

Somatic cell count in quarter foremilk of cows from small herds with a high level of subclinical mastitis

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ABSTRACT

Aseptic foremilk samples of 213 cows from 77 small farms were collected. Of 852 samples, 65.5% were infected. The most frequently isolated pathogens were *Streptococcus agalactiae* (22.7%), *Staphylococcus aureus* (18.8%) and *Staphylococcus epidermidis* (18.3%). Somatic cell count (SCC, in thousand/ml) in foremilk samples, determined by Fossomatic, ranged significantly ($P < 0.01$) between bacteria from 313' (non-infected) to 2128' cells/ml (infected by *Streptococcus* species). A high percentage of infected samples with no or weak SCC reaction was observed within all bacteriological findings. Thresholds of 120' cells, determined from a density distribution of SCC in uninfected and infected quarters, correctly detected 68% samples with 33 and 31% false negatives and false positives, respectively. Corresponding proportions at a threshold of 410' cells, estimated from healthy quarters were 55, 66 and 14%. The correlation coefficient between the SCC and the California Mastitis Test (CMT) was 0.61. For both SCC and CMT test the probability of misclassification for quarters infected with secondary pathogens was higher than those infected by streptococci.

KEY WORDS: mastitis, udder pathogens, SCC, CMT, threshold

INTRODUCTION

The subclinical form of mastitis shows no visible signs of intramammary infection, but the number of epithelial cells and leukocytes (called the somatic cell count — SCC) will be elevated.

The best method of diagnosing mastitis is bacteriological analysis of quarter foremilk in combination with SCC (IDF, 1987). In mass examinations, diagnosis has been limited to measuring bulk SCC levels alone (Gordon et al., 1980). Correct classification of milk samples into infected and uninfected groups depends on an SCC threshold; 500' cells/ml is commonly accepted as the

threshold value. A more recent proposal of the International Dairy Federation (1987) is lower (250' cells), especially when individual quarter samples are used for diagnosis.

The single most important factor affecting SCC in milk is the infection status of the mammary gland (Brolund, 1985). The SCC level of bacteriological findings differs between species of bacteria (Hogan et al., 1988). However, information on the relationship between SCC and the bacterial species present is sparse and unreliable (IDF, 1987; Timms and Schultz, 1984; Vecht and Wisselink, 1990).

Contrary to other countries, milk production in Poland is based on a large number of small herds with relatively poor hygienic conditions. High mastitis prevalence in such herds has been reported by several authors (e.g. Kozanecki and Kostrzyński, 1987; Kurek et. al., 1990), but there is little information concerning the degree of inflammatory response to the type of infection (Kostrzyński and Kozanecki, 1990). Moreover, while automatic counters (such as Fossomatic) are predominant for monitoring of SCC in most countries the indirect methods, like the California Mastitis Test (CMT), have until now been used alone. So far, no attempt has been made to investigate the validity of the SCC threshold with reference to small dairy herds.

The objectives of this study were 1. to examine the influence of udder pathogens on SCC and CMT score; and 2. to investigate the predictive value of SCC thresholds in detecting mastitis in small herds with high rates of bacterial infection.

MATERIAL AND METHODS

Milk which originated from 77 small farms (each from 2 to 7 cows) samples were obtained from cows with no clinical signs of mastitis. On these farms animals were kept in conditions typical for the central region of Poland. Cows were normally milked by hand and the milk yield was on the average of ca 3000 l per year. Most animals were in their 5–6 lactation and cows with colostral milk (7 days *post partum*) and those in late lactation (21 days *pre partum*) were excluded from the study.

A foremilk sample from each quarter was collected from all cows at the morning milking. Prior to collection the udder was washed thoroughly with water and dried with a towel. Teats were carefully cleaned with 70% ethanol. The first 4 milk streams were rejected, but the next ones were examined macroscopically for abnormalities. The CMT-test was carried out with a commercial reagent „Mastirapid” by mixing 2.5 cc of milk with 2.5 cc of reagent according to the Polish standard methodology (Wiśniowski, 1965). The reactions were graded into five categories: negative 1 (“-”), trace 2 (“- +”), positive 3 (“+”), positive

4 ("++") and positive 5 ("+++"). Milk samples were obtained aseptically from each quarter and put into sterile vials: one for bacterial culture evaluation and the second for somatic cell count determination. The samples were delivered to the laboratory within two hours and immediately analyzed. The bacteriological examination of milk was done with methods obligatory in Poland (Instruction, 1978). The samples were determined as infected if pathogens were present. Pathogens were classified as follows: major pathogens comprised of *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus* species (other than *Streptococcus agalactiae*) and minor pathogens comprised of *Streptococcus epidermidis* and micrococci.

For all samples the somatic cell count was determined by the Fossomatic apparatus (Foss-Electric, Hillerod, Denmark). The SCC were denoted in 1000 cells per 1 ml and transformed to a log scale with base 10 (LSCC) to allow statistical analysis because cell counts formed non-normal distributions with a pronounced skewness to the right. Where appropriate, the geometric mean (GSCC) was calculated from the LSCC.

In order to exclude systematic effects such as herd, parity and stage of lactation, data for each quarter were computed as the difference from the SCC of milk from the apparently healthiest quarter of the same cow (Mijnen et al., 1982). The healthiest quarter was the one which was pathogen-free and had the lowest SCC. Consequently the cell count difference of a certain quarter was computed as the actual cell count of that quarter minus the cell count of the quarter showing the lowest cell count. A total 363 comparisons were done.

In order to determine the threshold value of somatic cells, quarters originating from the same udder were divided into groups of healthy and mastitic, respectively. Based on means and standard deviations, the intersection points of the density distribution of somatic cells in healthy and mastitic quarters were determined. Infected quarters with cell counts below the estimated or proposed cell count threshold were termed false negatives (FN) while noninfected quarters with cell counts above that threshold were termed false positives (FP) (Andrews et al., 1983). A total 852 foremilk samples were collected from 213 udders.

RESULTS AND DISCUSSION

The low rate of contaminated samples (below 2%) indicates proper sampling procedures and that most of the bacteria isolated were recovered from the gland or the teat canal.

The overall distributions of bacteriological quarter status are shown in Table 1. Totally 34.5% of samples were classified as negative. Different species of pathogens causing udder infection were detected. Of 852 quarter foremilk

TABLE 1

Infection status of 852 quarters

Pathogen isolated ^{x)}	No.	Percent of	
		total	infected
None	294	34.5	—
Major			
<i>Streptococcus agalactiae</i>	127	15.0	22.7
Other streptococci	54	6.3	9.7
<i>Staphylococcus aureus</i>	105	12.3	18.8
Minor			
<i>Staphylococcus epidermidis</i>	102	12.0	18.3
Micrococcus sp.	53	6.2	9.5
Mixed	64	7.5	11.5
Other	53	6.2	9.5
Total infected	558	65.5	100.0

^{x)} Pathogens were isolated from a simple sample

samples 33.6% were positive for major pathogens and 18.2% were positive for minor pathogens. *Streptococcus agalactiae* was isolated in 22.7% and appeared to be the primary organism causing udder infection of the cows. The next most frequently isolated major pathogen was *Staphylococcus aureus* (18.8%). *Staphylococcus epidermidis* was the predominant minor pathogen (18.3%). Other mixed organisms as well as miscellaneous pathogens (if bacteriological status could not be confirmed, due to the presence of mixed cultures and organisms

TABLE 2

Arithmetic (A) and geometric (G) means of somatic cell count (x 1000/ml) in foremilk by bacteriological status of quarter (n = 735)

Pathogen	Mean of SCC	
	A	G \pm s.d.(log)
None	313	61 \pm 0.728 A
<i>Staphylococcus aureus</i>	683	168 \pm 0.749 B
<i>Streptococcus agalactiae</i>	1129	349 \pm 0.682 C
Streptococcus sp.	2182	512 \pm 0.825 C
<i>Staphylococcus epidermidis</i>	347	156 \pm 0.559 B
Micrococcus sp.	1023	118 \pm 0.793 AB
Major	1164	287 \pm 0.756
Minor	579	142 \pm 0.672
Total infected	958	224 \pm 0.741
Overall mean	700	132 \pm 0.787

A, B, C, D — Means with different superscripts are significantly different $P < 0.01$

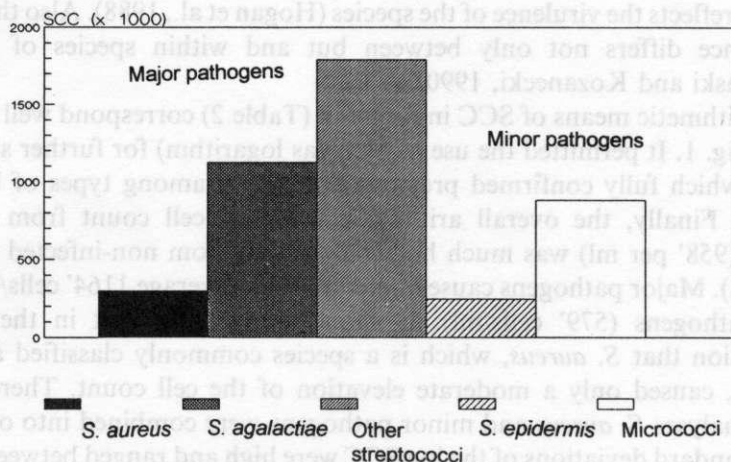


Fig. 1. Effect of various pathogens on cell count as difference with the healthiest quarter within udders.

causing sporadic infections) which consist at least 21% of total infected samples are shown in Table 1. The other Polish field studies (Malinowski et al., 1992; Kostrzyński and Kozanecki, 1990) also reported that *S. agalactiae* and *S. epidermidis* are the most common bacteria in milk.

Pathogens were isolated in all herds; prevalence ranged from 12 to 100%. In general, there were only 13 cows (6%) without bacteria. On the other hand, sixty three udders (30%) had pathogens in all of their quarters.

The level of overall bacterial infection presented here (65.5%) was high but it was typical for home milk production. For example, Malinowski et al. (1992) found the presence of bacteria (46.4%) in quarters when samples with only positive CMT reaction were examined. A very low rate of cows (6.9%) with no udder pathogens was also demonstrated by Dudko (1990).

The cell counts in samples from quarters with different udder health status are given in Fig. 1 and Table 2. Fig. 1 graphically express the real arithmetic differences in SCC with respect to pathogen agents. As expected, bacterial infection markedly influenced the SCC in foremilk. The presence of both *S. aureus* and *S. epidermidis* increased the cell number by not more than 300' cells/ml compared to the uninfected quarter within the udder.

A stronger SCC, reaction was observed for micrococci and streptococci. However, when streptococci were divided into species it was found that *S. agalactiae* was about 500' SCC higher than streptococci except for *S. agalactiae*. Other authors (Vecht et al., 1989) have found generally similar responses with

respect to bacterial infection. The cell count levels of different bacteria species probably reflects the virulence of the species (Hogan et al., 1988). Also the degree of virulence differs not only between but and within species of bacteria (Kostrzyński and Kozanecki, 1990).

The arithmetic means of SCC in foremilk (Table 2) correspond well with the data of Fig. 1. It permitted the use of SCC (as logarithm) for further statistical analysis which fully confirmed previous differences among types of bacterial infection. Finally, the overall arithmetic mean of cell count from infected quarters (958' per ml) was much higher than that from non-infected quarters (313' cells). Major pathogens cause higher SCC (on average 1164' cells/ml) than minor pathogens (579' cells/ml). It should be pointed out in the present investigation that *S. aureus*, which is a species commonly classified as major pathogen, caused only a moderate elevation of the cell count. Therefore, in further analyses *S. aureus* and minor pathogens were combined into one class.

The standard deviations of the log SCC were high and ranged between 11.5% (*S. epidermidis*) and 15.6% (micrococci). They were somewhat lower than those reported by Dohoo et al. (1981), Lindstrom (1983) and Brolund (1985). Two-thirds of measurements in non-infected quarters were below 100' and of 88% were below 500' cells/ml. On the other hand, only single quarters with established micrococci infections showed cell counts with the same levels as for streptococci infections, many quarters (29%) showed no cellular response at all (below 100' cells). Consequently, a high proportion of infected samples with no or trace reaction in SCC reduced the differences between means of positive and negative quarters. This suggests that it is difficult to estimate reliable threshold values.

In the study reported here, the thresholds were determined twofold based on the normal distribution of LSCC.

First, the intersection points of the density distribution of the somatic cells in healthy and mastitis quarters were determined (Fig. 2). The basic experimental approach was to compare at least two quarters of the same udders, non-infected and infected, by specific bacteria. In the cows infected by streptococci the intersection points reached 179' cells/ml, while in cows infected by other pathogens the critical cell count threshold was considerably lower (80' cells/ml). A pooled cut off was obtained at SCC = 120' cells/ml.

Next, considering that in a normally distributed population 98% of all samples are below a value corresponding to the mean plus 2 standard deviations, the value of 4.507 ± 0.55 for a LSCC of 134 healthy quarters (no pathogen and CMT reaction of 1 and 2) was used as estimates of upper limits for the healthy variation. The CMT is often used in investigations (e.g. by Dohoo et al., 1981) as diagnosis parameter of udder health. A corresponding threshold 410' cells/ml

TABLE 2

Percentage of quarters with somatic cell count	Threshold (cells/ml) ($\times 1000$)	Threshold (log units)
120	120	2.08
410	410	2.61
500	500	2.70

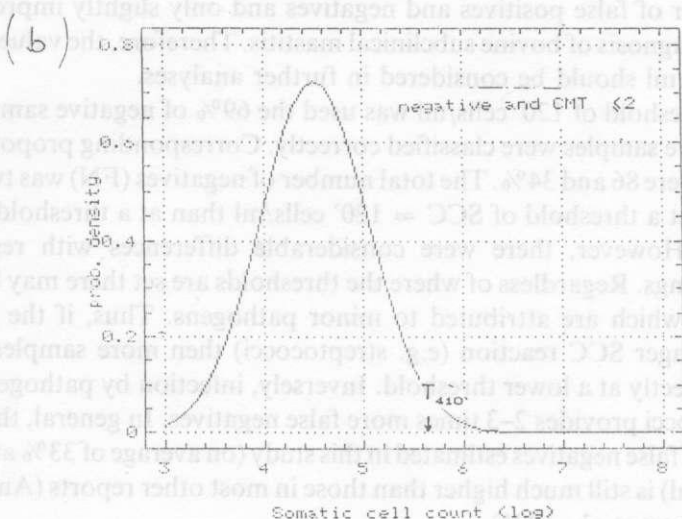
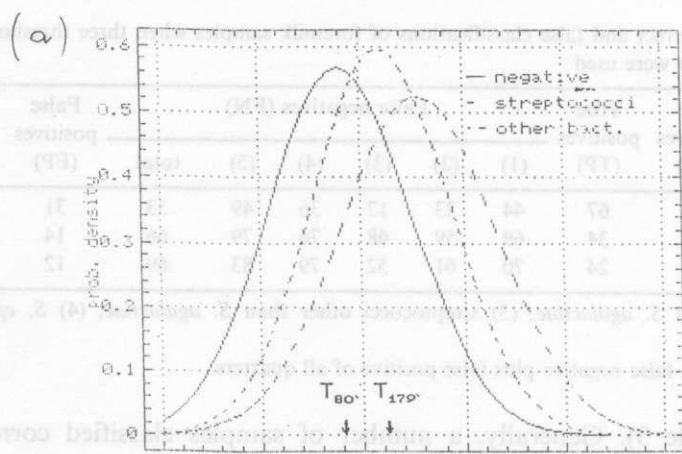


Fig. 2. Determination of threshold values ($t \times 1000$) for the cell count in foremilk based on the frequency distribution: (a) — of groups uninfected and infected by streptococci (T_{170}) and other bacteria (T_{80}); and (b) — only of healthy quarters (T_{410}) (no pathogens and CMT score ≤ 2).

was obtained which is also marked in Fig. 2. This value lies below standard cell count threshold of 500' cells per ml (IDF, 1987).

Two thresholds obtained from the present study, i.e. of 120' and 410' cells/ml as a well as of 500' from IDF (1987) were examined as indicators of subclinical

TABLE 3

Percentages of correct and false classifications of foremilk samples when three thresholds of the somatic cell count were used

Threshold (x 1000)	True negatives (TN)	True positives (TP)	False negatives (FN)						False positives (FP)	Error ^{x)}
			(1)	(2)	(3)	(4)	(5)	total		
120	69	67	44	23	17	36	49	33	31	32
410	86	34	69	59	48	76	79	66	14	45
500	88	24	70	61	52	79	83	69	12	46

(1) *S. aureus*, (2) *S. agalactiae*, (3) streptococci other than *S. agalactiae*, (4) *S. epidermidis*, (5) micrococci

^{x)} Error (total) — false negative plus false positive of all quarters

mastitis (Table 3). Generally, a number of samples classified correctly or incorrectly markedly changed with an elevation of threshold value from 120 to 410 cells/ml. The setting of higher SCC threshold (500 cells/ml) had little effect on the number of false positives and negatives and only slightly improved the accuracy of diagnosis of bovine subclinical mastitis. Therefore, the values of 120 and 410 cells/ml should be considered in further analyses.

When a threshold of 120 cells/ml was used the 69% of negative samples and 67% of positive samples were classified correctly. Corresponding proportions at 410 cells/ml were 86 and 34%. The total number of negatives (FN) was two times lower (33%) at a threshold of SCC = 120 cells/ml than at a threshold = 410 cells (66%). However, there were considerable differences with respect to bacterial findings. Regardless of where the thresholds are set there may be many FN, most of which are attributed to minor pathogens. Thus, if the bacteria caused a stronger SCC reaction (e.g. streptococci) then more samples will be classified correctly at a lower threshold. Inversely, infection by pathogens other than streptococci provides 2–3 times more false negatives. In general, the lowest percentages of false negatives estimated in this study (on average of 33% at SCC = 120 cells/ml) is still much higher than those in most other reports (Andrews et al., 1983; Dohoo et al., 1981).

It is quite possible that the high percentage of infected milk samples with no or weak SCC reaction could be caused by a streak of canal colonization or latent infections of the parenchyma (Heald et al., 1977). It is well known that most pathogens colonize the teat canal before they invade upper parts of the mammary glands (Schultze et al., 1978). According to Matilla (1985), subclinical infections by common pathogens often are accompanied by only a marginal increase in cell counts. The high prevalence also may indicate a high occurrence of short time infections which often are spontaneously eliminated (Timms and Schultz, 1984).

The number of non-infected quarters classified as infected (FP) diminished from 31 to 14% as SCC in foremilk increased from 120 to 410' cells/ml. Many biological (parity, stage of lactation, milk yield) and environmental herd (housing, feeding, milking procedures) factors can elevate the SCC in non-infected udders (Brolund, 1985). It is likely that higher lactation number (on average of 5-6) of cows and poor environment have been responsible for non-bacterial cells rising in the examined farms. Older cows have higher average cell counts than younger cows (Jaartsveld et al., 1983). In herds with extreme high/low milk yield the interpretation of the SCC in milk may also be difficult (Vecht et al., 1989).

The most important criterion in the selection of the somatic cell threshold is the intended use of the information. If the goal is to use the SCC information in mastitis control, the threshold must be set to express the true dynamics of mastitis, properly. It can say that in farms with high udder pathogen frequency, using 120' cells/ml as a threshold, will provide not more than 70% correct diagnosis. A large overlapping region between non-infected and infected samples prevents a reduction in misclassifications. A similar problem appeared in studies concerning the determination of the threshold value of SCC in large-scale farms. For instance, Sender et al. (1987) classified samples more correctly (with 82.6% at a threshold of 324' cells/ml) than in present study, but also showed the large overlapping region of SCC. Thus, it could be presumed the high variability of SCC within the herd is a common problem, both in large and small herds.

On the other hand, if the SCC program is oriented toward treatment of udder infection, it would be appropriate to set the threshold low enough to assure that no infections are missed and thus go untreated. However, in practice one cannot expect that any reference diagnosis can give a true picture of udder health. According Andrews et al. (1983), when thresholds are chosen for dry cow therapy, much higher numbers of false positives instead of false negatives can be tolerated. Moreover, the choice of the threshold value is dependent on the milk loss/treatment expenses ratio. Some authors (Andrews et al., 1983) reported that yield of milk from subclinically affected udders may be reduced by up to 45%, but no reliable Polish data are attainable for small farms. Using 410' instead of 120' cells/ml results in a lower proportion of false positives (14 vs 31%). The number of uninfected samples correctly diagnosed (around 90%) is often used as the threshold SCC (Batra and McAllister, 1984). The 55% correct classifications achieved in the present study with a SCC threshold of 410' cells/ml is not promising for the near future, but it may be representative for many Polish regions. Since mastitis is a multifactorial disease, further studies, apart from bacteria, on the influence of different environmental and management factors on the SCC level in quarter as well in udder and bulk milk are needed.

Monitoring of SCC in bulk milk, which has the place in most national programs, allows control of mastitis in a herd but it does not identify problem of cows. In commercial herds, an indirect method like the California Mastitis Test is often used in preliminary diagnosis of subclinical mastitis.

Comparison of Fossomatic and CMT data are shown in Table 4. The arithmetic average of somatic cells increased from 111 to 2136' per ml with an increase in the CMT score from 1 to 5, respectively. The geometric means derived from CMT=1 (negative) and CMT=2 (trace) were similar, whereas the

TABLE 4

Agreement of CMT and distribution of pathogens in relation to CMT score

CMT score ^A	Mean of SCC (x1000)		Agreement CMT	Distribution rate (%) of infection status						Percent of all quarters	
	A	G	%	(0)	(1)	(2)	(3)	(4)	(5)	infected	uninfected
1 (141)	111	41 ^A	91	22	20	10	7	27	19	54	46
2 (180)	147	54 ^A	20	34	16	13	18	22	28	45	55
3 (130)	427	99 ^B	12	22	12	19	15	18	17	53	47
4 (123)	679	262 ^C	18	10	23	25	28	16	13	73	24
5 (155)	2136	827 ^D	10	12	29	33	32	17	23	77	23
≤2 (321)	131	48 ^A	94	56	26	23	25	49	47	49	51
≥3 (414)	1142	288 ^B	41	44	74	77	75	51	53	69	31

(0) No pathogen, (1) *S. aureus*, (2) *S. agalactiae*, (3) streptococci other than *S. agalactiae*, (4) *S. epidermidis*, (5) micrococci

x/ CMT₁ ≤ 200; CMT₂ = 150 - 500; CMT₃ = 400 - 1500; CMT₄ = 800 - 5000; CMT₅ ≥ 5000

A, B, C, D — P < 0.01

A — arithmetic, G — geometric mean

differences among other scores was significant (P < 0.01). No bacteriological status x CMT score interaction, was detected. Comparison of CMT pattern with the arithmetic means for SCC obtained in present study demonstrate that lower values are assigned by Fossomatic than CMT. There is an agreement between CMT and Fossomatic scores, they decreased with increasing cell numbers to a low of 12% for CMT=5. However, if the test were grouped into two main classes (CMT ≤ 2 as negatives and CMT ≥ 3 as positives) a percentage of agreement CMT was 94 and 41%, respectively. In this study a phenotypic correlation of CMT score with somatic cell count was 0.61. A higher correlation (0.74) was found by Batra and McAllister (1984). Thus CMT is a rough method to estimate the cell count. However, as is shown in Table 4, the larger streptococci infection (75–77%) could be detected by a CMT score ≥ 3 than infection by other bacteria (51–74%).

In the present work the CMT score ≥ 3 as a threshold and the presence of microorganisms was used in a cause and effect model (IDF, 1987). Of 414 samples with a CMT score of 3–5, only 69% were mastitic and the remainder of the samples were classified as nonspecific mastitis. On the other hand 49% of infected samples still had a CMT score ≤ 2 (latent infection). Therefore, if individual measurements of CMT without laboratory diagnosis are used for selective antibiotic therapy, it must be accepted that appreciable numbers of infected cows will be treated and some infected cows will be not treated. From the present study it could be confirmed that the CMT test gives only an early indication of mastitis.

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STRESZCZENIE

Liczba elementów komórkowych w mleku z chłopskich obór z wysokim udziałem podklinicznych stanów zapalnych wymienia krów

Badaniami objęto 213 krów z 77 gospodarstw chłopskich. Próbkę mleka pobierano osobno z każdej ćwiartki wymienia. W mleku określono liczbę elementów komórkowych (SCC) przy pomocy aparatu Fossomatic i testu TOK oraz rodzaj zakażenia bakteryjnego. W 65,5% próbkach mleka stwierdzono obecność bakterii, spośród których najczęstszymi były *S. agalactiae* (22,7%), *S. aureus* (18,8%) i *S. epidermidis* (18,3%). Średni pomiar SCC, zależny od typu zakażenia, wahał się od 313 (mleko niezakażone) do 2128 tys./ml (mleko zakażone *S. species*), ale w znacznym procencie zakażonych próbek nie stwierdzono podwyższenia poziomu SCC. Wartość graniczna dla SCC obliczona dwoma sposobami wynosiła 120 lub 410 tys./ml. Pozwalała ona na prawidłowe zidentyfikowanie odpowiednio w 68 lub 55% próbek, ale jednocześnie 31 lub 14% niezakażonych próbek zostało mylnie uznanych za zakażone. Test TOK wykrywał bakterie w mleku z 51–77% dokładnością. Korelacja między SCC a TOK wynosiła 0,61. Niezależnie od metody mierzenia poziomu elementów komórkowych, podkliniczny stan zapalny wymienia można było przewidzieć najdokładniej w przypadku obecności paciorkowców.