

## USE OF CALIFORNIA MASTITIS TEST, SOMATIC CELLS COUNT AND BACTERIOLOGICAL FINDINGS IN DIAGNOSTICS OF SUBCLINICAL MASTITIS

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**Abstract:** We have performed diagnostics of sub clinical mastitis in three different cow breeds with comparison of California mastitis test results, somatic cells count at quarter level and with bacteriological findings confirmation in order to justify their appliance in mastitis diagnostics. In total, 90 cows or 360 quarters of mammary gland have been examined. In 63.3 % of the examined cows, with different racial origin, positive reaction to California mastitis test have been established. Usually, positive reaction have been found in milk of one and two quarters, with reaction intensity of one and two plus. SSC higher than 200.000 in 1 ml is established in 60 % of cases and the most often causes of mastitis were staphylococcae, streptococcae and mixed infections. With continuous use of this methods it is possible to timely detect presence of sub clinical mastitis and so to obtain satisfactory results in prevention and therapy of mastitis, as well as improvement in amount and quality of milk.

**Key words:** sub clinical mastitis, California mastitis test, somatic cells count, cow

### Introduction

Inflammation of mammary gland or mastitis is response of mammary gland to action of different internal and extrenal factors. During last few decades mastitis has become very expensive disease of dairy cows (*Kossabiati et al., 1997; Fourichon et al., 2001; Bennett et al., 2002*). It is established that there is high risk of developing subclinical mastitis in period of early lactation and high procent of intramammary infections in postpartum period (*De Viegher et al., 2005; Oliver et al., 2003; Trinidad et al., 1990*). In researches efforts are directed to improve

success of clinical mastitis treatment and supervision of subclinical mastitis in wish to obtain milk of high quality (Febre et al., 1999; Hillerton et al., 1998).

Identification and removing of intramammary infections in early period of lactation are significantly economically usefull. The occurrence of mastitis according to Barkem et al. (1999) special influence have management of the herd, including housing, diet and udder management. Status of udder infection can be expressed as clinical and subclinical mastitis. Clinical mastitis is characteristic by visible changes in milk with appearance of flakes or blobs and with appearance of oedema and pain in the udder. Subclinical mastitis has been defined as inflammation without clear signs. Forms of subclinical mastitis are: disorder od secretion, latent infection and chronically catarrhal mastitis. Subclinical mastitis is widespread disease in milk production where at every clinical case of mastitis, 15-40 subclinical cases appears (Kelly et al., 2002).

Inflammation of mammary gland is followed with increase of somatic cells count in milk (Rodriguez et al., 2000). In milk from healthy quarters (Antunac et al., 1997) somatic cells count (SCC) is less than 200.000 cells/ml, and it is made of epithelium cells and leukocytes (polimorphonuclear neutrophils, lymphocytes, macrophages and other cells). For detection of subclinical mastitis there are series of methods and tests, but the simplest and practically reliable is California Mastitis Test (CMT), (Dingvell et al., 2003; De Viegher et al., 2005) as determination of somatic cells by electronic counters.

Purpose of this research was to compare results of California Mastitis Test, findings of SCC and microbiological findings of mammary gland secretion from different breeds of cows as method for control and diagnostics of subclinical mastitis.

## Materials and Methods

Researches were performed in three herds in: black east friesean cows in large farm breeding near Sarajevo, simental cows in privat mini farm near Ilijaš and hereford cows in Han Pijesak farm whose milk is used only for feeding of calves by suckling without milking. Out of every breed 30 cows has been examined. Control of mammary gland health we performed with clinical examination of udder, milk testing from every quarter of udder using CMT as with milk sampling for SCC and bacteriological search. Using clinical methods of examination all changes which are incurred in tissue of mammary gland with acute or chronical inflammatory process are established. By inspection we looked at asimetry of quarters and differences in shape, development and teats position. With palpation from tip of the teats over milk cistern, we established irregularity of teat tip, thickening and swelling in teat channel and parenchyma comparing quarters between themselves.

After clinical examination of mammary gland we performed milk testing from every quarters by CMT (SOMA TEST and test plate manufactured by FARM

d.o.o.Vrbanovac RH). We have performed test during months April and May directly in test plates in stables, with cows immediately after clinical examination and before morning milking. Reagent is mixed with approximate same quantity of milk (1-2 ml) and with light circular motions of test plate results of changes are readable within 1-2 minutes. Milk samples rich with cellular elements gave visible changes within few seconds. Presentation of reaction was: negative ( - ) in cases where we had mixture of milk and reagent as homogenous transcendentally, suspected ( ± ) appearance of blobs and flakes which with further mixing disappear, positive ( + ) appearance of flakes that with further mixing concentrate in the middle, very positive reaction ( ++ ) where during mixing of reagent and milk thick viscous mass is created with separation of clear liquid and extremely positive reaction ( +++ ) where gelatine mass was created. Before taking samples for BSS determination and microbiological analysis teats were washed and disinfected with 70% alcohol and in a sterile container intended for SCC analyse we took 25 ml of milk and in a special sterile test tubes samples for microbiological examination. Each container and beaker are marked with number of udder quarter and number of animal.. Samples were taken from every udder quarter regardless whether CMT reacted positively or negatively. Analysis of SCC we have worked in Federal institute for agriculture Sarajevo using Fusomatic FC 6200 device with method of flow cytometry and microbiological milk examination with common methods.

All obtained results we processed with the method of absolute and relative frequency and their testing is done with Z – test for comparison at the level of significance  $\alpha = 0,05$

## Results

Research consisted of 90 cows of different breeds (Table 1). From the total number of examined cows positive reaction of California mastitis test was determined at 57 or 63.33% cows.

**Table 1. Number and percentage of cows according to breeds that positively reacted to the CMT**

Breeds of cows	Number of examined cows	Positive reaction	% positive
Simmental	30	16	53.3
Hereford	30	22	73.3
East Frisian	30	19	63.3
Total	90	57	63.3

The percentage of positive cows within the breed ranges from 53.3% to 73.3%. Results indicate the relative uniformity regardless of the obvious differences between breeds.

**Table 2. Number and percentage of udder quarters with positive reaction to the CMT**

Breed of cows	Number of positive cows	Examined udder quarters	Number of cows with positive reaction by quarter				Positive quarters		Negative quarters
			One	Two	Three	Four	number	%	number
Simmental	16	64	6	7	2	1	30	46.8	34
Hereford	22	88	2	10	7	3	55	62.5	33
East Frisian	19	76	4	10	1	4	43	56.0	33
Total	57	228	12	27	10	8	128	56.1	100

From the total number of examined quarters 128 has reacted positive to CMT or 56.1% of cases (Table 2). The most positive reactions were found in the milk of two quarters and least in milk of all four quarters. Within individual breeds, reaction of California mastitis test was quite uniform.

**Table 3. Reaction intensity of CMT in relation to the cow breeds and quarters**

Breed of cows	Total examined quarters	Quarters of the udder according to intensity of the test									
		-		±		+		++		+++	
		No.	%	No.	%	No.	%	No.	%	No.	%
Simmental	64	34	53.1	2	3.1	14	21.8	13	20.3	1	1.5
Hereford	88	33	37.5	6	6.8	31	35.2	15	17.0	3	3.4
East Frisian	76	33	43.4	3	3.9	22	28.9	16	21.0	2	2.6
Total	228	100	43.8	11	4.8	67	29.3	44	19.3	6	2.6

Reactions of the California mastitis test with mark ± (doubtful reaction) were represented in 4.8% of cases (Table 3). The greatest percentage of positive quarters was with one or two plus and it was 29.3% and 19.3% and the lowest positive percentage was recorded at the most intensive reaction with three plus, out of 2.6%. From the total number of tested quarters 43.8% showed a negative reaction. Viewed by breed most positive reactions to the mastitis test was recorded at Hereford cows.

**Table 4. Relationship between CMT and SCC in milk from quarters of different breed cows in %**

Breed of cows	Total examined quarters	Quarters of the udder according to intensity of the test									
		-		±		+		++		+++	
		No	%	No	%	No	%	No	%	No	%
Simmen.	64	34	53.1	2	3.1	14	21.8	13	20.3	1	1.5
Heref.	88	33	37.5	6	6.8	31	35.2	15	17.0	3	3.4
East. Frisi.	76	33	43.4	3	3.9	22	28.9	16	21.0	2	2.6
Total	228	100	43.8	11	4.8	67	29.3	44	19.3	6	2.6
Breed of cows	Total examined quarters	Quarters of the udder according to number of somatic cells in 1 ml.									
		200.000		200.000 - 400.000		500.000 - 1.500.00		2.000.000 - 5.000.000		Over 5.000.000	
		No	%	No	%	No	%	No	%	No	%
Simmen.	64	32	50.0	6	9.3	11	9.3	14	21.8	1	1.5
Heref.	88	28	31.8	8	9.0	30	34.0	17	19.31	5	5.6
East. Frisi.	76	31	40.7	5	6.5	22	28.9	15	19.7	3	3.9
Total	228	91	39.9	19	8.3	63	27.6	46	20.7	9	3.9

Reaction of the mastitis test with the mark ++ and +++ (Table 4) shows that in milk there is more than 500.000 thousand somatic cells and with mark ± and + less than the specified limits. From 228 samples of milk 100 of them gave a negative reaction to mastitis test and 91 samples were in limits up to 200.000 somatic cells in 1ml of milk. From the breeds, largest number of samples whose had over 200.00 somatic cells in 1ml is registered in Hereford breed which agrees with the reaction of CMT. Viewed by percentage difference between mastitis test, which amounted 56.1% and number of somatic cells larger than 200.000 in value of 60.0% talk about coincidences and justification of these methods in diagnostics of mastitis with obligatory bacteriological analysis of milk.

**Table 5. Results of CMT and bacteriological findings**

CMT	Total examined quarters	Bacteriological findings								% of matching
	Simmen.	negative	micrococcae	Nonpathogen staphillococce	Staph. aureus	streptococcae	Streptococcus .agalactie	Mixed infection	Accordance of results	
-	34	32					1	1	2	5.8
±	2	1		1					1	50.0
+	14	1	2	1	6	1	2	1	13	92.8
++	13		1	2	1	4	1	4	13	100.0
+++	1				1				1	100.0
	64	34	3	4	8	5	4	6	31	48.4
	Hereford									
-	33	28		2	1			3	5	15.1
±	6	2	2			1		1	4	66.6
+	31	5	4	7	5	3	2	5	26	83.8
++	15	1		2	7	1	1	3	14	93.3
+++	3				2		1		3	100.0
	88	36	6	11	15	4	4	12	52	59.0
	East Fris.									
-	33	31	1					1	2	6.0
±	3			3					3	100.0
+	22	1	3	1	7	2	3	5	21	95.4
++	16		2	3	4	6		1	16	100.0
+++	2					2			2	
	76	32	6	7	11	10	3	7	44	57.8
Total	228	101	15	22	34	20	11	25	127	55.7

Bacteriological findings (Table 5) confirmed the validity of application CMT in detection of subclinical mastitis. Of the total 228 samples 43.8% gave a negative reaction to CMT of which 75 had a positive bacteriological finding what draws our attention to the latent infection of the udder. Analyzing positive reactants on CMT out of them 128 only 8.5% gave a negative bacteriological findings. Most common cause of the mastitis were staphylococcae. streptococcae and mixed infections.

## Discussion

Past efforts in suppression of mastitis and control of mammary gland health status are based on detection of animals with disturbed secretion and identifying causes of such conditions. To prevent and reduce infections of mammary gland with pathogenic bacterias from environment it is necessary to take care of the complete production management (*Ferguson et al.. 2006; Kelmus et al.. 2006; Compton et al.. 2007*) what we support.

Inflammation of the mammary gland is usually characterized by increase of somatic cells in milk. At the level of cow (quarter) SCC is 200.000/ml in healthy non-infected quarter and can be lower than 100.000/ml (*Echlenbach et al.. 1971*). About 50% non-infected cows have SCC under 100.000/ml and 80% to 200.000 cells/ml. Every increase of SCC is considered as abnormal and indicate a mammary gland inflammation what is confirmed by our research. Comparing CMT and SCC (*Sargeant et al.. 2001*). CMT represent a suitable test for herd inspection and detection of subclinical mastitis caused by mastitis agents in 84% of cases. Bacteriological findings indicates that increase of CMT reaction increase also probability of infection and (*Pyrola et al.. 2003*) even in negative CMT reaction 25% of quarters are infected. suspicious 50%. positive 75%. very positive 90% and extremely positive 90-100% what is somewhat in consent with our research. Relationship of reaction correspondence between CMT and bacteriological findings range from 70 to 86% depending on the causative agent (*Sanford et al.. 2006*) while *Bastan at al. (2008)* gives data of correspondence of 85%. In our research percentage of correspondence between CMT and bacteriological findings is 55.7%. As most common isolated causative agents series of authors (*Bradley. 2002; Dingvell et al.. 2003; Fatur et al.. 2000; Milne et al.. 2002; Schukken et al.. 1989; Sol et al.. 2002*) cite staphylococcae and streptococcae. Our research coincide with studies by these authors but we noted in addition to listed pathogens mixed infection in 10.8% of cases.

## Conclusion

Based on the analysis of secretion from mammary gland using CM. determining SCC and bacteriological findings it can be concluded that:

CMT as SCC findings represent valuable diagnostic methods in detection of cows with secretion disorder. whose show no clinical signs of disease. Secretion disorder of mammary gland is detected by CMT in 63.3% of cows.

Difference between reaction of CMT and SCC at quarter level of 3.9% talks about justification of these methods in detection of mammary gland disorders and subclinical mastitis.

When negative reaction by CMT occurred, bacteriological analysis has found 7% infected quarters which indicates the presence of latent mastitis and in 8.5% of cases with positive reactions to CMT involved secretion disorder because bacteriological findings were negative.

The percentage of matching between CMT and bacteriological findings was 55.7%.

According to the types of isolated pathogens in the first place are staphylococcae, streptococcae and mixed infections.

We believe that with this method of mammary gland health status control and better participation of complete management in production it is possible to obtain satisfactory results in improving quantity and quality of milk and suppression of chronic and decrease of acute mastitis numbers.

## **Primena kalifornija mastitis testa, broja somatskih stanica i bakteriološkog nalaza u dijagnostici subkliničkih mastitisa**

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### **Rezime**

Dijagnostiku supkliničkih mastitisa kod tri različite pasmine krava vršili smo upoređivanjem rezultata Kalifornija mastitis testa, broja somatskih ćelija na nivou četvrti uz potvrdu bakteriološkim nalazom u cilju opravdanosti njihove primene u dijagnostici mastitisa. Ukupno je pregledano 90 krava ili 360 četvrti mlečne žlezde. U 63.3% slučajeva pregledanih krava različitog pasminskog porekla utvrđena je pozitivna reakcija na Kalifornija mastitis test. Najčešće smo pozitivnu reakciju ustanovili u mleku jedne i dve četvrti sa intenzitetom reakcije od jednog i dva plusa. BSS veći od 200.000 u 1 ml je ustanovljen u 60% slučajeva, a najzastupljeniji uzročnici mastitisa su bili stafilokoke, streptokoke i mešane infekcije. Kolinuiranom upotrebom ovih metoda moguće je blagovremeno otkriti prisutnost supkliničkih mastitisa i tako dobiti zadovoljavajuće rezultate u prevenciji i terapiji mastitisa kao i povećanju količine i poboljšanju kvaliteta mleka.

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