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

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Abstract

Influence of breed on somatic cell count (SCC) and occurrence of particular species was evaluated. The samples were collected both from farms with Holstein (H; 10 farms; 365 cows) breeding and from farms with Czech Fleckvieh (CF; 2 farms; 67 cows). The animals were investigated on occurrence of mastitis pathogens and SCC. The obtained dataset was statisticaly evaluated by analysis of variance to test main effects on logSCC with following testing of bacterial species impact. The occurrence of mastitis pathogens was compared between Holstein and Czech Fleckvieh. The relationship between SCC and incidence of isolated mastitis 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 mastitis 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 breed CF. SCC results showed the higher SCC in H breed compared to CF in case of both negative and positive cows as well (negative results = 192 H and 128 CF; positive results = 752 H and 282 $10^3 \times ml^{-1}$ CF) and significant impact of breed on logSCC (F=6.4, P=0.012). The significant effects of bacterial species were confirmed (F=13.63, P<0.001). Multiple comparison among groups of bacterial species showed significant differences between NEG and SAU, SAG, SUB (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).

Key words: Holstein; Czech Fleckvieh; mastitis species; somatic cell count

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. 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, Ayrshire, 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) (Kvapilik et al., 2012). Regarding to the milk yield, the highest was confirmed in H (8,808 kg), followed by Montbeliard, Braunvieh, Czech Fleckvieh (6,545 kg), Ayrshire, Jersey and Normande (5,602 kg). Pregnancy of heifers of both breeds (H and CF) were comparable (59%), but in cows with the first insemination was more successful in CF (44 %) than in H (38%).

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 (J) and Holstein Friesian (HF). SCC showed positive moderate relation with the temperament scores of Jersey ($r=0.67$; $P=0.0001$) and HF ($r=0.66$; $P=0.0001$). Vasilev et al. (2007) compared the results of individual SCC in breeds H, 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 $10^3 \times ml^{-1}$). Koç and Kizilkaya (2009) studied the influence of selected factors on the SCC and milk yield in Holstein Friesian (HF) and Brown Swiss breed (BS). The SCC mean was higher in HF breed (HF=451, BS=291 $10^3 \times ml^{-1}$) and the effect of breed on SCC was statistically significant ($P<0.01$). Ptak et al. (2009) evaluated genetic relationship between LSCS (lactation somatic

cell score) and udder traits in Polish Holstein-Fresian cows and found genetic correlations between LSCS and udder depth (rg=-0.17) and between LSCS and udder rear (rg=0.20).

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 (Tichacek et al., 2007; Schroeder, 2012a). The significant effect on SCC has especially milking hygiene and technological state of milking equipment (Hanus et al., 1997; Schroeder, 2012 b, c). 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 genetically fixed resistance against the subclinical and clinical mastitis disease (Shook, 2001; Caraviello, 2004; Eding et al., 2009). This can be observed and compared not only between breeds, but also between groups of primiparous half-sister cows after various sires within the breed or between fathers within breeds. However, Hanus et al. (2011) described no significant differences in logSCC among groups of half-sister cows on the first lactation according to fathers CF breed. Citek et al. (2011) found in the polymorphism CGIL4 between bulls (Czech Simmental and German Holstein) no significant differences in breeding value for SCC, as well. On the other hand, the BTA27 QTL was responsible for 18% of the genetic variability in the SCS (somatic cell score; Baes et al., 2010) and 22 QTL clarified most of the observed variability in the estimation of breeding values for SCS (Tal-Stein et al., 2010).

This knowledge could be practically useful for the deliberate increasing of innate resistance of dairy and dual purpose cattle against mastitis 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 CSN 57 0529), electrical conductivity, lactose, casein, whey proteins, the occurrence of pathogens, etc. become changed (worsened) along with the udder health worsening (Ali and Shook,1980; Shook, 1982; Reneau et al., 1988; Hanus et al., 1992; Ryan, 1993; Benda et al., 1997). Culling of cows because of udder disease is around 9% in the Czech Republic (Kvapilik 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, 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.

Material 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., 1998), 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 logSCC 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

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.

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).

Next to the significant impact of breed on logSCC (Figure 1; ANOVA, F=6.4, P=0.012) influence of bacterial species was significant, as well (ANOVA, F=13.63, 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).

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=1,274 $10^3 \times ml^{-1}$), 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=1,274 $10^3 \times ml^{-1}$), where the values for SAG are typically higher (Tab. 3, Figure 1). While the difference in SCC between two the most important pathogens (SAU and SUB) was not statistically significant (Tab. 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.

Discussion

Incidence of identified mastitis pathogens is in accordance with previous works (Vyletelova et al., 2010; Vyletelova et al., 2013). The occurrence of mastitis pathogens was evaluated since year 1996. It was found out that the most frequent species today are *S. uberis*, *S. aureus* and then coagulase-negative staphylococci (especially *S. haemolyticus*) while *S. agalactiae* compared to *S. uberis* was one of the main mastitis pathogen till year 2005. Similar results were described also by Bradley (2002), Pitkälä et al. (2004) or Kalmus et al., (2011) 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.).

In case of mastitis pathogen occurrence in relation to the breed, Nóbrega & Langoni (2011) described the similar results in relation to H and Jersey cows. However, they found

higher frequency of intramammary infection (IMI) in Jersey compared to Holstein cows during dry and rainy season as well. Their results also showed that envionmental pathogens were more frequently isolated from the breed Jersey. 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. Gencurova 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. Zavadilova et al., (2011) investigated the difference in the somatic cell score (SCS) during 1^{st} to 3^{rd} 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 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.

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 analyzes in order to support the mastitis prevention and improve milk quality.

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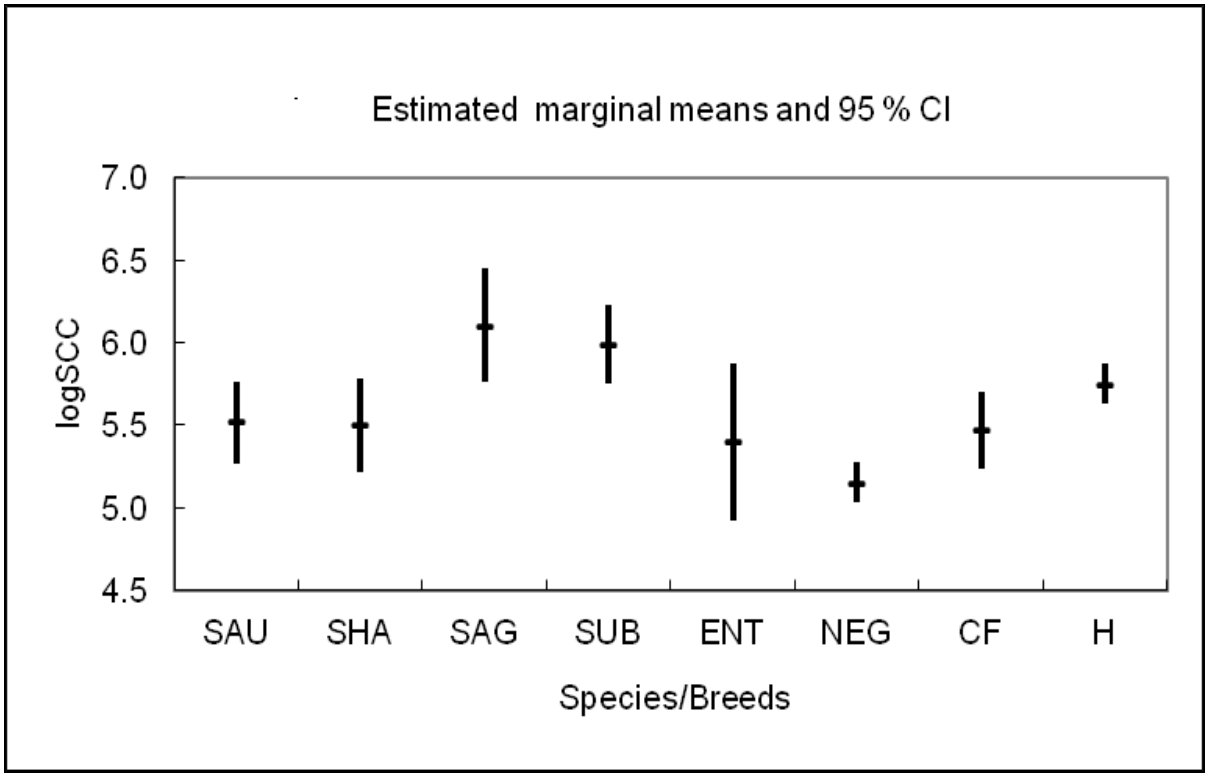
Table 1. Results on identified mastitis pathogens and SCC according to breed

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.I	54-24937	4.73-7.40	1981	13	19.4	11-3904	4.04-6.59	244
E. .faecalis	9	2.I	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

Figure 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

Table 2. LogSCC means and confidence intervals (ANOVA)

1. Species				
Dependent variable: logSCC				
Species	Mean	Standard error	95% Confidence interval	
			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				
Breed	Mean	Standard error	95% Confidence interval	
			Lower bound	Upper bound
CF	5.468	0.117	5.238	5.698
H	5.750	0.061	5.629	5.870

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	1.5338
SUB	ENT	0.5133	0.26335	0.374	-0.2406	1.2672
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

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