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CORRELATIONS AMONG THE SOMATIC CELL COUNT OF INDIVIDUAL BULK MILK, RESULT OF THE CALIFORNIA MASTITIS TEST AND BACTERIOLOGICAL STATUS OF THE UDDER IN DAIRY COWS

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In a survey of about 3000 dairy cows producing low somatic cell count (SCC) milk and kept on a large-scale dairy farm, California Mastitis Test (CMT) positivity was found in 2714 udder quarters of 1491 cows. Pathogenic microorganisms were isolated from 57.6% of these 2714 udder quarters during bacteriological examination. The commonest pathogens were coagulase-negative staphylococci (CNS, 41%) and *Staphylococcus aureus* (32.5%); however, udder infections caused by environmental streptococci (12.8%) and coliform bacteria (6.8%) were also common. All pathogens resulted in a significant increase of the SCC in individual bulk milk (IBM) samples. In the case of CNS, this SCC elevation in IBM was significantly lower than in the case of infection by the other pathogens. In spite of this, because of the high number of udder infections caused by CNS, the adverse effect exerted by CNS on dairy herds is considered to be substantial. It was found that 54.6% of all CMT-positive cows produced IBM of an SCC below 400 thousand per ml. The milk produced by 41% of the 315 cows excreting *S. aureus* also had an SCC below 400 thousand per ml. This poses a serious risk of infection to the healthy herdmates. At the same time, 11% of the infected cows produced IBM with an SCC below 100 thousand per ml. On the basis of these findings, only the regular analysis of SCC of IBM can be a reliable indicator of chronic intramammary infection. As the SCC of milk produced by CMT-positive cows (and especially of those excreting pathogens) tended to increase with advancing lactation, the authors suggest that an efficient drying-off therapy should be used to restore udder health and, whenever justified, culling of cows cannot be avoided either.

Key words: Mastitis, bovine, coagulase-negative staphylococci, somatic cell count, California Mastitis Test

Mastitis of cows is one of the diseases causing the most substantial economic losses to dairy farming. It lowers the milk yield and decreases the butter-

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fat, casein and, to a smaller extent, the lactose content of milk. In addition to other markers of inflammation, including elevated levels of certain serum proteins (such as albumin and α_1-antitrypsin) and ions (sodium and chloride) and increased activity of certain enzymes (e.g. N-acyl-glucosaminidase), the number of leukocytes originating from the blood, the so-called somatic cells, will also increase in the milk of mastitic cows (Sandholm, 1995; Huszenicza et al., 1997).

The SCC of IBM is measured by Fossomatic, a particle-counting method in the dairy industry. This method is based on the counting of the stained nuclei of cells (leukocytes with higher and epithelial cells with lower DNA content). The CMT is a simple method to estimate the DNA content of milk. A relatively high proportion of epithelial cells in the SCC may result in a lower CMT score (Huszenicza et al., 1997; Albert and Huszenicza, 2000).

The dairy industry regards the somatic cell count (SCC) as an indicator of outstanding importance among the qualification parameters of raw milk. Therefore, dairy producers should make every effort to ensure that the SCC of the milk produced by their herd is constantly at the lowest possible level and thus meets the qualification limits in force. In the practice this work means regular, herd-level mastitis control.

In most Hungarian dairy herds, individual bulk milk (IBM) samples are collected at the monthly milking trials in the framework of performance recording. These samples are then tested for several parameters including also the SCC. The resulting information may be useful for assessing the udder health status of dairy herds and may serve as a basis for the further examination and treatment of individual cows.

Cows producing IBM with low (< 400 thousand/ml) SCC are considered 'healthy'. However, even cows producing IBM of relatively low SCC may include animals affected by subacute or chronic subclinical mastitis in one (or two) of their udder quarters. In such animals, the SCC of the IBM samples remains below the limit because of the diluting effect of milk from the healthy udder quarters (Buelow et al., 1996). This may pose a serious risk to healthy herdmates, especially if a contagious pathogen (e.g. *S. aureus*) is present.

The objective of this trial was to study (1) the incidence of various intramammary pathogens, and (2) tendencies in SCC of IBM samples before and after sampling for bacteriology in cows with symptomless but CMT-positive udder quarters in a large-scale dairy herd.

Materials and methods

The study was conducted in a Hungarian dairy farm with 1800 black and white Holstein-Friesian cows. The farm has been implementing an own udder health programme for years and is free from *Streptococcus agalactiae* infection.

Cows diagnosed to be affected by *S. aureus* mastitis are identified and then culled. The average milk production per cow is 8600 kg per year. The SCC of the milk produced by the herd is permanently low (< 400,000/ml). The cows are kept under loose housing conditions, on deep litter. They are milked three times a day in a milking parlour equipped with BouMatic milking machine. In accordance with the usual Hungarian practice cows were fed a monodiet based on corn silage, alfalfa haylage and hay, which was complemented with concentrate depending on the level of milk production.

In March 2000 and 2001, a mastitis screening involving all milking cows (a total of about 3000 animals) was conducted. (1) All udder quarters of all cows were tested by the California Mastitis Test (Mastitest®, Phylaxia-Sanofi Co. Ltd.) according to the manufacturer's instructions, then (2) aseptic milk samples were collected for bacteriology from the positive quarters, i.e. those supposed to produce milk of elevated SCC. In addition, (3) the SCC data of individual bulk milk samples obtained during the regular monthly milking trials of the month closest to the date of the screening test (within approx. 1 week), as well as those of the 4 months preceding and the 2 months following the screening test (a total of 7 consecutive months) were collected. (The SCC was determined in the milk laboratory of the Hungarian Herd Recording Ltd. of Gödöllő, by the Fossomatic fluoro-optic technology.) Because of strict monitoring and elimination of acute clinical mastitis cases from the producing groups, the clinically healthy but CMT-positive cows in the producing groups were considered to have chronic/subacute subclinical mastitis and supposed to be infected at the IBM SCC sampling time. For comparative statistics the $\log_{10}$ of SCC were calculated. The obtained data were evaluated with the help of the SPSS 9.0 database-handling programme. The bacteriological examinations were performed according to the recommendations of Quinn et al. (1994) and Honkanen-Buzalski and Seuna (1995).

SCC data of the individual bulk milk of cows that were in the sick barn or calving barn during the test milking in the month of the screening test were not available; therefore, these animals were excluded from further evaluation irrespective of the result of the bacteriological examination. Assuming a measurement error, data of cows with a milk SCC stated as '0' were also disregarded.

Results and discussion

Isolated microorganisms and their distribution by udder quarter

Of the approx. 3000 dairy cows examined during the two screening tests, a total of 2714 udder quarters of 1491 cows (1454 udder quarters of 750 cows in the year 2000 and 1260 udder quarters of 741 cows in 2001) were CMT positive. This means that about 22% of the producing udder quarters and almost 50% of the cows were affected with mastitis of varying severity. Of these 2714 udder

quarters, 57.6% (n = 1564) yielded pathogenic microorganisms. The bacteriologically negative samples were predominantly sterile (n = 1116) or contaminated (n = 34) with mixed environmental microflora.

The species distribution of the isolated bacteria is presented in Table 1.

Coagulase-negative staphylococci (CNS) were isolated in the highest number, from 41% of the udder quarters shedding pathogens. This observation is consistent with data of others (Ruffo and Zecconi, 1994; Chaffer et al., 1999; Zecconi and Piccinini, 2002), who also found CNS to be the commonest mastitis pathogens in bacteriological surveys of several dairy herds. The role of CNS in the aetiology of mastitis was reported to increase when *S. aureus* infected cows were continuously culled from herds (Myllys et al., 1994).

As opposed to the expectations, udder quarters infected by *S. aureus* were found in a relatively high proportion of cows despite the specific mastitis control programme implemented in the herd. This bacterium accounted for 32.5% of all pathogens isolated. Projected to the entire cow population, this represented a *S. aureus* infection of about 10% prevalence at the time of the survey; however, this infection affected 'only' about 4.2% of all udder quarters milked.

Table 1

Isolated microorganisms and their distribution by udder quarter

	Total no. of udder quarters	LF	RF	LR	RR	Front quarters	Rear quarters
	n	n	n	n	n	%	%
	2714						
Positive for pathogens	1564	317	312	444	491	40	60
Negative for pathogens	1150	279	270	278	323	48	52
Staphylococcus aureus	508	100	95	145	168	38	62
CNS	640	127	126	179	208	40	60
Streptococcus spp.	200	41	43	60	56	42	58
Coliforms	107	22	24	35	26	43	57
Yeasts	42	10	13	11	8	55	45
Arcanobacterium pyogenes	15	3	1	5	6	27	73
Corynebacterium bovis	12	3	2	1	6	42	58
Other bacteria	17	2	4	2	9	35	65

LF = left front; RF = right front; LR = left rear; RR = right rear; CNS = coagulase-negative staphylococci

As is typical of dairy herds producing milk of low SCC (Hogan et al., 1989), besides the above-mentioned CNS streptococci of environmental origin (*Str. dysgalactiae* and *uberis,* 12.8%), so-called 'coliform' bacteria belonging to the *Enterobacteriaceae* family (6.8%) and yeasts (2.7%) were also commonly found, and other bacteria also occurred, though with a lower incidence.

In conformity with earlier observations (Lancelot et al., 1997; Langoni and Domingues, 1998) the different bacterial pathogens (*S. aureus,* CNS, streptococci, coliforms) were more commonly isolated from the rear udder quarters (Table 1).

Effect of different pathogens on the somatic cell count of milk

At the time of the survey, the non-weighted mean SCC of the herd was 430,000 per ml. This 430,000 per ml herd average arose from the 316,000/ml SCC of the milk of CMT-negative cows thus considered healthy and from the 646,000/ml SCC of the milk of CMT-positive cows thus classified as being affected by subclinical mastitis. While cows shedding the pathogen produced milk having a mean SCC of 707,000/ml, the mean SCC of the milk produced by cows negative for the pathogen (but positive by the CMT) was only 547,000/ml. At the same time, the extreme minimum and maximum values of the SCC indicate that the group classified as healthy and that classified as being affected by mastitis equally contain a rather large number of animals that have an SCC markedly deviating from the mean SCC typical of the group.

Studying the number of cows suffering from mastitis caused by different pathogens in proportion to the total number of cows milked, it can be established that CMT positivity affecting nearly 50% of the herd is divided between pathogen-shedding (29.76%) and pathogen-negative (19%) cows. *S. aureus* is responsible for about one-third of the confirmed udder infections that were detected in about 30% of all cows milked (Table 3). In the SCC category below 400,000/ml, the proportion of cows positive for pathogens was not negligible at all (15.2% in the present case; Table 3).

According to data reported by Mellenberger (1999), every 10% rise of udder infection affecting a herd will increase the SCC measured in tank milk by 100,000/ml as compared to the approx. 150,000/ml level that can be regarded as normal (Holdaway, 1990). As mentioned above, at the time of the present survey the non-weighted SCC mean of the herd was 430,000/ml. This is consistent with the findings of the bacteriological examination, i.e. that approx. 10% of the cows are affected by *S. aureus* infection and 20% of them have udder infection caused by other pathogens.

Comparing the effect of the different pathogens (Table 2), it is remarkable that in the case of the numerous CNS infections the SCC is significantly ($P < 0.001$) lower than in infections caused by other bacteria that occurred in an interpretable number of cases. This finding is consistent with the results reported by Oliver and Jayarao (1997). However, it should not be disregarded that the SCC elevated as a result of intramammary infection (IMI) with CNS is significantly higher than that of the milk produced by the uninfected udder quarters. According to Oliver and Jayarao (1997), CNS can permanently colonise the udder and cause SCC elevation associated with a milk production drop. In addition,

the studies of Trinidad et al. (1990) have revealed that, in addition to infiltration by white blood cells, connective tissue proliferation also commences in the infected udder. This often occurs already in pregnant heifers, lowering the subsequent performance of the animals. On the basis of all these facts the losses caused by CNS can be considered substantial in the herd studied in this survey.

Table 2

Mean somatic cell counts (SCC) in the case of different pathogens

	No. of cows n	Mean SCC (10^3/ml)	Min.	Max.	SD
SCC of all milked cows	2807	430	10	9978	767
Milked cows having bacteriologically examined SCC data	1560	646	15	9978	910
Healthy, not examined	1615	316	10	8078	633
Negative for pathogens	603	547	15	6744	845
Positive for pathogens	957	707	15	9978	944
Staphylococcus aureus	315	735	24	6569	811
Coagulase-negative staphylococci (CNS)	400	610	15	6796	908
Streptococcus spp.	117	815	24	6796	1014
Coliforms	65	769	27	4200	943
Yeasts	29	683	34	2623	669

The dissimilar effects exerted by different pathogens on SCC are apparent also from Table 3. Fifty-eight and 56% of cows infected by *S. aureus* and streptococci produce milk with an SCC exceeding 400,000 per ml. In contrast, only 40% of cows infected by CNS produced milk with an SCC higher than 400,000 per ml. Consistently with the figures presented in Table 2, these data clearly indicate that the elevation of the SCC is lower in cows infected with CNS. Nearly 50% of cows infected by coliform bacteria produced milk with an SCC exceeding the 400,000/ml limit.

Pathognomonic value of the somatic cell count

Table 3 presents the cows drawn under bacteriological examination divided into four groups depending on their SCC measured during the test milking. It can be stated that 54.6% of the Mastitest-positive cows produced milk with an SCC exceeding 400,000 per ml.

Table 3

Ratio of cows infected by different pathogens in the different somatic cell count categories

		Distribution according to pathogens				SCC categories in proportion of all milked cows	
		SCC categories					
	n	$< 10^5$/ml %	10^5–4×10^5/ml %	4×10^5–10^6/ml %	$>10^6$/ml %	$\leq 4\times10^5$/ml %	Total %
Positive for pathogens (CMT positive)	957	11	40	28	21	15.2	29.76
Negative for pathogens (CMT positive)	603	22	40	23	15	11.9	19
Staphylococcus aureus	315	7	35	35	23	4.1	9.9
Coagulase-negative staphylococci (CNS)	400	13	47	24	16	7.6	12.6
Streptococcus spp.	117	11	33	31	25	1.6	3.6
Coliforms	65	17	35	20	28	1.0	1.96
Yeasts	29	14	34	29	24	0.4	0.65

The group characterised by an SCC of 100,000 to 400,000 per ml contained a high percentage of both bacterium-shedding cows and cows negative for pathogens (40% each). At the same time, it can be seen that 11% of the cows shedding pathogens produced IBM with an SCC below 100,000 per ml. On the basis of these data, the measurement of SCC of IBM on a single occasion appears to be an unreliable indicator of mastitis. This statement is in harmony with the similar opinion of Zecconi and Piccinini (2002). However, the latter authors consider not only the SCC of individual bulk milk but also that of the udder-quarter milk to be of insufficient pathognomonic value. In their opinion, udder quarters infected by *S. aureus* often produce milk of an SCC below 200,000 per ml, especially at the beginning of lactation. Examining 7908 udder quarters infected primarily by environmental pathogens, Zecconi and Piccinini (2002) found that 20% of the infected udder quarters secreted milk of an SCC below 100,000 per ml. According to their data, mastitic cows can be identified successfully only by a combined evaluation of the SCC and bacteriological status of udder-quarter milk. In the present study, the CMT-negative udder quarters were not subjected to further bacteriological examination. The reason for this was that optimising the cost/benefit ratio is an important consideration in the practice, and the comprehensive bacteriological examination of all cows would involve dis-

proportionately high costs. However, our results indirectly support the data reported by Zecconi and Piccinini (2002). In this study, the mean SCC of the milk of cows found CMT negative, i.e. considered to be free of mastitis, was 316,000/ml. This figure is clearly higher than the SCC published in the literature as the maximum value defined as a criterion of a healthy udder (Holdaway, 1990). Thus, it is obvious that this group included cows producing milk of elevated SCC and having an udder possibly infected by pathogens. This false-negative diagnosis can be explained by the limit of sensitivity of the applied California Mastitis Test (~400,000–500,000/ml) and the subjectivity of the evaluation. These data indicate that accurate measurement of the somatic cell count in udder-quarter milk would be necessary.

Change of the somatic cell count over time

In both years of the survey, the SCC of the milk of cows regarded as having subclinical mastitis was followed up for 7 months (Table 4). The difference between the two study periods may primarily be explained by the changed pathogen spectrum (in the first year there was a higher incidence of *S. aureus,* streptococci and coliforms, while in the second year the number of samples negative for pathogens was higher. The ratio of the pathogen-free milk samples in the year 2000 and 2001 was 29 and 49%, respectively).

However, the continuous significant ($P < 0.001$) increase of the SCC of milk produced by bacterium-shedding cows could be observed in both years. This indicates that the average cow belonging to this category is continuously suffering from mastitis (or possibly becomes affected by a new disease on a regular basis), and her condition slowly deteriorates over time. This observation underlines the outstanding importance of an efficient drying-off therapy or, should such therapy prove unsuccessful, the necessity of culling such cows.

The elevation of SCC was not significant in cows that did not shed bacteria at the time of examination, although in absolute value the SCC found in such cows was also higher than that considered typical of healthy animals. This allows us to establish a more favourable prognosis for pathogen-negative cows in terms of the SCC. However, the data obtained in the present study fail to elucidate clearly the cause of the smaller or larger SCC elevation found for pathogen-negative udder quarters representing 40% of all udder quarters. It is possible that in some of these pathogen-negative cows a milder somatic cell elevation of an aetiology other than microbial mastitis occurs (e.g. as a result of mechanical or heat stress, fresh or late milking cows, etc.). However, in the other udder quarters probably mastitis of bacterial origin was present. Namely, in certain cases a mastitic udder quarter does not shed the pathogenic microorganism continuously in uniform numbers (e.g. *S. aureus;* Sears et al., 1990), and this may result in negative samples. For this reason a single bacteriological examination does not give results of full diagnostic value: e.g. in studies aimed at the detection of *S.*

aureus by bacteriological examination it is advisable to repeat the bacteriological examination after a few days or weeks or especially in the first few weeks after calving (Zecconi and Piccinini, 2002).

Table 4

Change of milk somatic cell count of cows included in the study during seven months

Total number of cows examined	1999–2000		2000–2001	
	n	SCC	n	SCC
November	488	653	498	415
December	537	567	561	437
January	653	647	628	542
February	714	693	677	464
March	791	734	769	555
April	690	713	706	633
May	631	759	621	571
Cows positive for pathogens				
November	346	635	256	434
December	387	577	284	477
January	468	678	315	579
February	514	723	347	488
March	564	784	391	597
April	490	750	357	659
May	446	804	323	648
Cows negative for pathogens				
November	197	594	242	395
December	202	518	277	396
January	241	567	313	505
February	245	607	330	439
March	225	607	378	512
April	224	646	349	606
May	212	635	298	487

Conclusions

The results of this study fully support our earlier observations (Baltay and Jánosi, 2001), according to which a substantial proportion of cows producing milk with an SCC below 400,000 per ml are affected by subclinical mastitis. Of the approx. 3000 dairy cows examined at the large-scale dairy farm studied by us, 1460 cows had at least one Mastitest-positive udder quarter, and 54.6% of these cows produced milk with an SCC below 400,000/ml, i.e. appeared healthy on the basis of the individual bulk milk SCC. The observation that 41% of the 315 cows excreting *S. aureus* also produced milk with an SCC below 400,000

per ml has particular practical importance. Such cows pose a serious risk of infection to the healthy herdmates.

Therefore, regular screening tests involving the entire herd should become an indispensable element of professional and efficient mastitis control. Such screening tests should include determination of the SCC of individual bulk and udder-quarter milk and the bacteriological examination of udder quarters with subclinical mastitis.

From the data obtained in this study it can be established that the rear udder quarters more often become affected by mastitis.

Different pathogens cause an SCC elevation of dissimilar degree. CNS cause a lower elevation of the SCC. Despite this fact, the adverse effect exerted by CNS on the bulk milk of the herd is substantial, as CNS are the commonest microorganisms causing subclinical mastitis on the farm. In addition to *S. aureus* and streptococci widely known to have high pathogenicity, the so-called coliform bacteria and yeasts also have a non-negligible role in the aetiology of subclinical mastitis occurring on the farm.

In view of the fact that the somatic cell count of the milk of cows with subclinical mastitis tends to increase with advancing lactation, an effective drying-off therapy must be used to restore udder health. Whenever such a measure is justified (e.g. if therapeutic attempts have failed), culling of cows based on individual evaluation of the affected animals cannot be avoided.

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