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From the State Veterinary Laboratory for Northern Norway, Harstad, Norway.

# CELL CONTENT IN GOAT'S MILK*

By

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PETTERSEN, KJELL-EIRIK: *Cell content in goat's milk.* Acta vet. scand. 1981, *22*, 226—237. — The geometric mean cell count of goat's milk in mid-lactation was $880 \times 10^3$ cells/ml and $690 \times 10^3$ cells/ml estimated by the Direct Microscopic Count (DMC) and the Electronic Cell Count (ECC), respectively. The numerous cell fragments in goat milk may have had an influence on the results obtained by the cell counting methods compared.

A highly significant correlation ($P < 0.001$) existed between the results obtained by the above mentioned methods, and the California Mastitis Test.

If routine surveys are done on milk samples of goats, a CMT score of 1, 2 or 3 should be regarded as normal in the mid-lactation. A higher CMT score may indicate an abnormal condition of the udder. In the diagnosis of mastitis in goats great attention should be paid to the difference in cell content between milk samples from halves of the same udder.

cell content; goat's milk; caprine mastitis.

Several types of somatic cells have been reported to be present in normal milk (*Schalm et al.* 1971). The content of cells increases in mastitic milk primarily due to an overwhelming number of leucocytes infiltrating from the blood. The diagnosis of mastitis is based on cytological and microbiological test methods (*Tolle* 1975, *Heeschen* 1978).

Various methods have been developed for estimating the cell content in milk (*Schalm et al.* 1971). The California Mastitis Test is an indirect method widely used for routine purposes in laboratories and under field conditions. The test is subjective and has strict limitations, primarily because of the wide overlap of cell ranges.

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Microscopic cell counting is a direct, visual method for determining the number of cells in milk. Today electronic cell counting is often used for somatic cells in milk. This is a very rapid and fairly accurate procedure, but still the microscopic method serves as a reference.

Few reports have dealt with the cell content in normal and mastitic milk of goats and with the correlation between different methods used to determine the cell content.

The aim of the present work was to study the cell content in goat's milk, and the relationship between three methods for estimating the cell content.

## MATERIAL AND METHODS

### *Samples*

The study was conducted in a herd of 60 goats. Milk samples of the udder halves at a total of 1972, were collected at weekly intervals during a lactation. The samples were drawn from healthy udders and from udders with mastitis. The period of study lasted from January to May and from September to November.

The milk samples were collected prior to the morning milking and were examined within 36 h.

Practically all parturitions took place in January and February. The daily milking was carried out with a pipeline milking system. Some of the goats were culled during the lactation period on account of diseases, injuries or low milk yield.

The lactation period was divided in three; Period 1: 0—14 days after parturition, Period 2: 15—120 days after parturition, Period 3: > 240 days after parturition.

### *Cytological examinations*

California Mastitis Test (CMT). The test was carried out at the laboratory using a method described by *Schalm & Noorlander* (1957). Reactions were graded 1, 2, 3, 4 or 5 in accordance with *Klastrup & Schmidt Madsen* (1974).

Direct Microscopic Count (DMC). 0.01 ml of each milk sample was spread over 1 $cm^2$ of a glass slide. The film was dried and stained with methylene blue according to *Prescott & Breed* (1910). The number of cells in 20 fields was counted and this figure was multiplied by 20,000 (working factor) to

give the number of cells per ml. All types of leucocytes and epithelial cells were counted.

Electronic Cell Count (ECC). Using a Coulter FM/ milk cell counter, the milk samples were subjected to a test described by *Tolle et al.* (1966)*.

*Bacteriological examinations*

For the bacteriological examination of milk samples agar containing 7 % bovine blood was used. The bacteria were identified by methods described by *Klastrup & Schmidt Madsen.*

*Udder health*

The occurrence of clinical mastitis was reported by the dairyman. All udder halves without clinical signs or not infected with Staphylococcus aureus were regarded as healthy.

*Statistical methods*

Statistical analyses were carried out by means of regression and analyses of variance. Arithmetic values were used in the calculation of the coefficients of variation between the DMC and ECC methods. Otherwise the geometric values were used (*Pearson et al.* 1970, *Dyrendahl* 1977, *Syrstad & Røn* 1978). The CMT results are always arithmetic values.

## RESULTS

*Cell content*

The geometric mean cell count of milk samples from healthy udders in mid-lactation (Period 2) was as shown in Table 1, $880 \times 10^3$ cells/ml and $690 \times 10^3$ cells/ml estimated by the DMC and ECC methods, respectively. The mean of the CMT scores obtained for the same milk samples was 2.7. The cell content in milk samples from healthy udder was significantly lower in mid-lactation, than in samples collected at the beginning and in the latter part of the lactation. The CMT scores from the periods 1 and 2 make exceptions since no significant difference was found between these values.

* This test was carried out at the National Veterinary Institute, Oslo, Norway.

Table 1. The cell content in milk from goats with healthy udders.

| Method | Cell content | | | | | | | | | F-values for differences in cell content between | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0—14 days after parturition (Period 1) | | | 15—120 days after parturition (Period 2) | | | > 240 days after parturition (Period 3) | | | | | |
| | n | $\bar{x}$ | s | n | $\bar{x}$ | s | n | $\bar{x}$ | s | periods 1 and 2 | periods 1 and 3 | periods 2 and 3 |
| DMC[a] | 154 | 1230 | 290 | 1380 | 880 | 240 | 432 | 3040 | 390 | 17.71*** | 66.22*** | 507.52*** |
| ECC[b] | 122 | 1040 | 290 | 1354 | 690 | 260 | 430 | 1940 | 430 | 19.90*** | 18.45*** | 279.36*** |
| CMT[c] | 154 | 2.5 | 1.5 | 1380 | 2.7 | 1.4 | 434 | 3.4 | 1.3 | 2.87 | 111.98*** | 229.99*** |

[a] Direct Microscopic Count — geometric mean ($10^3$/ml).
[b] Electronic Cell Count — geometric mean ($10^3$/ml).
[c] California Mastitis Test — arithmetic mean.
*** $P < 0.001$.

Table 2. Geometric means of the cell count ($10^3$/ml) obtained by direct microscopic count (DMC) and electronic cell count (ECC) by scores of the California Mastitis Test (CMT).

| CMT scores | DMC | | | ECC | | |
|---|---|---|---|---|---|---|
| | n | $\bar{x}$ | s | n | $\bar{x}$ | s |
| 1 | 531 | 690 | 260 | 526 | 490 | 230 |
| 2 | 243 | 800 | 230 | 235 | 610 | 250 |
| 3 | 324 | 820 | 220 | 213 | 620 | 230 |
| 4 | 501 | 1230 | 210 | 485 | 920 | 240 |
| 5 | 367 | 4520 | 310 | 351 | 3730 | 390 |

Corresponding to CMT scores 1, 2 and 3 only small differences were demonstrated in the estimated mean cell counts (Table 2). There was, however, a marked rise in the cell count when the CMT score increased from 3 to 4, and a very significant rise from 4 to 5. The histograms illustrated in Fig. 1 strongly support this. Furthermore there was a close correlation between the results estimated by DMC, ECC and CMT (Table 3).

A variation in cell counts was found in weekly milk samples from normal udder halves und halves with mastitis. A high CMT score (4 or 5) was sometimes found in mid-lactation, in both udder halves, even if there was no evidence of mastitis (Fig. 2).

The cell content in milk from udder halves suffering from clinical mastitis during the sampling week had a mean CMT score of $5.0 \pm 0.0$ while the mean cell count ($10^3$/ml) of DMC and ECC was estimated to be $8320 \pm 420$ and $9770 \pm 300$, respec-

Table 3. Correlations among the results obtained by direct microscopic count (DMC), electronic cell count (ECC) and California Mastitis Test (CMT).

| Methods compared | | n | Correlation coefficient |
|---|---|---|---|
| DMC (R) | ECC (R) | 954 | 0.825*** |
| DMC (L) | ECC (L) | 955 | 0.767*** |
| DMC (R) | CMT (R) | 984 | 0.512*** |
| DMC (L) | CMT (L) | 955 | 0.504*** |
| ECC (R) | CMT (R) | 988 | 0.509*** |
| ECC (L) | CMT (L) | 955 | 0.505*** |

R: Right half of the udder.
L: Left half of the udder.
*** $P < 0.001$.

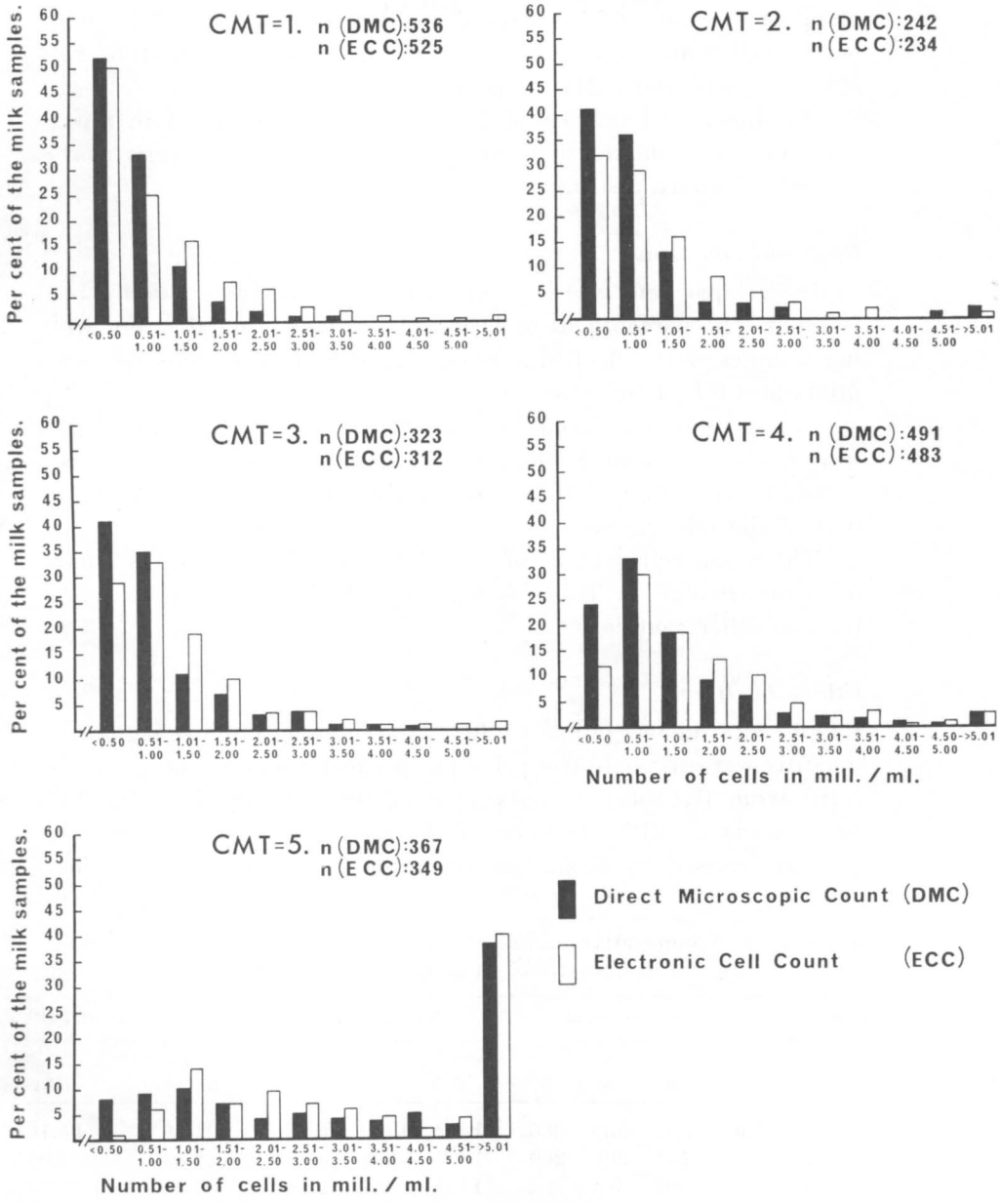


**Figure 1. Distribution of cell count against scores of California Mastitis Test (CMT).**

tively. The corresponding results obtained from the healthy gland of the same udder showed a mean CMT score of 2.1 ± 0.9 and a cell count ($10^3$/ml) of DMC and ECC estimated to be 620 ± 210 and 780 ± 240, respectively.

As shown in Figs. 3 and 4 the increased cell count in milk samples from glands with mastitis caused by S. aureus may persist for many weeks.

*Bacterial isolations*

In 71.1 per cent of the samples no microorganisms could be isolated. The percentages of bacterial findings from the remaining samples were 3.1 of S. aureus, 25.7 of Staphylococcus epidermidis and 0.1 of Streptococcus sp.

S. aureus was isolated from several weekly milk samples of nine goats and from a single milk sample of five goats. It was noted that infection with S. aureus always was confined to one half of the udder.

The mean cell content of samples containing S. aureus was, as demonstrated in Table 4, significantly higher than the cell level in other samples.

*Udder health*

Clinical mastitis. Eight goats showing clinical signs of mastitis were treated. The principal pathogen was S. aureus, isolated from five of the goats. One of the animals died due to gangrenous mastitis. In spite of therapy three cases of clinical mastitis caused by S. aureus continued in a subclinical form.

Table 4. Comparative results of the cell content and the bacteriological examinations on milk samples from goats.

| Method | S. aureus isolated | | | S. epidermidis isolated | | | No bacteria isolated | | | F-values for differences in cell content between | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | $\bar{x}$ | s | n | $\bar{x}$ | s | n | $\bar{x}$ | s | S. aur./ S. epid. | S. aur./ no bact. |
| DMC[a] | 62 | 3860 | 340 | 506 | 930 | 280 | 1402 | 1230 | 310 | 101.89*** | 61.16*** |
| ECC[b] | 59 | 4030 | 370 | 481 | 800 | 260 | 1362 | 860 | 340 | 129.62*** | 89.02*** |
| CMT[c] | 62 | 4.6 | 1.0 | 507 | 3.4 | 1.2 | 1403 | 2.7 | 1.5 | 53.29*** | 99.73*** |

[a] Direct Microscopic Count — geometric mean ($10^3$/ml).
[b] Electronic Cell Count — geometric mean ($10^3$/ml).
[c] California Mastitis Test.
*** $P < 0.001$.

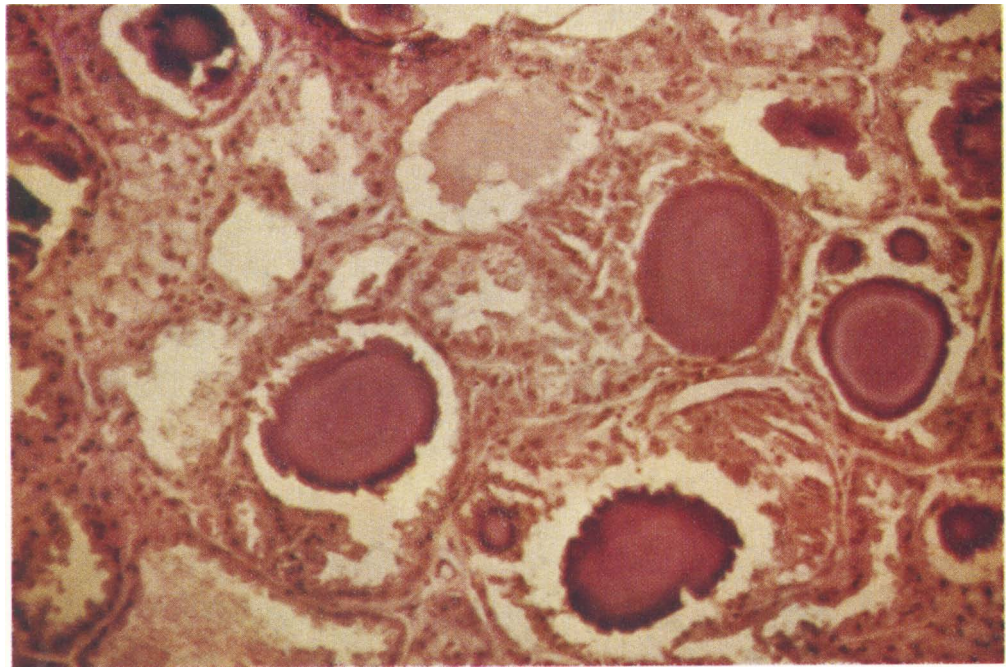

Figure 2. Histological picture of a mammary gland from a goat in mid-lactation with a CMT score of 4. There is no evidence of cellular infiltration in the tissue. The alveoli are normal with epithelial cells in different stages of secretion. A few corpora amylacea surrounded by a low epithelium with elongated nuclei are seen. Hematoxylin-Eosin × 400.

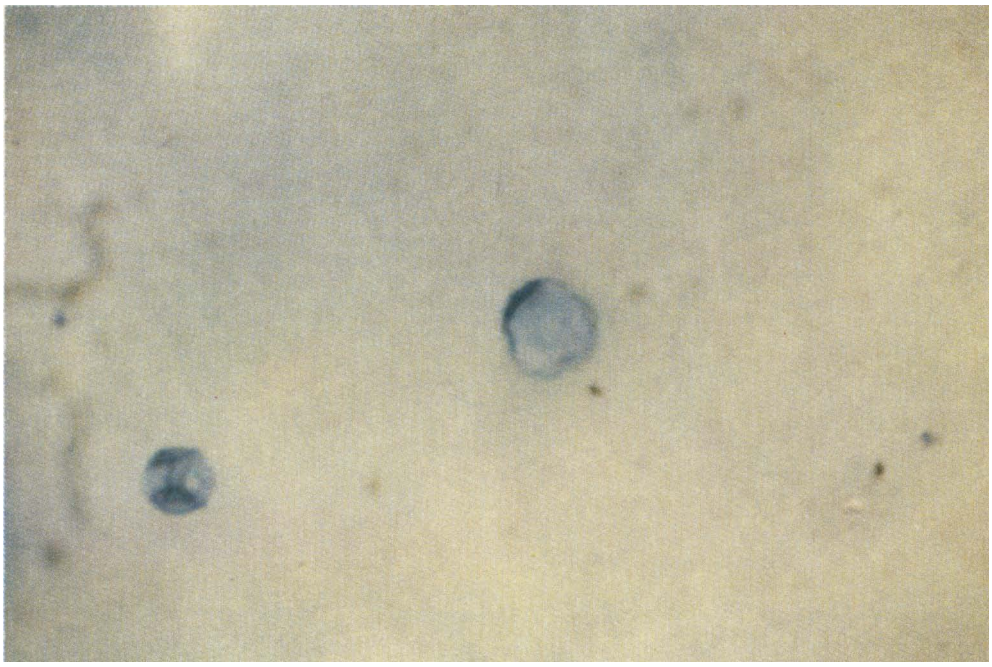

Figure 5. A smear from goat's milk stained with methylene blue, showing cellular fragments with no nucleus ("christiesomes") of the same size as a leucocyte. Methylene blue × 1000.

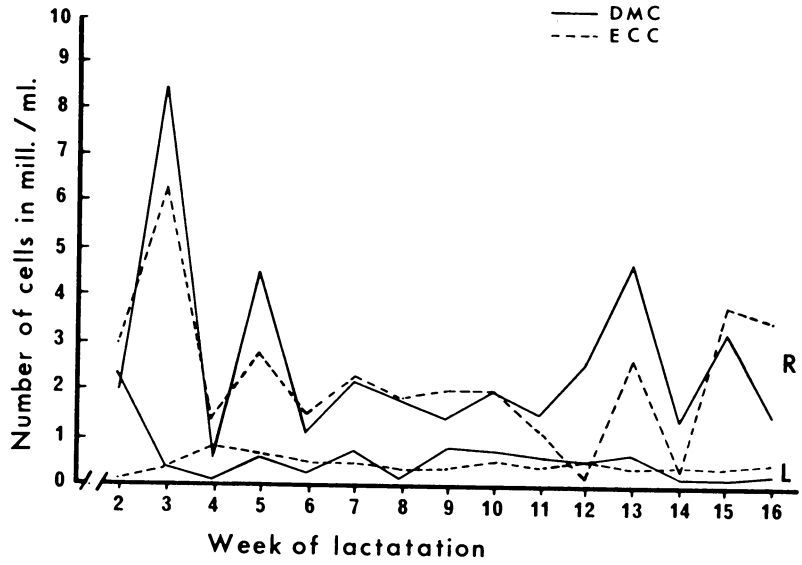


Figure 3. Direct Microscopic Count (DMC) and Electronic Cell Count (ECC) in weekly samples from right (R) and left (L) mammary gland of a goat showing subclinical mastitis in the right gland. S. aureus was isolated from the samples of the right gland in week 2 and 6.

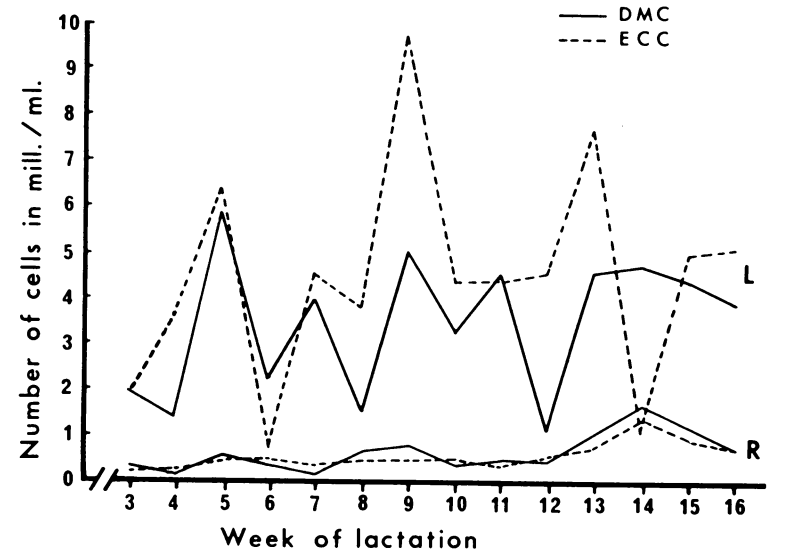


Figure 4. Direct Microscopic Count (DMC) and Electronic Cell Count (ECC) in weekly samples from right (R) and left (L) mammary gland of a goat showing clinical mastitis in the left gland in week 3. S. aureus was isolated from the samples of the left gland every week until the goat was culled.

For the remaining three goats the milk samples were culturally negative.

Subclinical mastitis. The study has demonstrated that four goats shedding S. aureus intermittently in the milk, always showed an increased cell count. No evidence of clinical mastitis was observed. Fig. 2 illustrates the bacterial isolation and cellular reaction in milk samples from a goat with subclinical mastitis. Although the cell count was above normal in every milk sample from the gland, S. aureus was present only in two of 16 samples.

## DISCUSSION

In the present work the mean cell count of milk samples from goats with healthy glands in mid-lactation were estimated by the DMC and ECC methods to be $880 \times 10^3$ cells/ml and $690 \times 10^3$ cells/ml, respectively. The results appear to agree fairly closely with a study made by *Okada* (1960) obtaining an average of $750 \times 10^3$ cells/ml in goat's milk. A Norwegian report (*Nesbakken* 1976) gave a mean cell count of $680 \times 10^3$ cells/ml.

The cell content of milk from non-mastitic goats in mid-lactation is higher than observed for cows in similar studies. *Klastrup & Schmidt Madsen* (1974) suggested $300 \times 10^3$ cells/ml as a limit between normal and abnormal udder secretion, while *Tolle* (1975) and *Heeschen* (1978) recommends $500 \times 10^3$ cells/ml. The CMT scores of 1 to 5 in milk samples from goats did not seem to correspond to the same cell count in milk from cows. The number of cells in milk from goats scoring 1, 2 or 3 is higher than that for cow's milk (*Schalm & Noorlander* 1957, *Schalm et al.* 1971, *Klastrup & Schmidt Madsen*).

A significant correlation was found between the cell count results obtained by the DMC, ECC and CMT. The high correlation between DMC and ECC, $r = 0.825$ and 0.767, found in milk samples from right and left glands respectively, is close to the coefficient of variation of 0.86 obtained by *Roguinsky* (1974).

Although a good correlation was obtained between the microscopic and electronic counts, the cell counts obtained by DMC tended to be higher than the corresponding figures of ECC. This is in contrast to bovine milk where higher cell counts were found by the electronic counting (*Pearson et al.* 1970, *Schalm et al.*).

The numerous cell fragments ("christiesomes") demonstrated in goat's milk (*Christie & Wooding* 1975, *Wooding et al.* 1977) may have had an influence on the results obtained by the cell counting (Fig. 5). In the DMC routine practice these particles are usually regarded as somatic cells. Probably they are counted as if they were somatic cells also in the ECC method.

The round cytoplasmatic structures in goat's milk described in an earlier study by *Schalm et al.* are probably identical with "christiesomes". These cytoplasmatic particles did not react with the CMT reagent, as they were without nucleus.

In the present work a distinct difference was found between the mean cell count in samples from Period 1 and Period 2, while no difference was demonstrated between the mean CMT score of the same periodes. This may be due to a higher content of "christiesomes" in early stages of lactation. The fact that "christiesomes" do not react with the CMT reagent and the possibility of counting these as cells, support this.

The cell count obtained in milk samples from the same udder half showed weekly variation. *Linzell & Peaker* (1972) made observations from day to day of milk yield and milk composition, including cell count, in healthy goats. In general the rise and fall in cell levels was parallel in samples from the same udder. Thus, if one gland deviates from the other, this may indicate a local effect. This is of importance in diagnosing mastitis in goats. For practical use a difference in CMT score $\geq 2$ between samples from halves of the same udder, seems to indicate an abnormal condition in the half showing the highest score.

Milk samples in which S. aureus was present, had a significantly higher cell content than the remaining samples. This may indicate that S. aureus has a considerable ability to initiate an inflammation of the goat's udder.

In the present work S. aureus was the most important pathogen associated with clinical mastitis in goats. This is in agreement with other studies (*Heidrich & Renk* 1967, *Jubb & Kennedy* 1970, *Schalm et al.*, *Plommet* 1974, *Smith & Roguinsky* 1977).

This study showed that sometimes no bacteria could be isolated from milk samples collected from chronic cases of mastitis caused by S. aureus. The reason may be an inadequate testing or that the goats shed the organism intermittently. Studies on cows (*Schalm et al.*) have demonstrated that bovine mammary glands infected with S. aureus may react in that way.

Although S. epidermidis was the most frequently isolated bacteria (25.7 %), no clinical mastitis due to S. epidermidis was observed. However, it cannot be ignored that S. epidermidis may have caused subclinical mastitis. *Holmberg* (1973) in an experimental study of S. epidermidis found the bacteria to be a genuine pathogen in the caprine udder. The local reactions appeared to be of relatively short duration. The bacteria were quickly eliminated from the gland and the elevated cell values decreased. *Smith & Roguinsky* isolated nonhemolytic staphylococci from 30 % of normal halves and from 22 % of halves of udders from goats with assorted clinical problems.

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## SAMMENDRAG

*Celleinnholdet i geitmelk.*

For geiter i laktasjonens hoveddel var det geometriske midlet av celletallet i melk henholdsvis $880 \cdot 10^3$ celler/ml ved direkte mikroskopisk celletelling (DMC) og $690 \cdot 10^3$ celler/ml ved elektronisk celletelling (ECC). Trolig har de tallrike cellefragmenter i geitmelk hatt betydning for resultatene oppnådd ved de metoder for celletelling som ble sammenlignet.

Det var signifikant korrelasjon mellom resultatene fra de ovenfor nevnte metoder og California Mastitis Test (CMT).

Ved rutineundersøkelser av speneprøver fra geit kan CMT reaksjoner på 1, 2 eller 3 i laktasjonens hoveddel betraktes som normalt. I diagnosen av mastitt på geit må det legges stor vekt på innbyrdes forskjell i celleinnholdet mellom speneprøver fra samme jur.

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