VETERINARSKI ARHIV 76 (2), 101-109, 2006

A comparison of indirect methods for diagnosis of subclinical mastitis in lactating dairy cows

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ŠPAKAUSKAS, V., I. KLIMIENĖ, A. MATUSEVIČIUS: A comparison of indirect methods for diagnosis of subclinical mastitis in lactating dairy cows. Vet. arhiv 76, 101-109, 2006.

ABSTRACT

A total of 1014 samples taken from Lithuanian cows at the end of milking were tested on several parameters: milk reaction with Mastest (developed, tested, and produced at the Lithuanian Veterinary Institute), California Mastitis Test (CMT), Bernburg, Profilac reagent, Eimü-milchtest. Somatic cell count (SCC) was established using the flow cytometry method and Anadis F device; electrical conductivity (EC) was measured using a Mastiidiindikaator device made in Estonia. Electrical conductivity of the samples taken from healthy cows was 4.5-6.0 mS/cm, while EC in the samples taken from cows with subclinical mastitis was 6.1-8.5 mS/cm. Even though electrical conductivity of milk produced by the cows with mastitis tends to increase, it does not always correlate with SCC. Mastest results correlate best (95.5-100%) somatic cell count in milk when reaction of milk with milk is rated to be 1, 2, and 5 points, while the poorest correlation (87.62%) is at a rating of 3 points. Mast test results correlate well with those of other tests. Total coefficient of correlation between Mastest and Bernburg reagent r = +0.985, between Mastest and Profilac r = +0.980, between Mastest and Eimü-milchtest r = +0.966. The best correlation (99.39-100%) between Mastest and CMT is at a rating of 1 and 5 points. At the intermediary ratings (2-4 points) test results correlate 95.96-99.05%. Overall correlation coefficient r = +0.997.

Key words: mastitis, somatic cell count, mastitis test, electrical conductivity

Introduction

Early diagnosis of mastitis is vital because changes in the udder tissue take place much earlier than they become apparent. Various methods based on physical and chemical changes of milk are used for diagnosis of subclinical mastitis (BATRA and MCALLISTER, 1984;

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ISSN 0372-5480
Printed in Croatia

EMANUELSON et al., 1987). Results obtained from separate testing of milk obtained from each of the udder quarters are more precise than those obtained by testing mixed milk from all quarters. Such tests should be sufficiently rapid to allow early diagnosis of subclinical mastitis and timely intervention with relevant prevention or treatment measures.

Among the simplest tests, which do not require any complex laboratory equipment, are solutions containing detergents. Detergents decrease surface tension, change the structure and conductivity of cell membrane and nucleus, disturb osmotic balance, block oxidizing and stimulate proteolytic enzymes, and increase milk viscosity (COFFEY et al., 1986; MIDDLETON et al., 2004; RUEGG and REINEMANN, 2002; SARGEANT et al., 2001). Subclinical mastitis in cows is diagnosed using superficial active agents (detergents) and indicators. The anion superficial active agents are the most useful for this purpose (HOLDAWAY et al., 1996; RUEGG and REINEMANN, 2002; SARGEANT et al., 2001; ŠPAKAUSKAS et al., 2001). The most commonly used superficial active agents of this group are those which, in solutions, dissociate into anions. For the production of tests, alkilsulphates, alkilsulphonates, alkilbensol-sulphonates, alkilarilsulphonates, and laurilsulphates are most often used (PYÖRÄLÄ, 2003; SARGEANT et al., 2001; ŠPAKAUSKAS et al., 1998). Modern mastitis tests allow for indirect determination of the number of somatic cells in milk (COFFEY et al., 1986; DOHOO and LESLIE, 1991; EMANUELSON and WEVER, 1989; GREINER et al., 2000; HARMON, 1994; KEHRLI and SHUSTER, 1994). As the inflammatory process develops in udder tissue the number of these cells (particularly leucocytes) in milk sharply increases. Due to inflammation response, white bodies migrate from the peripheral circulation to the udder. The count of these cells may reach up to several millions in 1mL of milk. As the count of these cells is the most liable to change, diagnostic tests of mastitis make possible diagnosis of mastitis in the early stage before clinical signs become apparent.

A new test for subclinical mastitis in cows has been developed and tested in the Lithuanian Veterinary Institute (ŠPAKAUSKAS et al., 1998). The effectiveness of this test has been compared to that of other tests.

Electrical conductivity (EC) of milk is determined by sodium, potassium, calcium, magnesium, chlorine, and other ions (BARTH and WORSTORFF, 2000; FERNANDO et al., 1985; HAMANN and ZECCONI, 1998; HAMANN and GYODI, 1999; SCHULZ and BEUCHE, 1990). Concentration of ions in secretion changes not only because of increased throughput of blood capillaries but also due to damaged active ion transport system. Secretory cells of mammary gland are distinguished by an active transport system when transporting Na^+ to the extracellular fluid and K^+ back into the cell. Na^+ and K^+ are transported from the secretory cells to milk in a passive manner. After disintegration of cells, ions contained in the extracellular fluid enter the alveolar chamber. Changes in ion concentration cause an increase in EC. EC may be used for mastitis diagnostics. However, data on the diagnostic value of this method is contradictory. According to some authors (HAMANN and GYODI, 2000; HILLERTON and WALTON, 1991), EC of healthy cow milk is 4.0-5.5 milisenses (mS)/cm. When EC increases to 6.0 mS/cm or more, pathological processes in the udder tissue may be suspected. Some authors point to a good correlation between EC and bacteriological and cytological tests (MUSSER et al., 1998; NIELEN et al., 1992), while others consider this method to be insufficiently sensitive (HAMANN and GYODI, 1999; HAMANN and GYODI, 2000; PYÖRÄLÄ, 2003; SANDHOLM, 1995). It has been found (MUSSER et al., 1998; NIELEN et al., 1992) that EC increases together with the temperature of a sample. Relationship with the temperature (15 °C and 40 °C) has been approximately linear, while increase coefficient has been 0.113 mS/cm (NIELEN et al., 1992). EC is also affected by other factors. Among such mastitis-related factors various authors indicate lactation stage (BARTH and WORSTORFF, 2000; FERNANDO et al., 1985); inter-herd differences (HAMANN and ZECCONI, 1998; HAMANN and GYODI, 1999): rutting (HAMANN and GYODI, 2000; HILLERTON and WALTON, 1991); changes during milking (MUSSER et al., 1998); milk fat (MUSSER et al., 1998); various diseases (NIELEN et al., 1995), etc. When diagnosing mastitis it is important to note the udder quarter with the highest EC compared to other quarters (FERNANDO et al., 1985; HAMANN and ZECCONI, 1998; NIELEN et al., 1995).

Materials and methods

A total of 1014 samples taken from Lithuanian cows at the end of milking were tested on several parameters: milk reaction with Mastest, CMT, Bernburg, Profilac reagent, Eimü-milchtest. SCC was established using Anadis F device; electrical conductivity (EC) was measured using a Mastiidiindikaator device made in Estonia. As EC depends on temperature we measured it in milk milked immediately prior to measuring.

Milk reaction with mastitis tests were conducted in the following way: special white plates were filled with 2 mL of test solution and mixed with 2 mL of examined milk. After turning a plate for 5-10 seconds, consistency and colour of the mixture were visually determined. According to changes in consistence and colour of the mixture the reaction was estimated using the 5-point system (adopted in Scandinavian countries): 1 point-consistency of mixture is homogenous, liquid, without visible changes; 2 points-insignificant flakes develop and disappear when the plate is rotated; 3 points-clots appear, the viscosity of the mixture increases; 4 points-the mixture becomes viscous, while rotating the plate a clot can be seen which localizes; when the rotation ceases the mixture disperses on the bottom; 5 points-the mixture becomes very viscous, a yolk-like clot is visible; when the content of the plate is poured out the clot extends and falls out.

Statistical analysis of the results was conducted using computerized variance statistics software Scientific Graph System Sigma Plot and Prism 3. Statistical significance of difference was evaluated according to the Student criterion. The level of significance used was $P<0.05$. Correlations between the dependent variables were calculated using Pearson's correlation.

Results

Fig. 1 reveals that EC of milk from healthy quarters varies from 4.3 mS/cm to 5.7 mS/cm. In cases of inflammation in the udder EC increases, varying from 6.1 mS/cm to 8.5 mS/cm. On the basis of EC, 794 (78.30%) of milk samples were classified as good, while on the basis of SCC (up to 0.3 mln./mL) only 625 (61.64%) were so classified. In 35 (3.45%) of samples, where SCC exceeded 0.5 mln./mL, EC was normal. Only 60 (5.91%) samples had EC of more than 7.1 mS/cm.

Table 1. Number of samples according to the reaction of "Mastest" with milk and somatic cell count according to the "Anadis" apparatus.

Somatic cell count, thous./mL	N° of samples	Reaction of "Mastest" with milk, points									
		1		2		3		4		5	
		N°	%	N°	%	N°	%	N°	%	N°	%
<200	72	46	63.89	26	36.11	-	-	-	-	-	-
201-300	234	202	86.32	32	13.68	-	-	-	-	-	-
301-400	96	56	58.33	36	37.51	4	4.16	-	-	-	-
401-500	96	26	27.08	50	52.09	20	20.83	-	-	-	-
501-1000	56	4	7.14	26	46.42	16	28.57	10	17.85	-	-
1001-1500	146	-	-	2	1.37	124	84.93	20	13.70	-	-
1501-2000	112	-	-	2	1.78	28	25.00	76	67.86	6	5.35
2001-2500	114	-	-	2	1.75	2	1.75	74	64.91	36	31.58
2501-3000	46	-	-	2	4.35	-	-	8	17.39	36	78.26
3001-3500	18	-	-	-	-	-	-	12	66.67	6	33.33
3501-4000	16	-	-	-	-	-	-	6	37.50	10	62.50
>4000	8	-	-	-	-	-	-	4	50.00	4	50.00

Somatic cell count dynamics and results of reactions of milk with Mastest are presented in Table 1. Data in Table 1 reveals that as milk consistency changes the somatic cell count in the milk increases. If somatic cell count is below 0.5 mln./mL, Mastest reaction is estimated at 1 and 2 points. As somatic cell count increases Mastest mixture points increase. When SCC is between 0.5 and 1.5 mln./mL Mastest reaction is mostly estimated at 3 points; when SCC reaches 1.5-2.5 mln./mL, electrical conductivity it is mostly assigned 4 points. The milk and test mixture was assigned 5 points when SCC was 2-5 mln./mL. Out of 1014 samples studied, in 614 cases reaction was estimated with 1 point. This is good quality milk from healthy cows in which, according to the data from the International Dairy Federation, other countries (and in this country) the number of somatic cells does not exceed 0.5 mln. cells/mL. There was a 99.34% correlation between Mastest results and SCC established by the Anadis device. In our study 178 milk samples were assigned 2 points. Because when

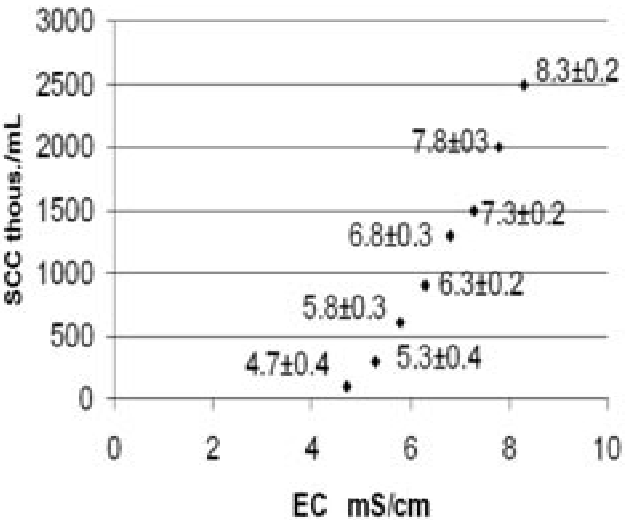

Fig. 1. Electrical conductivity and somatic cell count changes in cow milk

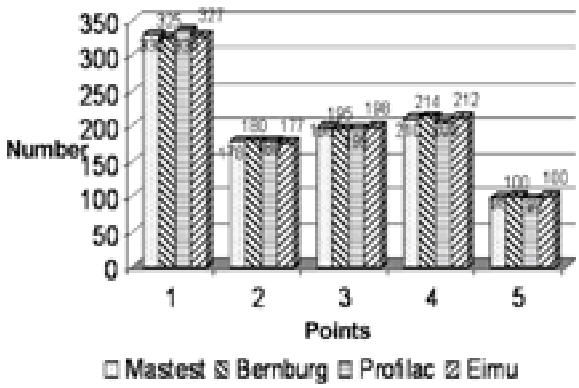

Fig. 2. Comparative activity of mastitis tests

Mastest reaction is estimated at 2 points the somatic cell count is up to 1 mln. cells/mL, and in 8 milk samples SCC was above 1 mln. cells/mL, it may be concluded that for 170 milk samples (95.50%) Mastests results and somatic cell count data correlate. The estimation by 3 points indicated the processes of inflammation in the udder resulting in increased SCC to over 1 mln./mL. Out of 194 milk samples, results of 24 samples were atypical for this situation (somatic cell count was below 0,5 mln. cells/mL). Thus, in this

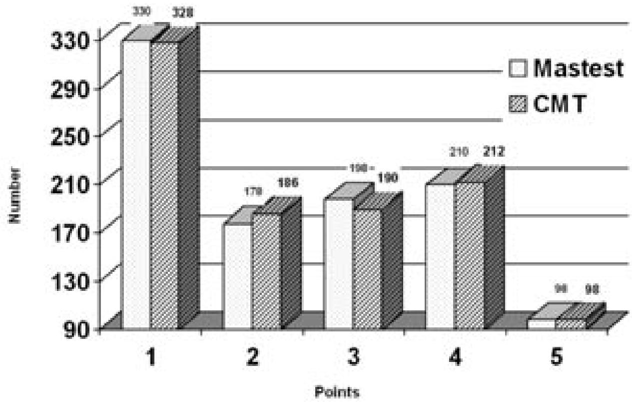

Fig. 3. Comparative activity of Mastest and CMT

case, Mastest results correlated with somatic cell count in 170 cases (87.62%). In the 4-point group there was a discrepancy between Mastest results and somatic cell count in 10 samples, and correspondence in 200 samples (95.24%). In the 5-point group there was a 100% correlation between Mastest results and SCC.

No significant differences in mixture consistence were found when milk samples with known SCC were subjected to Mastest, Bernburg, Profilac Reagent, and Eimü-milchtest (Fig. 2). The overall coefficient of correlation between Mastest and Bernburg reagent results r = +0.985; between Mastest and Profilac reagent r = +0,980; between Mastest and Eimü-milchtest r = 0.966.

Results of test effectiveness comparison indicate (Fig. 3) that Mastest and CMT results correlate best (99.39-100%) at 1 and 5 points. In case of intermediate ratings (2-4) points correlation is 95.69-99.05%. Overall correlation coefficient r = +0,997. Changes in colour (purple) and consistency of both tests and milk samples were readily noticeable visually.

Discussion

The results of the study indicate that increase in somatic cell count (particularly leukocyte) is the first indicator of the onset of inflammation in the udder. Only cells possessing nuclei interact with superficial active agents. During interaction between detergents and nuclei of somatic cells DNA is released, which increases milk viscosity (MIDDLETON et al., 2004; RUEGG and REINEMANN, 2002; SARGEANT et al., 2001). Research indicates that consistency of mixture of milk and reagents changes depending on the amount of detergent in the reagent. The reaction between detergent and milk was slightly affected by the pH of the solution. The tests work best in a weakly alkaline medium, where changes in mixture are maintained for more than 15 minutes. This may explain the linear relationship between mastitis test results and somatic cell count in milk. As somatic cell count increases, milk and mastitis test reaction rating in points increases because of the changes in milk viscosity, which are more pronounced in a weak alkaline medium. Various tests contain similar detergents. Accordingly, we found no significant differences.

According to our results, in most samples (61.7%) EC was 4.5-6.0 mS/cm, while EC exceeding 7.1 mS/cm was found only in 5.91% of samples. However, even though in cases of pronounced reaction with tests electrical conductivity of milk has a tendency to increase, it does not always correlate with other indices. Discrepancy of results may be explained by the fact that EC depends on the number of ions, the numbers of which in milk increase much later than SCC. Like other authors (HAMANN and GYODI, 2000; PYÖRÄLÄ, 2003; SANDHOLM, 1995), we believe that due to limited correlation with other known methods, the EC determination should not be used as the sole method of mastitis diagnostics with cows having high somatic cell count in their milk.

Conclusions

Electrical conductivity of milk samples taken from healthy cows (61.7%) was 4.3-5.7 mS/cm. Even though in cases of cows with subclinical mastitis electrical conductivity of milk increases to 6.1-8.5 mS/cm, it does not always correspond to the somatic cell count.

Mastests results have the highest correlation (95.5-100%) with somatic cell count when milk is rated at 1, 2, and 5 points, while the lowest correlation (87.62%) is at 3 points.

Mastests results correlate well (r = +0.957-0.997) with results of other tests (Bernburg, Profilac reagent, Eimü-milchtest). Mastest and CMT results correlate best (99.39-100%) at the rating of 1 and 5 points. At intermediate ratings (2-4 points) correlation of test results is 95.69-99.05%. Overall correlation coefficient is r = +0.997.

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Received: 25 October 2004
Accepted: 2 March 2006

ŠPAKAUSKAS, V., I. KLIMIENĖ, A. MATUSEVIČIUS: Usporedba neizravnih metoda dijagnosticiranja supkliničkog mastitisa u mliječnih krava u laktaciji. Vet. arhiv 76, 101-109, 2006.

SAŽETAK

Ukupno 1014 uzoraka mlijeka uzeto je od litavskih krava na kraju mužnje te pretraženo reakcijom mlijeka na Mastest (razvijen, testiran i proizveden na Litavskom Veterinarskom Institutu), kalifornijskim mastitis testom, Bernburg, Profilac reagens i Eimü-milchtestom. Broj somatskih stanica (BSS) utvrđen je protočnom citometrijom i Anadis F aparatom, a električna provodljivost je mjerena Mastindikatorom proizvedenim u Estoniji. Električna provodljivost uzoraka uzetih od zdravih krava iznosila je 4,5-6,0 mS/cm, dok je u krava sa supkliničkim mastitisom iznosila 6,1-8,5 mS/cm. Iako se električna provodljivost mlijeka krava s mastitisom može povećati, ona nije uvijek u korelaciji s BSS. Rezultati polučeni Mastestom u najvećoj su korelaciji (95,5-100%) s brojem somatskih stanica u mlijeku gdje se reakcija mlijeka s mlijekom ocjenjuje s 1,2 ili 5 bodova, dok je najslabija korelacija (87,62%) ocijenjena s 3 boda. Rezultati Mastesta u dobroj su korelaciji s rezultatima drugih testova. Ukupni koeficijent korelacije između Mastesta i Bernburg reagensa je r = +0,985, između Mastesta i Profilaca je r = +0,980, između Mastesta i Eimü-milchtest je r = + 0, 966. Najbolja korelacija (99,39-100%) ustanovljena je između Mastesta i kalifornijskog mastitis testa i ocjenjuje se s 1-5 bodova. Kod srednjih ocjena (2-4 boda), rezultati testa su 95,96-99,05%. Ukupni korelacijski koeficijent iznosio je r = + 0,997.

Ključne riječi: mastitis, broj somatskih stanica, mastitis test, električna provodljivost