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RELATIONSHIP BETWEEN SOMATIC CELL COUNT AND OCCURRENCE OF INTRAMAMMARY PATHOGENS IN DAIRY COWS

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The goal of this observation was to evaluate relationship between somatic cell count and intramammary pathogens occurrence in milk of dairy cows.

Somatic cell counting was performed by new on-farm commercial device Deleval Cell Counter (DCC) and laboratory Fossomatic cell counting (FSCC). 100 sensoric unchanged mixed milk samples collected during milking time on dairy farm were analysed in this study for detection of somatic cell count by both methods. Quarter milk samples (n=389) of all selected cows were cultured.

Increased somatic cell count was detected in 46 mixed milk samples by DCC and in 58 by FSCC. Of total quarter milk samples bacteria were determined in 76 (19.5 %). The most prevalent bacteria were Enterococcus spp. (26.3 %), followed by E. coli (25 %), A. viridans (15.7 %), coagulase-negative staphylococci (11.8 %), Proteus spp. (9.2 %), Streptococcus spp. (6.6 %) and S. intermedius (2.6 %). Contagious isolates (S. aureus) were detected in 3 quarter milk samples (4 %). Agreement between DCC and microbiological culture was found in 90 %, and between FSCC and bacteriological incidence in 84 %.

Higher SCC was detected in milk samples contaminated by bacteria than in healthy milk ($P<0.001$). Presence of individual species of intramammary pathogens was not related to different levels of SCC.

The presented data illustrated that bacteria are predominant causes of subclinical mastitis. However, in some milk samples with increased SCC no bacteria were detected. This means that it could have been caused by numerous other agents or factors for mastitis in dairy industry.

Keywords: BACTERIA, COWS, SOMATIC CELL COUNT

In spite of strong scientific effort in the prevalence of mastitis no important elimination of this disease in dairy industry all over the world has been reached. Severe intramammary inflammation remains major problem causing high losses of dairy farms. According to recent sources prevalence of mastitis has been extremely different in separate countries and regions. The incidence of mastitis in dairy herds results from a complex interaction between the infectious agents, poor management practices, genetic and environmental factors stressing the defence of udder. The most common potential risk factors for mastitis in dairy farms are classified as quarter, cow and environmental risk factors [5]. Numerous authors have pointed out risk factors for CM associated with farm management, hygiene management, the breeding environment, milking technology, feeding, the calving season and preventive health management [1]. In an individual herd, cow factors are responsible for the differences among cows in contracting CM. A great number of individual cow-specific risk factors for CM have been identified, including breed, parity, period of lactation, udder and teat morphology, age at first calving, milk leakage, udder oedema, milk production, somatic cells count, and reproductive disorders [7, 8, 12].

For rapid analysis of SCC many assays and devices were invented, whose principle is performed on the base of chemical reaction between DNA of white blood cells and testing reagent. The most commonly used on-farm rapid test is California mastitis test or its modifications. However this test has served only for a semi-quantitative analysis, and counting of accurate SCC cannot be performed. It yields only approximate value of SCC and subsequently degree of subclinical mastitis. Second on-farm test is Eimu Cell Check Test that has been more sensitive and determines lower SCC compared to requirements of farmers originated from West European countries, who prefer mean SCC below 100 000 SC/ml. However, ECC test is also a semi-quantitative without the accurate SCC in milk.

In commercial environment new devices are used that ensure accurate reliable counting of

somatic cells in milk samples. Delaval Cell counter is one of them and therefore it was tested in the field conditions, whereby benefits and liabilities were evaluated. Agreement between SCC analysed by DCC in the field and SCC analysed in the laboratories of The Breeding Services of the Slovak Republic was accomplished. Moreover, relationship among SCC detected by these methods with occurrence of bacteria and SCC in milk samples contaminated by individual species of intramammary pathogens was evaluated.

Materials and methods

Experimental dairy herd consisted of 335 lactating cows with average milk yield of 7,100 kg. All the animals were housed in a free stall system with the deep straw bedding. Of complete herd SCC was analysed in mixed milk samples and bacterial culture was performed in quarter milk samples of 100 dairy cows.

Somatic cell counting. Sensorically unaltered mixed milk samples were tested by Delaval Cell Counter (DCC, *DeLaval International AB*, Tumba, Sweden; Obr. 2) within 1 h after milking. DeLaval cell counter (DCC) is a portable device designed for on-farm somatic cell count (SCC) analysis in bovine milk within 1 min. Laboratory counting of SC was carried out in milk samples preserved with 0.1 % $K_2Cr_2O_7$ by fluoro-opto-electronic method in accredited laboratory of The Breeding Services of the Slovak Republic the following day (*Fossomatic™ FC*, Denmark). For the evaluation of SCC in milk samples we used a scale based on SCC. Milk samples with SCC up to 400 000 SC/ml were considered as a threshold or negative and samples above the 400 000/ml were considered positive.

Bacteriological analyses. Bacteriological examinations were performed to detect bacterial genus *Staphylococcus* spp., S*treptococcus* spp., *Enterococcus* spp., *Proteus* spp. and were evaluated according to commonly accepted rules. Milk samples (10 µl) were cultured at the respective veterinary practice according to their routine procedures, usually employing Columbia Blood Agar Base with 5 % of defibrinated blood, Staphylococcal medium N°110, Baird-Parker agar, Edwards Medium, Mac Conkey Agar (*Oxoid*, *OXOID Ltd.*, Basingstoke, Hants, UK) incubated at 37 °C for 18–24 h. Besides evaluation of bacterial growth characteristics another assays were used to bacterial species determination: pigment and coagulase production, catalase activity, haemolysis, Gram staining and other virulence factors. Commercial kits *STAPHYtest 24*; *STREPTOtest 24*, resp. *ENTEROtest 24* (*Erba Lachema*, Brno, Czech Republic) were used for the identification of bacteria. All kits were evaluated by the programme *TNW Pro-Auto 7.0®* (*Erba Lachema Ltd.*, Brno, Czech Republic) according to the manufacturer's instructions (*Erba Lachema*, Brno, Czech Republic). *S. aureus* was identified by means of typical colony morphology, and α- and β-hemolysis and positive coagulase reaction. Coagulase-negative staphylococci (CNS) were identified by typical colony morphology and negative coagulase reaction.

Statistical analyses. Agreement of increased SCC and occurrence of bacteria in milk was assessed by percentage. Moreover statistical analyses was tested between SCC in contaminated milk by intramammary pathogens by Student's *t*-test and relationship between SCC and bacterial species by ANOVA test.

Results and discussion

Increased somatic cell count was detected in 46 mixed milk samples by DCC and in 58 by FSCC. Of total quarter milk samples bacteria were determined in 76 samples (19.5 %) in 47 dairy cows. The most prevalent bacteria were *Enterococcus* spp. (26.3 %), followed by *E. coli* (25.0 %), *A. viridans* (15.8 %), coagulase-negative staphylococci (11.8 %), *Proteus* spp. (9.2 %), *Streptococcus* spp. (5.3 %) and *S. intermedius* (2.6 %). Contagious isolates (*S. aureus*) were detected in 3 quarter milk samples (3.9 %). Presence of individual species of intramammary pathogens was not related to different levels of SCC (table 1).

Agreement between DCC and microbiological culture was found in 90 %, and between FSCC and bacteriological incidence in 84 %. Higher mean SCC was detected in milk samples contaminated by bacteria than in healthy milk samples ($P<0.001$), whereby mean SSC of contaminated milk analysed by FSCC was 3514.8 and by DCC was 1549.8. Opposite mean SCC

of uncontaminated milk was 253 counted by FSCC and 187.4 by DCC (table 2).

Somatic cells are indicators of both resistance and susceptibility of cows to mastitis and can be used to monitor the level or occurrence of subclinical mastitis in herds or individual cows. SCC is a useful predictor of intramammary infection (IMI). An increased SCC in milk has a negative effect on the quality of raw milk [10]. Relationship between SCC and prevalence of subclinical mastitis and occurrence of intramammary pathogens has been observed in many studies. Based on obtained results closed relationship between increased SCC and IMI was noticed in our study, whereby SCC was higher ($P<0.001$) in infected milk. A study to determine the relationship between SCC and mastitis etiological agents was carried out by [4]. They reported that milk samples with SCC lower than 200,000 cells/ml were mostly (59.6 %) culture negative. Coagulase-negative staphylococci (CNS), *S. aureus* and *Streptococcus spp.* were mostly detected in samples with 200,000 to 2,000,000 of SCC/ml. However, in our study in milk infected by *S. aureus* mean SCC was lower than 200,000. Coagulase-negative staphylococci and *Streptococcus* spp. were detected in milk samples ranging from 1,219,000 to 2,376,667 SC/ml. In Poland observed samples having more than 2 million/ml of SCC were infected mainly with CAMP-negative and CAMP-positive streptococci and Gram negative bacilli. The highest SCC (≥10 million/ml) in foremilk samples was associated with intramammary infections caused by *Arcanobacterium pyogenes* (95.5 %), *Streptococcus agalactiae* (57.6 %) and Gram-negative microorganisms (46.5 %). Very high SCC (≥5 million/ml) was connected with infections caused by *Prototheca* sp. (64.5 %), yeast-like fungi (60.2 %) and *Streptococcus* sp. (55.1 %). *S. aureus* (76.2 %), CNS (84.2 %), Gram-positive bacilli (72.4 %) and *Corynebacterium* sp. (83.2 %) caused an increase in SCC that was smaller than 5 million/ml. Review by [2] makes it obvious that milk SCC (×1 000/ml) ranged from 27 to 600 in culture negative quarters, from 191 to 9 433 in quarters infected with *S. aureus*, from 561 to 4 758 in quarters infected with *Streptococcus agalactiae*, from 809 to 1 944 in quarters infected with *Streptococcus dysgalactiae*, from 851 to 1 085 in quarters infected with *Streptococcus uberis*, from 590 to 9 009 in quarters infected with coliforms, from 90 to 3 040 in quarters infected with CNS and from 128 to 1 352 in quarters infected with *C. bovis*. In South African study [9] of the eight pathogens that were quantified according to SCC levels, *S. dysgalactiae* had overally the highest SCC followed by *S. aureus* (STH and STA) and *S. agalactiae*. Although only small numbers of *T. pyogenes* were isolated in this data set, more than 84 % of these bacteria had SCC exceeding 200 000 cells/mL. However, some bacterial species were isolated at an SCC of below 200 000 cells/mL: *S. dysgalactiae* (4.2 %), *S. aureus* (STH) (5.9%) and *S. agalactiae* (9.0%). A study conducted by [6] showed that the mean SCC for samples identified with the CNS strains *S. chromogenes* and *S. hyicus* was 168 000 cells/mL and 193 000 cells/mL compared with 39 000 cells/mL from uninfected quarters. According to Brasilian study the geometric means of the bacteriological examination results were in milk samples with no

Table 1

Distribution of intramammary pathogens and SCC in dairy cows

Bacteria	Number of positive quarter milk samples	DCC (x±sd)	FSCC (x±sd)
Contagious pathogens	3 (3.9 %)	146.0	145.0
Staphylococcus aureus	3		
Environmental pathogens	73 (96.1 %)		
Enterococcus spp.	20	1833.1	4123.8
E. coli	19	1591.7	4349.8
Aerococcus viridans	12	1678.4	3363.9
CNS	9	1219.0	2377.8
S. epidermidis	5		
S. chromogenes	3		
S. warneri	1		
Proteus spp.	7	1710.3	3078.0
Streptococcus spp.	4	2736.7	5854.8
S. intermedius	2	231.0	335.5
Totally	76	1549.8	3461.8

Table 2

Milk SCC analysed by two methods

	DCC (x±sd)	FSCC (x±sd)
Milk with bacterial contamination	1549.8^{***}	3461.8^{***}
Healthy milk	187.4^{***}	253^{***}

Note: x — mean, sd — standard deviation.

growth 52,000, coagulase-negative staphylococci 85,000, *S. aureus* 587,000; other streptococci 432,000 and *S. agalactiae* (1,572,000; 333,000 [3]. In another study average SCC of the *A. viridans* infected cows was significantly higher (1000.0×10^3 cells/mL) ($P<0.01$) compared to healthy cows (72.4×10^3 cells/mL) [11].

Conclusion

The presented data illustrated that bacteria are predominant causes of subclinical mastitis. However in some milk samples with increased SCC no bacteria were detected. This suggests that it could have been caused by numerous other agents or factors for mastitis in dairy industry.

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