

The influence of intramammary antibiotic treatment, presence of bacteria, stage of lactation and parity in dairy goats as measured by the California Milk Cell Test and somatic cell counts

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ABSTRACT

KARZIS, J., DONKIN, E.F. & PETZER, I.M. 2007. The influence of intramammary antibiotic treatment, presence of bacteria, stage of lactation and parity in dairy goats as measured by the California Milk Cell Test and somatic cell counts. *Onderstepoort Journal of Veterinary Research*, 74:161–167

The California Milk Cell Test (CMCT) and somatic cell counts (SCC) on their own were not reliable methods in the identification of subclinical mastitis in the dairy goats studied and should be accompanied by microbiological tests. However, CMCT and SCC were indicators of irritation of the udder parenchyma. In healthy goats Spectrazol Milking Cow (Schering-Plough AH) caused the least and Curaclox LC (Norbrook (ARK AH)) the most irritation of parenchyma after intramammary treatment. The effects of Rilexine 200 LC (Logos Agvet (Virbac)) were intermediate. There was a highly significant difference (P < 0.001) in the mean log SCC between treated and control groups for goats treated with Curaclox LC and Rilexine 200 LC but no significant difference was present in the mean log SCC of treatment and control groups for goats treated with Spectrazol Milking Cow at the 07:00 and at 19:00 samplings. The CMCT was an indicator of the level of SCC in goat milk. The CMCT was more useful in confirming the absence of infection, rather than in diagnosing mastitis.

Keywords: California Milk Cell Test, dairy goats, intramammary antibiotics, mastitis, somatic cell count

INTRODUCTION

In dairy cows, the number of somatic cells per $m\ell$ of milk correlates with udder inflammation or the degree of irritation to the mammary parenchyma (International Dairy Federation 1973). The somatic cell count (SCC) in dairy cows is used as an indicator of the inflammatory response. The exact role that the SCC and California Milk Cell Test (CMCT) can play

in the diagnosis of subclinical mastitis in dairy goats, however, remains uncertain. The measurement of somatic cells in goat milk differs from that in cow milk because the presence of cytoplasmic particles gives a false count. The mammary gland of the goat produces milk by apocrine secretion and DNA-free cytoplasmic particles occur in the milk, which are similar in size to leukocytes. The Coulter Counter will give false readings as it does not distinguish between the cytoplasmic particles and the somatic cells. In addition, intact epithelial cells that are sloughed from acini and ducts are present in variable numbers in goat milk (Smith & Sherman 1994).

Therefore, milk from normal goats has a higher SCC than that of normal cows, and a diagnosis of udder inflammation should be based more on a leukocyte count than on the total SCC (Hinckley & Williams 1981). The normal cell count in goats has been es-

Accepted for publication 9 March 2007—Editor

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timated to be at an SCC either below 700 000 or below 1 000 000 per $m\ell$ (Smith & Sherman 1994).

Smith & Roguinsky (1977) proposed that 1.5 million or more leukocytes per m\ell of goat milk should be regarded as indicative of inflammation of mammary tissue i.e. mastitis. Radostits, Gay, Blood & Hinchcliffe (2000) found the use of SCC as a guide for diagnosis of mastitis in goats controversial due to the variation in SCC in infected and non-infected goats. A physiological threshold of 500 x 10³ cells per me has been suggested by Contreras, Sierra, Corrales, Sanchez & Marco (1996), while Kalogridou-Vassiliadou, Manolkidis & Tsigoida (1992) regarded a count of more than 1 million cells per $m\ell$ as positive for mastitis. Lerondelle, Richard & Issartial (1992) proposed a threshold of 0.8 x 106 for the diagnosis of udder infection. Karzis (2005) found that breed, stage of lactation, parity, season, management, farming systems, type of infective micro-organisms, intramammary treatment and the presence of intramammary infection all have an influence on the SCC.

The arithmetic and geometric means of SCC per m ℓ for each CMCT score were, respectively (Contreras et al. 1996):

- 312 x 10³ and 172 x 10³ for score 0 and traces
- 1014 x 10³ and 531 x 10³ for score 1
- 2912 x 10³ and 2051 x 10³ for score 2
- 4950 x 10³ and 4436 x 10³ for score 3

According to Contreras et al. (1996), the CMCT scores 2 and 3 discriminated between infected and uninfected glands. These results show that both tests (SCC and CMCT) could be used to detect a high percentage of true negative udder halves (uninfected udder halves with low SCC and negative CMCT results), but the percentage of false positive udder halves (uninfected udder halves with high SCC and positive CMCT results) were low. Smith & Sherman (1994) found CMCT more useful for confirming the absence of infection than for diagnosing mastitis in goats. Therefore, caution must be exercised when interpreting the results of CMCT in goats because a negative result may be a good indicator of the absence of infection, but a positive test does not always indicate infection (Lewter et al. 1984).

In this study the degree of udder irritation after intramammary treatment with three different antibiotics was evaluated by means of both the SCC and CMCT. The influence of udder infection, stage of lactation and parity on CMCT and SCC were also investigated. This was done to assess the effectiveness of these methods for diagnosing subclinical mastitis in dairy goats.

MATERIALS AND METHODS

Model system

Herds used in trials

Three trials were conducted. Trial 1 and 2 were conducted at the Faculty of Veterinary Science, University of Pretoria (Herd A), while Trial 3 was conducted on a commercial goat dairy in the Limpopo Province of South Africa (Herd B).

TRIAL 1

Herd A consisted of 14 lactating multiparous Saanen goats and was conducted over a period of 7 days. Thirteen of the goats were in early lactation, while one was in late lactation.

TRIAL 2

Trial 2 was conducted in Herd A over a period of 8 days using 14 lactating multiparous Saanen goats. Thirteen of the goats were in mid lactation, while one was in late lactation.

TRIAL 3

Trial 3 was conducted over a period of 8 days in Herd B, a large commercial herd (350 lactating goats) using 64 Saanen, Saanen/indigenous crossbreeds and Toggenburg goats. All of the goats were in either early or mid lactation; and 57 were in their first, and the rest in their second lactation.

In all trials, the results of clinical udder examination, milk production, age and stage of lactation were evaluated, and the selection of experimental animals was done by the principle of pairing. Daily milk yield was categorized as low (less than 1.3 ℓ), medium (1.3–1.5 ℓ) and high (more than 1.5 ℓ).

Sampling in all trials

Goats were milked at 12 hourly intervals and foremilk samples were taken aseptically from udder halves of all goats according to the procedure described by Giesecke, Du Preez & Petzer (1994). Udder halves were milked separately and milk yields were recorded.

Antibiotic treatment

Products investigated

All the products investigated are available commercially as intramammary infusions for the treatment of bovine mastitis. In Trial 1, six untreated goats were

used as controls, while eight goats were treated at 12 hourly intervals after three consecutive milkings with Curaclox LC (Pharmacia AH), containing 75 mg sodium ampicillin, 200 mg sodium cloxacillin and a blue dye per treatment. In Trial 2, seven goats were untreated controls and seven were treated at 12 hourly intervals after three consecutive milkings with Spectrazol Milking Cow (Schering-Plough Animal Health), containing 250 mg cefuroxime per treatment. Two products were investigated in Trial 3. Twenty untreated goats were used as controls and 20 were treated at 12 hourly intervals after three consecutive milkings with Rilexine (SA) 200 LC (Virbac), containing 100 mg cephalexin, 100 mg neomycin sulphate and 10 mg prednisolone per treatment, while 12 goats served as controls and 12 were treated at 12 hourly intervals after 3 consecutive milkings with Curaclox LC.

Administration of antibiotics

The entire content of the intramammary antibiotic syringe was injected aseptically via the teat canal into each udder half of the goats in the treatment groups at 12 hourly intervals after three consecutive milkings.

Tests performed on the goat milk

The CMCT is a chemical-physical technique for the evaluation of somatic cell numbers in milk. It depends on the chemical reaction between the CMCT reagent and the DNA (from the nuclei of somatic cells and leukocytes) in the milk. This affects the viscosity of the mixture, which is evaluated visually. The CMCT also gives an indication of the milk pH. A pH indicator in the CMCT reagent turns yellow for milk with an increased pH and purple for milk with a decreased pH. Mastitis tends to decrease milk pH (Giesecke *et al.* 1994). The CMCT was done in the

dairy according to standard procedures (Karzis 2005). Inter-half differences of more than 2 points between milk samples from udder halves of the same goat were taken as significant for the identification of inflammation (I.M. Petzer, unpublished data 2004).

The milk laboratory of the Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria performed the microbiological investigation and SCCs on milk samples. A Fossomatic 5000 (Rhine Ruhr) the semi-automatic cell counting machine was used for determining SCCs.

Data management

All data were entered and stored in Microsoft Excel and were analysed using the statistical programme GenStat (2003).

RESULTS

CMCT assessments

Table 1 shows the frequency of CMCT ratings for the different treatments.

Analysis of variance of somatic cell counts (SCC) of Trials 1–3

Table 2 provides the differences in log SCC between treatment and control groups, infected and non-infected udder halves, stage of lactation and also parity for both treatment times.

Statistical analysis of peak SCC values

The differences of peak SCC values between treatment groups (T1, T2 and T3) are shown in Tables 3–5.

TABLE 1 Frequency of CMCT ratings (%) for different treatments

Trial and products used	Rx	CMCT= 0	CMCT= 1	CMCT= 2 & 3	Total
Trial 1 Curaclox LC	C1	42 (21.9%)	129 (67.2%)	21 (10.9%)	192
	T1	67 (23.3%)	155 (53.8%)	66 (22.9%)	288
Trial 2 Spectrazol Milking Cow	C2	90 (37.8%)	116 (48.7%)	32 (13.4%)	238
	T2	99 (41.6%)	98 (41.2%)	41 (17.2%)	238
Trial 3 Curaclox LC & Rilexine 200 LC	C	443 (43.3%)	398 (38.9%)	183 (17.9%)	1 024
	T1	116 (30.2%)	161 (41.9%)	107 (27.9%)	384
	T3	242 (38.1%)	260 (40.9%)	134 (21.1%)	636
Trials 1 & 3	C	485 (39.9%)	527 (43.3%)	204 (16.8%)	1 216
Curaclox LC	T1	183 (27.2%)	316 (47.2%)	173 (25.7%)	672

 $(\chi^2 = 38.331; P < 0.0001; degrees of freedom = 2)$

TABLE 2 Differences in log SCC between treatment and control groups, infected and non-infected udder halves, stage of lactation and parity for both treatment times

Trial and products used	Time	Treated and non-treated	Infected and non-infected	Stage of lactation	Parity
Trial 1 Curaclox LC	07:00 19:00	P < 0.001 P < 0.001	P = 0.186 P = 0.355		P < 0.001 P < 0.001
Trial 2 Spectrazol Milking Cow	07:00 19:00	P = 0.227 P = 0.078	P = 0.320 P = 0.01		P < 0.001 P < 0.001
Trial 3 Curaclox LC & Rilexine	06:00 18:00	P < 0.001 P < 0.001	P = 0.41 P = 0.993		P = 0.937 P = 0.877
Trials 1 and 3 Curaclox LC	06:00 18:00	P < 0.001 P < 0.001	P = 0.257 P = 0.546	P < 0.001 P < 0.001	P < 0.001 P < 0.001

P is significant at the 5 % level (P < 0.05)

TABLE 3 Differences of transformed log SCC values at peak SCC between treatment groups: Curaclox LC (T1), Spectrazol Milking Cow (T2) and Rilexine 200 LC (T3)

Variate	Size	Mean (T1) <u>+</u> SD	Size	Mean (T2) <u>+</u> SD	Size	Mean (T3) ± SD	F probability
Log SCC	42	3.964 ± 0.3723	9	3.559 ± 0.5376	40	3.794 ± 0.3127	P = 0.006

P is significant at the 5 % level (P < 0.05)

TABLE 4 Differences in time (h) from first treatment to peak SCC between treatment groups

Variate	Size	Mean (T1) + SD	Size	Mean (T2) + SD	Size	Mean (T3) + SD	F probability
Start to peak time (h)	42	71.43 + 18.918	9	77.33 + 26.907	40	67.80 + 15.742	P = 0.340

P is significant at the 5 % level (P < 0.05)

TABLE 5 Differences in time (h) from peak SCC to return to baseline values between treatment groups

Variate	Size	Mean (T1) + SD	Size	Mean (T2) + SD	Size	Mean (T3) + SD	F probability
Peak to end time (h)	42	108.57 + 18.918	9	114.67 + 26.907	40	112.20 + 15.742	P = 0.543

P is significant at the 5 % level (P < 0.05)

DISCUSSION

California Milk Cell Test

The CMCT and SCC in cow milk are used to determine udder irritation (which is the inverse of tissue tolerance). Poutrel & Lerondelle (1983), however, showed that the CMCT is not an accurate indicator of udder infection in goats and does not correlate with the SCC. However according to Contreras *et al.* (1996), CMCT scores of 2 and 3 do discriminate between infected and uninfected udder halves.

Trial 1: Curaclox LC

The percentage of udder halves with a CMCT score of 1 was highest for the treated goats (53.8%), but the goats in the control group had a CMCT higher

than those in the treatment group C1 (67.2%). A moderate positive correlation was present between the SCC and CMCT for Trial 1 ($R^2 = 0.482$). This is in agreement with findings in cows, where CMCT can be used as an indicator of SCC.

The Chi-square test was highly significant (χ^2 = 12.7; P = 0.002; degrees of freedom = 2) between treated and controls indicating that the number of CMCT counts per category did depend on treatment (Table 1) and shows that treatment with Curaclox LC caused tissue irritation in goats.

Trial 2: Spectrazol Milking Cow

The Chi-square test showed no significant difference ($\chi^2 = 3.052$; P = 0.216; degrees of freedom = 2), indicating that neither the number of CMCT counts

per category nor the SCC were influenced by treatment with Spectrazol Milking Cow (Table 2). The percentage of udder halves with a CMCT score of 1 was highest for the control goats, (C2, 48.7%) while the CMCT scores 0 (41.6%) and 1 (41.2%) were almost similar for the treated goats (T2).

A negative linear correlation ($R^2 = -0.432$) was present between the SCC and CMCT, which shows that Spectrazol Milking Cow did not cause significant udder irritation. This is contrary to the results obtained after the use of Curaclox LC in Trial 1.

Trial 3: Curaclox LC & Rilexine 200 LC

The control group had the highest percentage of goat udder halves with a CMCT score of 0 while the treated goats from Trial 1 (Curaclox LC) and Trial 3 (Rilexine 200 LC) had the highest number of scores of 1. There was a significant difference between the number of CMCT scores of 0 and the number of CMCT scores of 1 ($\chi^2 = 10.165$) and between CMCT scores of 0 and CMCT scores of 2 and 3 together. $(\chi^2 = 25.791)$ (Table 1). No significant correlation was present between SCC and CMCT. The Chi-square test was highly significant ($\chi^2 = 26.745$; P < 0.0001; degrees of freedom = 4) indicating that the number of CMCT counts per category did depend on the nature of the treatment (Table 1). Both SCC and CMCT increased after antibiotic treatment for both Curaclox LC and Rilexine 200 LC. This is an indication of udder irritation caused by intramammary treatment with both of these antibiotics.

Trials 1 and 3: Curaclox LC

When the data from goats treated with Curaclox LC in both Trials 1 and 3 were analysed together, as in Trials 1 and 3 separately, the highest proportion of goats in the control group had a CMCT score of 0 while the highest proportion of goats in the treatment group (combined for Trials 1 and 3) had a CMCT score of 1. The proportions of udder halves with a CMCT score of 0 and a CMCT score of 1 (χ^2 = 16.701), the proportions of udder halves with a CMCT score of 0 and 2 and 3 together (χ^2 = 35.875) and the proportions of udder halves with a CMCT score of 1 and 2 and 3 together, (χ^2 = 7.314) were all significantly different (Table 1).

The Chi-square test was highly significant (χ^2 = 38.331; P<0.0001; degrees of freedom=2) between treatment and control groups of the combined data for Curaclox LC treatments, further indicating that the counts of CMCT did depend on the treatment (Table 1).

Somatic cell count

In the literature the SCCs in dairy goats differs considerably between studies and most of this variability remains unexplained. Droke, Paape & Di Carlo (1993) found that 8.6% of the producers in commercial goat dairy herds had SCC below 750 x 10³ cells per m ℓ and 34.5% below 1000 x 10³ cells per m ℓ . In a study by Zeng & Escobar (1996) the mean SCC was 930 x 10³ cells per mℓ for the entire lactation period, while 51% of these milk samples had an SCC of above 1000 x 10^3 cells per m ℓ . They also found that there was no significant influence of breed or milking method on SCC. Luengo, Sanchez, Corralez, Fernandez & Contreras (2004) obtained SCCs for non-infected udder halves which were considerably higher than those obtained for other dairy ruminants such as sheep (77 x 103 cells per ml) and cattle (14 x 10³ cells per m ℓ). The normal SCC of goat milk is thought to range from 270 to 2000 x 103 cells per m\ell according to Paape, Poutrel, Contreras, Marco & Capuco (2001), which makes its diagnostic application more difficult. Lerondelle et al. (1992) found that factors such as nutritional disorders and vaccination increased SCCs. In a study by Lerondelle & Poutrel (1984) mainly coagulase negative staphylococci (CNS) were isolated from milk. In the study reported here, insufficient numbers of different bacterial species were present to assess SCCs in relation to the numbers of bacteria present. However, the mean SCCs of infected and non-infected goats were evaluated.

Effect of treatment on SCC

In Trial 1 the increase in the SCC indicated tissue irritation in the udder caused by infusion of the intramammary antibiotic (Curaclox LC) (Table 2). There was no significant increase in SCC in Trial 2 but an irregular fluctuation in the mean SCC was present. This could have been a natural fluctuation, or as a result of stress caused by several power-failures of short duration which occurred during milking time. The failure of SCC to increase after treatment and the finding of no significant difference in mean SCC between goats in the treatment (T2) and control (C2) groups could indicate that Spectrazol Milking Cow caused little udder irritation. In Trial 3 there was a highly significant difference (P < 0.001) of mean log SCC between goats in treatment groups T1 and T3 and those in the control group (C), at both treatment times (Table 2). Both treatments caused udder irritation, and the reaction to Curaclox LC was more severe than to Rilexine 200 LC. In Trials 1 and 3 together using Curaclox LC, the mean SCC was

significantly higher (P < 0.001) for udder halves treated with Curaclox LC, than for udder halves of the control group. Similar results were obtained for Curaclox LC in Trial 1 and Trial 3 separately (Table 2).

Effect of the presence of bacteria on SCC

Much of the variation in SCC was not due to intramammary infection. Non-infected goats may have a SCC > 1000×10^3 cells per m ℓ . These variations mean that their value as a guide to diagnosis in this species is controversial (Radostits *et al.* 2000). Wilson, Stewart & Sears (1995) found that most of the difference in goats' SCC is not due to intramammary infection, as 77% of the variation was unexplained.

In Trial 1 the presence of bacteria did not significantly affect the mean log SCC at both treatment times (Table 2). In Trial 2 infected udder halves had a significantly higher mean log SCC (P = 0.01) than that of non-infected udder-halves at the evening milking (19:00) but not at 07:00 (P = 0.320) (Table 2). Similar results were obtained in Trial 3 where infected udder halves had significantly higher (P = 0.041) mean log SCC than non-infected udder-halves but at 06:00, and not at 18:00 (P = 0.993). Results obtained in the three trials were therefore not convincing that the presence of bacteria had a meaningful influence on the SCC of goat milk.

Effect of stage of lactation on SCC

In Trials 1 and 2 the goats were in similar stages of lactation. A highly significant (P < 0.001) difference of mean log SCC between early, mid and late lactation was present in the combined data for Curaclox LC in Trials 1 and 3 (Table 2). The mean SCC was high in early lactation, decreased in mid lactation and increased again in late lactation. This was not consistent with results from other studies, in which the SCC increased with increasing stage of lactation (Zeng & Escobar 1996; Paape et al. 2001; Luengo et al. 2004). Wilson et al. (1995) showed that there was an increased SCC with increasing stage of lactation in Alpine goats with or without intramammary infection.

Effect of parity on SCC

There was a highly significant difference (P < 0.001) of mean log SCC between udder halves for lactation numbers 2–5 and 7, at both treatment times in Trials 1 and 2 (Table 2). The mean SCC was high in the second lactation in both trials and peaked in the fifth and third lactations in Trials 1 and 2, respectively. This was not consistent with findings that goats

of the first and second lactation group had the lowest SCC (Luengo *et al.* 2004) or with the findings of Randy, Wildman, Caler & Tulloch (1988) who did not detect any influence of parity on goat milk SCC. Too few goats in the second lactation were present in Trial 3 to be able to compare mean log SCC between lactation numbers.

There was a moderate positive correlation between log SCC and parity ($R^2 = 0.467$) for combined data of goats treated with Curaclox LC in Trials 1 and 3.

Peak in SCC

There were significant differences between the log SCC peaks of Trial 1 (Curaclox LC), Trial 2 (Spectrazol Milking Cow) and Trial 3 (Rilexine 200 LC) (Table 3). This shows that the use of each product resulted in a unique peak value of log SCC. Goats treated with Curaclox LC showed the highest peak log SCC, and was followed by those treated with Rilexine 200 LC, while those treated with Spectrazol Milking Cow showed the lowest peak log SCC. This substantiates the finding that Spectrazol Milking Cow caused the least tissue irritation and Curaclox LC the most, as was indicated by the log SCC.

There was no significant difference in the time (h) from start of the trial to peak in SCC between treatment groups (Table 4), nor was any significant difference present in the time (h) from peak in SCC to the end of the trials (return to baseline values) between treatment groups (Table 5). This shows that treatment with all three products caused an increase in SCC at the same time, but the degree of tissue irritation differed between treatments.

In Trial 2 only nine udder halves from the group treated with Spectrazol Milking Cow had peaks in SCC, while the rest were similar to the untreated controls of the trial (Tables 3–5).

CONCLUSION

The correlation between CMCT and SCC was found to be inconsistent in the three trials. It varied from no correlation in Trial 3 to a moderate positive correlation in Trial 1 and a negative linear correlation in Trial 2. However the Chi-square test was highly significant for treatment and control groups in Trials 1 and 3, and the combined data of Trials 1 and 3 for Curaclox LC, indicating tissue irritation caused by treatment. No significant udder irritation was present in Trial 2 after intramammary treatment with Spectrazol Lactating Cow. It is evident from findings that different intramammary antibiotics cause different

degrees of tissue irritation and further studies are indicated.

There appears to be no consensus in the literature about the exact role of SCC as a tool for diagnosing mastitis in goats, but it is evident that many factors can influence the SCC.

Each product used in the three trials peaked at a unique log SCC value. This time of peak SCC was similar in the three treatment groups in that this time correlated with the intramammary treatment time.

Although infected udder halves had significantly higher mean log SCC in Trial 2 (19:00) and in Trial 3 (06:00) than non-infected udder halves, no significant difference was evident in Trial 1 (07:00 and 19:00), Trial 2 (07:00) and Trial 3 (18:00). The results obtained in the three trials are therefore inconclusive and do not show that the presence of udder infection influences the SCC of goat milk.

A highly significant difference of mean log SCC between early, mid and late lactation was present in the combined data for Curaclox LC in Trials 1 and 3. The mean SCC was high in early lactation, decreased in mid lactation and increased again in late lactation. There is a highly significant difference in mean log SCC between udder halves for different lactation numbers. The mean SCC is high in the second lactation in Trials 1 and 2 and peaked in the fifth and third lactations, respectively.

Further investigations are indicated to determine the influence of the sample type (foremilk, composite samples or strippings) and storage method of milk on the accuracy of SCC and withdrawal periods. Other tests such as the NAGase could be used in conjunction with SCC and microbiology to determine their use in the diagnosis of mastitis in dairy goats.

ACKNOWLEDGEMENTS

We thank the National Research Foundation for the funding of this research. We also thank Mr Tobie Fourie and the staff of the Limpopo Melkery; the staff of the Milk Laboratory, Faculty of Veterinary Science and that of the Faculty of Veterinary Science's Teaching Animal Unit for all their help, and Marie Smith of the Agricultural Research Council (ARC) Silverton for the statistical analysis.

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