

## RESEARCH ON SUBCLINICAL MASTITIS AND ITS ETHIOLOGY IN DIFFERENT BREEDS OF COWS

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**Abstract** – Detection of subclinical mastitis in five different breeds of cows were done on the territory of a municipality Ključ, through all four year seasons by using the California mastitis test, at the level of the udder's quarter with a certificate of bacteriological findings in order to justify their application in the diagnosis of mastitis. In total, 2150 cows of different breed composition: Simmental 1090, red Holstein 322, Holstein-Friesian 340, montafon 108 and various crossbreeds of 290 in total. After 1978 tested cows, 56.02% of cases had positive reactions to the California mastitis test. The most common positive reaction we found in the milk was in one or two quarters with the intensity of the reaction of one and two plus. The most common causes of mastitis were staphylococci, streptococci and mixed infections. Continuing use of these methods it is possible to timely detect the presence of subclinical mastitis and get satisfactory results in the prevention and treatment of mastitis as well as increasing the quantity and improving the quality of milk.

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

### Introduction

Inflammation of mammary gland or mastitis is response to effects of internal and outside factors. During last several decades mastitis has become very expensive disease in dairy cows (*Bennett et al., 1999; Fourichon et al., 2001; Kelmus et al.,2006*). Researchers had found, 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; Milne et al., 2002; Sol et al., 2002*). Goal of various researches was to put efforts to enhance success of healing against clinical mastitis and to monitor appearance of subclinical mastitis

for obtaining milk of high quality (*Fabre et al., 1999; Fleiss 1981*).

Identification and removal of intramammary infections in period of early lactation is important for economical benefits. Mastitis occurrence is according to Barkema et al. (1999) result of herd management, including rearing condition, nutrition and udder management. Udder infections can be expressed as clinical or subclinical mastitis. Clinical mastitis is characteristic because of visible changes in milk with appearance of flakes or beads and with signs of inflammation and pain. Subclinical mastitis is defined as inflammation without clear signs. Types of subclinical mastitis are: disorder of secretion, latent infection and chronic chatalar mastitis. Subclinical mastitis is most widespread disease in milk production, where upon one clinical case, comes new 15-40 subclinical cases (*Hillerton, 1998*).

For detection of subclinical mastitis there is array of methods and tests, but most simple and reliable is California Mastitis Test (CMT), (*De Viegheer et al., 2005; Dingvell et al., 2003*) as well as somatic cells counting with electronic counters.

Aim of this research was to establish representation of subclinical mastitis in researched area and to reveal their etiology with help of California Mastitis Test and microbiology results of mammary gland secretion from different breeds of cows through all seasons of the year.

## Materials and methods of work

Research in field conditions had been conducted in breeds from individual milk producers, as well as in the mini farms, capacity 5 – 15 cows in the area of one municipality.

This experiment have included 2150 cows of different breeds: simmental 1090, red holstein 322, holstein-friesian 340, montafon 108 and various cross breeds 290. Control of udder health have been performed with clinical examination, CMT tests for each quarter and milk sampling for bacteriological findings.

Clinical examination have revealed all changes that occurred in udder as result of acute or chronic inflammation.

After clinical examination, milk have been tested with CMT test from all quarters (SOMA TEST reagent and Test plate by FARM d.o.o.Vrbanovac RH). Examinations have been performed through all seasons of the year, before morning milking. Reagent for CMT test have been mixed with approximately same amount of milk (1-2 ml) and with easy circle moves of test plate reaction was read after 1-2 minutes. Milk samples rich with somatic cells gave visible changes within few seconds. Presentation of reaction was: negative ( - ) in cases where we had mixture homogenously blur, suspicious (±) in cases where mixture had flakes and beads that disappear with continuous mixing, positive ( + ) where present flakes are concentrated in the middle, very positive reaction ( ++ ) where during mixing

dense viscous mass separate from clear liquid part and extremely positive reaction (+++) where gelatinous mass is created.

Before milk sampling for microbiology analysis, teats were washed and disinfected with 70% alcohol and milk have been taken into sterile tubes. Every tube have been marked with number of quarter and animal. Samples have been taken from all quarters regardless for CMT findings and sent to laboratory for analysis.

All obtained results have been processed with Chi-quadrat test (*Trinidad et al., 1990*). During processing of results, category of cross breeds have been neglected, because of possibility biasa and for interpretation of results one less number of considered categories was used ( $df=n-1$ ).

## Results

From total of 2150 cows, 1978 were tested with CMT and 1108 cows or 56.02% had positive or suspected reaction and negative 870 or 43.98% (table 1).

**Table 1. Results of CMT reactions according to breeds and in total**

Breed	Number of CMT tested cows	Positive reaction		Negative reaction	
		number	%	number	%
Simmental	1003	519	51.74	484	48.26
Red holstein	296	195	65.88	101	34.12
Holstein-Friesian	313	200	63.90	113	36.10
Montafon	99	50	50.51	49	49.49
Cross breed	267	144	53.93	123	46.07
<b>Total</b>	<b>1978</b>	<b>1108</b>	<b>56.02</b>	<b>870</b>	<b>43.98</b>

Within certain breeds procent of CMT positive animals is 50.51-65.88%. In table 1. is visible relative equalization between certain breeds with exception of montafon and Holstein-Friesian cows as well as Montafon and Red holstein. Differences between other breeds are from 1.24% to 3.43%.

From 1108 positive cows, most disorders have been found in milk in one quarter (465 or 41.97%) and at least cows had positive all four quarters (123 or 11.10%). After 4432 examined quarters, suspected and positive reaction we had in 2166 quarters or 48.87% cases. According to seasons of the year most quarters had positive or suspected reaction in winter 50.74%, spring and summer were pretty much equal, while autumn period had least number of positive and suspected quarters 47.45%.

According to breeds (figure 1) the highest percent od disorders in secretion

in one quarter have been noted in Montafon breed (52%) and least in Simmental breed 36.22%, while in other breeds result was pretty much equal. Results with two quarters affected, highest disorder in secretion had Simmental 35.07%, then Montafon 34%, while least was in cross breeds 17.36%.

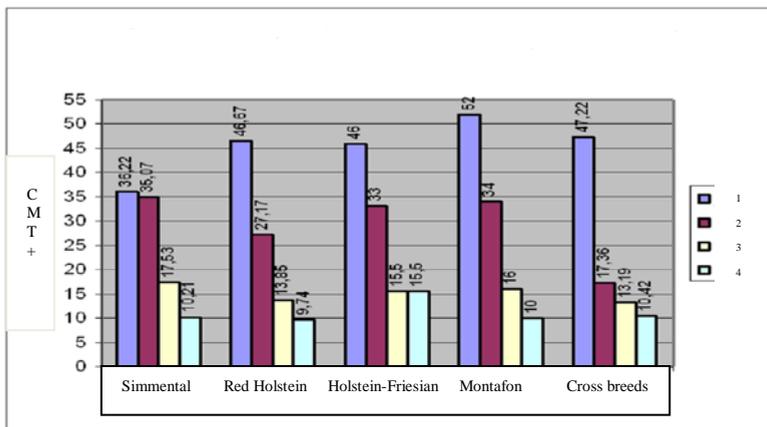


Figure 1. CMT positive results (%) according to number of affected quarters

The highest percent of positive with three quarters was in Simmental 17.53%, while in other breeds it was almost equal. When it comes to all four affected quarters, highest results have been found in Holstein-Friesian 15.5%, while other breeds are mostly equal.

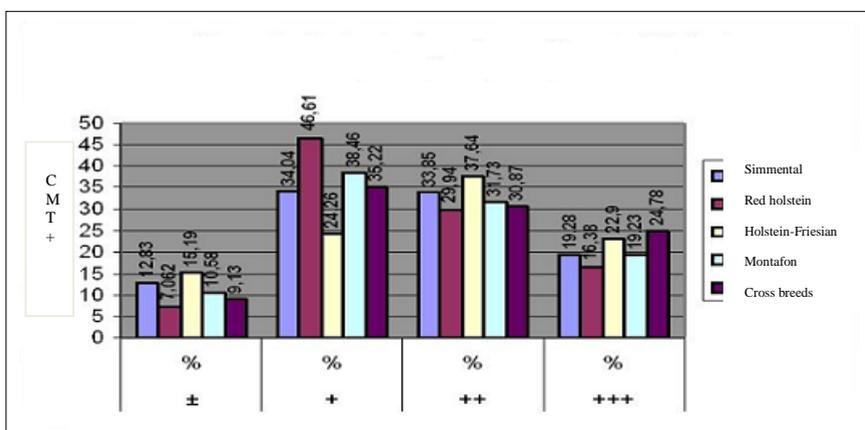


Figure 2. Number of positive quarters (%) according to CMT intensity in examined breeds

Percent of CMT reaction intensity related to breeds is least for suspicious reactions 7.062% to 15,19% (Figure 2). Most of the cows had one plus 24.26% to 46.61%, two plus 29.94% to 37.64% and with three plus 16.38% to 24.78%.

After analyzing 1400 bacteriological results, obtained from 350 cows during research (Figure 2) through seasons of the year, it is clear that during summer we had highest number of bacteriological positive samples. Most common isolates were staphylococci, streptococci, enterobacterias and mixed cultures.

**Table 2. Results of bacteriological findings through seasons of the year in %**

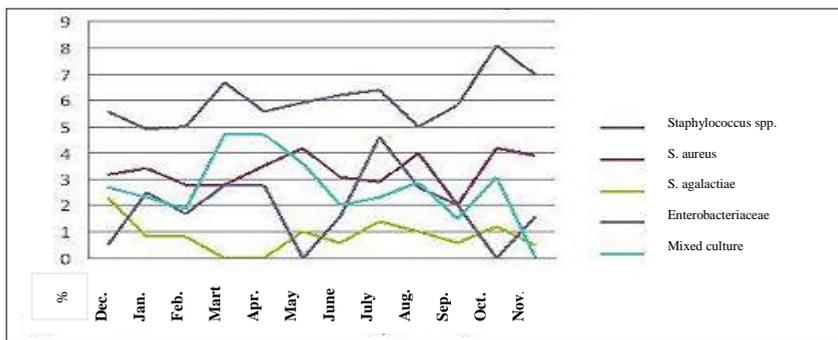
	Seasons of the year											
	Winter			Spring			Summer			Autumn		
	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Okt	Nov
<b>Negative findings</b>	<b>83</b>	<b>86,1</b>	<b>85,4</b>	<b>80,2</b>	<b>85,3</b>	<b>83</b>	<b>83,3</b>	<b>77,8</b>	<b>80,7</b>	<b>84,4</b>	<b>78,3</b>	<b>82,5</b>
Staphylococcus spp.	5,6	4,9	5	6,7	5,6	5,9	6,2	6,4	5	5,8	8,1	7
S. aureus	3,2	3,4	2,8	2,8	3,5	4,2	3,1	2,9	4	2	4,2	3,9
Streptococcus agalactiae	2,3	0,8	0,8	-	-	1	0,6	1,4	1	0,6	1,2	0,5
Streptococcus uberis	-	-	-	0,9	-	-	0,2	0,9	0,5	0,4	-	0,6
Streptococcus dysgalactiae	0,5	-	-	-	0,9	-	-	0,3	0,5	0,4	-	-
Other streptococci	2,2	-	2,4	1,9	2,7	2,4	2,7	3,4	2,7	1,7	5,1	3,9
Mixed culture (S. aureus, Nonpathogen stafilococci)	2,7	2,3	1,9	4,7	-	3,6	2	2,3	2,9	1,5	3,1	-
Enterobacterias (E colli, Proteus sp.)	0,5	2,5	1,7	2,8	1,4	-	1,6	4,6	2,7	2	-	1,6
Arcanobacterium pyogenes	-	-	-	-	0,6	-	0,2	-	-	0,2	-	-
<b>Number of tested samples</b>	<b>96</b>	<b>136</b>	<b>116</b>	<b>84</b>	<b>104</b>	<b>128</b>	<b>120</b>	<b>164</b>	<b>164</b>	<b>108</b>	<b>84</b>	<b>96</b>
<b>% of positive</b>	<b>17</b>	<b>13,9</b>	<b>14,6</b>	<b>19,8</b>	<b>14,7</b>	<b>17</b>	<b>16,7</b>	<b>22,2</b>	<b>19,3</b>	<b>15,6</b>	<b>21,7</b>	<b>17,5</b>

Results of statistical assessment related to number of quarters positive to CMT and breeds of cows, as well as relationship of number of quarters positive to CMT and season of the year did not fulfilled given criteria of statistical significance (table 3). In both cases zero hypothesis have been confirmed, which is about absence of researched relationship, respectively lesser or higher number

of quarters affected with subclinical mastitis (CMT+) appears independently of breed or season.

**Table 3: Results of statistical testing of relationship of number of quarters positive to CMT and breed of cows and season of the year**

Ho	Ha	X <sup>2</sup>	p vrijednost
Proportions of cows with one, two, three and four CMT+ udder quarters <b>are equal</b> in all breeds i.e. <b>there is no relationship</b> in number of CMT+ quarters with breed.	Proportions of cows with one, two, three and four CMT+ udder quarters <b>are not equal</b> in all breeds i.e. <b>there is relationship</b> in number of CMT+ quarters with breed.	12,6	0,181
Proportions of cows with one, two, three and four CMT+ udder quarters <b>are equal</b> for all seasons i.e. <b>there is no relationship</b> in number of CMT+ quarters and season.	Proportions of cows with one, two, three and four CMT+ udder quarters <b>are not equal</b> for all seasons i.e. <b>there is relationship</b> in number of CMT+ quarters and season.	1,2	0,999



**Figure 3. Percentage of different etiological agents that caused subclinical mastitis during the year**

Statistical significance in representation of different etiological causes of mastitis (Figure 3) during the year is not established, although mixed cultures were more prevalent in spring season, while enterobacterias in summer season, especially in July. Nonpathogenic staphylococcus were more prevalent in October and pathogenic staphylococcus in May, August and October.

## Discussion

Previous efforts in eradication of mastitis and control of udder's health status are based on detection of animals with disorders in milk secretion and identification of causal agents, that cause such conditions. To prevent and reduce udder infections with pathogenic bacterias from surroundings, it is necessary to take care for complete management in milk production (*Compton et al., 2007; Ferguson et al., 2007; Kelly 2002*). Mastitis is usually characterised with increase of SCC in milk. Every increase should be considered as abnormal and point to inflammatory condition (*Oliver et al. 1992*), which is confirmed by our results of CMT and bacteriological analyse. CMT represents suitable test for herd investigation and detection of subclinical mastitis caused by mastitis agents in 84% of cases. Bacteriological findings show that increase in CMT reaction, increase probability of infection (*Oliver et al. 1992*), while 25% of infection is present in negative CMT reaction, 50% in suspected CMT reaction, 75% in positive CMT reaction, 90% in very positive CMT reaction and 90-100% in pronouncedly positive reaction, which is in agreement with our research.

Ratio of CMT congruence and bacteriological findings is 70-80%, depending of causal agent (*Pyrola 2003*), while *Bastan et al. (2008)* gives information about congruence of 85%. As most often isolated causal agents more authors allege (*Bradley 2002; Dingvell et al., 2003; Fatur et al., 2000; Kossabiati and Esslemont 1997; Sanford et al., 2006; Schukken et al., 1989*) staphylococcae and streptococcae. Our research are matched with research of mentioned authors, but among mentioned causal agents we have noted mixed infection in different months of seasons in representation of 1,9 % to 3,6% of cases.

## Conclusions

Based on milk analyse with CMT and bacteriological examination we can conclude:

1. CMT represents valuable diagnostic method in detection of cows with disorder of secretion without clinical signs of disease.
2. After examination of 4432 quarters, positive reaction to CMT had 2166 or 48.87 %.
3. Disorders in mammary gland secretion, detected by CMT, depending of breed, ranged from 51.74% to 65.88% of cows.
4. According to season, the most quarters reacted in winter season, spring and summer season were equable, while autumn season is characterised with lowest number of suspected and positive quarters.
5. Most often isolated etiological agents were staphylococcae, streptococcae, enterobacteriaceae and mixed infections.

6. Percentage of subclinical mastitis is high in researched area and in next period, it is imperative to approach more organised in protection of udder health status through mastitis preventive care and education of farmers.

## **Istraživanje supkliničkih mastitisa i njihovih uzročnika kod različitih rasa krava**

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### **Rezime**

Otkrivanje supkliničkih mastitisa kod pet različitih rasa krava, vršili smo na području jedne opštine kroz sva četiri godišnja doba pomoću Kalifornija mastitis testa, na nivou četvrti uz potvrdu bakteriološkim nalazom u cilju opravdanosti njihove primene u dijagnostici mastitisa. Ukupno je pregledano 2150 krava različitih rasa: simentalac 1090, crveni holstein 322, holstein frizijsko 340, montafonac 108 i ukrštenih životinja 290 grla. Od 1978 testiranih krava u 56,02% slučajeva utvrđena je pozitivna reakcija na Kalifornija mastitis test. Najčešće je pozitivna reakcija ustanovljena u mleku jedne i dve četvrti sa intenzitetom reakcije od jednog i dva plusa, a najzastupljeniji uzročnici mastitisa su bili stafilokok, streptokok i mešane infekcije. Kontinuiranom 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.

### **References**

- BARKEMA H.W., VAN DER PLOEG J.D., SCHUKKEN Y.H., LAM T.J., BENEDICTUS G., BRAND A. (1999): Management style and its association with bulk milk somatic cell count and incidence rate of clinical mastitis. *J.Dairy Sci.* 82: 1655-63.
- BASTAN A., KACAR C., ACAR, B.D., SAHIN, M., CENGIZ, M. (2008): Investigation of the Incidence and Diagnosis of Subclinical Mastitis in Early Lactation Period Cows. *Turk. J. Vet. Anim. Sci.* 32 (2): 119-21.
- BENNETT R.M., CHRISTJANSEN, K., CLIFTON-HADLEY R.S. (1999): (Estimating the costs associated with endemic diseases of dairy cattle. *J.Dairy Res.* 66: 455-59.
- BRADLEY A.J. (2002): Bovine mastitis: An evolving disease. *The Veterinary journal* 164: 116-28.

- COMPTON C.W., HEUER C., PARKER K., MCDUGALL S. (2007 ): Epidemiology of mastitis in pasture grazed peripartum dairy heifers and its effects on productivity. *J. Dairy Sci.* 90(9): 4157-70.
- DE VIEGHER S., BARKEMA H.W., STRYHN H., OPSOMER G., DE KRUIF A. (2005 ): Impact of early lactation somatic cell count in heifers on milk yield over the first lactation. *J. Dairy Sci.* 88: 938-47.
- DINGVELL, R.T., LESLIE, K.E., SCHUKKEN, Y.H., SARGEANT, J.M., TIMMS, L.L. (2003): Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactation dairy cows. *Can. Vet. J.* 44: 413-16.
- FABRE J.M., BERTHELOT X., BOUSQUET E., BOSQUET G., LAUMONNIER G., SEEGER S. (1999): Traitement des mammites subcliniques en lactation: expérimentation d'un nouveau protocole dit "traitement en parallèle". *Bulletin des GTV* 1: 49-58.
- FATUR B., NIVES MAVSAR, VERDNIK M. (2000): Sezonska pojavnost subkliničkih mastitisa. XXII Savjetovanje s međunarodnim učešćem. Pliva i stočarska proizvodnja-Govodarstvo. Supetar.
- FERGUSON J.D., AZZARO G., GAMBINA M., LICITRA G. (2007 ): Prevalence of mastitis pathogens in Ragusa, Sicily from 2000 to 2006. *J. Dairy Sci.* 90 (12): 5798-813.
- FOURICHON C., BEUDEAU F., BAREILLE N., SEEGER S. (2001 ): Incidence of health disorders in dairy farming systems in western France. *Livestock Prod. Sci.* 68: 157-70.
- FLEISS J.L. (1981): *Methods for Rates and Proportions*. Wiley-Interscience, New York.
- HILLERTON J.E. (1998): Mastitis therapy is necessary for animal welfare. *Bulletin of the IDF* 330: 4-5.
- KELLY A.L. (2002): Test methods and standards. *Encyclopedia of Dairy Sciences*. Academic Press. 3: 1995 – 2002.
- KELMUS P., VILTROP A., AASMAE B., KASR K. (2006 ): Occurrence of clinical mastitis primiparous Estonian dairy cows in different housing conditions. *Acta. Vet. Scand.*10. 1186/1751-0147-48-21.
- KOSSABIATI M.A., ESSLEMONT R.J. (1997 ): The costs of production diseases in dairy herds in England. *Vet.J.* 154: 41-51.
- MILNE M.H., BARRET D.C., FITZPATRICK J.L., BIGGS A.M. (2002 ): Prevalence and aetiology of clinical mastitis on dairy farms in Devon. *Veterinary Record* 151: 241-243.
- OLIVER S.P., LEWIS M.J., GILLESPIE B.E., DOWLEN H.H. (1992): Influence of prepartum antibiotic therapy on intramammary infections in primigravid heifers during early lactation. *J. Dairy Sci.* 75: 406-08.
- PYROLA S.( 2003): Indicator of inflammation in diagnosis of mastitis. *Vet. Res.* 34, 565-578,

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SANFORD C.J., KEFFE G.P., SANCHEZ J., DINGWELL R.T., BARKEMA H.W., LESLIE K.E., DOHOO I.R. (2006 ): Testcharacteristics from latent-class models of the California Mastitis test. *Prevent. Vet. Medicine*, 77 (1-2): 96-108.

SCHUKKEN Y.H., SMIT J.A.H., GROMMERS J., VANDEGEER D., BRAND A. (1989): Effect of freezing on bacteriologic culturing of mastitis milk samples. *J. Dairy Sci.*, 72: 1900-906.

SOL J., SAMPIMON O.C., HARTMAN E., BARKEMA H.W. (2002): Effect of preculture freezing and incubation on bacteriological isolation from subclinical mastitis samples. *Vet. Microbiol.*, 85: 241-49.

TRINIDAD P., NICKERSON S.C., ALLEY T.K. (1990): Prevalence of intramammary infection and teat canal colonisation in unbreed and primigravid dairy heifers. *J. Dairy Sci.*, 73: 107.

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