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ANALYSIS OF THE SOMATIC CELL PATTERN IN MASTITIS - AFFECTED COWS ON THREE DAIRY FARMS IN VOJVODINA

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SUMMARY

Somatic cells (SCs) in milk, which include epithelial cells from the gland and blood cells, are present in milk during the normal course of milking. Increase in SCs is found in mastitis-affected dairy cows and can be a useful indicator for estimating mammary health and milk quality worldwide. The aim of this study was to determine whether there was a pattern of somatic cell count (SCC) in mastitis-affected cows on three different farms. The study was conducted on three dairy farms of high milk-producing cattle breeds in Vojvodina during 2021. Samples were taken from 15 cows from each farm, all of the cows being diagnosed with clinical and subclinical mastitis. The SCC in milk samples was determined by the microscopic reference method according to the standard (SRPS EN ISO 13366-1:2010) of the Institute for Standardization of Serbia. The value of SCC was considered as high if >200.000 cells/mL, as this is the threshold indicating secretion disorder. In order to examine the differences between the observed three farms, one-factor analysis of variance (ANOVA) was applied, while a post-hoc LSD test was used for determination of statistically significant differences between the SCC in cows on three different farms. The mean values of the SCC on Farms 1, 2 and 3 were 7,055,266.67, 2,619,893.33 and 552,000 cells/mL, respectively. Based on the results, a statistically significant difference ($p<0.05$) was established between Farms 1 and 2, as well as between Farms 1 and 3, while there was no statistically significant difference between Farms 2 and 3. Apart from mastitis, differences in the SCC on the farms could also be influenced by the cows' productivity, parity, lactation stage and breed, as well as poor management practices. Besides mastitis control, better hygiene and proper nutrition can help in reducing SCs in milk. In conclusion, establishing SCs pattern can provide useful information that may contribute to reducing SCs and developing differential SCs standards to help obtain milk with low SCs and consequently better dairy products with a longer shelf life.

Key words:
bacteria, cow, mastitis, somatic cells

Abbreviations:
WF - SCs – Somatic cells; SCC – Somatic cell count; DSCC – differential somatic cell count

INTRODUCTION

Mastitis is an inflammation of the mammary gland, regardless of the cause. It is characterized by physical and chemical changes in the milk, as well as pathological changes in the udder tissue (Radostits et al., 2007). It is found in dairy herds worldwide and it is one of the major health problems in intensive livestock production (Kurjogi & Kaliwal, 2011; Cobirka et al., 2020). In addition to affecting the health and welfare of the animals, mastitis leads to great economic losses on dairy farms related to increased treatment costs, reduced quality of produced milk,

decreased milk production due to withdrawal and cessation of lactation in severe cases (Shum et al., 2009; Hogeveen et al., 2011).

Mastitis has a complex etiology and different factors can affect the occurrence, duration and spread of this disease among cows (Ashraf & Imran, 2020). A number of studies have shown that various types of microorganisms can cause mastitis, but bacteria play the main role in its development (Ibrahim, 2017; Kibebew, 2017). Mastitis causing microorganisms can be divided into contagious and environment (Sharma et al., 2011). The most important contagious pathogens are *Staphylococcus aureus* and *Streptococcus agalactiae,* while the main way of transmission is from cow to cow during the process of milking (Williamson et al., 2022). Moreover, *Streptococcus uberis, Streptococcus dysgalactiae* and coliforms such as *Escherichia coli* and *Klebsiella* are the major pathogens that can be found in the habitat of dairy cows (Sharif et al., 2009). On the other hand, fungi are considered environmental agents, and the most common cause of fungal mastitis is *Candida* spp. (Costa et al., 1998; Krukowski & Saba, 2003). Microorganisms from the environment usually cause clinical mastitis that is easily diagnosed by clinical examination of the udder and the animal and it does not last long, while contagious microorganisms generally lead to subclinical mastitis that often goes unnoticed and affects a large number of cows in the herd, which is why this form of mastitis is a serious problem on farms (Sharif et al., 2009).

The presence of microorganisms triggers the inflammatory response of the mammary gland, and the main defense mechanism is the mammary SCs. Certain SCC is normally found in milk and 75% of these cells are leukocytes, macrophages, lymphocytes and erythrocytes, while the rest are epithelial cells (Sharma et al., 2011). During inflammation, SCC in the udder tissue increases, and the dominant cells are neutrophil granulocytes, which make up more than 90% of the total number of leukocytes in the mammary gland (Radostits et al., 2007). The SCC is higher the stronger the infection and the more severe the case of mastitis is (Kehrli & Shuster, 1994). This leads to lower milk yields and poorer milk quality (Khan & Khan, 2006).

The aim of the study was to determine whether there was a SCC pattern in mastitis-affected cows on three different farms located in the Province of Vojvodina, the Republic of Serbia, during 2021.

MATERIAL AND METHODS

The research was conducted on three farms of Holstein Friesian lactating cows in the area of the Province of Vojvodina, Republic of Serbia, during 2021. Samples were taken from 15 cows from each farm. All cows were diagnosed with clinical or subclinical mastitis. The animals were in the lactation phase and, according to the clinical examination, they had no other health problems apart from mastitis. Clinical mastitis was diagnosed by clinical and milk examination, while subclinical mastitis was diagnosed using SCC in the milk samples by California Mastitis Test. The milk samples taken from these animals were used for testing SCC and bacteriological testing. After disinfection and milking of the first streams, aseptic milk samples were taken for analysis during the morning milking. The samples were collected in sterile test tubes marked with the identification number of the animal and then stored at 4ºC. Further tests were performed in the milk hygiene laboratory of the Department of Veterinary Medicine of the Faculty of Agriculture, University of Novi Sad.

The samples were incubated for 48 h at 37 °C on 2% blood agar in order to determine the growth, biochemical and cultural characteristics of the microorganisms. The microbiological techniques outlined in the National Mastitis Council - NMC (2004) guidelines for diagnosing mammary gland infections were employed to isolate and identify bacterial strains from milk samples. A loopful of the milk sample was streaked on blood agar (Oxoid) and subsequently subcultured on selective media including mannitol salt agar, Edwards agar, Salmonella-Shigella agar and MacConkey agar. These plates were then incubated at 37°C for 24 hours, and during 24 to 48-hour period the colonies were observed for their appearance, color and any signs of hemolysis. In order to distinguish staphylococci from other Gram-positive cocci, we performed a combination of tests including the catalase test, mannitol fermentation test, coagulase test (positive or negative), hemolytic pattern and colony morphology. Further confirmation of isolates was achieved through biochemical tests, including oxidase activity, acid production (fermentation of lactose, sucrose and glucose), indole production, Voges–Proskauer reaction and hydrogen sulfide production. Analytical Profile Index API-20 tests (API, bioMérieux, Craponne, France) were used for confirmation of each strain. Staphylococci were isolated using media like blood agar, nutrient agar, Ziehl–Neelsen and Mannitol Salt Agar (MSA). For the isolation of *E. coli*, nutrient agar, MacConkey agar, and API 25 were used. Phenotypic characteristics for staphylococci were determined based on the presence of α and β hemolysis, while pink colonies with precipitation indicated the presence of *E. coli*. *Serratia* spp. were identified based on colony morphology, particularly the production of a red pigment. Edwards agar and the hydrolysis of esculin were used for identification of steptococci.

In order to isolate fungi, milk samples were seeded on Sabouraud agar and incubated at 25°C for three to five days. After incubation, the sown and incubated substrates were examined for the presence of fungi. Microscopic preparations with chlorlactophenol were made from Sabouraud agar. The final identification of yeasts (*Candida albicans, Cryptococcus neoformans, Trichosporon and Saccharomyces* sp.) was made with the commercial system "API 20 C AUX" manufactured by bioMérieux, Craponne, France.

The SCC in the collected milk samples was determined using the microscopic reference method according to the standard SRPS EN ISO 13366-1:2010 of the Institute for Standardization of Serbia. The SCC was established by spreading 0.01 mL of mixed milk from each sample over 1 cm^2 area on a glass slide. After air-drying and staining by Newman-Lampert stain, the slides were examined under a microscope. If the value of the SCC was higher than 200.000 cells/mL, it was considered as an indicator of secretion disorder.

The obtained results were statistically processed. The mean value of the SCC on the farms was calculated using Microsoft Office Excel (v2019). The differences between the observed three farms were examined by applying one-factor analysis of variance (ANOVA). The homogeneity of variances for the obtained data was checked using Levene's test. Finally, statistically significant differences in the SCC in cows between the farms were determined by applying a post-hoc LSD test.

RESULTS AND DISCUSSION

From the total of 45 milk samples collected from three farms, 27 (60%) were positive for the pathogen presence. In the remaining 18 (40%) samples, a causative agent was not cultured. More precisely, both Farm 1 and Farm 2 had 4 culture-negative samples, while Farm 3 had 10 samples without isolated pathogens. *Escherichia coli* was the most prevalent causative agent and was isolated in 8 samples. The largest number of positive samples with *E. coli* were isolated on Farm 1 (5 samples), while on Farm 2 and 3 there were 2 samples and 1 sample, respectively. Streptococci were also isolated in most cases and Table 1 shows their prevalence together with the presence of other isolated bacteria that occurred in smaller number of cases. The only fungal causative agent isolated was *Candida* spp. which was found in milk samples from Farm 2 and Farm 3 (3 samples on each).

Table 1. Isolated pathogens from milk samples of cows diagnosed with mastitis collected from the farms

Pathogens	Farm 1	Farm 2	Farm 3
Negative	4	4	10
Staphylococcus aureus	2	-	-
Coagulase-negative *Staphylococcus* spp.	-	1	-
Beta-haemolytic *Streptococcus* spp.	-	2	-
Streptococcus spp.	2	3	-
Streptococcus uberis	1	-	-
Streptococcus dysgalactiae	1	-	-
Escherichia coli	5	2	1
Serratia marcescens	-	-	1
Candida spp.	-	3	3

It is well known that the most frequently used method for diagnosing mastitis in dairy cows is measuring SCC (Ashraf & Imran, 2020). It was found that the production losses related to increase in SCC become more severe as lactation progresses (Hagnestam-Nielsen et al., 2009). High SCC present in milk is an important indicator of mammary gland health since SCs are involved in protecting the mammary gland from infection caused by microorganisms, which could lead to contagious and environmental mastitis (Li et al., 2014; Sharma et al., 2011). In our study, however, in 18 out the total of 45 milk samples a causative agent was not cultured. Similarly, in the study by Petzer et al. (2017) a large portion of samples with SCC exceeding 200 000 cells/mL were also culture-negative. More precisely, more than 30% of the samples with elevated SCC were culture-negative, indicating that SCC cannot be used on its own as a diagnostic method to identify bacteria-positive cultures (Petzer et al., 2017).

Table 2. SCC in milk (1000/ml) of cows with subclinical and clinical mastitis

Number of animal	Farm 1	Farm 2	Farm 3
1	1439	326	739
2	5922	798	446
3	7119	5692	715
4	20264	223	801
5	4004	609	304
6	8850	709	296
7	11183	271	261
8	356	208.8	387
9	2269	5140	560
10	12228	235.2	610
11	13443	3840	1060
12	1095	3840	676
13	208	5120	714
14	14133	11800	389
15	3316	486.4	322

The SCC in milk of cows with subclinical and clinical mastitis is given for all three farms in Table 2. The mean values of the SCC on Farms 1, 2 and 3 were 7,055,266.67, 2,619,893.33 and 552,000 cells/mL, respectively. The lowest SCC was recorded on Farm 3. It could be related to the fact that this was the farm with the highest number of culture-negative samples tested for mastitis causing pathogens. Based on these results, a statistically significant difference ($p<0.05$) in the SCC was determined between Farms 1 and 2, as well as between Farms 1 and 3. However, there was no statistically significant difference between Farms 2 and 3.

The existence of udder infection and the type of microorganisms that are involved are the main factors responsible for variations in the SCC, and mastitis-affected udder quarters have a significantly higher mean SCC than healthy ones (Sumon et al., 2020). Furthermore, *Staphylococcus aureus* is the major contagious pathogen in dairy herds and infections caused by these bacteria remain the major mastitis problem of dairy animals (Khan & Khan, 2006). In the study conducted by Boddie et al. (1987) SCC approached 20×106 cells/ml in quarters infected with *S. aureus* and over 13.6×106 cells/ml in those infected with coagulase-negative staphylococci and *Streptococcus* spp. These results indicate that contagious microorganisms produce larger increase in the SCC than environmental ones. However, a recent study has found decline in the udder infection caused by *S. aureus* and increase in the prevalence of Gram-negative pathogens (Ruegg, 2017). Furthermore, Gram-negative bacteria, such as *E. Coli* and *Klebsiella* spp., are typically identified by dairy producers to be responsible for increased mastitis cases and higher SCC values. Infections caused by these bacteria may reflect upon the severity of the signs and symptoms compared to the signs and symptoms of infections by Coagulase-negative staphylococci which also have proven to be associated with lower SCC (Wilson et al., 1997; Williamson et al., 2022). *E. Coli* is one of the most important causative pathogens of the environmental mastitis (Cobirka et al., 2020). In our study, it was the most prevalent pathogen isolated from the milk samples.

Besides bacteria, different pathogens can be isolated from milk samples with increased SCC, such as yeasts-like fungi and *Prototheca* sp. (Malinowski et al., 2006). In the study by Wilson et al. (1997) yeast had the average SCC between the lowest and highest groups. The incidence of yeast mastitis is usually low in dairy farms, but during the last decade it has increased significantly. Mastitis caused by fungi has been attributed to the therapy used for other causative agents using contaminated injectors, cannulas or antibiotic preparations (Dworecka-Kaszak et al., 2012). In general, although a small number of pathogens were isolated on all three farms, when looking at the total number of pathogens that were isolated and how each of them individually affected the SCC in milk, this might be the reason why the SCC was higher on Farm 1 compared to the Farm 2 and 3.

Moreover, it is well known that apart from mastitis, SCC in milk may be affected by various other factors (Davidov et al., 2014). The differences in SCC on dairy farms may be influenced by cows' productivity, parity, lactation phase and breed, stress, udder anatomy, season of the year, along with improper management practices and farmers education (Schepers et al., 1997; Rup et al., 2000; Uzmay et al., 2003; Yalçin et al., 2010). The increased number of mastitis cases, and thus also higher SCC in milk, may also be affected by increased lying time caused by lameness in

dairy cows (Garvey, 2022). However, the health of the udder and the hygiene of the milking process itself should be emphasized (Spahić-Bajrić et al., 2015). Indeed, in the occurrence of mastitis of various etiologies, the level of hygiene in the barn is of great importance (Hristov et al., 2005). Poor hygienic conditions during milking, along with omitting disinfection, especially of teats and milkers' hands, facilitate the contamination of milk from the environment. Apart from better hygiene and proper nutrition, mastitis control help in reducing SCs in milk. The purpose of the standard mastitis control program is to reduce the duration of current infections and to minimize the number of new ones (De Haas, 2003).

However, diagnosing individual cows by using the SCC patterns might not be the best method, because of the time needed to complete the pattern. For individual cases, bacteriological analysis of milk samples gives faster and more precise results. On the other hand, analyzing these SCC patterns at the herd level may indicate a dominant pathogen, which can help in establishing mastitis control programs specific for a certain pathogen. Control programs consist of guidelines aimed to control udder infections caused by contagious and environmental pathogens (De Haas, 2003). Owing to the implementation of mastitis control programs, the prevalence of mastitis caused by contagious pathogens has been decreased significantly over the past few decades on the global level (Shum et al., 2009).

In addition to SCC, future studies could include determination of differential somatic cell count (DSCC), which can be described as the combined proportion of neutrophils and lymphocytes presented as a percentage of the total. The study conducted on three different farms showed that data obtained from a big dataset using the relationship between SCC and DSCC in milk samples collected from separate udder quarters taken during different periods of time (at dry-off, after calving and at the lactation peak) could be an important contribution to improving predictive models for subclinical mammary gland infections which can be applied in dairy herd management (Dal Prà et al., 2022).

CONCLUSION

As mastitis is caused by a variety of pathogens and prevalence of mastitis is high in farms all over the world, especially in conditions of intensive milk production, it is necessary to develop an effective mastitis control program in order to achieve a profitable dairy business. Whether mastitis is caused by contagious or environmental pathogens, preventive measures are crucial in controlling these pathogens. Good environmental conditions, proper functioning of milking machines and milking hygiene are necessary for reducing the risks of mastitis and thus also SCC in milk. Establishing SCs pattern can provide useful information that may contribute to reducing SCs and developing differential SCs standards to help obtain milk with low SCs and consequently better dairy products with a longer shelf life.

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