Relationship between California Mastitis Test Scores and Somatic Cell Counts in Different Crossbred Dairy Cattle Genotypes

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***Abstract*—** *The objective of this study was to evaluate relationship between California Mastitis Test scores (CMT) and Somatic Cell Counts in different crossbred dairy cattle genotypes. A total of 152 milk samples were screened for mastitis using the California Mastitis Test (CMT) kit. Somatic Cell Count (SCC) in milk samples were analysed directly using microscopic method. Based on the analysis of CMT score, the study found out that 55.92 % of udder quarters were negative while 43.99 % were positive for subclinical mastitis. The Least Square Difference (LSD) for pairwise comparison between CMT scores and lactation stage were significantly different between First and second lactation at 0.25±0.11; second and third at 0.27±0.0118 at P≤ 0.05. The means of SCC among the breeds were significantly different at P≤0.05; Ayshires and Friesians (68,055±18.82 cells/ml); Ayshire and Guernsey (71,976±23.844 cells/ml); Friesians and Jerseys (64.863±21.429 cells/ml); and Guernsey and Jersey (68.78±25.952 cells/ml). In conclusion, this study provides baseline information in the area of selection for mastitis resistant breeds of dairy cattle. This study also strongly recommends the use of this technique in screening for somatic cell counts in udder quarters of crossbred dairy cattle*.

Keywords; Crossbred, Mastitis resistant, Somatic Cell Count, Somatic Cell Score.

I. INTRODUCTION

Good management practices especially on udder health are fundamental for quality and profitability of dairy production (Sadeghi & Amer, 2015; Gupta *et al*,. 2016). Somatic Cell Counts (SCC) are an important primary indicator of milk hygiene (Jingar *et al.,* 2017). They are also linked to the level of profitability of dairy enterprises (Hadrich *et al*,.2015; Holland *et al.,* 2015; Jadhav *et al.,* 2016). These cells include; macrophages, polymorphonuclear neutrophils, lymphocytes, and epithelial cells, which resulted from infections of udder quarters. The number of cells are usually reported in milk per millilitre (Division, 2018), and are used internationally to scrutinize/screen milk quality and udder health status in the dairy herds (Li *et al.,* 2014). The SCC above 310,000 cells/ml is the recommended value as a monitor of udder health status (Jadhav *et al.,* 2018). However, the threshold level for assessing and monitoring in dairy herds is often 2.0 x 10^5 cells/ml and below (Division, 2018). Somatic Cells play a critical role in the immune response of infected udder quarters of cows (Li *et al*., 2014; Azmi et al., 2017; Iraguha*et al.,* 2017). The increased somatic cell counts in the milk as a resultof udder tissues' inflammation, which subsequently affects the quality and quantity of milk produced (Division, 2018; Malik *et al*., 2018).

The presence of somatic cells in milk is attributed due to an infection of the mammary glands, which eventually results in mastitic condition of the udder quarters (Balaji *et al*., 2016). Subclinical mastitis does not show visible clinical symptoms, but can results in severe economic losses from discarded milk, the sudden death of cattle, cost of veterinary services, the decline in quality and milk produced (Sadeghi & Amer, 2015; Hadrich *et al*.,2018).

The low heritability of mastitis makes it unfavourable for selection of mastitis resistance breeds of dairy cattle in conventional breeding (Boas *et al.,* 2017). The heritability for milk production traits is usually moderate to high. Therefore, the low SCC could be employed in selection for mastitis resistance in crossbred dairy cattle in an attempt to reduce mastitis incidences the smallholder dairy farmers.

The clinical mastitis is genetically correlated to SCC. The correlation varies from 0.64 to 0.77. Udder health and SCC significantly affect the quality of milk produced by dairy cows (Bhutto *et al.,* 2012). The objective of the present study was to assess the relationship between California Mastitis Test scores (CMT) and Somatic Cell Counts in different crossbred dairy cattle genotypes.

II. MATERIALS AND METHODS

2.1 Study Area

The study was done in Kanyariri Veterinary Teaching Farm, which is a learning and research facility for the University of Nairobi. The farm sit on a 375acre piece of land in Lower Kabete located 2 kilometres West of the College of Agriculture and Veterinary Sciences and 15 kilometres from the Nairobi city centre. Kanyariri farm is on an elevation of 5,600 feet (1,700 meters) above sea level with an average temperature of 18.7˚C. It receives rainfall amount of about 869 mm per annual.

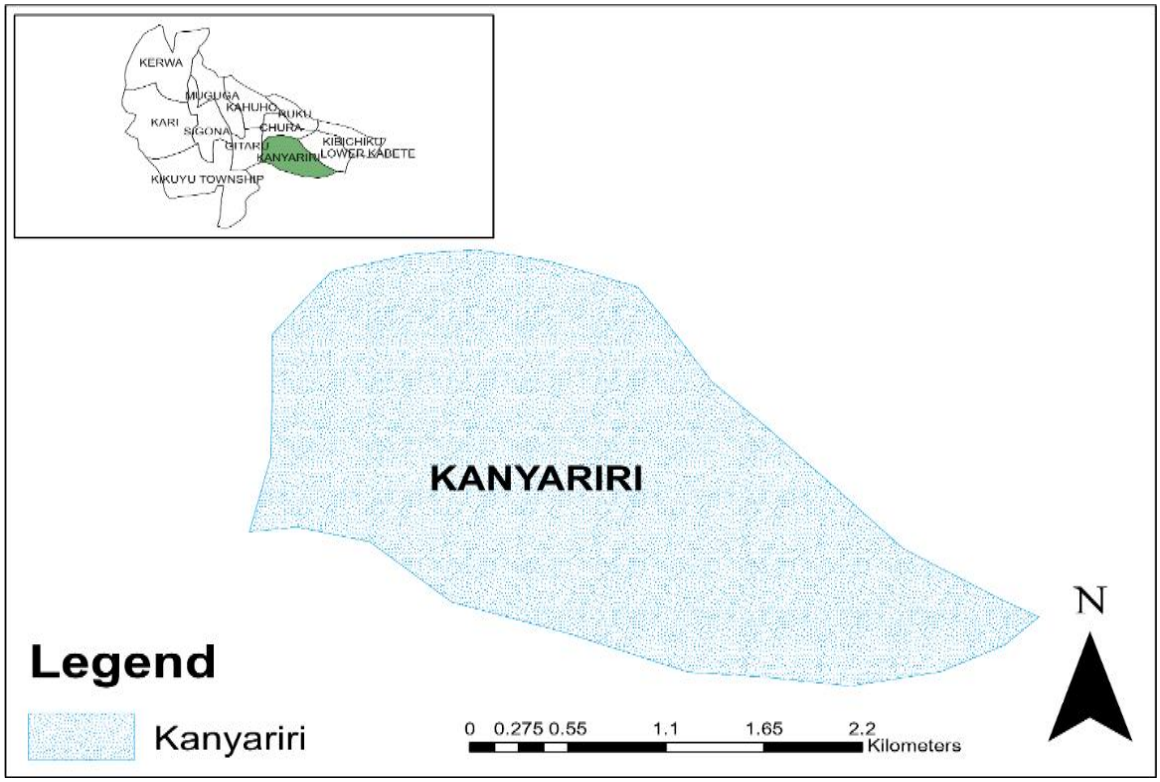

FIGURE 1: A Map of Kiambu showing Kanyariri Veterinary Farm – University of Nairobi

2.2 Sample collection

Thirty-eight crossbred cows were selected based on lactation. A total of 152 quarters from full udder quarters of the 38 cows were screened for mastitis using California Mastitis Test scores (Schalm, 1957). For SCC each of the udder quarters were cleaned using 75 percent ethanol before collecting 5ml of milk samples aseptically into 10ml screw cupped tubes. These were then transported at -20℃ to the Microbiology Laboratory in the Department of Food Science and Nutrition, Faculty of Agriculture, University of Nairobi. Milk samples were stored in a freezer at -21℃ before they could be analyzed for Somatic Cell Counts.

2.3 Microscopic procedure for SCC

The slides were labelled to match the sample container identification numbers. The slides were smeared with the milk samples, stained and allowed to dry (Prescott and Breed, 1910). They were later examined under Electric microscopy with the magnification of 100X (Ferronatto et al., 2018). Somatic Cell Counts were observed as dark-spots which were counted and recorded. To obtain Somatic Cell Scores, SCC were log transformed using the formula Log2 [SCC/100,000 +3] (Rupp *et al.*,1999; Yuan *et al.*, 2013).

2.4 Statistical analysis

The SCC data was analyzed using SPSS version 21.0. To calculate correlation coefficients, Tukey test, LSD and to test if there was a significant difference of breed effect on mastitis susceptibility traits; CMT Scores were analyzed using one-way ANOVA at $P \leq 0.05$ level of significance. The models below were used in testing the effect of breed on SCC.

1) $y_{ij} = \mu + G_i + e_{ij}$

Where y_{ij} is the effect of the SCS in the udder quarters**,** μ is the average for traits, G_i is the breed's effects, e_{ij} is the residual error. The analysis of the association among the breeds with SCC that reflects mastitis susceptibility traits was solved using the equation below;

2) $y_{ij} = \mu + G_i + p_t + e_{ij}$

Where y_{ij} is the observation of somatic cell count, μ is the overall mean of SCC, G_i and , p_t were the fixed effect of breed and the fixed effect of SCC respectively, and e_{ij} residual error

III. RESULTS

Figure 1. Presents the result of the 152 quarters examined for mastitis by California Mastitis Test (CMT) 55.92 % (N=85) were negative for mastitis with a somatic Cell Score range of 0 to 2.0 x 10^5 cells/ml. Those that tested positive for subclinical mastitis were trace (N = 65) and +1 score (N= 2) at 42.67 percent (2.10 x 10^5 cells/ml to 3.0 x 10^5 cells/ml) and 1.32 percent (3.10 x 10^5 cells/ml to 5.10 *x* 10^5cells/ml) respectively.

In Table 1; Tukey test was carried out to determine if there were significant differences between CMT scores and Somatic cell scores (SCS) using one-way ANOVA. Results revealed that there was a significant association between CMT and SCS at the P≤0.05. The analysis showed that all values were greater than High Significant Difference (HSD) values of 0.23, and the least significant difference (LSD) was greater than the mean of 0.17. Further, results indicated that both HSD and LSD were significantly different at P≤0.05. Generally, the result shows that there were significant association between CMT scores and SCS of crossbred dairy cattle genotypes.

TABLE 1
MULTIPLE COMPARISON OF THE EFFECT CMT AND SCS ACROSS UDDER'S QUARTERS OF THE CROSSBRED DAIRY CATTLE.

CMT SCORE	Normal	T	1^+ score
Normal (healthy)	—	0.863* ± 0.1	0.46* ± 0.0023
T	—	—	0.403* ± 0.101
1^+ score	—	—	—

Normal =Negative score for subclinical mastitis, T = Trace (subclinical mastitis), &1^+ score (subclinical mastitis)
**** The significant difference when P≤0.05 level of significance***
CMT Score = California Mastitis Test Scores, SCS = Somatic Cell Scores

Table 2; associations between the age of crossbred dairy cows and CMT scores were significantly different at P≤0.05. An increase of 2.1% in CMT results into an equal in the age. The linear associations between the age and CMT can be used in the prediction of subclinical mastitis.

TABLE 2
REGRESSION BETWEEN AGE (PREDICTOR) AND CMT SCORE OF MILK SAMPLE

ANOVA	SM	df	Mean Square	F	sign
Regression	1.125	1	1.125	4.16	0.043^b
Residual	40.553	150	0.27		
Total	41.678	151			

The independent variable is the Somatic cell count.
R = 0.164, R^2 = 0.027, Adjusted R^2 = 0.021, SE = 0.520, and Y = -0.036 + 1.701.

The table 3; presents that the mean of SCS and Lactation Stages for first and lactation stages (0.25±0.11) and second and third (0.27±0.0118) were significant at P≤0.05. Thus, there were significant association between SCS and lactation Stage, this can be used in predicting level of SCS in milk as per lactation stage. There was no association in somatic cell scores between first and second; and second and third lactation stages (Tables 3).

TABLE 3
MEAN DIFFERENCE BETWEEN SOMATIC CELL SCORE AND LACTATION STAGES OF THE CROSSBRED DAIRY CATTLE.

Lactation stage	First	Second	Third
	Mean ± SE	Mean ± SE	Mean ± SE
First	–	0.25* ± 0.11	0.021 ± 0.097
Second		–	0.27* ± 0.0118
Third			–

**** This indicated the result was significant at P≤0.05***

Figure 2. Four breeds of crossbred dairy cattle namely Ayshires, Friesian, Guernsey, and Jersey were used in this study. In figure 3 their mean distribution of Somatic Cell Counts and standard error mean were 246.64±15.177 cells/ml, 178.59±10.008 cells/ml, 174.67±13.212 cells/ml, and 243.45±21.997 cells/ml for each breed, respectively. The SCC varied from one breed to one another (Ayrshire = 1.06 x 10^5 to 4.0 x 10^5cells/ml; Friesian = 9.7 x $10^4$to 3.12 x 10^5cells/ml; Guernsey = 9.2 x $10^4$to 3.1 x 10^5 cells/ml; and Jersey = 9.2 x 10^4 to 3.94 x 10^5cells/ml).

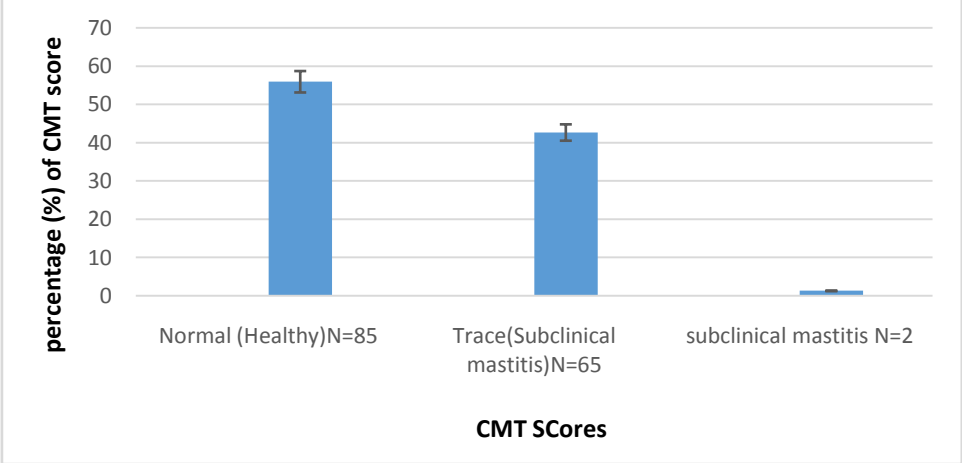

FIGURE 2: Infection status of lactating dairy cattle genotypes.

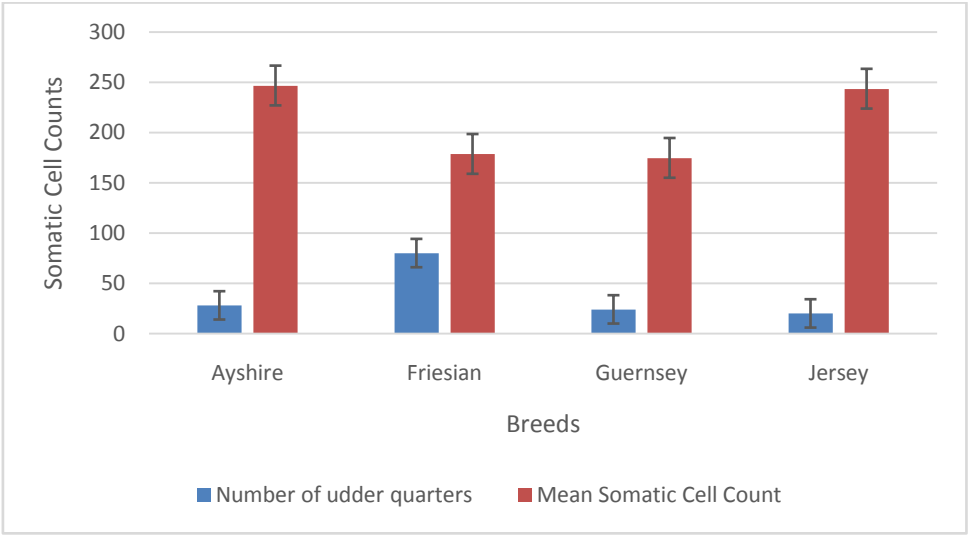

FIGURE 3: Number of udder quarters and Mean of Somatic Cell Counts of crossbred cows

Ayshire genotype of the crossbred dairy cattle had the highest numbers of Somatic Cell Counts (246.64±15.177 cells/ml), and Jersey genotype had the least amount of SCC (174.67±13.212 cells/ml). It implies that Ayshire genotype was highly susceptible to mastitis incidence and Jersey genotype was slightly resistant to mastitis.

Table 4; Means of SCC were significantly different between Ayshire and Friesian; Ayshire and Guernsey; Friesian and Jersey and Guernsey and Jersey at P≤0.05. Their means were found be to greater than 56.162 for the Tukey analysis. For the case of the Least Significant Differences (LSD) analysis, the means were elevated more than 23.69 for significant values at P≤0.05.

TABLE 4
PAIRWISE COMPARISON OF SOMATIC CELL COUNTS BETWEEN FOUR GENOTYPES OF CROSSBRED DAIRY COWS.

Breed	Friesian	Guernsey	Jersey
	Mean ± SE	Mean ± SE	Mean ± SE
Ayshire	68.055* ± 18.821	71.97*± 23.844	3.193 ± 25.095
Friesian	–	3.921 ± 19.949	64.863* ± 21.429
Guernsey		–	68.78* ± 25.952
Jersey			–

The mean is significant at P≤0.05, SEM; standard error mean

The result showed that there were significant differences in term of SCC between Ayshire and Friesian; Ayshire and Guernsey; Friesian and Jersey and Guernsey and Jersey. However, the differences between Ayshire and Jersey; and Friesian and Guernsey genotypes were small, and therefore, they were no significant.

The correlation between Somatic Cell Counts and udder quarters were significantly correlated at P≤0.01. The higher scored were in Left front and Right hind quarters while as Left hind and Right front quarters were moderate (Table 5).

TABLE 5
CORRELATION BETWEEN SOMATIC CELL SCORE AND THE UDDER QUARTERS OF CROSSBRED DAIRY CATTLE GENOTYPES.

somatic cell count (x1000 cells/ml)				
Quarter	**Left front**	**Left hind**	**Right front**	**Right hind**
Left front	0.831**	0.993**	0.742**	0.751**
Left hind	1.00**	0.848**	0.937**	0.904**
Right front	0.900**	0.763**	0.942**	1.00**
Right hind	0.902**	0.761**	0.938**	1.00**

Correlation is significant when P≤0.01 level of significance (2-tailed)

IV. DISCUSSION

4.1 California Mastitis Test scores

The California mastitis test scores for the udder quarters were tested 55.92% Negative for mastitis and 44.08% positive subclinical. Figure 2 results were in disagreement with the findings of Hoque and Das (2014), who stated that CMT results are 25% Negative and 75% positive for subclinical mastitis. The differences in results can be attributed to factors such as climatic variations, geographical, and sometimes breed differences. Hoque and Das, (2014) also reported that CMT results positive for subclinical mastitis are usually less than 25% as compared to the findings of this study, which were 44.08%. These are contrary to the findings of this study, as shown in Figure 2. Iraguha *et al*., 2017 also reported that 60% and above of the udder quarters were positive for subclinical mastitis. The aforementioned is contrary to the findings of this study (Figure 2).

CMT is a simple technique which is affordable, reliable, economically viable and which requires minimum expertise to apply (Holland *et al.*,2018). This factor makes CMT a tool which can be applied by most smallholder dairy farmers for early detection of mastitis. Early detection helps farmers to prevent mastitis incidence. Thus improving hygiene as well as the quality and quantity of the milk they produce, this is in agreement with Abebe et al., 2016; Kandeel *et al.*,2018.

The associations between California Mastitis Test (CMT) score and Somatic Cell Score (SCS) across the udder quarters were significantly associated. This is in agreement with Das et al. (2018) which stated CMT scores and Somatic cell scores varied significantly across udder quarters. The associations between CMT scores and SCS could be utilized as a marker-assisted in selective breeding for mastitis resistance. Meredith et al., 2012; Republic, 2017 reported that SCS can be applied as pseudo phenotypic trait for selection of mastitis resistance, because SCS has higher heritability as compared to both mastitis and SCC.

The Least Significant Differences (LSD) of mean differences between California Mastitis Test (CMT) scores and lactation stage were significantly different at $P \leq 0.05$. These results are in agreement with Jadhav *et al*. (2018)

The regression between age and CMT score revealed that they were significantly different at $P \leq 0.05$. Table 3 This could be utilized by dairy farmers as a tool to predict the level of somatic cell count through the application of the California mastitis test reaction at a particular corresponding age of a dairy cow. Optimal distributions of quarter CMT scores for the healthy cows were similar at $P \leq 0.01$ and $P \leq 0.05$, respectively, this is line with Morin *et al.(*2018).

4.2 Somatic Cell Counts

This study showed that number of Somatic Cells for healthy cows were between o to 200,000 cells/ml, this is not agreement with Hemati Doust *et al.* (2014) which that the number range between 250,000 cells/ml and 300,000 cells/ml on average.

In table 3; Tukey analysis of the amount of Somatic Cell Count (SCC) in the udders' quarters among the breeds, Ayshire and Friesian were 68.055*±18.821 cells/ml, Ayshire and Guernsey were 71.97*±23.844 cells/ml, and Friesian and Jersey were 64.863*±21.429 cells/ml, and there were no significant differences between Ayshire and Jersey had 3.193±25.095 cells/ml and Friesian and Guernsey were 3.921±19.949 cells/ml. The ability of a breed to produces significant amounts of somatic cells in milk is treated an indicator of robust immune responses to mastitis infections. This ability is used as a first line of response to infection and is used as an intervention before the disease worsens. Therefore, an increase in Somatic Cell Counts in milk can facilitate a rapid and effective response to an intramammary infection (IMI) in dairy herds (Hussein *et al.*, 2018).

Table 5; There was a strong correlation between somatic cell counts and udder quarters. The SCC varied progressively among udder quarters at $P \leq 0.01$, as previously reported by Das et al. (2018) that SCC increases across the udder quarters.

4.3 Somatic cell count as an indicator of udder health.

In dairy herds, somatic cell counts should be taken regularly in order to minimize mastitis incidences. This is vital in monitoring and evaluation of the udder health status and intramammary infection (IMI) incidence in dairy herds (Hussein et al., 2018). The direct microscopic examination is the most suitable and straightforward technique for evaluating the level of somatic cell counts in milk. The percentages of macrophages in milk can be reckoned for differential somatic cell counts (Jadhav *et al.,* 2016). In figure 2 the amount of somatic cell count for Ayrshires and Jersey were slightly above the threshold 246,640 cells/ml and 243,4500 cells/ml respectively, and the somatic cell count for Friesian and Guernsey were below the threshold level 178,590 and 174,670 cells/ml respectively.

However, the decline in milk production has also been attributed as results of other factors such as physiological aspects of lactation, Feeds, Climatic condition and geographical location of an area that influences the production of milk (Sharma *et al*.,2011). The occurrence of mastitis in mammary glands trigger inflammation of udder tissues which eventually interrupts release oxytocin hormone in udder during milking hence less milk produced. Therefore, somatic cell count in udder quarters is taken as an important trait that may be used in selective breeding for mastitis resistance in dairy herds. This is because of their higher heritability as compared to clinical mastitis. Somatic cell score in e udder quarters is also another strategy which can be explored in a selection of mastitis resistant breeds of dairy cows.

V. CONCLUSIONS

The study found out that CMT and SCS were significantly associated across all the udder quarters of these crossbred dairy cattle. Therefore, they can be used to predict amount of Somatic Cells presence in milk. It was also found out that an increase in age of crossbred cow results in a proportionate increase in California mastitis Test scores. The amount of somatic cell

counts varies from one breed to another, and they were significant associated among the four genotypes of crossbred dairy cattle.

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