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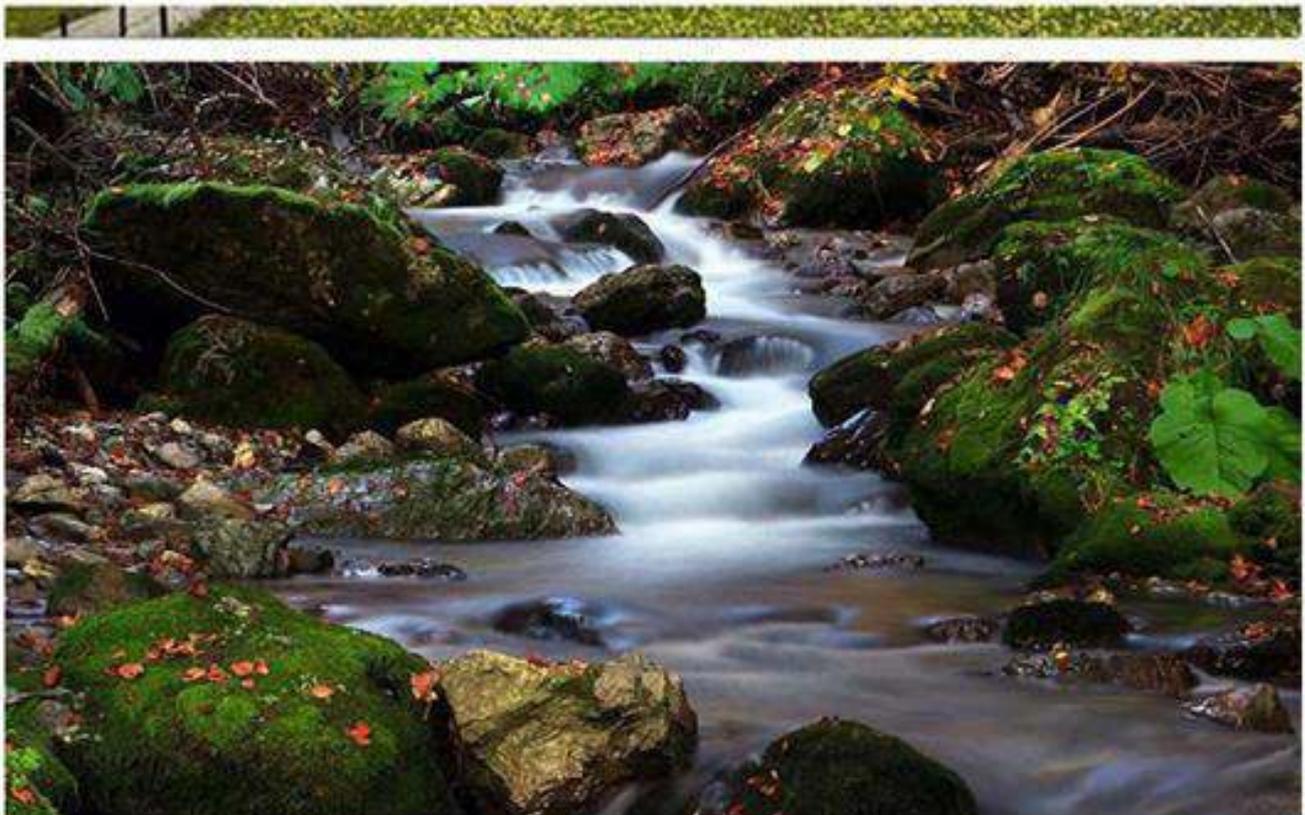


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Article

Indirect method for detection of Subclinical Mastitis in Dairy cows

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Abstract: Monitoring the health status of the mammary gland is an essential part in the process of safe milk production. The aim of the research was to evaluate the usefulness of the California Mastitis Test (CMT) which is an indirect method for detecting subclinical mastitis in dairy cows and it is used as a farm screening test. The efficacy of CMT for diagnosis of the subclinical mastitis was determined by comparing the results with LACTOSCAN SCC instrument. A total of 18 milk samples were examined. The results of our research indicate that there is a good correlation between the results of the California Mastitis Test and the total number of somatic cells, i.e. the test showed 75% sensitivity and 70% specificity.

Keywords: mastitis, SCC, CMT

1. Introduction

Mastitis is a common problem in dairy cattle, which results with increased somatic cells, changes in milk composition, and increased farm costs (Asfaw and Negash, 2017). In addition to the higher number of somatic cells, mastitis milk is usually characterized by increased total number of bacteria compared to the normal milk (Forback *et al.* 2010).

Somatic cells are mainly milk-secreting epithelial cells that have been shed from the lining of the gland and white blood cells (leukocytes) that have entered the mammary gland in response to injury or infection (Dairyman's digest, 2009). Approximately 98% of somatic cells are leukocytes, which number increases as a result of bacterial invasion, the remaining 2% being epithelial cells, which are the result of regeneration of the udder (Kocoski, 2011). In high SCC milk and infected quarters, the concentrations of non-casein fractions, sodium, chloride, and free fatty acid were higher ($p < 0.05$), while the casein content, lactose, casein-to-total protein, potassium, and calcium were lower ($p < 0.05$) compared to normal quarters (Ogola *et al.* 2007). In addition, mastitis is accompanied by physical, chemical, pathological, and bacteriological changes in milk and glandular tissues (Sharma *et al.* 2010).

California mastitis test (CMT) is an indirect method to measure the somatic cells in the milk sample on the farm level. CMT it is quick, cheap, and simple and that it is an „animal side test“ (Sharma *et al.* 2010). On the other hand, a reliable direct method of measuring somatic cells is by using an automatic cell counter. The advantage with an automatic counter is that it is objective and accurate (Salvador *et al.* 2013).

2. Materials and Methods

The research was conducted during the summer period on a farm in the Pelagonia region; the analyses were performed in the laboratory for quality and safety of milk and dairy products, at the Faculty of biotechnical Sciences - Bitola.

In total 18 samples were analyzed. The CMT results for each teat were reported for every observation, randomly taken during morning milking. The basis for this test is lysis of somatic cells by the CMT reagent to precipitate the DNA and proteins contained in the cells. On the basis of the viscosity change, the sample can be semi quantitatively scored to allow for sample comparison and to

facilitate communication of the severity. The obtained results were evaluated for a time period of 30 seconds as 0 (negative), T (trace), 1+, 2 ++ or 3 +++ (Sharma *et al.* 2010).

Also, additional sample was taken for total number of somatic cells which were performed on the LACTOSCAN SCC on the same day. Samples were placed in a sterile plastic bottle with Broad Spectrum Microtabs II preservative (one tablet for 40 ml of milk) and transferred to a laboratory at 4 °C with portable refrigerator.

Animals were considered positive for mastitis when the CMT score was $\geq 1+$, while the number of somatic cells was $\geq 200,000$ / ml. Sensitivity and specificity were calculated to determine the suitability of CMT score cut-points for predicting intra mammary infection (Sharma *et al.* 2010). The results obtained with LACTOSCAN SCC were taken as a standard method.

3. Results

Comparison of CMT results against SCC/ml is shown in table 1. Apparently, low SCC is not an assurance that the result of CMT will be negative (sample 15,16 and 17). Results showed that in samples with SCC less than 200.000 cells per ml the CMT results can be positive (1+). This observation may be related to the subjectivity involved in the interpretation of the test result.

Table 1 - Comparison of CMT results against SCC/ml

Sample number	SCC/ml	CMT score	Result
1.	46,000	0	TN
2.	553,000	3+	TP
3.	35,000	0	TN
4.	1,441,000	3+	TP
5.	16,500	0	TN
6.	5,000	0	TN
7.	174,000	T	TN
8.	111,500	T	TN
9.	240,000	T	FN
10.	1,532,000	3+	TP
11.	278,500	T	FN
12.	621,500	2+	TP
13.	305,500	1+	TP
14.	72,000	0	TN
15.	1,000	1+	FP
16.	123,000	1+	FP
17.	23,000	1+	FP
18.	830,000	2+	TP

The values derived in Table 2 were used for the calculation of sensitivity (Sn) and specificity (Sp) of CMT. Results showed that CMT had 75% Sn and 70% Sp. Hence, the probability that a mastitis infected animal can be identified through CMT is 75%. Likewise, the likelihood of correctly identifying non-mastitis animal is 70%.

Table 2 - Table of the results obtained using CMT for detection of subclinical mastitis

Test	Positive		Negative	
	TP	FP	TN	FN
	9		9	
CMT	6	3	7	2
	Sensitivity 75%		Specificity 70%	

TP=true positive, FP=false positive, TN=true negative & FN=false negative

Compared to some of the studies done before, results showed that the Sn and Sp of CMT reported in this study are comparable. The obtained results are in accordance with the examinations of Sharma *et al.* (2010) where the specificity of CMT is 59.70%, and the sensitivity is 86.07%, as well as with the examinations of Galifi *et al.* (2017) where the specificity of CMT of 82.05% and sensitivity of 78.57% was determined. The close difference with the results of the other studies may indicate that CMT test can be used on farm level for estimating the subclinical mastitis. The results indicate that CMT has satisfactory sensitivity and specificity for predicting subclinical mastitis, which confirms the importance of using CMT as a screening test at the farm level (Galifi *et al.* 2017).

4. Conclusions

Indirect diagnostic methods, such as CMT, can be used by farmers to identify infected quarters, because mastitis is one of the most common disease in the dairy farm which causes large economic losses, so early diagnosis and prevention of subclinical mastitis must be a priority for each dairy farmer. Cost of mastitis can be divided in different categories including: milk production losses, drugs, discarded milk, veterinarian, labor, milk quality and other. According to the obtained results, it can be noticed that there is a good correlation between these two methods, i.e. CMT is a reliable diagnostic method on a farm level.

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