and nutritional values of milk, which are important for human nutrition, may be changed with a systemic or mammary gland infection of host animals. High SCC in milk reduces the quality of both milk and dairy products, affects milk shelf life and flavor, and deteriorates the physicochemical properties of milk (4). Mastitic milk contains both pathogens and bacterial toxins and its consumption may directly or indirectly increase the risk of food-borne illnesses.

Different methods have been suggested for detection of subclinical mastitis, such as the California mastitis test (CMT), SCC, some biochemical methods, the presence of pathogens in the milk, and electrical conductivity (EC) (5,6).

EC, which increases during the infection of dairy cows, is also one of the diagnostic methods for detection of subclinical mastitis. EC is determined by the concentration of anions and cations. According to Kitchen (7), mastitis increases the EC of milk because of changes in ionic concentrations. As a result of the damage to the udder tissue, concentrations of lactose and K^+ decrease, and concentrations of Na^+ and Cl^- increase.

This study was conducted to determine the effectiveness of the EC method on the diagnosis of subclinical mastitis in dairy cows, compared with SCC and the CMT, and also to designate the effect of these values on the amount of total viable bacteria, density, freezing point, and mineral substances in the acquisition of healthy milk for consumer rights.

Materials and methods

The study was conducted in a part of the Thrace region of Turkey, which is located about 150 km northwest of İstanbul. A total of 386 milk samples were collected from quarters of 188 cows at 10 different small holder dairy farms, and were examined using the CMT, EC, SCC, and total viable bacterial count (TVBC). The density, freezing point, and total mineral substances were also investigated.

Study methodology: Clinical examination of the udder and screening using the CMT were carried out on farms for 320 lactating cows in order to determine subclinical mastitic cows. CMT positive milk samples (CMT +, ++, +++) were defined as subclinical mastitis suspected samples and were collected carefully using all of the material for bacteriological and physicochemical examinations.

Clinical inspection of the udder: Udders of the cows were examined visually and by palpating for the presence of any lesions, such as redness, pain, heat, and swelling. Moreover, milk samples from each quarter was taken and checked for any change in color and consistency.

Milk sample collection: Milk samples were collected according to the National Mastitis Council (8). After a quarter had been cleaned up by removing any possible dirt and washed with tap water, the teat end was dried and swabbed with cotton soaked in 70% ethyl alcohol. Approximately 100 mL of milk was then collected aseptically from subclinical (CMT positive) mastitic cows into sterile bottles, after discarding the first 3 milking streams. Samples from each quarter were transported to the laboratory of the Faculty of Veterinary Medicine, İstanbul University, in an ice-cooled box and analyzed immediately (max. 4 h after collection) for microbiological and physicochemical parameters.

CMT: This test was done according to the method described by Schalm and Noorlander (9), at cowside, by mixing an equal volume of milk with a 1:1000 dilution of 3% sodium lauryl sulphate and bromocresol (DeLaval, Cardiff, UK). Each quarter's milk sample was placed in 1 clean well of a plastic test paddle, divided into 4 separate wells. As the plate was rotated gently, any color changes or formation of a viscous gel were interpreted. Scores were given within the range 0-3, with 0 for no reaction, 1 for a weak positive, 2 for a distinct positive, and 3 for a strong positive.

SCC: Somatic cell counts were performed within 24 h of collection using a Fossomatic 90 instrument (Foss Electric, Hillerod, Denmark) after heat treatment at 40 °C for 15 min (10).

TVBC: 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 (11).

Physicochemical analysis: The amount of electrical conductivity (EC), density, freezing point, and mineral substances were determined using a LactoStar Milk Analyzer (Funke Gerber, Germany).

Statistical analysis: The General Linear Model procedure (PROC GLM) of SPSS 13.0 (SPSS Inc. IL, USA: Chicago) was used to analyze the data and to determine the least squares means (LSM), standard errors (SE), and the significant differences among means. Significance of differences was defined as $P < 0.05$ and if the effect of CMT levels was found to be significant, Duncan's multiple range test was used to evaluate the significance of the difference. Pearson correlations among defined characteristics were also estimated using SPSS 13.0.

Results

Results of analyzed milk samples are shown in Tables 1 and 2. Of the samples, 258 (66.85%) were evaluated as CMT (+), 85 (22.02%) were CMT (++), and 43 (11.13%) were CMT (+++). The mean EC and SCC were 25.71, 28.02, and 29.63 mS and 249453, 1167058, and 2108139, respectively, according to their CMT results. The total viable bacteria counts ranged from 3.4771 to 6.9395, from 3.4771 to 7.3617, and from 4.7782 to 7.5315 log CFU/mL, respectively. The average amount of mineral substances were 0.65%, 0.70%, and 0.76%; the density values were 1.031, 1.0297, and 1.029; and the freezing points were –0.5281, –0.5285, and –0.5282, respectively. The correlation between the parameters of subclinical mastitis suspected milk is explained in Table 3. The correlations were –0.001, –0.002, and –0.451 between the freezing point and the CMT, SCC, and TVBC, respectively.

Discussion

The economic impact of both the clinical and subclinical forms of mastitis is large in the current dairy industry. Losses occur from decreased milk production, treatment, labor costs, non-deliverable milk, veterinary fees, increased risk of subsequent mastitis, risk of culling or death of the cow, and

Table 1. Means and standard errors (SE) of EC, SCC, TVBC, and some quality parameters of subclinical mastitis suspected milk, depending on CMT levels.

Characteristics	CMT (+)	CMT (++)	CMT (+++)	Significance
SCC (1000 cells/mL)	$249.453^{c} \pm 14.061$	$1167.058^{b} \pm 68.712$	$2108.139^{a} \pm 64.354$	***
TVB (log CFU/mL)	$5.7777^{c} \pm 0.493$	$6.5797^{b} \pm 0.567$	$7.1872^{a} \pm 0.615$	***
Density (g/mL)	$1.0310^{a} \pm 0.0001$	$1.0297^{b} \pm 0.0003$	$1.0290^{b} \pm 0.0004$	***
Freezing points (°C)	–0.5281 ± 0.005	–0.5285 ± 0.006	–0.5282 ± 0.007	NS
EC (mS)	$25.71^{c} \pm 0.184$	$28.02^{b} \pm 0.422$	$29.63^{a} \pm 0.698$	***
Mineral substances (%)	$0.6537^{c} \pm 0.004$	$0.7088^{b} \pm 0.010$	$0.7628^{a} \pm 0.018$	***

Mean values within the same row with different superscript small letters are different.
(***): $P < 0.001$ NS: Not significant ($P > 0.05$)

Table 2. Minimum and maximum values of EC, SCC, TVBC, and some quality parameters of subclinical mastitis suspected milk.

Characteristics	n	**SCC (1000 cells/mL)**		**TVBC (log CFU/mL)**		**Density (g/mL)**		**Freezing points (°C)**		**EC (mS)**		**Mineral substances (%)**	
		Min	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
CMT (+)	258	30	1500	3.4771	6.9395	1.0180	1.0380	–0.777	–0.419	19.66	37.46	0.51	0.89
CMT (++)	85	100	2800	3.4771	7.3617	1.0180	1.0370	–0.773	–0.427	22.24	40.98	0.57	1.06
CMT (+++)	43	1000	2900	4.7782	7.5315	1.0214	1.0350	–0.678	–0.449	20.76	41.65	0.53	1.08
Overall mean	386	659.244 ± 37.648		6.4697 ± 0.5486		1.0305 ± 0.0001		–0.5282 ± 0.0035		26.65 ± 0.1866		0.6779 ± 0.0046	

Table 3. The coefficient of correlation between EC, SCC, TVBC, CMT, and some quality parameters of subclinical mastitis suspected milk.

Characteristics	**EC (mS)**	**SCC**	**TVBC (log CFU/mL)**	**Density (g/mL)**	**Freezing points (°C)**	**Mineral substances (%)**	**CMT**
EC (mS)	1.00	0.399***	0.323***	–0.615***	0.228***	0.911***	0.384***
SCC (cell/mL)	0.399***	1.00	0.682***	–0.274***	–0.002	0.431***	0.863***
TVB (log CFU/mL)	0.323***	0.682***	1.00	–0.185***	–0.039	0.325***	0.720***
Density (g/mL)	–0.615***	–0.274***	–0.185***	1.00	–0.303***	–0.536***	–0.233***
Freezing points (°C)	0.228***	–0.002	–0.039	–0.303***	1.00	0.203***	–0.001
Mineral substances (%)	0.911***	0.431***	0.325***	–0.536***	0.203***	1.00	0.411***
CMT	0.384***	0.863***	0.720***	–0.233***	–0.001	0.411***	1.00

*** Correlation is significant at the level of $P < 0.001$.

reduced milk quality and milk price (1,12). In addition, low quality milk can consist of pathogens and their toxins, which are risky for human health.

The quickest and simplest way to identify the intramammary infections (IMIs) in dairy cows is using defined parameters within either SCC or CMT scores. The CMT is a rapid and inexpensive test to indirectly determine the somatic cell concentration in milk (13) and is a practical and easy method for demonstrating IMIs by testing milk samples on-farm (14). Somatic cells are always present in milk and they increase due to mammary gland infections.

SCC in healthy cow's milk is between 50,000 and 100,000 cells/mL. If the SCC exceeds 200,000 cells/mL, it is considered unhealthy for consumers. High SCC in milk reduces the quality of both milk and dairy products, and also affects milk shelf-life and flavor (4). Due to human health and animal welfare concerns, several countries (EU nations and Switzerland) set 400,000 cells/mL as the upper limit for SCC in milk.

In the present study, 258 (66.85%) milk samples were evaluated as CMT (+), 85 (22.02%) were CMT (++), and 43 (11.13%) were CMT (+++). All of the samples were accepted as suspected for subclinical mastitis and had an average SCC of 249,453, 1,167,058, and 2,108,139, respectively, according to their CMT results. The total viable bacteria counts ranged from 3.4771 to 6.9395, from 3.4771 to 7.3617, and from 4.7782 to 7.5315 log CFU/mL, respectively. Risvanli and Kalkan (15) examined 271 subclinical mastitic udder lobes and 8.12%, 22.88%, and 69.00% of the udder lobes were CMT (+), CMT (++), and CMT (+++), respectively. They stated that the microbiological isolation rate was 60.89% in all of the udder lobes and the mean SCCs of the milk samples were 313,001, 559,007, and 1,563,618, respectively, according to CMT results. However, Baştan et al. (16) analyzed 344 subclinical mastitis suspected milk samples and found that 250 were CMT (+) and 94 were CMT (–). Similarly, Baştan et al. (5) indicated that out of 183 milk samples from 49 cows, on 3 different farms, 50 (27.3%) were CMT (–), while 40 (21.8 %), 56 (30.6%), and 37 (20.2%) were CMT (+), (++), and (+++), respectively. The SCCs were 100-200,000, 200-300,000, 300,000-1,000,000, and above 1,000,000 cell/mL, respectively, in the groups of CMT (–), CMT (+), CMT (++), and CMT (+++). Lafi (6) examined a total of 1210 milk samples from 46 flocks near Irbid, Jordan, and 719 (59.4%) did not have any bacterial growth; 91% (654/719) and 80% (570/719) of these samples had less than 1.00×10^6 cells/mL and ≤2+ CMT score, respectively. He added that in 472 milk samples (39.1%), the bacterial culture yielded at least 1 pathogen. He found that 9% (44/493) of the infected glands had SCC less than 1.00×10^6 cells/mL and none had <3+ CMT score. Bhutto et al. (17) explained that 507 (53%) of 960 subclinical intramammary infection milk samples were CMT (–), while 192 (20%), 146 (15%), 64 (7%), and 51 (5%) were CMT (+), (++), (+++), and (++++), respectively. They emphasized that the SCCs were $<150 \times 10^3$, 151-250 $\times 10^3$, 251-500 $\times 10^3$, 501-750 $\times 10^3$, and 751->1000 $\times 10^3$, respectively. Out of 308 mammary quarters of 77 cows, Kuplulu et al. (18) determined 165 (53.58%) to be CMT (–) and 143 (46.42%) to be CMT (+). They added that, according to microbiological investigations, 143 quarters were infected by bacteria, most of which were staphylococcus, streptococcus, and *E. coli*.

EC of milk has been introduced as an indicator trait for mastitis (19). The EC is determined by the concentrations of anions and cations. The altered concentrations of Na^+, K^+, and Cl^- in mastitic milk cause the EC of milk to be increased (7). Typical EC of normal milk appears to be between 4.0 and 5.5 mS/cm at 25 °C. If the EC is higher than 5.6 mS/cm, it means the cow suffers from mastitis or the milk is suspected of mastitis. The EC of milk has also been expressed as a concentration of NaCl with the same conductivity as the examined milk (12).

The mean EC of 386 analyzed milk samples was 25.71, 28.02, and 29.63 mS according to their CMT results, respectively, for CMT +, ++, and +++. As the CMT positive results supported the presence of subclinical mastitis, the increase in SCC resulted in an increase of the EC. Likewise, Baştan et al. (5) determined that the mean conductivity of 183 milk samples was 5.410, 6.317, 6.867, and 7.850 mS/cm, respectively, for CMT –, +, ++, and +++. Tekeli et al. (20) stated that the EC value varied depending on milking intervals and EC of the analyzed milk samples was 4.52, 5.14, and 7.38 mS/cm, respectively, depending on CMT levels. Kuplulu et al. (18) indicated that according to the results taken by the microbiological investigations of 308 quarters of subclinical mastitic cows, the milk from quarters infected subclinically with *S. aureus* had a higher electrical conductivity (7.5 mS/cm) than the milk from quarters with subclinical *Strep. uberis*, *Strep. dysgalaetia*, and *E. coli* infections with normal EC (5.24, 5.54, and 4.86 mS/cm, respectively).

Norberg et al. (19) explained that the mean EC of healthy cows was 4.87 mS/cm, while the mean conductivity of subclinical and clinical mastitic cows was 5.37 and 6.44 mS/cm, respectively. Bruckmaier et al. (21) determined that electrical conductivity was higher in infected quarters and added that the mean EC of log SCC/mL >6.0 milk was 6.06 mS/cm, while the mean EC of log SCC/mL between 5.6 and 6.0 was 5.84 mS/cm. Ilie et al. (22) stated that 50 dairy cows were classified as healthy/clinically infected or subclinically infected, and EC was measured repeatedly from samples collected at milking. For the subclinically infected quarters (n = 115), the mean EC was 5.42 mS/cm, while it was 4.53 mS/cm for the healthy (n = 21) and 6.31 mS/cm for clinically infected cows (n = 52). Additionally, Janzekovic et al. (23) indicated that the conductivity in individual quarters of 102 lactating cows (Black and white, Simmental, and Brown Swiss breed) on 7 farms was <5.5 mS/cm for healthy udders and >6.5 mS/cm for subclinical udders. According to the researchers findings (19,21-23), electrical conductivity is an indicator of subclinical mastitis when it is above >5.5 mS/cm. This increase in EC value results from the alteration of the concentration of mineral substances.

The average amount of mineral substances was 0.65%, 0.70%, and 0.76%; the density was 1.031, 1.0297, and 1.029; and the freezing points were –0.5281, –0.5285, and –0.5282, respectively, in the present study. Similarly, Matei et al. (24) reported that in 127 analyzed cows, the number of somatic cells counts (450,000-1,200,000 cell/mL of milk) increased with decreases of the freezing point (–0.570 to –0.639). For subclinical mastitis, cow's milk showed an increase in milk chlorides, while the density of the milk was between 1.028 and 1.0345. Wendorff et al. (25) emphasized that milk has a fairly constant freezing point, which is affected by the solutes in its solvent. Mitchell (26) determined that lactose, chloride, citrate, and lactic acid accounted for 79% to 86% of the total freezing point depression, and added that subclinical mastitis showed a significant increase in chloride with no significant effect on the freezing point. In addition to this, Bruckmaier et al. (21) stated that the concentrations of Na and Cl were higher in infected quarters, while K was lower, which resulted in high electrical conductivity in infected quarters. El Zubeir et al. (27) indicated that the levels of minerals (Ca, Na, K, Mg, P, Fe, Zn, and Cu) for the subclinical mastitic milk samples revealed a positive correlation between iron, calcium, and magnesium, while significant a negative correlation was found between sodium and potassium, and they concluded that the minerals from the milk of cows can be used as indicators of mastitis infection. Likewise, Ogola et al. (28) stated that in 396 analyzed quarter milk samples from lactating crossbred cows (Holstein-Zebu), 56% of these quarters had intramammary infection, with an overall mean SCC of 619,000 cells/mL for infected quarters and increased concentrations of sodium, and chloride ($P < 0.05$), while potassium and calcium were lower ($P < 0.05$). Pyörälä (29) also reported that the main changes in the production and composition of milk caused by mastitis was seen in the concentration of the milk components. The amount of SCC, EC, sodium, and chloride increased, while calcium, magnesium, phosphorus, zinc, and potassium decreased. Shitandi et al. (30) reported that intramammary infection was recorded in 68.1% of the 72 quarter milk samples from 18 lactating, crossbred (Holstein-Zebu), small-scale dairy cows and added that the concentrations of sodium and chloride were higher ($P < 0.01$), while potassium and calcium were lower in infected quarters. They emphasized that sodium content was positively correlated with chloride content ($r = 0.77$, $P < 0.0001$), but negatively correlated with K content ($r = –0.61$, $P < 0.0001$).

The correlation between the parameters of subclinical mastitis suspected milk of the present study is explained in Table 3. There was a significant positive correlation ($P < 0.001$) between CMT, SCC, TVBC, EC, and mineral substances, while a significant negative correlation was found between density and CMT, SCC, EC, TVBC, freezing point, and mineral substances, and also a negative correlation was determined in the freezing point and CMT, SCC, and TVBC ($r = –0.001$, $–0.002$, and $–0.451$, respectively, $P > 0.05$).

The early diagnosis and prevention of subclinical mastitis must be a priority for each dairy holder, because of the acquisition of good quality milk and the prevention of economical losses. The electrical conductivity of milk is an indicator for the diagnosis of subclinical mastitis, used together favorably with other methods. EC is determined by the concentration of anions and cations based on the ionic changes that occur during inflammation of the udders. Measuring the EC of milk could be an early warning system for udder health monitoring while implanting to the milking system. If the cow is affected by mastitis, the sodium and chloride concentrations would increase, also determining an increment in EC value. Electrical conductivity exhibits a high correlation with CMT and SCC scores, while SCC and EC are influenced by parity, age, stage of lactation, season, stress, milking interval, and environmental factors.

It is concluded that EC showed similarity with CMT and SCC in the detection of subclinical mastitis; furthermore, its reliability would further increase when used together with the other diagnostic methods.

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