Bulgarian Journal of Agricultural Science, 20 (No 6) 2014, 1477-1482
Agricultural Academy

STAPHYLOCOCCUS AUREUS AND OTHER PATHOGENS IN RELATION TO BREED OF CATTLE AND SOMATIC CELL COUNT

M. VYLETELOVA-KLIMESOVA[1], O. HANUS[1], A. DUFEK[2], I. NEMECKOVA[1], J. HORACEK[3], A. PONIZIL[3] and L. NEJESCHLEBOVA[3]
[1] *Dairy Research Institute, Ke Dvoru 12a, Prague, Czech Republic*
[2] *Agriresearch Rapotín, Výzkumníků 267, Vikýřovice, Czech Republic*
[3] *Agritec Plant Research, Zemědělská 16, Šumperk, Czech Republic*

Abstract

VYLETELOVA-KLIMESOVA, M., O. HANUS, A. DUFEK, I. NEMECKOVA, J. HORACEK, A. PONIZIL and and L. NEJESCHLEBOVA, 2014. *Staphylococcus aureus* and other pathogens in relation to breed of cattle and somatic cell count. *Bulg. J. Agric. Sci.,* 20: 1477-1482

Influence of breed on somatic cell count (SCC) and occurrence of particular species was evaluated. The samples were collected from farms with Holstein (H; 10 farms - 365 cows) breeding and from farms with Czech Fleckvieh (CF; 2 farms - 67 cows). The obtained dataset was statisticaly evaluated by analysis of variance. The occurrence of pathogens was compared between H and CF. The relationship between SCC and incidence of pathogens was determined as well. It is evident that more positive cows were in breed H compared to CF (H 41.6%; CF 26.9%). The most frequent pathogens in H breed were *S. aureus* (13.7%), *S. uberis* (9.6%), *S. haemolyticus* (8.5%), *S. agalactiae* (6.9%), *E. faecalis* (2.5%) and *E. faecium* (0.8%), while only *S. uberis* (19.4%), *S. haemolyticus* (6%) and *S. aureus* (1.5%) were found in CF. SCC results showed the higher SCC in H breed in case of both negative and positive cows as well and significant impact of breed on logSCC ($F = 6.4$, $P = 0.012$). The significant effects of bacterial species were confirmed ($F = 13.6$, $P < 0.001$). Multiple comparison among groups of bacterial species showed significant differences between negative and *S. aureus*, *S. agalactiae*, *S. uberis* ($P = 0.012$, $P < 0.001$, $P < 0.001$). Differences between negative and other pathogens were not signifiant.

Key words: raw milk; Holstein; Czech Fleckvieh; pathogens; somatic cell count

Abbreviations: H - Holstein, CF - Czech Fleckvieh, SCC - somatic cell count, TCM - total count of microorganisms, BC - Brown cattle, SC - Simmental cattle, J - Jersey, CSN - Czech Norm Standards, MRSA - methicillin-resistant *Staphylococcus aureus*, SPSS - Statistical Package for the Social Sciences, ANOVA - Analysis of variance , IMI - intramammary infection, SCS - somatic cell score, n - count, Tukey´s HSD - Tukey‘s honestly significant difference **test**, SAU - *Staphylococcus aureus*, SAG - *Streptococcus agalactiae*, SHA - *Staphylococcus haemolyticus*, SUB - *S. uberis*, ENT - *E. faecalis* and *E. faecium*, NEG - negative, xg - geometric mean, P - level of significance (probability of zero hypothesis validity), F - value of F-test criterion, CI - confidence interval, d - difference

Introduction

The selection of breed according to milk yield was carried out since the beginning of the first dairy farms. Although genetic analyses did not exist dairy farmers tried to breed animals of those breeds that showed primarily higher milk yield. Later, the attention has been focused (in relation to the breed) on the health indicators of raw milk as a raw material (food pathogens, mastitis pathogens, somatic cells count etc.). Currently, the most common breed in the Czech Republic and other countries is breed Holstein (H). However, dairy cows of Czech Fleckvieh (CF) and others such as Montbeliard, Ayr-

E-mail: marcela.vyletelova@seznam.cz

shire, Jersey, Braunvieh, Normande and others are bred in the Czech Republic, as well. The ratio of animals bred in the Czech Republic is 58 (H): 42 (CF) - (Kvapilík et al., 2012). Regarding to the milk yield, the highest was confirmed in H (8808 kg), followed by Montbeliard, Braunvieh, Czech Fleckvieh (6545 kg), Ayrshire, Jersey and Normande (5602 kg).

The main health and hygiene indicators of milk are the somatic cell count (SCC) and the total count of microorganisms (TCM), which may indicate the presence of mastitis disease. Differences between SCC, TCM and mastitis diseases in different breeds were and are the subject of research monitoring. Orbán et al. (2011) observed the possible relationship between personality traits and SCC in Jersey and Holstein Friesian. SCC showed positive moderate relation with the temperament scores of Jersey ($r = 0.67$; $P = 0.0001$) and Holstein Friesian ($r = 0.66$; $P = 0.0001$). Vasilev et al. (2007) compared the results of individual SCC in breeds Holstein, Brown cattle (BC) and Simmental cattle (SC) at the same degree of contamination of the udder, and found that SCC was lowest in individual samples of the breed H, then BC and SC (means SCC: H = 183 to 286, BC = 196 to 294 and SC = 274 to 342 $10^3 \times ml^{-1}$).

Due to pathogenic infection of the mammary gland, the mastitits disease can be identified as a primary factor for SCC growth and milk quality worsening (Ticháček et al., 2007; Schroeder, 2012a). The significant effect on SCC has especially milking hygiene and technological state of milking equipment (Hanuš and Ticháček, 1997; Schroeder, 2012b; Schroeder, 2012c). However, there are also secondary factors that may affect SCC and milk quality, e.g. lactation parameters or breed. With regard to the breed, it may be a geneticaly fixed resistance against the subclinical and clinical mastitis disease (Shook, 2001; Caraviello, 2004; Eding et al., 2009).

This knowledge could be practically useful for the deliberate increasing of innate resistance of dairy and dual purpose cattle against mastitis pathogens using conventional methods of breeding work. At present, this goal is monitored by determination and offer of breeding values (of breeding precious animals) for characters such as SCC (secondary functional and health indicators) or for morphological characters udder and related resistance to mastitis (Caraviello, 2004; Eding et al., 2009; Urioste et al., 2011).

In general, regardless of the breed, number of indicators of milk quality as the SCC (according to the Czech standard), electrical conductivity, lactose, casein, whey proteins, the occurrence of pathogens, etc. become changed (worsened) along with the udder health worsening (CSN 57 0529, 1993; Ali and Shook, 1980; Reneau et al., 1988; Hanuš et al., 1992; Ryan, 1993; Benda et al., 1997). Culling of cows because of udder disease is around 9% in the Czech Republic (Kvapilík et al., 2012). The resistant of pathogenic strains to antimicrobial agents can contribute to this figure, as well. In recent years, an unfavorable trend of increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) is worldwide recorded (Le Blanc et al., 2007). This may represent a significant economic burden. Regarding significant effets of cattle breed on SCC, the possibility of the breed influence on the incidence of mammary gland bacterial infection could be expected.

The aim of this work was to evaluate the influence of the most common breeds in the Czech Republic (H and CF) on the incidence of mastitis infection in order to support potential preventive procedures.

Materials and Methods

The samples were collected from the farms with Holstein breed (H; 10 farms; 365 animals) and from the farms with Czech Fleckvieh breed (CF; 2 farms; 67 animals). All these farms were suspicious of mastitis troubles owing to occurrence of milking equipment disorders. Average milk yield (305 days in milk) of included herds varied from 5,000 to 8,000 kg per lactation. Number of sampled cows varied from 12 to 32 per herd. According to lactation number there was taken 30% of primiparous and 70% of multiparous dairy cows into account in herd group. In the lactation group there was included 50% of dairy cows in first third of lactation and 50% in second third of lactation. In this way only economically important period of lactation in terms of milk yield was and the end of lactation was not evaluated. Dairy cows were selected also regarding SCC into lactation groups (first; second and others; animal selection for mastitis advisory service purposes was following: 25% of animals had SCC ≤ 250 $10^3 \times ml^{-1}$ and 75% SCC ≥ 800 $10^3 \times ml^{-1}$). The method of farm and animal selection was comparable among herds. The samples came from all teats (composite samples).

The animals were investigated on occurrence of mastitis pathogens and the somatic cell count (SCC $10^3 \times ml^{-1}$) – (Benda et al., 1997). The milk samples were inoculated on the surface of Blood Agar (Oxoid, Basingstoke, UK), Edwards Agar and Endo Agar (HiMedia, Bombay, India) and cultivated at 36°C/24 h. The suspected colonies were inoculated on the Blood Agar at 36°C/24 h. The isolated species were identified by biochemical tests of STAPHYtest, STREPTOtest, ENTEROtest and identification program TNW Pro 7.0 (Erba Lachema, s.r.o., Brno, Czech Republic). In addition, all identified strains *S. aureus* were confirmed by the multiplex PCR method for the detection of the species specific fragment SA442 (Martineau et al., 1988), then examined for antimicrobial susceptibility by disk diffusion method with oxacillin (1 μg) antibiotic disk (Oxoid, Basingstoke, UK) and

were screened for the presence of *mecA* gene which encodes the resistance to methicillin (Boşgelmez-Tinaz et al., 2006).

SCC was investigated using fluoro-opto-electronic method on rotation disc calibrated according to the results of direct microscopy method (Fossomatic 90, Foss Electric, Denmark).

The obtained dataset was statisticaly evaluated by SPSS 16.0 for Windows using ANOVA to test the main effects of 6 bacterial species and 2 breeds on log SCC in one model followed by Tukey HSD for multiple comparison of bacterial species impact. Interaction between effects wasn´t included into the model because some species had not been occured in CF breed. The occurrence of mastitis pathogens was compared between H and CF. Influence of breed (H and CF) on SCC and on occurrence of identified species was evaluted. The relationship between SCC and incidence of isolated mastitis pathogens was determined as well.

Results and Discussion

There are results of identified mastitis pathogens isolated from milk of breed Holstein and Czech Fleckvieh in the Table 1. It is evident from the results that more positive cows were in breed H compared to CF (H 41.6%; CF 26.9%). The most frequent mastitis pathogens in Holstein breed were bacteria genus *Staphylococcus* (*S. aureus* 13.7%; *S. haemolyticus* 8.5%), follow *Streptococcus* spp. (*S. uberis* 9.6%; *S. agalactiae* 6.6%) and *Enterococcus* spp. (*E. faecalis* 2.5%; *E. faecium* 0.8%), while only *S. uberis* (19.4%), *S. haemolyticus* (6%) and *S. aureus* (1.5%) were found in breed CF. No *S. aureus* strain was identified as MRSA. Incidence of identified mastitis pathogens is in accordance with previous works by Vyletělová et al. (2010; 2013). They found the most frequent species today *S. uberis*, *S. aureus* and then coagulase-negative staphylococci (especially *S. haemolyticus*). Similar results were described also by other authors who stated *S. aureus*, *E. coli*, *S. uberis*, coagulase-negative staphylococci, *Corynebacterium bovis* and *S. agalactiae* as the main species depending on the type of mastitis (contagious, clinical, subclinical, environmental, etc.) – (Kalmus et al., 2001; Bradley, 2002; Pitkälä et al., 2004). Nóbrega and Langoni (2011) described the similar results in relation to H and Jersey cows in case of mastitis pathogen occurrence in relation to the breed. However, they found higher frequency of intramammary infection (IMI) in Jersey. Their results also showed that enviionmental pathogens were more frequently isolated from the breed Jersey.

The results on SCC are shown in the Table 1, as well. SCC values were log-transformed in all analyses. The higher SCC in H breed compared to CF breed in case of both negative and positive cows is evident from the results (geometric mean in negative results = 192 H and 128 CF, in positive results = 752 H and 282 $10^3 \times ml^{-1}$ CF). Nóbrega and Langoni (2011) described also the higher SCC in H compared to Jersey (J) in the dry and rainy season as well (dry season: marginal means H 282 and J 260 $10^3 \times ml^{-1}$; rainy season: 313 and 266 $10^3 \times ml^{-1}$, respectively), whereas the season had no significant effect on SCC. Genčurová et al. (1993) found similar results for the influence of breed (CF and H) on SCC. They found out that the breed had a significant effect on the SCC ($P < 0.01$), the higher SCC showed H compared to CF breed. Zavadilová et al. (2011) investigated the difference in the somatic cell score (SCS) during 1st to 3rd lactation between breeds CF and H on the basis of genetic characteristics and environment. The differences between these two breeds in the monitored lactation were not significant. SCS was higher in the breed H in all of lactations, and the average results of SCS were 3.4, 3.78 and 4.13, while in the breed CF were SCS 3.16, 3.68 and 4.01. The

Table 1
Results on identified mastitis pathogens and SCC according to breed

Species	H (n = 365)					CF (n = 67)				
	n	%	SCC ($10^3 \times ml^{-1}$)	logSCC	xg ($10^3 \times ml^{-1}$)	n	%	SCC ($10^3 \times ml^{-1}$)	logSCC	xg ($10^3 \times ml^{-1}$)
S. sureus	50	13.7	10-46663	4.00 - 7.67	446	1	1.5	687	5.84	687
S. haemolyticus	31	8.5	11-20752	4.04-7.32	410	4	6	52-3042	4.72-6.48	359
S. agalactiae	24	6.9	87-29436	4.94-7.47	1759	neg.	-	-	-	-
S. uberis	35	9.1	54-24937	4.73-7.40	1981	13	19.4	11-3904	4.04-6.59	244
E. faecalis	9	2.1	10-2561	4.00-3.41	323	neg.	-	-	-	-
E. faecium	3	0.8	201-861	5.30-5.94	417	neg.	-	-	-	-
positive	152	41.6	10-46663	4.00 - 7.67	752	18	26.9	11-3904	4.04-6.59	282
negative	213	58.4	3-24945	3.48-7.40	192	49	73.1	7.97	3.85-6.53	128

Xg=geometric mean; SCC=somatic cell count; H=Holstein; CF=Czech Fleckvieh

influence of heritability on SCS was higher in the breed H than in the breed CF (H 0.10 to 0.14; CF 0.10 to 0.11) as well.

Next to the significant impact of breed on logSCC (Figure 1; ANOVA, F = 6.4, P = 0.012) influence of bacterial species on SCC was significant, as well (ANOVA, F = 13.6, P < 0.001). Estimated marginal means and 95% CI (confidence interval) are shown on the Figure 1. Multiple comparison among groups of bacterial species showed significant differences between NEG and SAU, SAG, SUB (Tukey HSD, P = 0.012, P < 0.001, P < 0.001, resp.). Differences between NEG and SHA or ENT were not significant (P = 0.124, P = 0.816, respectively).

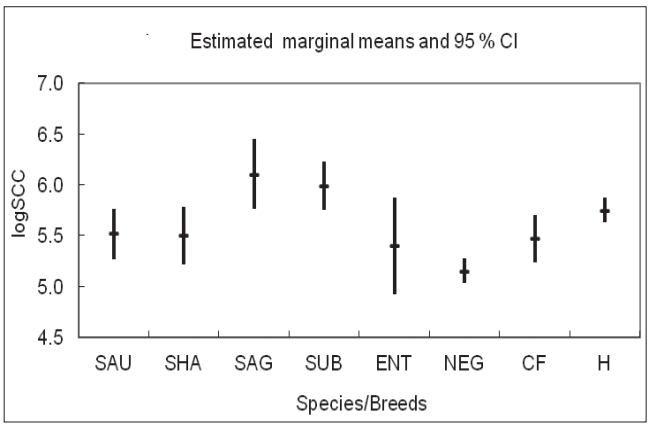

Fig. 1. Relationship between SCC and occurrence of bacterial species (ANOVA)

logSCC means = in H and CF breeds in total;
SAU – *S. aureus* (5.518); SHA – *S. haemolyticus* (5.498);
SAG – *S. agalactiae* (6.105); SUB – *S. uberis* (5.986); ENT – *E. faecalis* and *E. faecium* (5.397);
NEG – negative (5.150); CF – Czech Fleckvieh;
H – Holstein; SCC – somatic cells count;
CI – confidence interval

In case of positive findings, the highest mean value of logSCC (according to ANOVA) was found in *S. agalactiae* (6.105; geometric mean xg SCC = 1274 $10^3 \times ml^{-1}$), followed by economically significant *S. uberis* (5.986; xg SCC = 968 $10^3 \times ml^{-1}$) and practically the most important pathogen *S. aureus* (5.518; xg SCC = 330 $10^3 \times ml^{-1}$). The logSCC levels were lower for potentially risk pathogen *S. haemolyticus* (5498; xg SCC = 315 $10^3 \times ml^{-1}$) and the lowest for *E. faecalis* and *E. faecium* (5.397; xg SCC = 249 $10^3 \times ml^{-1}$) – see Table 2.

Table 3 shows the detailed results on multiple comparison among bacterial groups. The difference in the number of logSCC mean of negative group (5.15; xg SCC = 141 $10^3 \times ml^{-1}$, Figure 1) and mastitis species is statistically significant for *S. aureus* (mean differences d = 0.41, P = 0.012), followed by *S. agalactiae* (d = 1.01, P = 0.000) and *S. uberis* (d = 0.81, P = 0.000).

There is a significant difference in SCC (P = 0.041) between two groups of contagious pathogens SAG (xg = 330 $10^3 \times ml^{-1}$) and SAU (xg = 1274 $10^3 \times ml^{-1}$), where the values for SAG are typically higher (Table 3, Figure 1). While the difference in SCC between two the most important pathogens (SAU and SUB) was not statistically significant (Table 3; P = 0.151). This can represent the practical problem in identification, respectively diagnosis of subclinical mastitis etiology SAU (as the most economically important species) only according to SCC.

Table 2
LogSCC means and confidence intervals (ANOVA)

1. Species				
Dependent variable: logSCC			95% Confidence interval	
Species	Mean	Standard error	Lower bound	Upper bound
SAU	5.518	0.126	5.270	5.766
SHA	5.498	0.144	5.214	5.782
SAG	6.105	0.176	5.759	6.450
SUB	5.986	0.120	5.749	6.223
ENT	5.397	0.242	4.921	5.872
NEG	5.150	0.061	5.030	5.271
2. Breed				
Dependent variable: logSCC			95% Confidence interval	
Breed	Mean	Standard error	Lower bound	Upper bound
CF	5.468	0.117	5.238	5.698
H	5.750	0.061	5.629	5.870

The differences in the incidence of SCC and pathogens (except *S. uberis*) at higher frequencies of pathogens and higher values of SCC at Holstein breed can be justified as follows: a) in the case of SCC is a confirmation of the fact regularly surveyed also by other authors, so it is a general and reliably verified trend; b) in the case of mastitis pathogens, studies of breeding efect are infrequent due to laboriousness and cost significantly limited, so there are only more or less probable estimations. However, both the above mentioned findings are with regard to the pathogenesis of mastitis in a logical and straight accordance, and the results are thus mutually confirmed, which increases the probability of correctness of conclusion. A higher incidence of pathogens in H (and also SCC) may be justified also by possible influence of genetic potential for higher milk yield in H, which can lead to significantly higher metabolic load on the body (higher physiological demands on animals) and the higher susceptibility to infection (e.g. longer milking brings the higher flabbiness of teat circle constrictor and weakening of the natural immune mechanisms). Hypothetically, this can also be considered (because of higher milk yield and massive outflow of Ca from the body in the top of lactation) of the effect of the increased production of cortisol adrenal cortex and his known immunosuppressive effects (e.g. the higher incidence of postpartum paresis), which may result in a higher incidence of pathogens in the mammary gland and the consequent an increase in SCC.

Conclusion

From results there is evident that more positive cows were in breed H compared to CF (H 41.6%; CF 26.9%). We have also comfirmed the higher SCC in H breed compared to CF breed in case of both negative and positive cows and the significant impact of breed on log SCC ($F = 6.4$, $P = 0.012$). The influence of bacterial species on SCC was significant, as well ($F = 13.6$, $P < 0.001$). The results and statistical evaluation of this work may have a significant importance in the design of algorithms for identifying programs concerning mammary gland health of dairy cows and in the control of milk yield with regular individual SCC analyses in order to support the mastitis prevention and milk quality improvement.

Acknowledgements

This research was supported by the Ministry of Agriculture of the Czech Republic, National Agency for Agricultural Research, in the programme Complex Sustainable Systems, Project No. NAZV KUS QJ1210301, NAZV KUS QJ1230044, NAZV KUS QJ1210284 and institutional support No. RO0312.

References

Ali, A. and G. E. Shook, 1980. An optimum transformation for somatic cells concentration in milk. *Journal of Dairy Science*, **63**: 487-490.

Table 3
Multiple comparison (logSCC, Tukey HSD)

(I) Species	(J) Species	Mean difference (I-J)	Standard error	Significance	95% Confidence interval	
					Lower bound	Upper bound
SAU	SHA	0.0465	0.17910	1.000	-0.4662	0.5592
	SAG	**-0.5922***	0.20198	**0.041**	-1.1704	-0.0140
	SUB	-0.3976	0.16409	0.151	-0.8674	0.0721
	ENT	0.1157	0.26179	0.998	-0.6338	0.8651
SHA	SAG	**-0.6387***	0.21625	**0.039**	-1.2578	-0.0196
	SUB	-0.4441	0.18136	0.142	-0.9633	0.0751
	ENT	0.0692	0.27295	1.000	-0.7122	0.8506
SAG	SUB	0.1946	0.20399	0.932	-0.3894	0.7786
	ENT	0.7079	0.28848	0.141	-0.1180	
SUB	ENT	0.5133	0.26335	0.374	-0.2406	
NEG	SAU	**-0.4146***	0.12488	**0.012**	-0.7721	-0.0571
	SHA	0.3681	0.14684	0.124	-0.7885	0.0523
	SAG	**-1.0068***	0.17402	**0.000**	-1.5050	-0.5087
	SUB	**-0.8123***	0.12811	**0.000**	-1.1790	-0.4455
	ENT	-0.2989	0.24088	0.816	-0.9885	0.3906

*=statistically significant

Benda, P., M. Vyletělová and A. Ticháček, 1997. A method of prevalence estimation of intramammary *Staphylococcus aureus* and *Streptococcus agalactiae* infection in herds by examination of bulk milk samples. *Veterinary Medicine – Czech,* **42**: 101-109.

Boşgelmez-Tinaz, G., S. Ulusoy, B. Aridogan and F. Coskun-Ari, 2006. Evaluation of different methods to detect oxacillin resistance in *Staphylococcus aureus* and their clinical laboratory utility. *European Journal of Clinical Microbiology and Infectious Diseases*, **25**: 410-412.

Bradley, A. J., 2002. Bovine mastitis: An evolving disease. *Veterinary Journal*, **163**: 1-13.

Caraviello, D. Z., 2004. Selection for clinical mastitis and somatic cell count. Reproduction and Genetics 613. The Babcock Institute for International Dairy Research and Development, *University of Wisconsin-Madison*, pp. 1-6.

CSN 57 0529:1993. Raw cow milk (in Czech), Czech Standards Institute Prague, 1993.

Eding, H., Y. de Haas and G. de Jong, 2009. Predicting mastitis resistance breeding values for somatic cell count indicator traits. In: Proceedings of the Interbull Meeting, *40*, Barcelona-Spain, 2009, pp. 21-25.

Genčurová, V., O. Hanuš, B. Gabriel and I. Žváčková, 1993. Somatic cell count in milk in relation to some breeding factors. *Czech Journal of Animal Science*, AUTHOR1!!! Volume???: 359-361.

Hanuš, O. and A. Ticháček, 1997. Analysis of milking technique effect on somatic cell counts. *Stočarstvo Animal Husbandry*, **51**: 121-128.

Hanuš, O., I. Žváčková, V. Genčurová and B. Gabriel, 1992. A relationship between milk lactose content and indicators of the mammary gland health in the first third of lactation. *Veterinary Medicine - Czech*, **37**: 595-604.

K**almus, P., B. Aasmäe, A. Kärssin, T. Orro and K. Kask, 2001.** Udder pathogens and their resistance to antimicrobial agents in dairy cows in Estonia. *Acta Veterinaria Scandinavica***, 53: 4.**

Kvapilík, J., Z. Růžička and P. Bucek, 2012. Annual. Cattle breeding in Czech Republic. Main results and indicators for 2011. Prague. *Czech-Moravia Breeders Association*, pp. 30-44.

Le Blanc, D. M., E. M. Reece, J. B. Horton and J. E. Janis, 2007. Increasing incidence of methicillin-resistant *Staphylococcus aureus* in hand infections: a 3-year county hospital experience. *Plastic and Reconstructive Surgery*, **119**: 935-40.

Martineau, F., F. J. Picard, P. H. Roy, M. Ouellette and M. G. Bergeron, 1988. Species-specific and ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus*. *Journal of Clinical Microbiology*, **36**: 618-623.

Nóbrega, D. B. and H. Langoni, 2011. Breed and season influence on milk quality parameters and in mastitis occurrence. *Pesquisa Veterinária Brasileira*, **31**: 1045-1052.

Orbán, M., K. K. Gaál, F. Pajor, A. Szentléleki, P. Póti, J. Tőzsér and L. Gulyás, 2011. Effect of temperament of Jersey and Holstein Friesian cows on milk production traits and somatic cell count. *Archiv für Tierzucht*, **54**: 594-599.

Pitkälä, A., M. Haveri, S. Pyörälä, V. Myllys and T. Honkanen-Buzalski, 2004. Bovine mastitis in Finland 2001 - prevalence, distribution of bacteria, and antimicrobial resistance. *Journal of Dairy Science*, **87**: 2433-2441.

Reneau, J. K., R. D. Appleman, G. R. Steuernagel and J. W. Mudge, 1988. Somatic cell count. An effective tool in controlling mastitis. Agricultural Extension Service, *University of Minnesota*, AG-FO-0447.

Ryan, D. P., 1993. Cell count interpretation. *IDF Mastitis Newsletter*, **134**: 12-15.

Schroeder, J. W., 2012a. Bovine Mastitis and Milk Management. Mastitis Control Programs. *North Dakota State University,* Extension Service. AS1129, 2012, pp. 1-16.

Schroeder, J. W., 2012b. Troubleshooting a Mastitis Problem Herd. *North Dakota State University*, Extension Service. AS1128, pp. 1-8.

Schroeder, J. W., 2012c. Proper milking techniques. *North Dakota State University*, Extension Service AS1126, pp. 1-2.

Shook, G. E., 2001. Breeding, Selection and Somatic Cell Counts: Where Are We Today? *National Mastitis Council,* Annual Meeting Proceedings, pp. 113-127.

Shook, G. E., 1982. Approaches to summarizing somatic cell count which improve interpretability. *National Mastitis Council*, Louisville, Kentucky, pp. 1–17.

Ticháček, A., M. Bjelka, O. Hanuš, P. Kopunecz, P. Olejník, L. Pavlata, A. Pechová and A. Ponížil, 2007. Advisory service as safety tool in milk primary production. *Agritec Šumperk*, 88 pp.

Urioste, J. I., J. Franzen, J. J. Windig and E. Strandberg, 2011. Genetic variability of alternative somatic cell count traits and their relationship with clinical and subclinical mastitis. Stavanger Norway. *Interbull Bulletin*, **44**: 204-209.

Vasilev, N., D. Dinev, Y. Mitev, M. Koleva and Ch. Miteva, 2007. Hygiene status of dairy cows rezed in a spacious building and resulting quality of produced milk. *Trakia Journal of Science*, **5**: 47-51.

Vyletělová, M., L. Nejeschlebová and O. Hanuš, 2010. Monitoring of the main mastitis pathogens. *Náš chov*, **2**: 68-71.

Vyletělová, M., O. Hanuš, L. Hasoňová, P. Roubal, I. Manga and L. Nejeschlebová, 2013. Occurrence of mastitis pathogens in relation to somatic cells. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, **5**: 1505-1511.

Zavadilová, I., J. Wolf, M. Štipková, E. Němcová and J. Jamrozik, 2011. Genetic parameters for somatic cell score in the first free lactations of Czech Holstein and Fleckvieh breeds using a random regression model. *Czech Journal of Animal Science*, **56**: 251-260.

Received March, 4, 2014; accepted for printing October, 2, 2014.