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DIRECT AND INDIRECT MEASUREMENT OF SOMATIC CELL COUNT IN DETECTING SUBCLINICAL MASTITIS IN GOATS

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Abstract

This study determined subclinical mastitis in goats thru direct microscopic somatic cell count (DMSCC) and California Mastitis Test (CMT) and how well direct (DMSCC) and indirect (CMT) measurement of SCC correspond in detecting subclinical mastitis in goats. Results show that DMSCC and CMT have correlation coefficient (r) of 0.986 in detecting subclinical mastitis in goats. The apocrine type of milk secretion in goats result in high somatic cell counts that generally alter the well-accepted relationship between somatic cell count (SCC) and the level of intramammary infection of goats. SCC in goats' milk is mostly much higher than in cows' milk and still be accounted to come from healthy udder. CMT has the advantage as "animal side" test that does not need to be skilled in order to use the instrument, however confirmation should be done by bacterial examination.

Keywords: Goats; Somatic cell count, California Mastitis Test; Subclinical mastitis.

INTRODUCTION

Breeding and production of goats (Capra hircus) in the Philippines is an important source of income for smallholder farmers (Montes et al. 2008). Goats require simple management and cheap production inputs as compared to swine and poultry (Alcedo et al., 2015), are easy to handle (Monteiro et al. 2018) and can subsist on crop residues or any locally available crop forages (Alcedo et al., 2015).

Mastitis is predominant and valuable disease in dairy goat production that needs diagnosis and control (Persson and Olofsson, 2011) that affects both quantity and quality of milk (Mishra et al. 2018). Compared to clinical mastitis, in which there is manifestation of inflammation of mammary gland (Cobirka et al., 2020), subclinical mastitis (SCM) often goes unnoticed, hence it is left unattended (Petlane et al., 2012). It is one of most challenging diseases (Gelasakis et al. 2016) that accounts to 20-30% prevalence per lactation (Rupp et al., 2014). SCM makes the milk unfit for processing (Petlane et al., 2012) due to physical, chemical, microbiological, and pathological changes in the milk (Mishra et al. 2018).

Somatic cells are mostly milk-secreting epithelial cells that have been shed from the lining of the mammary gland and white blood cells (leukocytes) that have entered the gland in reaction to injury or infection (Sharma et al., 2011). Because of the direct relationship between inflammatory cells and intramammary infection, milk SCC have been the most widely used measurement to monitor udder health (Kaskous et al., 2023; Jimenez-Granado et al., 2014; Rampino et al., 2006). Milk somatic cells are usually made up of leucocytes (75%) and epithelial cells (25%). Elevated number of leucocytes is a reaction to bacterial infection, tissue injury and stress; and an increased SCC in milk implies low quality of raw milk (Sharma et al.,

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2011).

SCC can be performed by direct microscopic method of somatic cell count (DMSCC) with the use of Levowitz-Weber or Modified Methylene Blue stain that can be applied to diagnose subclinical mastitis in goats. Levowitz-Weber stain has the advantage of demonstrating leukocytes and lysing the fat globules of milk while staining the somatic cells (Coles, 1986). Cows release milk through merocrine secretion as compared to apocrine in goats (Podhorecká et al., 2021) and this apocrine secretion had been accounted for higher SCC in goats than in cows (Rupp et al., 2019; Robertson and Muller, 2005).

CMT is well known indirect method of measuring SCC and its main advantages is that it is fast and easy to perform "animal side" test (Persson and Olofsson, 2011) that can provide a practical means to identify inflammatory infections (Robertson and Muller, 2005) in the field. It contains bromcresol purple that act as pH indicator that react to the DNA content of the milk (Pyorala, 2003) but does not react with cytoplasmic particles, that can favorably be used under practical farm conditions to indicate intra-mammary inflammation in the udder of the goat (Robertson and Muller, 2005). Literature is scant on the correlation coefficient of DMSCC and CMT. This study aimed to establish the correlation coefficient value of DMSCC and CMT in detecting subclinical mastitis in goats.

MATERIALS AND METHODS

Selection of animals

Lactating goats were identified from the commercial goat farm in Luzon, Philippines. These animals were enlisted in the study by using their number or tag together with their corresponding farm records.

Aseptic Milk Collection

Milk samples were obtained aseptically by hand milking. The udder and teats were cleansed and wiped with clean white cloth before taking the samples. The first 3-5 squirts of milk were discarded and approximately 5-10 milliliters of milk were collected according to the standard milk sampling techniques described by Sears et al., (1993). For the CMT test, 5-10ml of milk were squirted directly to the paddle then another 5-10ml were taken from each teat for direct microscopic count.

Labeling of Samples

The collection tubes were labeled properly using the animal number or tag in order to accurately identify the samples that correspond to the animal. Labeling was done first before commencing with the milk collection.

Sample Storage and Transport

Milk samples (approximately 10ml) were collected in sterile tubes and kept on ice chest during transport to the laboratory of College of Agriculture and Veterinary Medicine, President Ramon Magsaysay State University, Zambales, Philippines.

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Mastitis Detection

California Mastitis Test (CMT) or (Indirect SCC).

CMT liquid concentrate was purchased from a seller of California Mastitis Test kit in the Philippines and then diluted based on the indicated instructions written in the label. The first streams of milk were discarded and then a small amount of milk is put into the paddle. The paddle is tilted almost vertically so that only 2 to 1 teaspoon of milk remains in each cup. An equal amount of CMT reagent is added to the milk and swirled for about 15 seconds. Observation of the reaction was done immediately to see if there is any thickening of the milk. The more thickness, the higher the SCC. The reaction scores are: **N** (negative) with average somatic cell count of 100,000 cell per milliliter, **T** (trace) with 300,000 cells/ml, when there is any trace amount of gelling on the paddle; **1** with 500,000 – 1,500,000 cells/ml, when there is thickening of the milk, but not clumping together; **2** with more than 2,000,000 cells/ml, if it thickens and sticks to the paddle; and **3** with 8,100,000 cells/ml (Scruton et al., 2010; Escobar, 1999).

Direct Microscopic Somatic Cell Count (DMSCC).

Direct somatic cell count was performed by spreading 0.01 ml of thoroughly mixed milk from each sample over 1cm^2 area on a glass slide. The slides were left to air-dry on a flat surface and then stained by Modified Methylene Blue stain and examined microscopically using the procedure outlined by Coles (1986). Twenty (20) microscopic fields using high power oil-immersion objective (100X) were used to count the somatic cells in each field and the average was multiplied with microscopic factor (MF) of 300,000 as proposed by Coles (1986) was used per sample. The SCC data will be categorized as subclinically mastitic if SCC>1,500,000 and non-mastitic if SCC<1,500,000 cells/ml.

Analysis and Interpretation of Data

Direct Microscopic Somatic Cell Count values were compared to the California Mastitis Test scores obtained in classifying mastitis of goats. Results of the tests were analyzed descriptively and presented in table for interpretation. Correlation coefficient (r) analysis using Microsoft Excel (Microsoft Office 2013) was performed to analyze the data obtained using the formula:

$$r - \frac{SS_{xy}}{\sqrt{SS_{xx}SS_{yy}}}$$

where:

$SS_{xy} = \Sigma_i(x_i-\bar{x})\,(y_i-\bar{y})$ = sum of products of y and x

$SS_{xx} = \Sigma_i(x_i-\bar{x})^2$ = sum of squares of x

$SS_{yy} = \Sigma_i(y_i-\bar{y})^2$ = sum of squares of y

n = sample size

$\bar{y}$ and $\bar{x}$ = arithmetic means of y and x

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Values for r also range between –1 and 1, inclusively (Kaps and Lamberson, 2004).

RESULTS AND DISCUSSION

A total of twenty six (26) available goat milk samples were tested with California Mastitis Test (CMT) and Direct Microscopic Somatic Cell Count (DMSCC).

Results of the test for CMT and their corresponding CMT interpretation designed for goats and its equivalent estimated cells per mL is presented in Table 1. Whereas the average somatic cell counts for each sample for the DMSCC together with their corresponding Microscopic Factor values and interpretation is presented in Table 2.

Table 1. California Mastitis Test Results

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
CMT Result	1+	T	N	N	T	T	**3+**	N	1+	1+	1+	**2+**	N	T	N	1+	T	N	T	N	N	1+	1+	N	T	T
Cells /mL	1000 000	300 000	100 000	100 000	300 000	300 000	8000 000	100 000	1000 000	1000 000	1000 000	2000 000	100 000	300 000	100 000	1000 000	300 000	100 000	300 000	100 000	100 000	1000 000	1000 000	100 000	300 000	300 000

Table 2. Direct Microscopic Count Results

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
DMC Result	3.35	1.85	0.4	0.25	1.1	1.9	37	0.85	3.7	2.5	4.35	13.85	0.85	1.7	0.95	3.5	1.6	0.45	1.05	0.2	0.2	4.15	2.25	0.9	2.1	1.45
X300 000 MF	1005 000	555 000	120 000	750 00	330 000	570 000	1110 0000	255 000	1110 000	750 000	1305 000	4155 000	255 000	510 000	285 000	1050 000	480 000	135 000	315 000	600 00	600 00	1245 000	675 000	275 000	630 000	435 000
SCC Result	Non-M	Non-M	Non-M	Non M	Non-M	Non-M	**M**	Non-M	Non-M	Non-M	Non-M	**M**	Non-M	Non-M	Non-M	Non-M	Non-M	Non-M	Non-M	Non-M	Non-M	Non-M	Non-M	Non-M	Non-M	Non-m

Correlation coefficient (r) = 0.986

The values encoded in Microsoft Excel for the estimated cells per mL of the test scores of CMT was based on the data proposed by Scruton et al. (2010) and Escobar (1999) as presented in the Materials and Methods. Both CMT and DMSCC had identified 2 samples to be positive of mastitis out of 26 milk samples. Calculated correlation coefficient (r) of the two diagnostic tests is 0.986 which means the strength of relationship between CMT and DMSCC in detecting subclinical mastitis is 98.6%. The very high relationship result of CMT and DMSCC in this study is in conformation to the findings of Persson and Olofsson (2011) and Kalogridou-Vassiliadou et al. (1992) where their studies had resulted to substantial agreement in detecting intramammary infection in goats.

Adjustments to the CMT scoring particularly in rising the cut-off point of non-mastitic until the score of 1 (Escobar, 1999) as well as the modification of SCC until 1,500,000 cells to be non-mastitic (Scruton et al. 2010) could be attributed principally to the apocrine type of milk secretion of goats (Podhorecká et al., 2021). On this type of secretion, the apical portion of

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milk-secreting cells are pinched off together with the milk as compared to merocrine secretion in cows where there is no cell damage accounted to the secretion (Escobar, 1999). Robertson and Muller (2005), Rupp et al. (2019) and Podhorecká et al. (2021) emphasized that the apocrine kind of milk secretion in goats, could result in high somatic cell counts that normally include a high percentage of non-DNA containing cytoplasmic particles that generally distort the well-accepted relationship between SCC and the level of udder infection that occurs in cow's milk. They pointed out that in goat's milk, the SCC is extremely variable and generally much higher than in cow's milk. Intrinsic and extrinsic factors such as stage of lactation, method of milking, seasonality and food, and lactation number may influence the SCC results (Jiménez-Granado et al., 2014). In addition, Persson and Olofsson (2011) pointed out that somatic cell count is difficult to interpret in goat's eventhough it is the most widely used indicator of udder health. Compared to cows, SCC in goat milk is fairly high also in the healthy mammary gland (Rupp et al., 2019) and it increases throughout the lactation and also with parity. California Mastitis Test has been used to diagnose mastitis in dairy animals and has the advantage as "animal side" test compared to DMSCC that requires laboratory tools such as microscope. Furthermore, it does not need to be a skilled one in order to use CMT. Robertson and Muller (2005) expressed that CMT can be satisfactorily be utilized under practical farm circumstances to indicate intra-mammary inflammation in the udder of the goat, however positive findings should be confirmed by bacterial examination.

SUMMARY, CONCLUSION AND RECOMMENDATION

DMSCC and CMT have correlation coefficient (r) of 0.986 which means they have very strong strength of relationship in detecting subclinical mastitis in goats. The apocrine type of milk secretion in goats could result in high SCC that largely alter the commonly accepted relationship between SCC and the level of udder infection that occurs in milk of cows. The SCC in goat's milk is mostly much higher than in cow's milk and factors such as stage of lactation, estrus, method of milking, season, breed and lactation number may influence the SCC results. CMT has the advantage as "animal side" test compared to DMSCC and it does not need to be skilled in order to use the test or instrument, however positive findings of intra-mammary infection should be confirmed by bacterial examination.

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