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California Mastitis Test and Somatic Cell Counts as Indicators of Intramammary Infection in Dairy Goat in Kenya.

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Abstract
Intramammary Infections in goats is an important and costly disease. Subclinical Mastitis (SCM) constitutes the greater part of this problem. Several methods are available for diagnosis of SCM.
The objective of this study was to evaluate the application of somatic cells measured indirectly by CMT, and directly by SCC – as possible markers for IMI in dairy goat mastitis.
CMT was performed randomly on 138 udder halves at milking site, while in the laboratory SCC was conducted on 239 samples. Bacterial culture was done on 250 samples.
The results of CMT showed that 12.3% of samples were negative, while 30.4%, 23.2% and 34.1% recorded +ve 1, 2, 3 respectively. The SCC ranged between 248 x 10^6 and 1693 x 10^6, with a mean of 869 x 10^6. The key bacterial isolates were *Staph. aureus* 58%, *E.coli* 40.5%. A statistical analysis to determine the strength and direction of association between CMT & SCC indicated a positive but not statistically significant correlation. ANOVA across the key bacterial isolates showed all bacteria falling between CMT mean scores of 2 and 3. The SCC showed that the key bacteria isolated had mean scores 86169 x 10^6 for *E. coli* and 8810 x 10^6 for *Staph. aureus.*
There is a consensus amongst researchers in this area that a CMT scores of 2 and above are indicative of infection in goat milk. The results of this study on *Staph. aureus* and *E. coli*, and the fact that 73.9% of *E. coli* and 68.5% of *Staph. aureus* fall in the range 500,000 and 1 million cells, SCC was an accurate determinant of infection.

Key words: Somatic cell counts, goat mastitis.

Introduction

Intramammary Infections (IMI) in goats constitute an enormous animal health problem by altering milk composition, lowering hygienic value of milk and causing economic losses. A great deal of IMI in dairy goats can be assigned to the group of subclinical mastitis with no outward clinical symptoms (Stuhr & Aulrich, 2010).
Leukocytes (Somatic Cells) migrate into the mammary tissue to provide the first immunological line of defense against bacteria that penetrate the physical barrier of the teat canal. It is therefore generally accepted that concentration of somatic cells in milk is directly related to the infection status of the udder, and no other factor(s) influences milk SCC to the degree bacterial infections do. Therefore the day to day management of the dairy goat infection status of the herd can be monitored effectively through SCC in bulk or individually (Escobar, 2007). Normal goat milk has higher SCC than that of the cow due to cytoplasm particles and epithelial cells shed along with milk (Haskell, 2005).
The assessment of udder health in goats, as in the cow, has been based on detection of elevation of somatic cells, using California Mastitis Test (CMT). The CMT reagent reacts with the DNA material of somatic cells to form a gel. The CMT is graded subjectively as Negative (O) Trace, positive (+ve) (1, 2, 3). Best results are obtained when CMT is conducted just before milking after stimulation of let down and discarding fore milk. CMT is regarded as an indirect Somatic Cells measurement. A direct determination of somatic cells (Somatic Cell Count-SCC) can be done in several ways, e.g. by use of Improved Neubauer Chamber – for a total Leukocyte Count (Shearer & Harris Jrn., 2006).
The objective of this study was to compare the use of CMT & SCC against bacterial culture, regarded as the gold standard test on dairy goat milk, in dairy goats kept by smallholder farmers in Kenya.

2.0 MATERIALS AND METHODS

Milk was collected from each udder half of only lactating does for bacteriological analysis. This was performed according to accredited standards (Carter, 1990 and Hogan *et al.*, 1999), from each half udder a milk sample of 0.01 ml was spread onto blood-agar plates containing 5% of washed sheep red blood cells onto MacConkey plates. Direct and enrichment cultures were incubated at 37^0C for 12 hours. Selection of colonies from

subcultures was done according to their predominance and homogeneity. All blood agar plates that showed no growth were re-examined 48 hours and 72 hours of incubation while fast growing non-haemolytic colonies were sub-cultured on nutrient agar (oxoid).

A California Mastitis Test (CMT) was performed at the milking site and graded according to increasing viscosity, where the highest viscosity was 3, and the lowest was 1, using the standard CMT kit (Berry E., & Broughan J., 2007). Direct Somatic Cell Count (SCC) was done using the Improved Neubauer Chamber (AO, American Optical, USA).

3.0 RESULTS

3.1 California Mastitis Test (CMT)

CMT was conducted on 138 milk samples (17) 12.3% of the samples graded as negative, (42) 30.4% were graded as 1, (32) 23.2% were 2, while (47) 34.1% were graded as 3, as in Table 1.

3.2 Somatic Cell Counts (SCC)

A total of 239 milk samples were analyzed for SCC. Table 2 summarizes the SCC in actual counts, and the corresponding log 6. The lowest SCC was 248,371 (248 x 10^6) the highest was 1,693,440 (86592 x 10^6), with a mean count of 869,522.87 (1693 x 10^6). Table 3 shows the distribution of SCC based on classes of 500,000 cells /ml.

3.3 CMT and SCC

Pearson's correlation co-efficient was applied to determine the strength and direction of the association between CMT and SCC as in Table 4.

This analysis showed the CMT and SCC, r = 0.080, p(0.417) > 0.05. There was a positive geometrical correlation, even though not statistically significant. Further analysis using independent samples t-test with CMT scores 1, 2, 3 collapsed into one category as +ve CMT, and 0 as another category was tested against SCC mean scores.

Table 5 summarizes the t-test results. The t-test results showed that combined CMT (1, 2, 3) had a higher SCC, mean score of 0.895 x 10^6, compared to CMT categorized as 0, which had SCC mean score of 0.870 x 10^6, however, a p(0.667) >0.05 was not statistically significant.

3.4 Bacteriology

In Table 6 gives the occurrence of various groups of microorganisms based on morphology and physiology were isolated from each half-udder. The gram positive *cocci* group constituted 42% while gram negative were about 27%.

3.4.1 Key bacterial isolates

The key bacteria isolated from 131 milk samples are indicated in Table 7, with *Staph. aureus* as the most dominant at (76) 58%, *E coli* (53) 40.5%, *Streptococcus* (2) 1.5%.

3.4.2 Comparison between CMT and bacteriology

Table 8 summarizes descriptive statistics of CMT scores across the key bacteria isolates based on one way analysis of variances (ANOVA). All bacteria isolated recorded CMT scores of between 2 and 3. CMT scores as shown in Table 8 indicate the sensitivity and specificity of CMT as indicator of IMI for the key organisms, *Staph. aureus* and *E. coli*. CMT 1 was associated with freedom of IMI while CMT ≥ 2 was associated with IMI.

3.4.3 SCC and bacteria

Table 9 is a cross-tabulation of the SCC against the two key bacterial isolates, *E. coli* and *Staph. aureus*. An independent sample t-test was used to determine if the SCC mean scores between the two unrelated bacteria differed significantly.

Table 10 summarizes the t-test result which indicates p (0.632) at >0.05 significance level. The SCC showed no significant difference due to the type of bacteria.

4.0 DISCUSSION

4.1 California Mastitis Test (CMT)

California mastitis test scores conducted on 138 milk samples ranged from 0 (12.3%), 1(30.4%), 2 (23.2%) and 3 (34.1%). According to Haskell (2005) and Ylva & Olofsson (2011) a CMT score of trace or 1 (one) indicates a healthy udder half, but at 2(two) and 3 (three) one must consider it infected. According to Shearer and Harris (2003) scores of 2 ≥ or ≤ 3 are indicative of infection. Pearson and Olofsson (2011) and McDougall & Prosser (2010), in their evaluation of direct and indirect measurement of somatic cell count as indicator of Intramammary Infection (IMI) in dairy goats concurred with the view that a CMT score of 1 was associated with freedom from Intramammary Infection (IMI) while a CMT score of 2 was indicative of IMI.

In the current study, considering the CMT scores in relation to the key bacterial isolates, i.e. *Staph. aureus and E. coli* had a CMT score of between 2 and 3 (Table 16). CMT was therefore a reliable measure of intramammary infection in relation to the key bacterial isolates.

4.2 Somatic cell count

The SCC conducted on 239 udder halves ranged between 248,371 cells/ml and 1,693,440, with a mean of 869,522. The use of SCC is one of the most established methods for diagnosis of udder health in cows (Paape *et al.,* 2007; Stuhr & Aulrich, 2010). Unfortunately SCC has not yet been established as a proven marker for Sub Clinical Mastitis (SCM) in dairy goats due to factors like parity, stage of lactation, estrus and breed which contribute significantly to levels of SCC in milk. Furthermore, Mycoplasma infections can lead to higher SCC in goat milk (Corrales *et al.,* 2004). It has also been documented that Caprine Arthritis Encephalitis virus (CAE) may lead to higher SCC, though regarded as a minor contributor (Bergonier *et al.,* 2003).

Souza *et al.,* (2009) examined bulk milk samples of 1,400 dairy goats resulting in a mean score of 779,000 cells/ml, while in a different study by Jendretzke (Stuhr & Aulrich, 2010) a mean score of 990 10^3 was established.

In the European Union (EU) the SCC threshold for raw cow milk was set at 400 10^3 (EC, 2004), but so far no limit values in EU exist for goat milk (Paape *et al.,* 2007). Nevertheless, some national thresholds exist for bulk milk ranging between 750 10^3 to 1 million cells/ml (Pirisi *et al.,* 2007).

A universal definition of a cell number threshold in goat milk to distinguish between healthy and infected udder does not exist. Only in the United States the SCC in bulk goat milk is not allowed to exceed 1 million cells / ml (US/Public Health Service, 2003). The findings in this study and elsewhere on SCC studies and the factors mentioned above that might influence SCC in goat milk must be considered when setting SCC criteria for assessing the quality of goat milk. Leitner *et al.,* (2008) proposed that differentiation between high, medium and low quality of bulk goat milk needs to be established as follows: high quality milk should have a SCC of < 800 10^3 cells/ml, associated with infection rate of 25%, medium quality milk should have < 1.5 10^6 cells/ml, associated with infection rate ranging between 25% and 50% while low quality milk should have a SCC of > 1.5 10^6 cells/ml. Goat milk having a cell count of > 3.5 10^6 should be regarded as unsafe for human consumption. Each one of the proposed categories should be verified under different production systems / conditions, in various countries.

In this study 73.9% of *E. coli* and 68.5% of *Staph. aureus* infection fell within the SCC range of 500,000 and 1 million cells/ml, with mean SCC for each of these key organisms 861,690 cells/ml and 881,008 cells/ml respectively. These figures are in agreement with findings elsewhere, especially the proposed quality grading by Leitner *et al.,* (2008). The range of SCC in this study in general, and the mean values for determining the key bacterial isolates therefore concur with results from studies elsewhere.

4.3 Bacterial isolates

The preliminary bacterial culture, showed predominance of gram positive (+ve) colli, 42%, and gram negative (– ve) rods, 27%. The objective of this study was to focus on key mastitis causing organisms. Therefore, as shown in Table 6 *Staph. aureus,* 30% and *E. coli,* 21% (Total 51%) became the focus. The contagious *Staph. aureus* has been documented as one of the most dominant and serious cause of goat mastitis.

In Kenya, a study was carried out in goats in Nyeri established Staphylococcus as the dominant isolates (63.6%), with *Staph. aureus* constituting 22.7% of all bacterial isolates (Ndegwa *et al.,*2000). Studies elsewhere reaffirm the dominance of *Staph. aureus* (Moroni *et al.,* 2007; Pearson & Olofsson, 2011, Stuhr & Aulrich, 2010). In Europe and USA the contagious IMI tends to be controlled by high level of standards of milking hygiene. However, amongst smallholder goat farmers in Kenya standards of hygiene are low and therefore the high incidence of *Staph. aureus*.

E. coli, a coliform is an environmental (faecal) bacteria present at all times on all dairy farms capable of living in bedding, saw dust, shavings especially in-hygienic environments (Ingalls, 2003).

5.0 CONCLUSION

Somatic cells, measured indirectly by CMT at score ≥2, and directly SCC can predict IMI of goats. Thus goat farmers can be recommended to use CMT as a "goat – side" test for subclinical mastitis. CMT is regarded as a quick, cheap and simple as an "animal side" test, even though some authors claim it is unreliable for IMI diagnosis in goat milk (Bergonier *et al.,* 2003; Schaeren & Maurer, 2006).

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Table 1: California Mastitis Test

CMT Level	Frequency	Percentage (%)
Negative (0)	17	12.3
Mild (1)	42	30.4
Moderate (2)	32	23.2
Heavy (3)	47	34.1
Total	**138**	**100**

Table 2: Somatic Cell Counts (SCC)

	SCC	SCC log ^{6}
N	239	239
Mean	869,522.87	.86,952 x 10^6
Standard deviation	206,609.32	.206,609 x 10^6
Range	1,445,069	1.455 x 10^6
Minimum	248,371	.248 x 10^6
Maximum	1,693,440	1.693 x 10^6

Table 3: Distribution of SCC

Levels of SCC	Frequency	Percentage (%)
<500,000	4	1.7
500,000 - 1,000,000	172	72
1,000,000 - 1,500,000	62	25.9
1,500,000 - 2,000,000	1	0.4
Total	**239**	**100**

Table 4: Correlation of CMT and SCC

		Somatic Cell Count (SCC)	California Mastitis Test
Somatic Cell Count (SCC) log ^{6}	Pearson Correlation	1	0.08
	Sig. (2 - tailed)		0.417
	N	239	104
California Mastitis Test	Pearson Correlation	0.08	1
	Sig. (2 - tailed)	0.417	
	N	104	138

Table 5: T-test Results

CMT Levels	N	Somatic Cell Count (log^6) mean score	Std. Dev.	t-value	df	Sig.(2 tailed)
Negative / None (-)	13	0.87017	0.255266	-0.431	102	0.667
Positive (+)	91	0.89546	0.188979			

Table 6: Bacteriology

	Frequency	Percentage (%)
E. coli	53	21
Staph. aureus	76	30
Bacilli	48	19
Streptococcus	2	1
No growth	71	28
Total	**250**	**100**

Table 7: Bacterial Isolates

	Frequency	Percentage (%)
E. coli	53	40.5
Staph. aureus	76	58
Streptococcus	2	1.5
Total	**131**	**100**

Table 8: CMT and Bacteria

	N	CMT Mean	St. Dev.	St. Error	Minimum	Maximum
E. coli	24	2.17	0.963	0.197	0	3
Staph. aureus	37	2	0.972	0.16	0	3
Streptococcus	2	3	0	0	3	3
Total	**63**	**2.1**	**0.962**	**0.121**	**0**	**3**

Table 9: Summary Statistics of SCC and bacteria

Statistics	*E. coli*		*Staph. aureus*	
	Somatic Cell Count (SCC)	Somatic Cell Count (SCC) $\log^6$	Somatic Cell Count (SCC)	Somatic Cell Count (SCC) $\log^6$
N	46	46	54	54
Mean	861690.99	0.86169	881008.8	0.88101
St. Deviation	193298.911	0.193299	206127.4	0.206127
Range	824141	0.824	835430	0.835
Minimum	485453	0.485	462874	0.463
Maximum	1309594	1.31	1298304	1.298

Table 10: SCC across the type of bacteria

Bacteria	N	Somatic Cell Count log^6 mean score	St. Dev.	*t-value*	*df*	*Sig. (2 tailed)*
E. coli	46	.86169 x 10^6	0.193299	-0.481	98	0.632
Staph. aureus	54	.88101 x 10^6	0.206127			

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