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DETECTION OF SUBCLINICAL MASTITIS IN DAIRY COWS USING CALIFORNIA AND DRAMINSKI MASTITIS TEST

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Abstract: Control of udder health is an essential element in the process of safe milk production. Thus through the mastitis control program dairy farms regularly conduct measures of detection and prevention of udder diseases. Subclinical mastitis is an important disease of dairy cows causing economic losses and physical and chemical changes in milk. The aim of this research was to evaluate the usefulness of the California and the Draminski mastitis test to detect the subclinical mastitis in dairy cows. The efficacy of indirect mastitis tests for diagnosis of the subclinical mastitis was determined by comparing results of mastitis tests with bacteriological findings. The experiment was conducted on two dairy farms (farm A and farm B) Holstein - Friesian breed. A total of 245 quarter milk samples were examined, 95 quarter milk samples with the California mastitis test from farm A and 150 quarter milk samples with the Draminski mastitis test from farm B. A quarter milk samples for bacteriological analysis were taken aseptically during the morning milking in sterile test tubes. On farm A, bacteria growth has not been detected in 46.32% (44/95) quarter milk samples, while on farm B negative bacteriological findings have been found in 50.67% samples (76/150). In present study, sensitivity of the California mastitis test (78.57%) is higher than sensitivity of the Draminski mastitis test (74.32%). The specificity of the California mastitis test and the Draminski mastitis test is 82.05% and 30.26%, respectively. Efficacy of the California mastitis test in detection of the subclinical mastitis in dairy cows is better than that of the Draminski mastitis test, since accuracy of the California mastitis test has been higher.

Key words: California mastitis test, Draminski mastitis test, subclinical mastitis, cow

Introduction

In modern livestock industry, mastitis is one of the most important diseases of dairy cows which cause a huge production loss. Mastitis is an inflammation of the mammary gland that leads to physical and chemical changes in milk and affects on the production of dairy cows (*Khan and Khan, 2006; Boboš et al., 2013*). Early detection of mastitis is important for dairy farmers to reduce economic losses which are associated with reduction in yield, increased treatment costs and discarded milk (*Bhutto et al., 2012*). Mastitis most common occurs in one of two forms - a clinical or a subclinical infection. Detection of clinical mastitis is easy, because of the visible changes in the affected mammary gland and its secretion, while diagnosis of subclinical is problematic since cow shows no physical symptoms. The milk can appears normal during subclinical mastitis, but more common can notice the increase somatic cell count and concentration of certain ions, Na^+, K^+, and Cl^-.

Various screening methods are used for diagnosis of subclinical mastitis during lactation, based on physical and chemical changes of milk (*Sharma et al., 2010*). They have differences with respect to accuracy (sensitivity and specificity) and cost (*Pyorala, 2003; Fosgate et al., 2013*). According to the International Dairy Federation (IDF) recommendations, detection of mastitis is based on the somatic cell count and microbiological status of the udder quarter. However, somatic cell count increased in the first week postcalving and may remain high up to the first month of lactation (*Atakan, 2008*) and again increased towards the end of lactation as normal physiological condition (*Sharma and Pandey, 2011*). The definitive diagnosis of mastitis requires the isolation of pathogenic bacteria, but this is an expensive method which requires time. Besides that, this method does not provide a measure of the degree of inflammation associated with the infection. The California mastitis test, first described and used by Schalm and Noorlander in 1957, is a simple, quick, inexpensive and rapid test that accurately predict the somatic cell count in milk (*Bhutto et al., 2012*). The California mastitis test is based upon the amount of cellular nuclear protein present in the milk samples. The number of somatic cells in milk increases as the inflammatory process develops in udder tissue. Electrical conductivity/resistance of milk has been used as indicator of mastitis since four decades, and it has a positive correlation with somatic cell count. Electrical conductivity is determined by the concentration of anions and cations in milk. As a results of the damage to the udder tissue during mastitis, concentration of lactose and potassium decrease, and concentration of sodium and chloride increase. Hand-held meters, such as Draminski mastitis test, have been promoted as a screening tool for subclinical mastitis in some countries (*Fosgate et al., 2013*). However, data on the diagnostic value of this method is contradictory. Some authors point to a good correlation between electrical conductivity and

bacteriological tests (*Nielen et al., 1992*), while others consider this method to be insufficiently sensitive (*Pyörälä, 2003*).

The aim of this research was to evaluate and compare the usefulness of the California and the Draminski mastitis test to correctly detect the subclinical mastitis in dairy cows.

Material and Methods

The experiment was conducted on two dairy farms (farm A and farm B) Holstein - Friesian breed in Autonomous Province of Vojvodina, Republic of Serbia. General condition and udder health status were evaluated by clinical examination of animals. Udders were examined visually and by palpating for the presence of any udder changes (redness, swelling, pain, heat). Also, milk samples from each quarters were examined for the presence of flakes and clots. Animals with visible signs of mastitis were not included in the study. Immediately after clinical examination and before milk sampling for bacteriological analysis, milk testing from each udder quarter was performed using California mastitis test and Draminski mastitis test for detection of subclinical mastitis. A total of 245 quarter milk samples were examined, 95 quarter milk samples with the California mastitis test from farm A and 150 quarter milk samples with the Draminski mastitis test from farm B.

California mastitis test was carried out according to the method described by *Schalm and Noorlander (1957)*, at cowside by mixing gently an equal volume of milk with reagent (2 mL). Milk colour changes or formation of a viscular gel are readable within 1-2 minutes. Based on the reactions, the results were graded as negative (-), trace (T), weak positive (+), distinct positive (++) and strong positive reaction (+++).

Draminski mastitis test mesaures electrical resistance of milk. Concentration of sodium and chloride ions increases in milk from infected quarters which lead to decreased milk electrical resistance. The results of the Draminski mastitis test were interpreted according to the manufacturer's instructions (above 300 units - high quality and healthy milk; the incidence of subclinical mastitis is very low; between 300 and 250 units - progressively increasing incidence of subclinical infection as the readings decrease; below 250 - indication of a rapid increase in the severity of infection as subclinical mastitis progresses to clinical states).

A quarter milk samples for bacteriological analysis were taken aseptically during the morning milking in sterile test tubes. Before sampling, teats were washed and desinfected with 70% alcohol. Each sample was marked with cow's identification number and teat from which sample was collected (fore - left, fore -

right, rear - left, rear - right), and submitted to the laboratory for microbiological examination at refrigerator temperature.

From each samples, 0,1 mL of milk was plated on Columbia blood agar base with 5% defibrinated ovine blood, MacConkey agar and Sabouraud dextrose agar. Plated were incubated for 24h to 48h (bacteria) and 5 days (yeasts. mould) at 37°C under aerobic conditions. Bacterial colonies were determined 24, 48 and 72 hours after incubation. The isolates were identified according to their cultural characteristics (shape, size and structure) and physiological features (Gram straining, pigment formation, catalase test, CAMP test, coagulase test).

Characteristics of the California mastitis test and the Draminski mastitis test were determined using the milk bacteriological culture as a gold standard control. Percent sensitivity, specificity and accuracy were calculated by the formulae of *Sharma et al. (2010).*

Sensitivity = TN / TP + FN x 100

Specificity = TN / FP + TN x 100

Accuracy = TP +TN / TP + FP + FN + TN x 100

TP - true positive; FP - false positive; TN - true negative; FN - false negative

Results and discussion

The study included 245 quarter milk samples from dairy cows without clinical signs. After incubation of milk samples the bacterial colonies were determined. On farm A, the California mastitis test was used for detection of subclinical mastitis, and a total of 95 quarter milk samples were examined. Results of the California mastitis test revealed that out of 95 quarter milk samples, the number of samples showing true positive, true negative, false positive and false negative were 44 (86.27%), 32 (72.73%), 7 (13.73%), 12 (27.27%) respectively. These results correspond with the findings of *Sharma et al. (2010)* and *Badiuzzaman et al. (2015).* In our research, the California mastitis test has high percentage sensitivity and specificity (Table 1). *Sargeant et al. (2001)* concluded that the California mastitis test could be used in dairy herd monitoring program as a screening test to detect cows with intramammary infection. On the other side, *Rice (1981)* amounts as disadvantages of the California mastitis test false positive reaction during early and late lactation period.

Table 1. Percentage accuracy of the California mastitis test used for the detection of subclinical mastitis on farm A taking cultural test as standard

Test	California mastitis test		Cultural isolation	
	N	%	N	%
Positive samples	51	53.68	51	53.68
True positive	44	86.27	51	100
False positive	7	13.73	/	/
True negative	32	72.73	44	100
False negative	12	27.27	/	/
Total samples examined	95		95	
Sensitivity (%)	78.57		100	
Specificity (%)	82.05		100	
Accuracy (%)	80		100	

The Draminski mastitis test was used for determination of milk electrical conductivity on farm B. Results of electrical conductivity with Draminski mastitis test revealed that out of 150 quarter milk samples, 72% (108/150) quarter milk samples showing positive and 28% (42/150) quarter milk samples negative results (Table 2). These results correspond with the findings of *Galfi et al. (2015). Chahar (2007)* and *Langer et al. (2014)* have reported lower percentage of true positive cases detected by Draminski mastitis test (38% and 7.6% respectively). *Langer et al. (2014)* indicated that detection of subclinical mastitis with hand-held electrical conductivity meter was very low. *Henningsson et al. (2005)* indicated that milk electrical conductivity is determined by type and concentration of ions, interactive influence of the ions and components contributing to milk viscosity (protein, fat, lactose). During subclinical mastitis, concentration of sodium and chloride ions increases which leads to increased electrical conductivity in milk (*Kitchen, 1981*). Research of *Norberg et al. (2004)* pointed that cows with subclinical mastitis may not always show an increased electrical conductivity of milk from the infected quarter, but the variation in electrical conductivity of milk from infected udder quarter may be larger than variation in electrical conductivity of milk from healthy quarters, while *Sheldrake el al. (1983)* indicated that higher values of electrical conductivity of milk in infected quarters can be noticed only in that quarter. *Morsi et al (2000)* indicated that milk chlorine percentage alone cannot judge the presence of mastitis as it usually give high results in colostrums or at late stage of lactation. Many factors have influence on the measurement of milk electrical conductivity such as breed, lactation stage, age of cow, oestrus, milk temperature, pH and fat concentration in milk (*Biggadike et al., 2000*).

Table 2. Percentage accuracy of the Draminski mastitis test used for the detection of subclinical mastitis on farm B taking cultural test as standard

Test	Draminski mastitis test		Cultural isolation	
	N	%	N	%
Positive samples	108	72	76	50.67
True positive	55	50.93	76	100
False positive	53	49.07	/	/
True negative	23	54.76	74	100
False negative	19	45.24	/	/
Total samples examined	150		150	
Sensitivity (%)	74.32		100	
Specificity (%)	30.26		100	
Accuracy (%)	52		100	

Dingwell et al. (2003) and *Midleton et al. (2004)* indicated in their research that an ideal screening method for detection of subclinical mastitis would have maximum sensitivity to minimize the proportion of false negative results, and also a reasonable degree of specificity to reduce the number of false positive results. Our results suggest that the California mastitis test and the Draminski mastitis test had good sensitivity as predictors of subclinical mastitis. The California mastitis had good specificity, too, but the Draminski mastitis had low specificity, what is its main weakness. Low specificity of the test leads to incorrectly identification a high percentage of udder quarter as infected. Many authors mentioned that the California mastitis test is the most sensitive and specific indirect test for detection subclinical mastitis in dairy cows (*Iqbal et al., 2006; Joshi and Gokhale, 2006*). *Galfi (2016)* pointed that the California mastitis test has higher sensitivity and specificity than Draminski mastitis test and it is better method in detection of subclinical mastitis.

Conclusion

Indirect diagnostic methods, such as the California mastitis test and the Draminski mastitis test, can be used by dairy farmers to identify infected udder quarters and help them to avoid economic losses of mastitis. Our research indicated that the efficacy of the California mastitis test is better than that of the Draminski mastitis test, since accuracy of the California mastitis test has been higher. The California mastitis test represent valuable diagnostic methods in detection of subclinical mastitis in dairy cows, while Draminski mastitis test should not be used as the sole method.

Detekcija subkliničkog mastitisa kod visokomlečnih krava primenom Kalifornija i Draminski mastitis testa

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Rezime

Kontrola zdravlja vimena krava je neophodan element u procesu proizvodnje zdravstveno bezbednog mleka, te se na farmama visokomlečnih krava, kroz program kontrole mastitisa, redovno sprovode mere otkrivanja i prevencije bolesti vimena. Subklinički mastitis je važno oboljenje visokomlečnih krava koje izaziva ekonomske gubitke i promene u fizičkim i hemijskim osobinama mleka. Cilj ovog istraživanja je da se proceni mogućnost primene Kalifornia i draminski mastitis testa za otkrivanje subkliničkih mastitisa. Efikasnost ovih testova je potvrđivana poređenjem rezultata testa sa bakteriološkim nalazima. Eksperiment je sproveden na dve farme krava (farma A i farma B) Holštajn frizijske rase. Ukupno je pregledano 245 pojedinačnih uzoraka mleka, 95 primenom Kalifornia mastitis testa na farmi A i 150 primenom Draminski mastitis testa, na farmi B. Pojedinačni uzorci mleka za bakteriološku analizu su uzeti aseptično za vreme jutarnje muže u sterilne epruvete. Na farmi A rast bakterija nije dokazan u 46,32% (44/95) uzoraka, a na farmi B u 50,67% (76/150) uzoraka. U sprovedenom istraživanju, senzitivnost Kalifornija mastitis testa (78.57%) je veća nego senzitivnost Draminski mastitis testa (74.32%). Specifičnost Kalifornija mastitis testa i Draminski mastitis testa je 82.05% i 30.26%. Efikasnost Kalifornija mastitis testa u detekciji subkliničkih mastitisa kod visokomlečnih krava je veća nego Draminski mastitis testa, jer je validnost Kalifornija mastitis testa bila veća.

Ključne reči: Kalifornija mastitis test, Draminski mastitis test, subklinički mastitis, krava

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