RESEARCH ARTICLE
JIVA 20 (1) April 2022
https://doi.org/10.55296/JIVA/20.1.2022.39-47

Received: 19/02/2022
Accepted: 15/03/2022

EVALUATION OF TWO DIFFERENT METHODS TO ESTIMATE THE SOMATIC CELL COUNT OF BOVINE MILK

Krupa Rose Jose[1]*, Vijayakumar K.[2], Justin Davis K.[3]and Shyma V. H.[3]

Ph.D scholar[1], Professor and Head[2], Assistant Professor[3]
Department of Veterinary Epidemiology and Preventive Medicine,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680651
**Corresponding author: krupaputhuparampil@gmail.com*

ABSTRACT

The number of somatic cells in milk is a useful indicator for the presence of an inflammatory process in mammary glands. Besides, it acts as a key factor in the evaluation of udder health, milk hygiene and milk quality. Recent advances in technology have led to the development of numerous easy to use, automatic on farm methods with acceptable repeatability and accuracy for measuring somatic cell count (SCC) in large dairies as well as in small holder systems. However, studies regarding their acceptability, accuracy and reproducibility are scarce. Hence, we assessed the direct microscopic approach using Broadhurst Paley stain and an automated commercial somatic cell counter (DeLaval cell counter DCC, Sweden) in estimating the somatic cell count of bovine milk. The study revealed that out of 381 quarters examined using Broadhurst Paley stain, 254 (66.66 per cent) were found to have a SCC above 2 lakh cells per millilitre whereas only 245 (64.3 per cent) quarters had a SCC above two lakhs when examined using Delaval cell counter.

Keywords: Broadhurst Paley stain, Delaval DCC, Mastitis, Somatic cell count

INTRODUCTION

Milk somatic cell count is a long-established and internationally accepted barometer of milk quality which in turn reflects the udder health status. Somatic cells are mainly the milk-secreting epithelial cells that have been shed from the lining of the gland and white blood cells (leukocytes) that have entered the mammary gland in response to injury or infection (Dairyman's digest, 2009). When there is bacterial infection, tissue damage or other inflammatory processes affecting the mammary tissue, the number of SCC in milk increases dramatically (Rysanek *et al.*, 2001; Lindmark-Mansson *et al*., 2006) due to the transfer of white blood cells from the blood to the mammary gland. In addition, the relative proportions of cell types present in milk changes significantly,

with an increase in PMN level (up to 90 per cent). Their primary function is to protect the udder from bacterial challenge (Kehrli and Shuster, 1994).

An elevated SCC is an indicator of udder infection, mostly subclinical mastitis which often remains obscure in a herd and should be considered as a risk for spread of mastitis pathogens, both within and between herds. It has also been associated with changes in milk components such as lactose, fat, casein etc (Harmon, 1994; Kelly *et al*., 2000; Somers *et al*., 2003; Lindmark-Mansson *et al*., 2006). These modifications in milk composition, often results in altered manufacturing properties (Santos *et al*., 2003), shorter (or decreased) shelf life and adverse milk flavour. This in turn leads to changes in quality of milk, heavy economic losses and often have significant impact on the export of milk and milk products.

In most developed countries, strict legislation for monitoring the minimum SCC level is in place to ensure the milk quality as well as the price of raw milk (Jayarao *et al*., 2004; Ott and Novak, 2001; Schukken *et al*., 1992). The Indian diary industry, being the world leader in milk production and consumption of dairy products has immense prospects for progress and could be geared to meet the export challenge if a major thrust is placed on assurance of the safety and quality of the exported milk and milk products. A major strategic intervention towards this involves screening dairy animals for milk SCC at periodic intervals and proper monitoring of milk quality based on SCC. This gives a broad picture of the udder health status of the animal/herd, increases the quality of our milk and milk products and forms the basis for taking future management decisions so that we could meet the export challenge.

At present several approaches have been utilised to determine the SCC, which includes the direct microscopic somatic cell counting (DMSCC), electronic particle counting, and fluoro-optic electronic cell counting by using disc cytometry or flow cytometry.However, studies regarding their acceptability, accuracy and reproducibility are scarce. Hence, the present study was conducted to compare the direct microscopic approach and commercial somatic cell counter in terms of estimating somatic cell count in bovine milk samples.

MATERIALS AND METHODS

A total of 98 apparently healthy cross bred dairy cows belonging to different age groups (3 to 12 years), parity (1 to 5) and stages of lactation (early, mid, late), maintained under uniform management conditions in an organised dairy farm were

included in the study group**.** Prior to sample collection, detailed clinical examination of the animals were done to rule out clinical mastitis.

Sample Collection

Teats were cleaned and swabbed with 70 per cent ethanol and the midstream lacteal secretion from each quarter were aseptically drawn separately into sterile plastic screw-capped vials. Samples collected were properly labelled, placed securely in an insulated ice box and transported to laboratory as quickly as possible.

Estimation of somatic cells

Somatic cells per ml of milk were estimated by Prescott and Breed method (1910) using Broadhurst Paley triple step staining (Schalm *et al*., 1971) as well as by an automated commercial cell counter (DeLaval cell counter DCC, Sweden).

Preparation of milk films

The milk samples were mixed 15 to 25 times to obtain a uniform distribution of cells. The samples were allowed to stand for two to five minutes to permit air bubbles to rise and foam to disappear. Each microscopic slide was identified by giving a unique number. A level surface was selected and the slide was placed over the template to outline one sq.cm area.Ten microliter of milk was placed exactly in the centre of the one sq.cm area and spread evenly to cover the area delineated by the template. The films were dried at room temperature protecting them from dust and flies.

Preparation of Broadhurst Paley stain

Dissolved 1.5 g of methylene blue in 250 ml of hot 70 per cent ethyl alcohol. Added 10 ml of saturated alcoholic basic fuchsin solution (10g in 100 ml of 95% ethyl alcohol) followed by 5 ml of aniline. The solution was shaken well while keeping it warm. To this warm solution 15 ml of dilute sulphuric acid (5 ml concentrated sulphuric acid in 95 ml of distilled water) was added, mixed well, warmed and filtered. Fifty millilitres of hot distilled water was added to every 100 ml of the filtrate and mixed well. The stain is stored in a glass stoppered bottle under refrigeration.

Staining

The air-dried slides were placed on the slide rack, defattened using xylene and fixed using 95 per cent ethyl alcohol. The defattened, fixed slides were stained by Broadhurst Paley stain for four minutes, washed in water, air dried and examined under oil immersion objective of the microscope.

Counting of cells

Stained films were examined under oil immersion objective and the number of cells in 25 random fields were counted. The fields were selected by moving the slide horizontally from one edge of the film through the center to the opposite edge and then, repeated in a vertical direction. All nucleated somatic cells within a field including those at periphery with more than 50 per cent of cell body in view as well as free nuclei representing more than 50 per cent of the nuclear material were counted. Whereas, cytoplasmic mass without nucleus and small cell fragments with little nuclear material were not counted.

Calibration of the microscope and estimation of somatic cell count

The diameter of the microscopic field was measured using a stage micrometer slide ruled in 0.1 and 0.01 mm, under the oil immersion objective. The diameter was measured up to two decimal points and the area of the field was calculated using the formula πr^2.

Microscopic Factor (MF) = Area of the smear in mm^2

Area of the microscopic field

The diameter was 0.165, then r = 0.0825

So,

$$MF = \frac{100}{3.14 \times 0.0825^2} = 4,679.01 \approx 4,600$$

Since 0.01 ml of milk was taken the total number of cells per ml of milk could be calculated by using the formula,

Cell count per ml of milk = Average no. of cells per field x MF x 10Cell count per ml of milk = Average no. of cells per field x MF x 100

The SCC value greater than 2,00,000 cells per ml of milk was taken as criterion to declare the animal as subclinically infected.

Estimation of somatic cell count using DeLaval cell counter

Estimation of somatic cell counts of individual quarter samples were performed using an automated commercial somatic cell counter (DeLaval cell counter DCC, Sweden) as per manufacturer's guidelines.

RESULTS AND DISCUSSION

Somatic cell count is a measure of the milk secreting epithelial cells that have been shed from the lining of the mammary gland and leukocytes that have entered the gland in response to injury or inflammation (Jadhav *et al.*, 2018). It is the most significant indicator reflecting the udder health status and therefore an important component in the assessment of aspects of milk quality, hygiene and control of mastitis at the cow, herd and population level (Schukken *et al.*, 2003). Recent advances in technology have

led to numerous new techniques by which the milk SCC can be estimated.

The reference method for determination of somatic cell count is by direct microscopic analysis. By this method, the milk somatic cells could be visualized under the oil immersion objective of a microscope either using Newman's stain, Levowitz Weber modification of the Newman Lampert stain, Broadhurst Paley stain etc. The Broadhurst Paley staining is a triple step process that involves defattening, fixing and staining. Here the milk solids stain pink, mononuclear cells - deep blue, PMN leukocytes - pale blue and bacteria are either deep blue or light blue (Fig. a). An important advantage of this method is that unlike in Newman's stain where both the milk solids as well as somatic cells stain blue, here the somatic cells stand clearly against a light background so that counting becomes easier. Moreover, this approach does not require any costly equipment, except a light microscope. However, the major drawback of this method is that it is time consuming and requires well trained staff to maintain its accuracy and reproducibility. Moreover, the milk films may come off during rinsing and if the intensity of the stain is on light side, the PMN cells may be overlooked (Schalm *et al*., 1971).

The DeLaval cell counter (DCC; DeLaval International AB, Tumba, Sweden) (Fig. b) isan automated, portable, battery-powered optical cell counter that provides results in less than one minute (about 40 seconds/sample). A cassette containing minute amounts of propidium iodide (PI) as the fluorescent stain is utilized to collect the milk sample. The piston attached to one end of the cassette helps to transport the sample to a counting window which is exposed to an LED light source. Cell nuclei emit fluorescent signals that are captured in an image and used to calculate SCC in milk. The DCC has proven to be an effective tool for the determination of SCC in raw bovine milk (DeLaval, 2005).

In this study, milk samples were

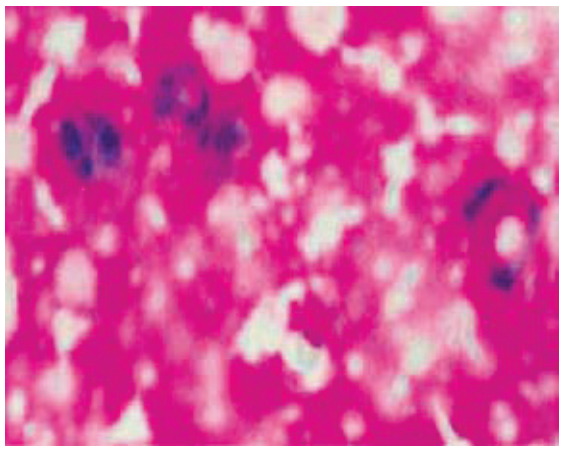

Fig.a: Milk smear, DMSCC; Stain – BroadhurstPaley stain, 1000X

Fig. b: DeLaval Cell counter and cassette

collected from 98 apparently healthy cross bred lactating animals without any visible abnormality on udder or milk. Eleven cows (11.2 per cent) had blind mammary quarter and hence the results for 375 quarters were included in the analyses. Each individual quarter sample were analysed separately by both the direct microscopic method using Broadhurst Paley stain as well as by anDelavalcell counter.

The cut-off value for SCC to declare SCM is not uniform across the globe, and each country has its own regulatory SCC limits. According to the FDA regulations, the legal milk SCC limit for cows in United states is 7.5 lakh cells per millilitre. However, in European countries, New Zealand, Australia and Canada, the cut off values is 4 lakh cells per millilitre. In India, there are no such regulatory SCC standards for milk production (Jadhav *et al*., 2018). A detailed review of literature (Ghosh *et al*., 2004; Samanta *et al*., 2006; De and Mukherjee, 2009; Singh and Garg, 2012; Tarate *et al*., 2012; Gera and Guha, 2012) revealed that a wide variation of SCC limits ranging from 1.22 lakh cells to 15.51 lakh cells per millilitre of milk are being used to describe the health status of cows in India. However, the majority of the observations range from two lakhs to more than or equal to 4 lakh cells per millilitre. Hence, in the present study, a SCC value of more than 2 lakh cells per millilitre was considered to declare SCM.

Out of the 381 quarters examined for DMSCC using Broadhurst Paley stain, 254 (66.66 per cent) were found to have a SCC above 2 lakh cells per millilitre whereas only 245 (64.3 per cent) quarters had a SCC above two lakhs when examined using Delaval cell counter (Table 1).

The disparity in SCC values estimated by DMSCC and automated Delaval cell counter could be attributed to the tendency of artifacts to get stained and the problem of cell aggregation that may arise during DMSCC that leads to the uncertainty in the number of cells counted (Alhussein and Dang, 2018). Despite the fact that the Delaval cell counter is user-friendly and does not require the submission of samples to a laboratory, one of the major factors that prevent the widespread use of Delaval cell counter is the requirement of

Table 1: Comparison of different methods to estimate somatic cell count

Sl. No.	Test	Positive (%)	Negative (%)	Total
1.	Direct Microscopic somaticcell count	254 (66.67)	127 (33.33)	381
2.	Automated commercial cell counter (DeLaval cell counter DCC, Sweden)	245 (64.3)	136 (35.7)	381

an expensive equipment and single use cassettes. This seems unaffordable to most of the landless and marginal farmers which comprises a majority of the Indian dairy sector. However, the use of an accurate technique for the measurement of SCC could be of immense use in large dairy herds which maintain more than 100 lactating cows wherein quality assurance will be an added attribute in the strive towards clean milk production.

CONCLUSION

Determination of the somatic cell count in milk is a key factor in evaluation of milk quality and udder health. It is inevitable that more complete and dynamic udder health programs and monitoring systems encompassing somatic cell counts are to be developed and implemented. A comprehensive understanding on the relationship between the SCC and the milk yield at quarter, cow, herd and population level needs to be developed. Other factors affecting the SCC, such as age, breed, parity, stage of lactation, milk output, season and temperature humidity index, must be taken into account. These will help to establish a regulatory SCC standard for milk production and thereby strengthens the milk quality monitoring system at the grass root level so that our milk and milk products are accepted internationally and gives the producer a higher price.

ETHICAL APPROVAL AND CONSENT STATEMENT

Oral consent was taken from the farm manager before sample collection. There is no specific law in India that requires the permission from ethics committee for collecting milk samples as a part of disease investigation and herd health management programmes.

CONFLICTS OF INTEREST

There were no conflicts of interest reported by the author (s).

ACKNOWLEDGEMENT

This paper is a part of the M.V.Sc. thesis submitted by the first author to the Kerala Veterinary and Animal Sciences University. The authors are thankful to the Kerala Veterinary and Animal Sciences University for providing the facilities needed for carrying out the research.

REFERENCES

Alhussein, C.R. and Dang, J.W. 2018. Biofilm formation in Staphylococcus implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials. *Biomaterials*. **33(26)**: 5967-5982.

Dairyman's digest. 2009. Why should you know about somatic cell counts?. Winter Issue.

DeLaval. 2005. Available at:http://www.delaval-us.com/Products/Milking Equipment/Milk Quality/DCC/default.htm

De, U.K. and Mukherjee, R.2009. Prevalence of mastitis in crossbred cows. *Indian Vet. J*. **86**:858-859.

Gera, S. and Guha, A. 2012. Effect of subclinical mastitis on milk biochemical constituents in crossbred cows. *Indian Vet. J*.**89**: 33-34.

Ghosh, C.P., Nagpaul, P. K. and Shiv Prasad. 2004. Subclinical mastitis in cattle and buffalo and its impact on somatic cell count and milk composition. *Indian J. Dairy Sci*.**57**: 329-333.

Harmon, R.J. 1994. Physiology of mastitis and factors affecting somatic cell counts. J. Dairy Sci. **77**:2103-2112

Jadhav, P.V., Das, D.N., Suresh, K.P. and Shome, B.R. 2018. Threshold somatic cell count for delineation of subclinical mastitis cases. *Vet. World*.**11(6)**: 789.

Jayarao, B.M., S. R. Pillai, A. A. Sawant, D. R. Wolfgang, and N. V. Hegde. 2004. Guideline for monitoring bulk tank milk somatic cell and bacterial counts. *J. Dairy Sci*. **87**:3561–3573.

Kehrli Jr, M.E. and Shuster, D.E. 1994. Factors affecting milk somatic cells and their role in health of the bovine mammary gland. *J. Dairy Sci,77*(2): 619-627.

Kelly, A.L., Tiernan, D., O'Sullivan, C. and Joyce, P. 2000. Correlation between bovine milk somatic cell count and polymorphonuclear leucocyte level for samples of bulk milk and milk from individual cows. *J. Dairy Sci*.**83**: 300–304.

Lindmark-Månsson, H., Bränning, C., Alden, G. and Paulsson, M., 2006. Relationship between somatic cell count, individual leukocyte populations and milk components in bovine udder quarter milk. *Int. Dairy J.* **16(7)**: 717-727.

Ott, S.L., and P. R. Novak. 2001. Association of herd productivity and bulk tank somatic cell counts in U.S. dairy herds in 1996. *J. Am. Vet. Med. Assoc*. **218**:1325–1330.

Prescott, S.C. and Breed, R.S. 1910. The determination of the number of body cells in milk by a direct method. *J. Inf. Dis*. **7**:632.

Rysanek, D., Vladimir B., Zbysek, S. and Miroslav, T. 2001. Variations among Unbred Heifers in the Activities of Polymorphonuclear Leucocytes from

the Mammary Gland and Blood. *J. Vet. Med.* **48(1)**:31-41.

Samanta, A., Prasad, S. and Ghosh, C.P. 2006. Incidence of subclinical mastitis in Karan Swiss and Karan Fries crossbred cows. *Indian J Dairy Sci*. **57**: 329-333.

Santos, M.V., Ma, Y. and Barbano, D.M. 2003. Effect of somatic cell count on proteolysis and lipolysis in pasteurized fluid milk during shelf-life storage. *J. Dairy Sci.* **86**: 2491-2503.

Schukken, Y. H., K. E. Leslie, A. J. Weersink, and S. W. Martin. 1992. Ontario bulk-milk somatic cell count reduction program. 2. Dynamics of bulk-milk somatic cell counts. *J. Dairy Sci*. **75**:3359–3366.

Schukken, Y. H., Y. T. Grohn, B. McDermott, and J. J. McDermott. 2003. Analysis of correlated discrete observations: Background, examples and solutions. *Prev. Vet. Med*. **59**:223–240.

Schalm, O.W., Carrol, E.J. and Jain, N.C. 1971. Bovine Mastitis. (1st Edn), Lea and Febiger, Philadelphia, U.S.A. 360p.

Somers, J.M., O'Brien, B., Meaney, W.J. and Kelly, A.L. 2003 Heterogeneity of proteolytic enzyme activities in milk samples of different somatic cell count. *J. Dairy Res*. **70**: 45–50.

Singh H.M. and Garg, H.M. 2012. Effects of antibiotic dry cow therapy and internal teat sealant (Teatseal) on milk somatic cell counts, clinical, and subclinical mastitis in early lactation. *J. Anim. Sci.* **94**: 76-77.

Tarate, S.B., Jadhav, P.V., Ghadhigaonkar, D.D., Khade, P., Bhatt, V., Khan, A., Joshi, C.G. and Rank, D.N. 2012. Evaluation of breed- dependent variation in microbial load during scbclinical mastitis in cattle using Real Time PCR. *Indian J Dairy Sci*. **65**: 60-65.