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RELATIONSHIP BETWEEN MASTITIS CAUSATIVE PATHOGENS AND SOMATIC CELL COUNTS IN MILK OF DAIRY COWS

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ABSTRACT

Milk somatic cell count is a key component of national and international regulation for milk quality and an indicator of udder health and of the prevalence of clinical and subclinical mastitis in dairy herds. The objective of this study was to evaluate the presence of mastitis pathogens in milk samples differed by somatic cell count (SCC) in microbiologically positive samples. Also frequency of distribution of samples differed by SCC were studied in non infected samples as well. The milk samples were collected from individual quarters from the dairy farms located in Nitra region with problematic udder health of herd for SCC and bacteriological analysis. Totally, 390 milk samples were examined, and 288 (73.85%) positive milk samples were detected. Four SCC groups of samples (<100, <100<SCC<200, <200<SCC<400 and >400×10^3/ml) were used to identify presence of microorganisms in positive samples. The most frequently isolated pathogens in samples with high SCC >400×10^3/ml according to year were Coagulase-negative Staphylococci (29.11 %) in 2012, followed by *Staphylococcus aureus* (28.0%) in 2010, yeasts (24.05%) in 2012, *Escherichia coli* (22.78%) in 2012, *Bacillus sp*. (20%) in 2010 and *Pseudomonas aerugenosa* (11.88%) in 2011. Coagulase-negative Staphylococci (66.67%) were the predominantly identified in the samples with low SCC <100×10^3 cells/ml, followed by *Bacillus spp* (50%), *Entrococcus spp.* (33.33%) and *Staphylococcus aureus* (16.67%) and *E. coli* (16.67%). The results of this study indicated that the SCC of individual milk samples corresponded with the health status of the udder of dairy cows represented by presence of mastitis microorganisms in milk. However, the contamination of milk samples could be also connected with low SCC. On the ohter side the samples with high SCC were found out without presence of microorganism. The further study is needed to identify the reason of high SCC in milk from negative samples.

Keywords: bovine mastitis; microorganism; somatic cell count, milk

INTRODUCTION

Milk somatic cell count is a key component of national and international regulation for milk quality and an indicator of udder health and of the prevalence of clinical and subclinical mastitis in dairy herds **(Sundekilde et al., 2012)**. The trade with milk and milk product could be thus possible influenced by milk quality related to consumer demands **(Kubicová and Dobák, 2012)**.

The majority of mastitis cases are caused by only a few common bacterial pathogens namely, *Staph.* spp., *Strep.* spp., Coliforms and *Actinomyces pyogenes* **(Sharma, 2010)**. While in a report by **Kumar et al., (2009)** *Strep. dysgalactiae* was major (50.00 %) organism isolated from the cases of subclinical mastitis in cows followed by *Staph. aureus* and others. Most of the intramammary infections (IMIs) arise during the process of milking or within 2 hours after it, i.e. to the time when the teat canal is fully closed. Microbial contamination of milk before and after preparation of the udder for milking was described by **Tančin et al., (2006)**.

SCC represent the number of cells in milk (representing mainly blood white cells during an immune response of mammary tissue) **(Sarikaya et al., 2006)** and indicate intramammary infection (IMI) when elevated **(Reksen et al., 2008)**. However, SCC can be affected by several factors such as stage of lactation, age, stress of the animals, time and frequency of milking, management, seasons, and mainly IMIs **(Schwarz et al., 2010; Tančin, 2013)**. SCC is used as a diagnostic tool to monitor subclinical mastitis in dairy herd's worldwide **(Schukken et al., 2003)**. SCC could be also important for comparison of different milking systems **(Mikulová, 2011)**.

The normal composition of milk somatic cells varies with the type of secretion or lactation cycle. Normally, in milk from a healthy mammary gland, the SCC is below 100×10^3 cells/ml, or even lowers **(Leitner et al., 2003)**. The high SCC is mostly related to the presence of microorganism in the udder but also the type of microbes could affect the SCC in milk **(Ariznabarreta et al., 2002)**. On the other side in some cases the high SCC was detected in milk samples without presence of microorganisms **(McDougall et al., 2001)**. Therefore, mastitis should be detected in a reliable and timely fashion based on SCC values or bacteriological culture; otherwise subclinical mastitis could develop into a clinical disease **(Hallén Sandgren et al., 2008)**.

Generally, the increase of somatic cells may lead to the greater risk of raw cow's milk contaminated by pathogens and antibiotic residues. Furthermore, high somatic cells count raises the suspicion that the raw food is produced under poorer standards of hygiene and from unhealthy cows **(Zajác et al., 2007, 2012)**.

The frequency of distribution of pathogens in positive milk samples we have recently published in work of **Idriss et al. (2013)** without evaluation of possible relationship to SCC. The aim of this study was to evaluate the prevalence of mastitis pathogens in milk samples in dependence of SCC. Also frequency of distribution of samples differed by somatic cells were studied in non infected samples as well.

MATERIAL AND METHODOLOGY

The study was conducted during the period from 2010 - 2012 in a surroundings Nitra region in Slovakia, which is located about 100 kms east from Bratislava. A total of 390 milk samples were collected from quarters of dairy cows at different dairy farms, and somatic cell count and pathogenic bacteria were examined. The samples were collected from farms with high bulk tank SCC and consequently from cows with possible problems with udder health **(Idriss et al., 2013)**.

Somatic cell count analysis

Somatic cell counts were performed within 24 h of collection using a Fossomatic 90 instrument (Foss Electric, Hillerod, Denmark) after heat treatment at 40 ℃ for 15 min.

Categories of somatic cell count (SCC)

SCC is considered as health indicator. Healthy and recovered cows were assumed to have SCC $<200\times10^3$ cells/ml, and cows with IMIs were assumed to have SCC $>400\times10^3$ cells/ml**.** Therefore, SCC were categorized as low when the uncorrected SCC measured was $<200\times10^3$ cells/ml, and high when the uncorrected SCC was $>400\times10^3$ cells/ml. An intermediate category was defined for SCC 200- 400×10^3 cells/ml. The frequency of milk samples distribution in above mentioned sorting criteria was calculated. Also the frequency of distribution of samples in above mentioned three SCC groups were evaluated in non-infected samples.

On the base of above SCC four groups of microbiologically positive samples only (<100, <100<SCC<200, <200<SCC<400 and $>400\times10^3$/ml) were created to identify effect of microorganisms.

Milk sample collection and laboratory analysis

Milk samples were collected from quarters immediately before a.m. milking as recommended by **Riekerink et al. (2007)**. After a quarter had been cleaned up by removing any possible dirt and washed with tap water, the teat end was dried and swabbed with cotton soaked in 70% ethylalcohol. Approximately 100 ml of milk was then collected aseptically into sterile bottles, after discarding the first 3 milking streams. Milk samples from each quarter were transported to the Animal Production Research Center Laboratory in an ice cooled box at 4 ℃ and analysed immediately (max. 4 h after collection) either for identification of the clinical mastitis pathogen or to determine somatic cell count (SCC). The milk samples were investigated for pathogenic mastitis accredited to a valid procedure of IDF **(IDF, 1981)**.

Statistical evaluation of data was done by Excel program to calculate frequency of distribution.

RESULTS

Distributions of somatic cell count (SCC)

The frequency of distributions of SCC in whole milk samples of dairy cow's was described in Table 1. Most milk samples were incorporated in group SCC $>400\times10^3$/ml. During the individual years the distribution was: the least in 2010 with 67.06%, followed by 2011with 68.56% and a high frequency in 2012 with 70.27%. A low frequencies of samples in SCC group 200- 400×10^3/ml were 12.94% and 11.86% in 2010 and 2011 respectively. While, in 2012 the frequency of SCC increased linearly from 12.61% (SCC $<200\times10^3$/ml to 70.27% (SCC $>400\times10^3$/ml).

Table 2 shows the frequency of distributions of SCC in microbiologically positive milk samples of dairy cows. The most samples were found out in a group of high SCC $>400\times10^3$/ml with 79.86%, followed by the SCC from 200-400×10^3/ml with 9.03%, SCC from 100-200×10^3/ml with 7.29% and the least samples in SCC $<100\times10^3$/ml with 3.82%. The obtained results showed that the year 2010 was the worst, with 81.97% of samples with SCC $>400\times10^3$/ml, and the best year were 2011 with 77.69% of samples. The lower percentage of SCC in 2011 - 2012 with SCC $<100\times10^3$/ml and from 100- 200×10^3/ml were 4.62 and 11.54% and 3.09 and 4.12 respectively. In 2010 the frequency of SCC increased linearly from 3.28% (SCC $<100\times10^3$/ml) to 81.97% (SCC $>400\times10^3$/ml).

Distributions of bacteriological results

The results of distributions of mastitis pathogens and SCC groups in milk dairy cows showed that the type of micro-organisms could affect the SCC in milk. The high frequency of mastitis pathogenic in high SCC $>400\times10^3$/ml group of samples depending on the year were Coagulase Negative Staphylococci (CNS), *Staph. aureus*, yeasts, *E. Coli*, *Bacillus sp*. and *Pseudomonas aerugenosa*. CNS also was the most frequently isolated pathogens in a group of samples with low SCC $<100\times10^3$ cells/ml, followed by *Bacillus* spp, *Entrococcus* spp. and *Staph. aureus* and *E. coli*. (Table 3).

E. coli and *Corynebacterium pyogenes* were the most frequency isolated pathogenic with 50.0% for each one with low SCC from 100-200×10^3/ml, followed by CNS (42.86%), other pathogenic (28.57%) and *S. chromogenes* (23.81%). CNS (66.67%) was the predominantly identified in the samples with SCC $<100\times10^3$ cells/ml, followed by *Bacillus spp* (50.0%), *Entrococcus spp.* (33.33%) and *S. aureus* and *E. coli* were (16.67%) for each one.

Table 4 shows the frequency distributions of *microbiologically* negative milk samples of dairy cows. There is no mastitis pathogens detected in 102 milk samples (microbiologically negative milk samples), and 92.29% of total negative samples incorporated into the groups of SCC $<200\times10^3$ cells/ml and f 200 - 400 $\times10^3$ cells/ml. Only 6.73% of samples were incorporated within the group of SCC $>400\times10^3$ cells/ml. In the group

Table 1 Frequency distributions of SCC in quarter milk samples of dairy cows

year	Number of samples (N)	Frequency of distribution (SCC in 10^3 cell/ml) in % from quarters		
	N	<200	200-400	>400
2010	85	20	12.94	67.06
2011	194	19.59	11.86	68.56
2012	111	12.61	17.12	70.27
Total	390	17.69	13.59	68.72

(SCC) - Somatic cell count, (%) - the percentage of SCC in milk sample

Table 2 Frequency distributions of microbiologically positive quarter milk samples of dairy cows in relation to SCC

year	Number of samples (N)	Frequency of distribution (SCC in 10^3 cell/ml) in %			
	N	<100	100-200	200-400	>400
2010	61	3.28	3.28	11.48	81.97
2011	130	4.62	11.54	6.15	77.69
2012	97	3.09	4.12	11.34	81.44
Total	288	3.82	7.29	9.03	79.86

(SCC) - Somatic cell count, (%) - the percentage of SCC in milk sample

>400×10^3/ml rose SCC linearly according to year from 4.17% (2010) to 12.5% (2012).

DISCUSSION

Most milk samples in studied years were found out in a group of high SCC >400×10^3/ml (around 70%). These results could be explained by taking samples from problematic farms and cows and also correspond to the findings in the same samples where we demonstrated high percentage of infected samples by microorganisms **(Idriss et al., 2013)**. In another study in milk with geometric mean composite SCC >50 000 cells/ml, nearly 65% samples had at least one culture-positive quarter **(Piepers et al., 2007)**.

In our study, when only infected samples were evaluated the percentage of samples with high SCC (>400×10^3/ml) was around 80%, which support last mentioned authors. It could be highly expected result because microorganism's presence stimulates the immunity of udder by release of somatic cells from blood into the milk **(Leitner et al., 2003; Schwarz et al., 2011)**. On the other side, we could demonstrate in samples with low SCC (<200×10^3 cells/ml) the presence of microorganisms in positive samples. It is difficult to conclude that whether the pathogens isolated from quarter foremilk samples with low SCC originated from contamination or whether they caused an IMI as pointed out by **Schwarz et al., (2010)**.

In our findings, the environmental microorganisms were the most frequent found out in samples with low SCC, though in 2011 only, there were two samples infected by *S. aureus* in SCC groups below 200×10^3 cells/ml. This finding is difficult to explain because *S. aureus* and *St. agalactiae* (contagious bacteria) are associated with high SCC in milk of ewes **(Ariznabarreta et al., 2002)**. SCC around 400×10^3 cells/ml was mainly related to the presence of minor pathogens (coagulase-negative staphylococci, *Corynebacterium bovis*, enterococci, and *Bacillus* spp.) **(Riekerink et al., 2007)**.

Low and high SCC in milk infected by CNS could be related to sensitivity to novobiocin as the possible criterion associated with staphylococci pathogenicity **(Ariznabarreta et al., 2002)**. Isolates of novobiocin-resistant CNS, micrococci, and Corynebacteria were associated to low values of log SCC (4.85 to 5.20). In contrast, infection by novobiocin-sensitive CNS, streptococci, and enterococci organisms was related to a sharp inflammatory response with high SCC **(Ariznabarreta et al., 2002)**. However, the prevalence of mastitis pathogens in the SCC range from 1,000 to ≤100,000 cells/ml was 8.5% (5.51% minor pathogens, 2.01% major pathogens, and 0.98% other pathogens) **(Schwarz et al., 2010)**. CNS is currently the most isolated pathogens from milk samples and usually related with high SCC **(Piepers et al., 2007)**.

Table 3 Frequency distributions of mastitis pathogens in relation to SCC in milk of dairy cows from microbiologically positive quarters

Frequency	**Mastitis pathogens in relation to SCC in 10^3 cell/ml in %**											
year	2010				2011				2012			
Mastitis pathogenic	<100	100-200	200-400	>400	<100	100-200	200-400	>400	<100	100-200	200-400	>400
Staphylococcus aureus				28.00	16.67	4.76		18.81				2.53
Strep. agalactiae				2.00				4.95				
Strep. uberis			22.22	6.00				4.95			9.09	5.06
Escherichia Coli		50.00	11.11	4.00	16.67	19.05	25.00	10.89		28.57	27.27	22.78
***Entrococcus* spp.**					16.67	4.76	12.00	3.96	33.33			7.59
***Bacillus* spp.**	50.00		11.11	20.00	16.67			4.95			9.09	6.33
C. pyogenes		50.00	11.11	6.00		19.05	12.00					
CNS	50.00		44.44	12.00	33.33	14.29	50.00	22.77	66.67	42.86	36.36	29.11
Pseud. aeruginosa								11.88				
S. epidermidis				8.00				5.94			18.18	2.53
S. chromogenes				8.00		23.81		3.96				
Yeasts				2.00				1.98				24.05
Others				4.00		14.29		4.95		28.57		
Number of samples	2	2	9	50	6	21	8	101	3	7	11	79

CNS - Coagulase Negative Staphylococci; C. *pyogenes* - *Corynebacterium pyogenes; S.* - *Staphylococcus;* SCC - Somatic cell count; others - (different types of bacteria and mold).

Table 4 Frequency distributions of microbiologically negative milk samples of dairy cows in relation to SCC

year	Number of samples	Frequency of distribution (SCC in 10^3 cell/ml) in %		
		<200	200-400	>400
2010	24	58.33	37.5	4.17
2011	62	31.96	48.44	6.25
2012	16	56.25	31.25	12.50
Total	102	49.02	43.27	6.73

(SCC) - Somatic cell count, (%) - the percentage of SCC in milk sample

In a study conducted by **Gonzalez-Rodriguez et al., (1995)**, they found that CNS were the most frequently isolated bacterial group, and gave lower SCC values compared with *Coagulase-positive staphylococci* and streptococci. Those results are comparable to our findings.

Bradley (2002) reported that *E. coli* was the most common cause of clinical mastitis in well-managed dairy herds with low milk SCC in the U. K. which was not found out by **Schwarz et al., (2010)**. Our results showed also occasionally occurrence of *E. coli* in low SCC group.

Low SCC in microbiologically positive milk samples could be related with possible effect of selection for improved mastitis resistance. Selection for lower 2 lactation average somatic cell count is expected to decrease the incidence of pathogen-specific mastitis, especially for *S. uberis*, *S. dysgalactiae*, and CNS and, to a lesser extent, for *S. aureus* and *E. coli* **(Sorensen et al., 2009)**.

Mastitis due to fungi and yeast are uncommon or rare. We could demonstrate also low occurrence of yeast in milk samples except in year 2012. Additionally, their presence was connected with high SCC. The comparable result was found by **De Casia dos Santos** and **Marin (2005)** 25.4% samples with high SCC.

As we could expect in a group of microorganisms free samples (microbiologically negative milk samples, Table 4) there were low percentage of samples with high SCC. However, the percentage of samples in intermediate group was relatively high when health of udder is taking into account. Threshold of 200×10^3 cells/ml has been recommended to differentiate between infected and uninfected quarters or cows **(Schukken et al., 2003)**. In bacteriologically negative milk samples the SCC was low **(Laevens et al., 1997)**. **McDougall et al., (2001)** stated that the high SCC value in the absence of bacterial growth on blood agar may be due to microorganism, such as Mycoplasma, or due non-bacterial causes, including physiological factors. We also assume that high SCC in milk samples free of microorganisms could be explained by possible consumption or killing the microorganism by white cells.

CONCLUSION

The results of this study indicated that the SCC of individual milk samples corresponded with the health status of the udder of dairy cows represented by presence of mastitis microorgqanisms in milk. However, the contamination of milk samples by microorganisms could be also connected with low SCC. On the ohter side the samples with high SCC were found out without presence of microorganism. The further study is needed to identify the reason of high SCC in milk from negative samples.

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