JOURNAL OF AGRICULTURE AND PLANT SCIENCES, JAPS, Vol 16, No. 2, 2018

Manuscript received 29.08.2018
Accepted: 20.09.2018

In print: ISSN 2545-4447
On line: ISSN 2545-4455
UDC: 636.2.09:[618.19-002:618.19-083
Original scientific paper

MONITORING UDDER HEALTH AND MILK HYGIENE ON-FARM USING QUICK SCREENING METHODS

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Abstract

In this paper the use of on-farm screening methods for monitoring udder health and milk quality are discussed. Special attention was given to the evaluation of the usefulness of California mastitis test (CMT) as quick field screening test for detection of udder quarters with an intra-mammary infections caused by major mastitis pathogens. Application of CMT in dairy herd health management in period of early lactation is illustrated through the two years cross sectional study that was carried out to screening the quarter milk samples with abnormal milk secretion (AMS) and using of microbiological culture for detection of inframammary infections (IMI). The quarter milk samples were obtained in two periods of early lactation: the period from calving until 21st day in lactation and period from 22nd to 42nd day in lactation. The quarter level prevalence of AMS and IMI in the first 21 days in lactation was 5.33% and 4.03%, and up to the 42 days in lactation the prevalence of AMS and IMI was 5.45% and 4.38%, respectively. The prevalence of AMS and IMI from udder quarters that show a positive reaction on CMT in the first 21 days in lactation was 56.96 and 55.42; and 55.42 and 44.58 in the period from 22nd to 42nd day in lactation, respectively. The results indicated that positive CMT reaction in early lactation may be a good indicator for IMI; there was a significant association between the frequency of isolation of major pathogens and the CMT score in milk samples obtained in the period of early lactation (Pearson's $\chi 2=240.031$, df=9, $P<0.001$).

Key words: *California mastitis test, dairy cows, intra-mammary infection*

INTRODUCTION

Milk composition and microbiological characteristics are important factors for the dairy farmer (raw milk quality), dairy industry (technological process and quality of dairy products), and consumer (nutritional quality and safety). In most dairy systems, it is assumed that farmers, informed by the official organization in their country or region, have the responsibility to deliver milk that is of sufficient quality (Hogeveen et al., 2010). Although regulations dealing with milk quality standards differ between dairy-producing countries, the general agreement is that abnormal milk, as the presence of flakes, clots, or other gross alterations in milk appearance, or highly elevated Somatic Cell Count (SCC) or injured udders should be excluded from milk supplied for human consumption.

Control of inflammation of the mammary gland and the reduction in their appearance in the herd is based on the number of somatic cells in milk and the incidence of clinical mastitis (IDF, 1997). The measurement used most commonly to detect subclinical mastitis is the SCC of milk. Thus, in order to minimize the appearance of mammary inflammation, the main goal should be the number of somatic cells below 200.000 / ml of milk, and the incidence of clinical mastitis below 20% (Pyorala, 2003; Schukken et al., 2003). According to the National legislation in the Republic of Macedonia (FVA, 26/2012) milk from uninfected mammary glands contains ≤100.000 somatic cells per millilitre. A milk SCC from 200.000 - 400.000/ml is a clear indication that milk has reduced manufacturing properties,

which means is not for consummation. This, an increase in the SCC of milk is a reasonably good indicator of inflammation in the udder.

Identifying cows with clinical mastitis (CM) involves visual inspection of the udder and manual check of the foremilk from each quarter at each milking, and regular implementation of screening methods for early detection of cows with elevated SCC, like *California Mastitis Test* (CMT), measurements of milk conductivity, and the presence of chlorides and sodium in milk as results of udder inflammation (Sharma et al., 2011). However, increasing herd sizes, reliance on less-skilled labour, and an increasing emphasis on lowering bulk milk SCC levels (Lacy-Hulbert et al., 2010) are all factors contributing to an increased demand for more consistent and less labour-intensive methods to help farmers manage mastitis and bulk milk SCC levels. Comparing to culturing methods and determination of SCC, the field screening methods for diagnosis of mastitis are easy and routine methods that give prior information for antibiotic treatment of infected udder quarters and early drying off. Among the others, CMT is widely used for on-farm detection of mastitis in dairy herds (Sharma et al., 2011). Calderon and Rodrigues (2008) reported some insufficiency of the CMT regarding their sensitivity and specificity in the determination of IMI. The main weakness of the CMT is its low specificity for determination of udder quarters infected with major or minor mastitis pathogens. The CMT is a rapid and inexpensive test to determine indirectly the somatic cell concentration in milk and is a practical, easy method for demonstrating IMI by testing milk samples on-farm. In general, there is no ideal screening test for prompt and quick diagnosis of IMI. Culturing examination is the "gold standard" for detection of infected udder quarters, but very often these methods are very expensive, time consuming for routine screening followed by lack for on-farm assessment (Sargeant et al., 2001). An ideal screening method would have maximum sensitivity to minimize the proportion of false-negative results, and a reasonable degree of specificity to reduce the number of false positive results (Dingwell et al., 2003; Midleton et al., 2004). Accurate mastitis detection and effective mastitis control strategies have an influent economic impact on dairy farms followed by sustainable milk production (Wallace et al., 2002).

The intensive systems for breeding of dairy cows in the Republic of Macedonia practice a free posture system, in accordance with the National legislation for Animal Welfare (FVA, 149/2014). Such breeding technology requires the existence of central milking parlour on farms. This segment of complex objects of farms for milking cows that is the one of the key control points for reduction of incidence of mammary gland inflammation. Trajchev and Nakov (2009) suggested mastitis control programme that outlines these procedures: systematic examination of herd for detection clinical and subclinical forms of mastitis; laboratory tests for identification of mastitis causative microorganisms and selection of most appropriate antimicrobial substance for cow therapy; application of suitable therapy and procedures for mastitis cow treatment; and preventive measures on farms, which is consist of standard methods and procedures, with main goals to prevent or reduce the influence of new intramammary infections in the herds.

The purpose of the study was to determine whether or not individual cow quarter milk CMT score, collected in the period of early lactation, from calving to 42nd day in, could indicate whether or not these quarters were infected by pathogens associated with subclinical mastitis. Testing the cows in one dairy herd, quarter milk samples were subjected to CMT under field (cow-side) conditions and the same samples were examined microbiologically.

MATERIALS AND METHODS

A two year cross sectional survey was carried out in a commercial dairy farm localized in the southeaster part of Macedonia. Cows were kept in a loose housing system and were feeding with an ordinary diet which differed according to the stage of pregnancy and lactation period. The feeds included corn and grass silage, hay, commercial concentrate (maize, wheat, barley) as well as vitamin and mineral premixes. Data were collected from 211 cows that were all starting their 1st or a subsequent lactation, and that had at least 3 functional quarters and a total of 844 quarter CMT and culture results were available for analysis. Milking of cows was performed twice daily in milking parlour with exception of cows in the first 45 days in lactation which were

milked three times daily. Pre-milking and post-milking hygiene measurements were practiced permanently. The udder health status was followed by calving to 42nd day in lactation. This period of early lactation was subdivided into 2 periods: the period from calving until 21st day in lactation and period from 22nd to 42nd day in lactation. The screening of udder health status was done on a quarter level using California Mastitis Test (CMT) as predicted tool for detection of quarters with abnormal milk secretion (Schalm и Noorlander, 1957). The test was performed at cow-side by mixing an equal volume of milk with a 1:1000 dilution of 3% sodium lauryl sulfate and bromocresol purple. Each quarter milk sample from the cow was placed in one clean well of a white plastic test paddle divided into four separate wells, one for each quarter sample. As the plate was rotated gently, any colour changes or formation of a viscous gel were interpreted by the authors above: scores were given within the range 0–4, with 0 for no reaction, 1 for a trace, 2 a weak positive, 3 a distinctly positive and 4 a strong positive.

Samples for bacteriological culture from each quarter positive to CMT were collected aseptically in sterile 10 mL tubes, without additives according to the National Mastitis Council (NMC, 2001) and kept at 4°C during transportation. Samples were analysed within 12 hours of collection. Bacterial species were identified according to the standard microbiological procedure using a certificated methodology based on the National Mastitis Council standards (NMC, 2001). A minimum of five colonies of the same type of bacterium was recorded as bacteriological positive, and growth of more than two types of bacterial colonies was categorized as mixed growth. No bacterial growth was recorded when fewer than five colony-forming units were detected during 48 h of incubation.

Based on results from screening using CMT and bacteriological culturing, all cows in the observed population were allocated into three groups: healthy cows without udder health problems, cows with persistent abnormal milk secretion (AMS) and cows with persistent intramammary infection (IMI). The difference in prevalence of udder quarters with AMS and IMI and their significance was estimated by the Chi-square test.

RESULTS AND DISCUSSION

The results from a quick screening test and the prevalence of udder quarter health disorders in an observed population of dairy cows during the early lactation are shown in Table 1.

Table 1. The quarter level prevalence of abnormal milk secretion (AMS) and intra-mammary infections (IMI) in dairy herd during the period of early lactation

Time of sampling	Total quarters tested	*CMT(-) quarters		**CMT(+) quarters		AMS		IMI	
	n	n	%	n	%	n	%	n	%
***Day_21	844	765	90.64	79	9.36	45	5.33	34	4.03
****Day_42	844	761	90.17	83	9.83	46	5.45	37	4.38

*negative reaction on California mastitis test (CMT)
** positive reaction on California mastitis test (CMT)
***period in lactation from calving until 21st day in lactation
****period in lactation from 22nd to 42nd day in lactation

Based on the results from the field screening test (CMT), the total prevalence of udder health disorders on the quarter level was 9.36% and 9.83%, respectively for the period from calving until 21st day in lactation and period from 22nd to 42nd day in lactation. Overall, the prevalence of udder quarters with AMS in these data was 5.33% and 5.45%, while the prevalence of quarters with IMI was 4.03% and 4.38%, respectively for the period from calving until 21st day in lactation and period from 22nd to 42nd day in lactation.

In Table 2 are shown the ratios between udder quarters with AMS and IMI versus udder quarters that have positive CMT reaction.

Table 2. The ratio of udder quarter with AMS and IMI relating to the udder quarters with positive reaction on California mastitis test (CMT+)

Time of sampling	CMT(+) quarters	AMS	AMS	IMI	IMI
	n	n	%	n	%
Day_21	79	45	56.96	34	43.04
Day_42	83	46	55.42	37	44.58

The prevalence of quarters with IMI from the screened udder quarters that were showed a positive reaction on CMT was high, 43.04% in the period from calving until the 21st day in lactation and 44.58% in the period from 22nd to 42nd day in lactation. There was a significant association between the frequency of isolation of major pathogens and the CMT score in milk samples obtained in the period of early lactation ($\chi 2=240.031$, df=9, $P<0.001$).

A major health problem in the dairy industry in the Republic of Macedonia is mastitis, either clinical or subclinical. Therefore, one of the most important objectives of the mastitis control programme in dairy herds is the control of raw milk quality and safety. Milk with clinical mastitis is easy to recognize, but when the milk of cows with subclinical mastitis without visible changes, is accidentally mixed into bulk milk, it enters the food chain and can be hazardous to human's health.

In the recent years, there is an increasing focus on milk quality and hygiene in the dairy industry. The main goal of legislation for milk quality is to stimulate farmers to produce hygienically proper milk. The basic criteria for assessing the safety of raw cow's milk are lying down in the "legislation on the special requirements for safety and hygiene and the manner and procedure for performing official controls of milk and dairy products" (FVA, 2012). According to national legislation in the Republic of Macedonia for the quality of raw milk (FVA, 2012), the milk is classified in terms of the total number of bacteria and the total number of somatic cells. According to the total number of microorganisms, raw milk is classified into three classes: extra class (≤100.000/ml), first class (100.001/ml – 700.000/ml) and second class (700.001/ml – 1.500.000/ml). According to the total number of somatic cells, raw milk also is classified into three classes: extra class (≤400.000/ml), first class (400.001/ml – 500.000/ml) and second class (500.001/ml – 600.000/ml). Increased SCC is associated with reductions in casein, milk fat, and lactose; increased enzymatic activity; and reduced quality and yield of dairy products (Schukken et al, 2003).

Mammary gland infection is the most important factor affecting SCC in milk in the subclinical mastitis by increasing the number of somatic cells in milk (Bachaya et al., 2011). As IMI is usually followed by an influx of leucocytes and other macrophages into the milk, an increase in its SCC has been used widely as indicating mastitis. Implementing good management practices at the begging of lactation is essential for good milk quality in the subsequent period of lactation and also, for evaluating udder health before the administration of antibiotic therapies. Preventing clinical mastitis in early lactation, decreasing the amount of discarded milk, and reducing the bulk milk SCC are some of the potential benefits. However, as there is growing consumer resistance to using antibiotics in all food animal production systems, it may be desirable to reduce the number of cows that need treatment by identifying and treating only those with IMI.

An ideal diagnostic tool to select quarters or heifers for pre-calving treatment should be quick, easy to perform on-farm, reliable and inexpensive. Milk bacteriology does not correspond to those criteria. The CMT is a rapid and inexpensive test to indirectly determine the somatic cell concentration in milk (Midleton et al., 2004) and is a practical, easy method for demonstrating IMI by testing milk samples on-farm (Dingwell et al., 2003). However, differences in CMT or SCC scores could also be associated with other factors such as the age of cows and environmental factors. Previous research had suggested that SCC is related with parity, a period of lactation, milking frequency and milk yield

(Hand et al., 2012). Some other researchers did not find any relationship between the physiological status of cow and SCC in milk from healthy cows (Charffeddine et al., 1997).

Whilst an increase in CMT score corresponds with an increase in SCC, it is uncertain whether or not CMT or SCC scores can reflect accurately IMI due to specific pathogens. If either SCC or CMT could be used reliably to identify subclinical mastitis in lactating cows, they might be useful in identifying such affected quarters that require antibiotic treatment and early drying off (Barkema et al., 1998).

The sensitivity of CMT on the quarter level in the period of early lactation according to the results obtained in our research was 43.04% and 44.58%, respectively for the period from calving until the 21st day in lactation and the period from 22nd to 42nd day in lactation. The sensitivity and specificity of CMT reported in the literature is variable (Pyorala, 2003). For example, at 3 days in milk (DIM), Sargeant et al. (2001) found that sensitivity and specificity to identify any IMI at a quarter level were 57% and 56%, respectively. On the other hand, Vijaya Reddy et al. (1998) reported a sensitivity of 71% and a specificity of 75%. Sensitivity and specificity are improved when only major pathogens are considered (Sargeant et al., 2001; Dingwell et al., 2003).

In the present study, we hoped to determine whether the CMT scores for individual quarter milk samples could be used as a screening method to identify mammary glands with subclinical infections. Our results suggest that overall there was a significant association between the frequency of isolation of major and minor pathogens and the CMT score in milk samples obtained in the period of early lactation. The present results also indicated that quarters infected with a major pathogen were more likely to have higher CMT scores than those infected with either minor pathogens or uninfected (previously published results Trajchev et al., 2017). These results agree with those reported by Kivaria et al. (2004), which suggested that CMT scores of 2+ or more were associated with an increased risk of infection with S. aureus. Sargeant et al. (2001) reported that the CMT had a useful surveillance role in dairy herd monitoring programs to detect cows with IMI caused by major pathogens. The results obtained by Saidi (2013) showed a good correlation between the results of CMT and isolation for the identification of intra-mammary infections in cows.

Ruegg and Reiman (2002), Kivaria et al. (2004) and Rasmussen et al. (2005) summarized that the CMT is still the superior screening diagnostic aid for subclinical mastitis, while bacteriological examination is still the most suitable for identifying mastitis but not feasible as a routine test to identify subclinical mastitis because some logistical and financial constraints limit its use especially in developing countries.

CONCLUDING REMARKS

All farmers in the Republic of Macedonia should aspire to the legislation for milk quality control to produce more quality milk. The milk producer or, where appropriate, the operator who collects the milk should ensure compliance with the specific safety requirements for the production of raw milk: animal health requirements for the production of raw milk for human consumption, requirements for premises and equipment of hygiene of the holdings for production of raw milk, hygiene requirements during milking, collection and transport and requirements for raw milk. Producing high quality milk requires effective udder health programmes at a herd level.

The early mastitis detection in the dairy herd has many potential benefits, out of which it will allow implementation of proactive management strategies that will avoid negative effects of disease and will lead to better milk quality.

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СЛЕДЕЊЕ НА ЗДРАВСТВЕНИОТ СТАТУС И ХИГИЕНАТА НА МЛЕЧНАТА ЖЛЕЗДА СО КОРИСТЕЊЕ НА БРЗИ СКРИНИНГ ТЕСТОВИ

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Резиме

Во овој труд беше дискутирана употребата на брзите скрининг тестови на фарма за млечни крави како алатка за следење на здравствениот статус и квалитетот на добиеното сурово млеко. Направени се двегодишни проспективни истражувања за оценување на карактеристиките на Калифорнија маститис тестот (КМТ) во периодот на рана лактација како брз теренски скриниг тест за откривање на инфицираните четвртинки од млечната жлезда со патогени микроорганизми кои предизвикуваат мастит. Истражувањето опфаќаше скрининг на пробите млеко од четвртинките на млечната жлезда на кравите со цел откривање на четвртинките со нарушена секреција на млеко (КМТ+) и употребата на микробиолошки методи за откривање на инфицираните четвртинки од млечната жлезда (ИМИ). Пробите млеко беа собирани во два периода од лактацијата на кравите: периодот од почетокот на лактацијата до 21. ден во лактацијата и периодот од 22. до 42. ден во лактацијата. Преваленцијата на КМТ+ и ИМИ во периодот од почетокот на лактацијата до 21. ден во лактацијата изнесуваше 5,33% и 4,03%, соодветно, а во периодот од 22. до 42. ден во лактацијата преваленцијата на КМТ+ и ИМИ изнесуваше 5,45% и 4,38%, соодветно. Преваленцијата на КМТ+ и ИМИ од четвртинките на млечната жлезда кои покажаа позитивна реакција на КМТ во периодот од почетокот на лактацијата до 21. ден во лактацијата изнесуваше 56,96 и 55,42; додека преваленцијата на КМТ+ и ИМИ во периодот од 22. до 42. ден во лактацијата изнесуваше 55,42 и 44,58; соодветно. Добиените резултати укажуваат дека позитивната реакција на КМТ во периодот на раната лактација кај млечните крави претставува добар индикатор за откривање на инфицираните четвртинки на млечната жлезда. Постоеше статистички значајна поврзаност помеѓу присуството на патогени микроорганизми во пробите млеко од четвртинките на млечната жлезда земани во периодот на рана лактација и позитивната реакција на Калифорнија маститис тестот (χ^2=240,031; df=9, P<0,001).

Клучни зборови: *Калифорнија маститис тест, млечни крави, инфекција на млечната жлезда*