Hayvancılık Araştırma Dergisi (1999) 9,1-2:77-80

STUDIES on DETECTION of AETIOLOGY of SUBCLINICAL MASTITIS in COWS USING by ELECTRICAL CONDUCTIVITY METER

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Elektriksel geçirgenlik aletinden yararlanarak ineklerde subklinik mastitisin etiyolojisinin belirlenmesi üzerine çalışmalar.

SUMMARY

According to California Mastitis Test (CMT), Electrical Conductivity (EC) test , Direct Microscopic Somatic Cell Counts (DMSCC) and microbiological analysis results, 51 subclinical mastitic and 27 normal foremilk samples were included in this study. In the mastitic foremilk samples, mean EC values were 6.86 ± 0.20 ms/cm in the *Staphylococcus spp.* infection , 6.55 ± 0.21 ms/cm in the *Streptococcus spp.* infection and 6.22 ± 0.38 ms/cm in the *Escherichia coli* infection. In the normal fore milk samples the mean EC value was 4.68 ± 0.065 ms/cm. Although, there was statistically significant difference in EC values between subclinical mastitis and normal fore milk samples, there was no relation between EC values and causative organisms.

In conclusion, the present study indicates that electrical conductivity meter is useful in the diagnosis of subclinical mastitis clinically, but it is unsuitable for detection of aetiology of subclinical mastitis.

KEY WORDS: Cow, mastitis, aetiology, diagnosis, electrical conductivity.

ÖZET

Kalifornia Mastitis Test (CMT), Elektriksel Geçirgenlik (EG) testi, Direkt Mikroskobik Somatik Hücre Sayımı (DMSHS) ve mikrobiyolojik muayene sonuçlarına göre belirlenen 51 subklinik mastitisli ve 27 normal ilk süt örneği çalışmaya alındı. Mastitisli ilk süt örneklerinin ort. EG değerleri *Staphylococcus spp.* infeksiyonunda 6.86 ± 0.20ms/cm, *Streptococcus spp.* infeksiyonunda 6.55 ± 0.21ms/cm ve *Escherichia coli* infeksiyonunda 6.22 ± 0.38ms/cm olarak belirlendi. Normal ilk süt örneklerinin ort. EG değeri 4.68 ± 0.065 ms/cm olarak bulundu. Subklinik mastitisli ve normal ilk süt örneklerinin EG değerleri arasında istatistiksel olarak önemli farklılıklar bulunmasına rağmen, EG değerleri ve etiyolojik etkenler arasında bir ilişki saptanmadı.

Sonuç olarak, bu çalışma elektriksel geçirgenlik aletinin klinik olarak subklinik mastitislerin teşhisinde yararlı olduğunu, fakat subklinik mastitislerin etiyolojilerinin belirlenmesinde faydalı olmadığını göstermektedir.

ANAHTAR KELİMELER: İnek, mastitis, etiyoloji, teşhis, elektriksel konduktivite.

INTRODUCTION

Subclinic mastitis that affects mammary tissues, composition and quantity of milk cannot be diagnosed by inspection and clinical examination, but it can be diagnosed by demonsrating the increased number of leucocytes, variation in biochemical values and isolation of pathogenic agents. In dairy herds subclinic mastitis seen more widespread than clinic mastitis and diminishes the production of milk in mammary quarters. In addition, milk originating from cows with subclinic mastitis reduces the quality of milk products. Therefore; its economic importance has been getting higher (Alaçam 1991, Kitchen 1981).

Mastitis can be diagnosed by demonsrating compositional changes in milk. These changes can be determined by diagnostic tests and these are: 1) direct and indirect tests demonstrating increased number of leucocytes, 2) tests showing increased permeability between the blood and milk compartments, 3) tests based on ionic changes, 4) test indicating celluler disruption and 5) tests demonsrating decreased synthetic capacity of the mammary gland epithelium (Mattila 1985, Sandholm and Mattila 1986).

Direct and indirect Somatic Cell Count (SCC) methods are extensively used to determine subclinical mastitic milks (International Dairy Federation 1979, Kitchen 1981, Uysal 1985). Many reports (Alaçam et al. 1988, International Dairy Federation 1979, Nak 1994, Unal et al. 1995, 1996) indicate that SCC is a sensitive indicator of inflammation and has gained wide acceptance for this purpose.

Mastitis causes damage of epithelial cells, increase of capillar permeability and osmotic pressure

Yayına Kabul Tarihi: 03.05.1999
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in milk. Milk from glands with mastitis shows an increase in the levels of sodium and chloride ions (Kitchen 1981, Sandholm and Mattila 1986, Schalm et al. 1971, Rao 1990). This increase in sodium and chloride levels results in a higher Electrical Conductivity (EC) in milk. Researches were done to evaluate the efficacy and economic aspects of EC test (Emanuelson et al. 1987, Green and Middleton 1984, Isaksson 1987, Kang 1984, Swarup et al. 1989, Tekeli et al. 1993, Ünal et al. 1996).

Investigations showing that subclinical mastitic milks had a higher EC than normal milks were done to determine the relation between EC values of subclinical mastitic milks and their aetiological agents (Chamings *et al.* 1984, Hillerton and Walton 1991, Kangasniemi et al. 1987). Aetiology of subclinical mastitis can only be detected by microbiological analysis. Therefore, isolation and identification of bacterial pathogens in milk are important (Aydın and Akay 1984). Most investigations (Alaçam 1988, Arda and İstanbulluoğlu 1980, Bozkır 1985, Kahraman et al. 1991, Kaya et al. 1993, Nizamlıoğlu et al. 1989, Pir et al. 1983, Türütoğlu et al. 1995, Ulusoy et al. 1985, Ünal et al. 1995, Ünal et al. 1996) explained that *Stapylococcus aureus, Staphy. epidermidis, Streptococcus agalactiae, Str. dysgalactiae, Str. uberis, Corynebacterium bovis, Actinomyces pyogenes, micrococcus sp., Escherichia coli, Enterobacter aerogenes, Klebsiella pneumonia, Citrobacter freundii, Proteus vulgaris, Pr. mirabilis, Pseudomonas aeruginosa, Candida albicans* are isolated in mastitic milks.

The aim of this study is to show if there is a relation between EC values of subclinical mastitic milks and their aetiological agents.

MATERIALS and METHODS

In the present study, a total of 423 Holstein from Faculty of Veterinary Medicine, University of Uludağ and some commercial dairy farms in Bursa located in the north-western Anatolia was used as research materials. All cows were 2-10 years old, mid-lactating and maintained under identical feeding programs. California Mastitis Test (CMT) was performed for milk samples obtained from all cows. According to these test results, 39 mammary quarters showing " - " reactions were identified as free from subclinical mastitis. 122 mammary quarters showing " ? , 1+ , 2+ " reactions were identified as subclinical mastitis.

CMT and Direct Microscopic Somatic Cell Count (DMSCC) were done as described in literatures (Nak 1994, Schalm et al. 1971). The EC of milk was measured in the foremilk samples, by a hand-held conductivity meter-"Milk Checker" (Drintal İnst. Ltd. Tokyo, Japan) (Ünal et al. 1996).

For microbiological analysis, strict precautions were taken to avoid contamination at the time of sample collection of samples were transported to laboratory under ice-packs. Homogenized milk samples were plated on 7% ewe blood agar. The plates were incubated at 37°C in aerobic condition for 24-48 hours. After incubation, only pure cultures were evaluated. Colony and microscopic chracteristics of microorganisms were examined. Then, they were replated to suitable fluid media (nutrient broth-nutrient broth with serum) and other cahracteristics were investigated. Identification was done by the standart procedures (Akay and Aydın 1984, Baron and Finegolg 1990, Disping and Amtsberg 1988, İzgür 1984).

Differences among groups were compared by the analysis of variance, When differences appeared Tukey test was applied (Sümbüloğlu and Sümbüloğlu 1994).

RESULTS

Research results were summarized in Table 1 and 2. According to microbiological analysis results, 51 subclinical mastitic milk samples from which *Staphylococcus spp., Streptococcus spp.* and *E. coli* were isolated purely and 27 normal milk samples that did not show any microbial development were used to evaluate this study. 51 out of 122 (41.8%) milk samples provided from suspicious mammary quarters for subclinical mastitis showed aerobic agent developments. In 23 of them *Staph. aureus* (45.09%), in 12 of them *Str. agalactiae* (23.52%), in 4 of them *Stsr. dysgalactiae* (7.84%), in 4 of them *Staphylococcus spp.* (coagulase-negative), in 5 of them *E. coli* (9.8%) and in 3 of them *Str. uberis* (5.88%) were isolated. Also, 27 out of 39 milk samples provided from healthy cows showed no sign of any microbial development.

Table 1. Distribution and Statistical Comparison of DMSCC and EC Values of Subclinic Mastitic Milks and Normal Milks According to CMT Results.

Test Name	CMT(-) X±SH	CMT(?) X±SH	CMT(1+) X±SH	CMT(2+) X±SH	F
SCC(cell/ml)	44074.07 ± 9109^{a}	571764.7±39493^{a}	1287333.3±146832^{b}	2783157.8±323207^{c}	53.95***
E.C.(ms/cm^{x})	4.68±0.065^{a}	6.29±0.22^{b,c}	6.41±0.18^{c}	7.25±0.24^{d}	44.07***

^{x} milisimens /centimeter

*** In rows, the different letter superscripts indicate that mean values are different, p<0.001.

Table 2. The Statistical Comparison of EC Values According to the Microorganism Genus and Species Among Themselves and With EC Values of the Normal Milks.

Microorganism genus	Staphylococcus		Streptococcus			Escherichia	Control	F
E.C. (ms/cm)	6.86 ±0.20[a]		6.55±0.21[a]			6.22±0.38[a]	4.68±0.065[b]	34.08* **
Microorganism Species	*Staph. aureus*	*Staphylococcus spp.* (coagulaz-negatif)	*Str. agalactiae*	*Str. uberis*	*Str. dysgalactiae*	*E.coli*	Control	
E.C. (ms/cm)	6.80±0.23[a]	7.25±0.39[a]	6.25±0.18[a]	6.76±0.24[a]	7.32±0.78[a]	6.22±0.38[a]	4.68±0.065[b]	18.861 ***

*** In rows, the different letter superscripts indicate that mean values are different, P< 0.001.

DISCUSSION

Colonization of the bovine mammary glands by pathogenic bacteria results in a series of events which lead to major alterations in the composition of the milk secreted from tissue cells. Initially, elevated levels of pathogenic bacteria occur, but this is closely followed by marked increases in the number of somatic cells present. Then, total milk yield falls as a result of impaired synthetic ability of the secretory tissue and marked changes take place in the levels of nearly all major and minor elements of the milk (International Dairy Federation 1979, Kitchen 1981, Mattila 1985, Sandholm and Mattila 1986, Schalm et al. 1971).

Many reports (Alaçam et al. 1988, Nak 1994, Ünal et al. 1995, 1996) have showed that CMT and DMSCC tests are reliable for distinguishing the normal and subclinical mastitic milks. In this study too, it was found that DMSCC values of milk samples from CMT " - " showed lower values than 500.000 cells/ml value which is accepted by International Dairy Federation (IDF) (1979) as an index for mastitis. In addition, DMSCC values of milk samples from " ?,1+,2+ " showed higher value than IDF index value. Statistically, differences between control and CMT mean values were found not significant but differences among the other group's mean values were found significant.

In most researches (Isaksson 1987, Kang 1984, Swarup *et al.* 1989, Tekeli *et al.* 1993, Ünal *et al.* 1996) using EC values of milks for mastitis detection, it was shown that there were differences between milk samples from normal and subclinical mastitic mammary quarters. In this study, EC values among the groups determined according to the CMT results were compared statistically and the differences between CMT " - " and each of CMT " ?,1+,2+ " showed P<0.001 value.

Kang and Shin (1985), in the subclinical mastitic foremilk samples, found EC values which were 63.9 mM-NaCl, 60.5 mM-NaCl and 57.0 mM-NaCl for *E. coli, Streptococcus* and *Staphylococcus spp.* respectively. However, Mottram (1991) explained that Staphylococcal and Streptococcal infections were detectable by the change in the conductivity but EC meter was not useful for detecting coliform infections. In this study, it was found that the difference between the subclinical mastitic milks caused by *Staphylococcus spp., Streptococcus spp.* and *Escherichia sp.* and control group was significant for EC values. Nevertheless, the differences among subclinical mastitic milks caused by *Staphylococcus spp., Streptococcus spp.* and *Escherichia sp.* group were not significant for EC values.

When the studies based on the species of microorganisms, milk from quarters infected with *Staphy. aureus* had a higher mean conductivity level than milk from quarters infected with *Str. uberis.* In addition, Mottram (1991) explained that EC meter was unable to detect subclinical infections of *Str. uberis.* Also, Chamings et al. (1984) found that milk from quarters infected with *Str. agalactiae* had a higher mean conductivity level than milk from quarters infected with *Staph. aureus* in two herds. On the other hand, in the same study opposite results were obtained from other herds. Moreover, in another study (Kangasniemi et al. 1987), it was expressed that EC values were the highest for milk from quarters infected with *Str. dysgalactiae* and *Staph. aureus* follows this microorganism. In our study, when the EC values of microorganism species were compared with the EC values of control groups, the differences were found important statistically but when the EC values were compared among microorganism species, the differences were not important statistically.

In conclusion, this study implies that electrical conductivity meter is practical for the diagnosis of subclinical mastitis clinically; however, it can not be used for the detection of aetiological agents of subclinical mastitis.

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