VETERINARSKI ARHIV 82 (5), 411-422, 2012

Cell content in milk from cows with *S. aureus* intramammary infection

Miroslav Benić[1]*, Boris Habrun[1], Gordan Kompes[1], Željko Mihaljević[1], Željko Cvetnić[1], Marijan Cergolj[2], and Nino Maćešić[2]

[1]*Croatian Veterinary Institute, Zagreb, Croatia*

[2]*Reproduction and Obstetrics Clinic, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

BENIĆ, M., B. HABRUN, G. KOMPES, Ž. MIHALJEVIĆ, Ž. CVETNIĆ, M. CERGOLJ, N. MAĆEŠIĆ: Cell content in milk from cows with *S. aureus* intramammary infection. Vet. arhiv 82, 411-422, 2012.

ABSTRACT

Somatic cell count, viability of cells and percentages of the cell subsets (CD45, granulocytes, monocytes/macrophages, CD4, CD8 and B lymphocytes) were determined in milk from uninfected cow udder quarters (n = 7) and from quarters infected with *S. aureus* (n = 18). Cell subpopulations were double labelled with monoclonal antibodies and determined by flow cytometry. Percentages of epithelial cells, polymorphonuclear granulocytes, monocytes and lymphocytes were determined by light microscopy in stained smears. The SCC, percentages of cells labelled with antibodies specific to CD45, granulocytes, CD4 and CD8 epitope were statistically different between infected and uninfected quarters. The percentages of cells obtained by light microscopy were statistically different between the infected and uninfected groups. The percentages of granulocytes in infected animals were associated with the age and gravidity of the animals. Within the infected group, the percentage of CD8 varied statistically between imported replacement cows and animals raised on the farm. Values of epithelial cells and CD45- were moderately correlated (Pearson's r = 0.47). The percentages of granulocytes obtained by the two methods were also moderately correlated (Pearson's r = 0.52).

Key words: milk, leukocytes, intramammary infection, *S. aureus*

Introduction

S. aureus is the most commonly isolated pathogen from cow's milk. It is part of the normal micro flora living on the udder and teat skin. *S. aureus* occasionally colonizes internal parts of the mammary gland, causing clinical or subclinical mastitis. Staphylococcal mastitis more frequently occurs in multiparous than in primiparous

*Corresponding author:

Dr. sc. Miroslav Benić, DVM, Croatian Veterinary Institute, Savska 143, 10000 Zagreb, Croatia, Phone: +385 1 612 3610; E-mail: benic@veinst.hr

ISSN 0372-5480

Printed in Croatia

cows (SOL et al., 2000). Spreading of the pathogen from infected to uninfected animals occurs during milking through the teat cups, milking equipment and the milker's hands. Furthermore the risk of the infection is high even during the interval between milking if the teat orifice is incompletely closed (FITZPATRICK, 2001). *S. aureus* produces toxins and enzymes such as hemolysin, leukocidin, enterotoxin, dermonecrotoxin, coagulase etc. The host organism forms a fibrous membrane around the focci which protect bacterial cells from antibiotics, making attempts at therapy doubtful.

The reasons for the high prevalence of *S. aureus* mastitis are its rapid spread between and within dairy herds, as well as the low cure rate (SANDHOLM et al., 1990; MYLLYS et al., 1997). The chronic nature of the staphylococcal udder infections is probably due to the metabolic products of bacterial cells, which interfere with the development of protective immunity (FERENS et al., 1998).

According to the many authors, macrophages represent a major part of the cell population from milk synthesised in a healthy mammary gland. Other cell types found in the milk from healthy udders are epithelial cells, T and B lymphocytes and neutrophilic leukocytes (CONCHA et al., 1986; MILLER et al., 1990; MILLER et al., 1991). An exception to this rule is the colostral period and late lactation when the polymorphonuclear leukocytes (PMN) become the most numerous cell types (MILLER et al., 1991). Similarly, PMN, whose task is phagocytosis, dominates over other the cell types in the cell population from an infected mammary gland (SLADEK and RYŠANEK, 2001; SMITS et al., 2000). The cell population found in milk originates from the blood or from the tissue surrounding the alveoli (FITZPATRICK, 2001).

A consequence of mammary gland infection is leukocyte infiltration and inflammation of the gland. If the pathogen is not eliminated, the leukocytes, mostly PMNs, pass massively from the blood stream toward the infected tissue in order to eliminate the pathogen. However, the complex process of elimination of the pathogen is sometimes unsuccessful, resulting in a chronic infection (LEITNER et al., 2000).

Somatic cells play an important role in the defence mechanism of the mammary gland. According to some authors, it seems that the proportion of the cell types, or subtypes, rather that their total number, is the factor which defines the outcome of the infection, which may be its cure, a chronic infection or the death of the animal (FITZPATRICK, 2001). It seems that the proportion of different cell types is influenced by the species or the strain of the pathogen (SOLTYS and QUINN, 1999). The diagnostic significance of some cell fractions has been documented from milk. Hence a ratio of CD4:CD8 lymphocytes in the milk is a more reliable sign for differentiation between early and late phases of infection than SCC or bacteriological examination (RIVAS et al., 2000).

Furthermore, an increased number of T lymphocytes in the milk during the later phase of lactation is the result of the increased number of activated CD8 lymphocytes (TAYLOR

et al., 1994; PARK et al., 1992; ASAI et al., 1998; ASAI et al., 2000). CD4 and CD8 lymphocyte subsets accumulate in the tissue around the milk alveoli during early lactation. It seems that lymphocytes containing CD8 play a role in maintaining alveolar integrity, as they are visible in the epithelial cells in milk alveoli (YAMAGUCHI et al., 1999). The CD8 subset is significantly higher during mastitis caused artificially with *S. aureus* (PARK et al., 1992). Milk from udder chronically infected with *S. aureus* contains a significantly higher number of cells including CD4, CD8 and macrophages (LEITNER et al., 2000). Conversely, the CD4 subset dominates over CD8 during the drying off period (ASAI et al., 1998).

The purpose of the research is to describe the cell population pattern in udder secretion from quarters naturally infected with *Staphylococcus aureus* and to compare cell percentages obtained by two different methods. Furthermore, it seeks to check if the cell population pattern is associated with management and physiological factors.

Materials and methods

Animal population: Animals (n = 25) were selected from 23 small dairy herds. The herd size ranged from 4 to 16 lactating animals. The cows were of the Simmental and Holstein-Friesian breed or their crosses. In a small proportion of herds the cows were kept permanently tied up while in other herds the cows spent a certain amount of time in pasture. The stalls were bedded with straw, hay or sawdust. The average production ranged between 4,000 - 7,000 litres in standardised lactation. Cows were fed on hay, pasture, green forage, silage, haylage, crushed grains or complete feed for dairy cows.

Sampling. Samples for microbiological examination were taken aseptically before the morning milking three times, with a 7-day interval between. The first few streams were discarded. The teat ends were disinfected with cotton swabs soaked with 70% ethanol. Samples were taken in sterile tubes and transported to a laboratory on ice within two hours. A bacteriological examination was carried out according to recommendations (National mastitis council, 1999).

Identification of *S. aureus* was performed based on colony morphology, pigmentation, catalase-test, and the ability to produce coagulase and to grow on Baird Parker agar.

Samples for cytological examination were taken during the last visit and placed in a sterile glass bottle (500 mL volume) and transported on ice to the laboratory within two hours.

Data concerning the origin of the animals, breed, age, pregnancy, milking type, udder sanitation practice, pasture and feeding were gathered, either in an interview or from farm documentation.

Sample preparation. Samples were warmed up to room temperature and homogenised by gentle shaking. 50 mL of the volume was used for somatic cell counting. The remaining part of the sample was used for preparation of a cell suspension.

Samples for cell counting were warmed up to 37 ºC and analysed by Fossomatic 5000 basic (FossElectric, Denmark).

Preparation of cell suspension for flow cytometry. A homogenised sample was diluted with a double volume of phosphate buffered saline (PBS) with the addition of 20 mM EDTA in a sterile bottle. The diluted milk was centrifuged in sterile glass tubes at 1000×g for 20 minutes at a temperature of 4 ºC. The supernatant was discarded using a vacuum pump, while the sediment was resuspended in PBS with 10 mM EDTA.

Cell concentration and viability were determined in the prepared suspensions. The concentration was determined by mixing 10 μL of the suspension with the same quantity of Türk´s reagent and was counted in a Neubauer chamber. Viability was determined by mixing 10 μL of the suspension with the same quantity of trypan blue in a Neubauer chamber.

Antibodies. The cells were double labelled with anti-CD45 (IgG2a isotype, CACTB 51A) and one of the IgG1 isotype (anti-granulocyte, clone MM 20 A; antimonocyte, clone BAQ 151A; anti CD4, clone CACT 138A; anti CD8, clone CACT 80C and anti B lymphocyte, clone LCT 27A) (VMRD Inc. USA). Secondary polyclonal antibodies conjugated with phycoerythrin-cyanid 5 (tricolor, goat anti-mouse IgG1) (CALTAG, USA) and fluorescein izothyocianat (FITC) (goat F(ab)2 anti-mouse IgG2a were used (Southern Biotech. USA).

Mouse IgG1 and mouse IgG2a were used as isotype controls (Dako, Denmark).

Labelling of cells. 100 μL of the cell suspension containing 10^6 cells was pippeted into plastic tubes. The primary antibody concentration in the cell suspensions was 2 μL in 100 μL of the final volume. Mouse IgG1 and IgG2a immunoglobulin (Dako, Denmark) were used as an isotype control. The tubes were vortexed and incubated for 20 minutes at +4 ºC. After incubation, the cells were washed with 2 mL of PBS and centrifuged. The supernatant was discarded and 5 μL of the secondary goat anti-mouse IgG2a antibody, labelled with a fluorescein isothyocianat, was added. The cells were vortexed and incubated 15 minutes at +4 °C in the dark. After washing the cells with 2 mL of PBS, centrifugation and discarding the supernatant, 2.5 μL of goat anti-mouse IgG1 labelled with tricolor was added onto the sediment and vortexed. The cells were incubated for 15 minutes at +4 °C in the dark. The incubated cells were washed three times with 2 mL of PBS. After the last washing the cells were diluted with 0.5 mL PBS, vortexed and analysed by a flow cytometer. A total of 10,000 events were measured. All the steps with secondary antibodies were carried out avoiding direct light.

Flow cytometry. The flow cytometry was performed by FacsCalibur (Becton Dickinson). The cells were characterised by granularity, size and intensity of fluorescence. The raw data were stored as list-mode files and processed using the CellQuest programme.

Gates. Gates were determined using a cell suspension from fresh bovine blood. Secondary antibodies were titrated in order to define the optimal concentration.

Cell staining. Cells smears were prepared using Cytospin and stained by the Pappanicolau method in an automatic device. Stained smears were air dried, glass covered and examined with microscope HAL 100 (Zeiss). Between 150 and 200 cells were determined per smear according to the size and shape of the cells, the size and shape of the nucleus and the coloration and intensity of coloration of cell parts, using 600× and 1000× magnification.

Statistical analysis. Statistical analysis was carried out using STATA 6.0 (Stata corporation, USA). Univariate statistical analysis was performed using the *t*-test, and the non-parametric Mann-Whitney and Kruskall Wallis test to check the observed difference between the groups. Univariate statistical analysis was used to check the significance of the differences between the two groups.

The Spearman correlation coefficient was used to express the correlation between values for epithelial cells and granulocytes obtained by the two methods.

Results

The results of the quantification of cell subsets from milk by flow cytometry are presented in Table 1.

Table 1. The average cell percentages and standard deviations obtained by flow cytometry

Cell fraction	Uninfected (n = 7) $\overline{X}$ ± SD	*S. aureus* (n = 18) $\overline{X}$ ± SD	P
SCC (×10^3)	45.71 ± 29.20	1100.89 ± 598.56	0.0001
Viability (%)	85.71 ± 14.74	78.56 ± 12.62	NS
CD45+ (%)	49.79 ± 19.92	79.39 ± 9.85	0.003
Granulocytes (%)	27.29 ± 23.87	79.94 ± 25.48	0.0005
Monocytes (%)	4.5 ± 5.28	10.12 ± 11.56	NS
CD4+ (%)	8.71 ± 8.94	2.76 ± 3.21	0.03
CD8+ (%)	23.07 ± 14.08	9.25 ± 6.01	0.03
B lymphocytes (%)	2.86 ± 3.81	1.62 ± 2.15	NS

NS - not significant

The results of cell quantification by light microscopy are presented in Table 2.

Table 2. The percentages of cells obtained by light microscopy

Cells	Uninfected (n = 7) $\overline{X}$ ± SD	*S. aureus* (n = 18) $\overline{X}$ ± SD	P
Granulocytes (%)	15.66 ± 2.51	61.43 ± 9.56	0.0001
Monocytes (%)	26.14 ± 5.11	20.34 ± 4.54	0.03
Lymphocytes (%)	22.66 ± 6.86	11.74 ± 4.83	0.001
Epithelial cells (%)	35.61 ± 7.95	5.52 ± 2.8	0.0001

The association of management and individual factors with certain cell subsets was checked in the infected group. Statistically significant associations are presented in Table 3.

Table 3. The association of cell fractions with certain management factors in milk from quarters infected with *S. aureus*

Cell fraction	Factor	Values defining the groups	$\overline{X}$ ± SD	P
CD4	SCC	$<6\times10^5$	0.25 ± 0.41	0.01
		$>6\times10^5$	4.01 ± 3.26	
Granulocytes	Age	≤ 7 years	75 ± 23.5	0.03
		> 7 years	91.55 ± 3.35	
	Pregnancy	Pregnant	75 ± 23.4	0.02
		Non-pregnant	91.5 ± 3.8	
CD8	Origin of animals	Rose on farm	7.54 ± 5.19	0.04
		External source	13.7 ± 6.21	
	Teat dipping	Yes	14.12 ± 6.91	0.06
		No	7.85 ± 5.18	

Discussion

Despite preventive measures and plenty of antibacterial medication, mastitis remains a major cause of economic losses in dairy production. Hence the immune system of the mammary gland is an object of research whose aim is to gain insight into the defence mechanism of the udder against pathogens, through the quantification of the cell subsets in milk.

In this research we checked the proportion of selected cell subsets in milk from uninfected mammary glands and those infected with *S. aureus*. Furthermore we statistically checked the influence of management factors on selected cell subsets in milk.

Expectedly the SCC was significantly higher in infected compared to the uninfected glands (P<0.0001), due to the massive infiltration of the infected gland(s).

In contrast to the some authors (SCHRÖDER and HAMMAN, 2005) viability did not differ between the infected and uninfected glands. However, these authors used a different methodology.

CD45 epitope is common for leukocytes and we used it to differentiate leukocytes from epithelial cells. The percentage of CD45 positive cells was significantly different between uninfected and infected animals and was in concordance with similar studies even if different antibodies were used (LEITNER et al., 2000).

Polymorphonuclear granulocytes (PMN) are the most numerous fractions of the cell population in inflamed mammary glands, due to the chemo-attraction. Massive infiltration of an acutely inflamed gland with PMN occurs within a few hours (KEHRLI and SCHUSTER, 1993). However the major part of naturally acquired infections of mammary glands passes without visible clinical signs of inflammation (SMITH et al., 1985). In this research the proportion of cells labelled with the antibody MM 20A in the granulocyte gate varied significantly from 27.3% in uninfected to 80% in infected animals. Similar results were obtained in other studies (LEITNER et al., 2000; RIOLLET et al., 2001) in spite of different antibodies used for labelling.

The percentage of monocytes labelled with a specific antibody in this research varied between 1.25-12.13% and did not vary significantly between infected and uninfected animals. It should be emphasised that in the case of monocytes, the results from different studies differ as for any other cell type. The percentage of viable monocytes with molecular structures on their surface able to bind to a specific antibody depends upon the handling of the sample, since sampling and transport in plastic bottles causes the destruction of monocytes (SCHRÖDER and HAMANN, 2005). We used sterile glass bottles for sampling, hence any toxic influence on the monocyte population was excluded. An unexpectedly low expression of binding site for BAQ 151A was observed in the monocytes from uninfected quarters, since this fraction has been reported as the most numerous in the uninfected quarters in other research.

The CD4 epitope is expressed in helper T lymphocytes. In the milk from the uninfected cows the proportion of CD4+ lymphocytes changes according to the stage of lactation and reaches its highest value at the end of lactation (PARK et al., 1992; TAYLOR et al., 1994).

The percentage of CD4 labelled cells was statistically different between uninfected and infected udder quarters. However the direction of the difference was opposite to that obtained in similar research (SOLTYS and QUINN, 1999; LEITNER et al., 2000; RIOLLET et al., 2001).

We compared statistically the percentage of CD4 labelled cells in uninfected with infected animals, separately for those with less than 6×10^5 and more than 6×10^5 somatic cells per millilitre. The obtained values are shown in Table 3. The observed values were

significantly different between uninfected and infected animals, with even SCC $<6\times10^5$ between two groups of infected ($P<0.01$). However if the SCC exceeded this value the percentage of CD4 was no longer statistically different from the uninfected group. The reason for the lower value of CD4+ cells might be caused by the suppressive CD8+ lymphocytes, since the proliferation of CD4 lymphocytes is decreased in mammary glands infected with *S. aureus* due to the higher activity of suppressive CD8 lymphocytes (PARK et al., 1993; SHAFER-WEAVER and SORDILLO, 1997).

The CD8 binding site appears on the surface of the cytotoxic and suppressive lymphocytes. Their number in cow's milk from healthy quarters is higher than CD4, in contrast to the infected gland (TAYLOR et al., 1997; SOLTYS and QUINN, 1999). The highest values are observed at the end of lactation (PARK et al., 1992). Our results are in accordance with the results from other studies, although the absolute values are somewhat different due to the methodology applied and monoclonal antibodies used.

The percentage of cells labelled with antibodies specific to B lymphocytes was similar to the results from other studies. They are rarely found in milk although they represent a significant proportion of the lymphocyte population in peripheral blood (TAYLOR et al., 1994). Nevertheless the plasma cells, the activated form of B lymphocytes, are the most numerous cell type found in histological examination around the Furstenberg's rosette (NICKERSON and PANKEY, 1983).

The value of the CD4:CD8 ratio was less than one and in accordance with values obtained in similar studies. Other studies indicated that the ratio is less than one after calving and during the whole lactation, while during drying off it exceeds one, even reaching three (ASAI et al., 1998).

The defence of mammary glands is influenced by many intrinsic and extrinsic factors. The immune system is able to resist infection and destroy the pathogen. However in some situations cows become more or less prone to infection. Susceptibility to udder infection in cows is higher in late pregnancy and soon after calving as a consequence of general immunosuppression. The direct and indirect influence of certain factors on the number of granulocytes in cow's milk and susceptibility to udder infections has been described in literature (KELLY et al., 2000).

The age of cows was associated with the percentage of granulocytes in infected animals ($P = 0.03$). The observed percentages are presented in Table 3. The reason for differences between the age groups could be the lower killing ability of granulocytes against *S. aureus* (MEHRZAD et al., 2009) in the older group. The difference observed between pregnant and non-pregnant cows was also statistically significant ($P = 0.02$), which may be caused by the stage of lactation.

The percentage of CD8 labelled cells was associated with the origin of animals reaching higher values in replacement animals from external sources than in animals calved and raised on the farm (P = 0.04). Post milking sanitation of udders showed a mild association with the percentage of CD8+ lymphocytes. Hence, in the group of animals with ordinal post milking teat dipping, the percentage of CD8 was higher than in non-dipped teats (P = 0.06).

A common problem of milk cell enumeration in stained smears is differentiation between macrophages and some types of epithelial cells. Furthermore the results depend on the technical side of the preparation of the cell suspension. Results can be compared only if the samples are prepared in the same manner. The relative proportion of cells is even changed if the suspension is washed twice instead of three times i.e. the proportion of lymphocytes is larger, while the proportion of granulocytes is smaller (SCHRÖDER and HAMMAN, 2005). Same authors stated that the results of flow cytometry are incomparable with the staining method because the different age stages of the same cells do not bind monoclonal antibodies.

The proportions of macrophages obtained in our research are similar to other similar research (SCHRÖDER and HAMMAN, 2005). However the proportion of granulocytes is somewhat lower while the proportion of lymphocytes are somewhat higher that could be the influenced by washing since we washed cells only ones.

The percentage of cells which did not bind anti-CD45 antibodies in this research varied between 8 and 76%, while the percentage of epithelial cells obtained by light microscopy varied between 2 and 48%. The values obtained by the two methods were moderately correlated. Overall, Spearman's correlation coefficient of CD45- and epithelial cells obtained by light microscopy was $r_s = 0.47$ (P = 0.016).

The overall proportions of granulocytes obtained by the two methods were also moderately correlated ($r_s = 0.52$, P = 0.0075) confirming the rules postulated concerning incomparability of results obtained by different methods.

Conclusion

The proportions of certain cell subsets in milk were different between infected and uninfected animals, regardless of the method of enumeration applied. Nevertheless some management and individual factors were able to influence the proportion of some cell subsets. Values relying on the same cell fractions obtained by different methods are incomparable.

References

ASAI, K., K. KAI, H. RIKIISHI, S. SUGAWARA, Y. MARUYAMA, T. YAMAGUCHI, M. OHTA, K. KUMAGAI (1998): Variation in $CD4^+$ T and $CD8^+$ T lymphocyte subpopulations in

bovine mammary gland secretions during lactating and non-lactating periods. Vet. Immunol. Immmunopathol. 65, 51-61.

ASAI, K., Y. KOMINE, T. KOZUTSUMI, T. YAMAGUCHI, K. KOMINE, K. KUMAGAI (2000): Predominant subpopulations of T lymphocytes in the mammary gland secretions during lactations and intraepithelial T lymphocytes in the intestine of dairy cows. Vet. Immunol. Immunopathol. 73, 233-240.

CONCHA, C., O. HOLMBERG, G. ASTROM (1986): Cells found in non-infected and Staphylococcus-infected bovine mammary quarters and their ability to phagocytose fluorescent microspheres. Z. Vet-Med. B 33, 371-378.

FERENS, W. A., W. C. DAVIS, M. J. HAMILTON, Y. H. PARK, C. F. DEOBALD, L. FOX, G. BOHACH (1998): Activation of bovine lymphocyte subpopulations by staphylococcal enterotoxin C. Infect. Immun, 66, 573-580.

FITZPATRICK, L. J. (2001): Milk somatic cells - what do they do? Proceedings of the British Mastitis Conference Garstang, P. 56-62 Institute for Animal Health/Milk Development Council.

KEHRLI, M. E., D. E. SCHUSTER (1994): Factors affecting milk somatic cells and their role in health of the bovine mammary gland. J. Dairy Sci. 77, 619-627.

KELLY, A. L., D TIERNAN, C. O. SULLIVAN, P. JOICE (2000): Correlation between bovine milk somatic cell count and polymorphonuclear leukocyte level for samples of bulk milk and milk from individual cows. J. Dairy Sci. 83, 300-304.

LEITNER, G., E. SHOSHANI, O. KRIFUCKS, M. CHAFFER, A. SARAN (2000): Milk leukocyte population patterns in bovine udder infection of different aetiology. J. Vet. Med. B 47, 581-589.

MEHRZAD, J., L. DUCHATEAU, C. BURVENICH (2009): Phagocytic and bactericidal activity of blood and milk-resident neutrophils against *Staphylococcus aureus* in primiparous and multiparous cows during early lactation. Vet. Microbiol. 134, 106-112.

MILLER, R. H., M. J. PAAPE, L. A. FULTON (1991): Variation in milk somatic cells of heifers at first calving. J. Dairy Sci. 74, 3782-3790.

MILLER, R. H., M. J. PAAPE, R. R. PETERS, M. D. YOUNG (1990): Total and differential somatic cell counts and N-acetyl-β-D-glucosaminidase activity in mammary secretions during dry period. J. Dairy Sci. 73, 1751-1755.

MYLLYS, V., J. RIDELL, J. BJÖRKROTH, I. BIESE, S. PYÖRÄLÄ (1997): Persistence in bovine mastitis of *Staphylococcus aureus* clones as assessed by random amplified polymorphic DNA analysis, ribotyping and biotyping. Vet. Microbiol. 57, 245-251.

NATIONAL MASTITIS COUNCIL (1996): Current Concepts Of Bovine Mastitis. 4th ed., Arlington, VA.

NICKERSON, S. C., J. W. PANKEY (1983): Cytologic observations of the bovine teat end. Am. J. Vet. Res. 44, 1433-1441.

PARK, Y. H., L. K. FOX, M. J. HAMILTON, W. C. DAVIS (1992): Bovine mononuclear leukocyte subpopulations in peripheral blood and mammary gland secretions during lactation. J. Dairy Sci. 75, 998-1006.

PARK, Y. H., L. K. FOX, M. J. HAMILTON, W. C. DAVIS (1993): Suppression of proliferative response of BoCD4+ T lymphocytes by activated BoCD8+ T lymphocytes in the mammary gland of cows with *Staphylococcus aureus* mastitis. Vet. Immunol. Immunopathol. 36, 137-151.

RIOLLET, C., P. RAINARD, B. POUTREL (2001): Cell subpopulations and cytokine expression in cow milk in response to chronic *Staphylococcus aureus* infection. J. Dairy Sci. 84, 1077-1084.

RIVAS, A. L, F.W. QUIMBY, O. COKSAYGAN, L. OLMSTEAD, D. H. LEIN (2000): Longitudinal evaluation of CD4+ and CD8+ peripheral blood and mammary gland lymphocytes in cows experimentally inoculated with *Staphylococcus aureus*. Can. J. Vet. Res. 64, 232-237.

SANDHOLM, M., L. KAARTINEN, S. PYORALA (1990): Bovine mastitis - why does antibiotic therapy not always work? An overview. J. Vet. Pharmacol. Therap. 13, 248-260.

SCHRÖDER, A. C., J. HAMANN (2005): The influence of technical factors on differential cell count in milk. J. Dairy Res. 72**,** 153-158.

SHAFER-WEAVER, K. A., L. M. SORDILLO (1997): Bovine $CD8^+$ suppressor lymphocytes alter immune responsiveness during the postpartum period. Vet. Immunol. Immunopath. 56, 53-64.

SLADEK Z., D. RYSANEK (2001): Apoptosis of neutrophilic granulocytes of bovine virgin mammary gland in scanning electron microscopy. Vet. Med. - Czech 46, 185-191.

SMITH, K. L., D. A. TODHUNTER, P. S. SCHOENBERGER (1985): Environmental mastitis: cause, prevalence, prevention. J. Dairy Sci. 68, 1531-1553.

SMITS, E., C. BURVENICH, A. J. GUIDRY, A. MASSART-LEËN (2000): Adhesion receptor CD11/CD18 contributes to neutrophil diapedesis across the bovine blood-milk barrier. Vet. Immunol. Immunopathol. 73, 255-265.

SOL, J., O. C. SAMPIMON, H. W. BERKEMA, Y. H. SCHUKKEN (2000): Factors associated with cure after therapy of clinical mastitis caused by *Staphylococcus aureus*. J. Dairy Sci. 83, 278-284.

SOLTYS, J., M. T. QUINN (1999): Selective recruitment of T-cell subsets to the udder during staphylococcal and streptococcal mastitis: analysis of lymphocyte subsets and adhesion molecule expression. Infect. Immun. 67, 6293-6302.

TAYLOR, B. C, R. G. KEEFE, J. D. DELLINGER, Y. NAKAMURA, J. S. CULLOR, J. L. STOTT (1997): T cell populations and cytokine expression in milk derived from normal and bacteria-infected bovine mammary glands. Cell Immunol. 182, 68-76.

TAYLOR, B. C., J. D. DELLINGER, J. S. CULLOR, J. L. STOTT (1994): Bovine milk lymphocytes display the phenotype of memory T cells and are predominantly $CD8^+$. Cell Immunol. 156, 245-253.

YAMAGUCHI, T., M. HIRATSUKA, K. ASAI, K. KAI, K. KUMAGAI (1999): Differential distribution of T lymphocyte subpopulations in the bovine mammary gland during lactation. J. Dairy Sci. 82, 1459-1464.

Received: 19 October 2011
Accepted: 17 April 2012

BENIĆ, M., B. HABRUN, G. KOMPES, Ž. MIHALJEVIĆ, Ž. CVETNIĆ, M. CERGOLJ, N. MAĆEŠIĆ: Somatske stanice u mlijeku krava kod intramamarne zaraze bakterijom *S. aureus*. Vet. arhiv 82, 411-422, 2012.

SAŽETAK

U radu su prikazani rezultati istraživanja broja somatskih stanica, udjela živih stanica te staničnih subpopulacija (CD45, granulocita, monocita/makrofaga, CD4, CD8 i B limfocita) u mlijeku nezaraženih četvrti vimena krava (n = 7) te u sekretu mliječne žlijezde iz četvrti vimena zaraženih bakterijom *S. aureus* (n = 18). Subpopulacije stanica označene su monoklonskim protutijelima neizravnim postupkom i analizirane protočnim citometrom. Udjeli epitelnih stanica, polimorfonuklearnih granulocita, monocita i limfocita utvrđeni su svjetlosnim mikroskopom u obojenim razmascima staničnoga taloga. Vrijednosti broja somatskih stanica, postotaka stanica označenih monoklonskim protutijelima specifičnim za biljeg CD45, granulocite, CD4 i CD8 biljeg, statistički su se razlikovale između zaraženih i nezaraženih četvrti vimena. Udjeli pojedinih stanica dobiveni brojenjem svjetlosnim mikroskopom statistički su se razlikovali između zaražene i nezaražene skupine. Postoci granulocita unutar zaražene skupine krava statistički su bili povezani s dobi krave i s bređosti. Unutar iste skupine krava uočena je statistički značajna razlika u zastupljenosti limfocita s izraženim biljegom CD8 između krava oteljenih na farmi i uvezenih životinja. Vrijednosti udjela epitelnih stanica dobivenih određivanjem pomoću svjetlosnog mikroskopa i stanica bez CD45 biljega bile su umjereno povezane (Pearsonov r = 0,47). Vrijednosti broja granulocita dobivenih dvjema različitim metodama također su bile umjereno povezane (Pearsonov r = 0,52).

Ključne riječi: mlijeko, leukociti, intramamarna zaraza, *S. aureus*