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Optimizing Mastitis Diagnosis: Beyond SCCs

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Abbreviations: SCC: Somatic cell count; SCM: Subclinical mastitis; CM: Chronic mastitis; DSCC: Differential somatic cell count; F-DSCC: FOSS DSCC

Introduction

Dairy farming is a multi-dollar industry, and the profits of the industry are linked to the udder-health and milk production on the farm animals. The development of the dairy sector, while bringing many benefits, has also led to increased concerns about animal health issues like mastitis. This condition significantly reduces milk production, causing financial losses to dairy farmers. This also comes along with another challenge of lack of efficient diagnostic tools to detect mastitis at an early phase.

As per the latest data the most efficient way to diagnose mastitis and its type is either the complete somatic cell count (SCC) or the Californian Mastitis Test. SCC is quantified as the number of cells per milk, including all the cellular materials, predominantly immune cells (polymorphonuclear cells or PMN, lymphocytes and macrophages). The SCC determines the quality of milk, the lower the value the better is the quality. The average SCC in milk of all US dairies has been below 180,000 [1], indicating that in the coming years SCC will be less than 100,000. Presently the US and European Union limits 400,000 SCC in milk.

How does SCC play a biggest role in mastitis? When a new infection occurs, there is a shift in the proportion of immune cells, with a notable increase in neutrophils. For example, in case of healthy glands (no pathogens) and SCC < 100,000, the percentage of PMN, lymphocytes and macrophages (detected using microscopy) were 10%, 67% and 23% respectively [2]. In severe mastitis, SCC >400,000, the percentage of PMN, lymphocytes and macrophages changed to 82%, 5% and 13% [3]. Considering somatic cells as the base, animal having SCC less than 100,000 is healthy, SCC between 200,000 to 300,000 is the SCM and above 400,000 is the the mastitis [4]. An effective method for predicting new infections in bovine mammary gland was detected by seeing a shift from below to above the threshold of 200,000 with low sensitivity (0.4), but notably high specificity (0.92) [5].

Several studies have advocated the use of differential somatic cell count (DSCC) to monitor proportions of different immune cells in milk. Owing to limitations of machine to monitor DSCC at large scale and farm level, limits usefulness in detecting early mastitis or SCM. Two machines that measure differential somatic cell counts are FOSS DSCC (F-DSCC) and QScout system. The F-DSCC is an advanced tool used for measuring differential somatic cell count in milk. Unlike traditional SCC, DSCC differentiates the proportion of neutrophils, lymphocytes, and macrophages, providing deeper insights into under health. By detecting shifts in immune cell proportions, such as increased neutrophils during infection, F-DSCC helps in identifying SCM early and improving herd management [6]. The QScout® MLD (milk leukocyte differential), developed by Advanced Animal Diagnostics (USA), is an innovative tool used for early detection of SCM in dairy cattle [7]. It analyzes milk samples on-farm to detect SCC and shift in proportions of immune cell types, particularly neutrophils.

In field conditions, the clinical presentation of mastitis may differ from expectations. An animal that appears completely healthy, showing no visible signs of inflammation in the udder,

can still have SCC >400,000. This elevated SCC, despite the absence of clinical symptoms, often goes unnoticed because the under seems normal. In this situation, the DSCC could be helpful in detecting new infections based on the shift in proportions of immune cells. Moreover, the additional parameters can also be established to form a more definitive conclusion about the disease stage. Incorporating other parameters such as cell size and the percentage of dead cells would provide comparative data, helping differentiate the type of mastitis. Based on cell size, the PMN typically ranging from 10-12 µm, lymphocytes ranges from 7-10 µm, whereas macrophages are the large immune cells of 12-15 µm and play key roles in chronic stages of mastitis [8].

In a hypothetical scenario (Figure 1), we are comparing three cows under similar conditions (age, parity, lactation stage, and milk yield) but with differing SCC and average cell size. The SCC of Animal-1 milk is 100,000 with an average cell size of 8 µm (primarily lymphocytes, say 67%) and that of animal- 2 is 450,000 with an average cell size of 12 µm (PMN, say 82% cells). Likewise, in Animal-3 the SCC is 400,000 with an average cells size of 14 µm (macrophage, say 75% cells). Animal-1's SCC is indicative of healthy stage along with a smaller average cell size of < 8 µm, suggests a predominance of lymphocytes, which are generally around 7-10 µm in size. Animal-2 has much higher SCC of 400,000 and a larger average cell size of > 8.0 µm, indicating a predominance of PMNs, which are typically 8-10 µm PMNs, particularly neutrophils, are the first responders during acute inflammation, rapidly migrating to sites of infection to combat invading pathogens. Animal-3 has also SCC 400,000 but the average cell size of >12 µm and the predominance of macrophages (75%) suggest a different stage of mastitis. Macrophages are generally larger (12-15 µm) and are critical in the later stages of inflammation, where they help to resolve infection and clear out dead cells and debris. Based on the cell types and their sizes, it can be inferred that Animal-1 may not have infection, Animal-2 is undergoing an acute infection, and Animal-3 is in a later stage, potentially moving towards recovery but still experiencing chronic inflammation. Thus, SCC can be the indicator of early and chronic mastitis depending upon the average cell size and predominance of proportions of immune cells.

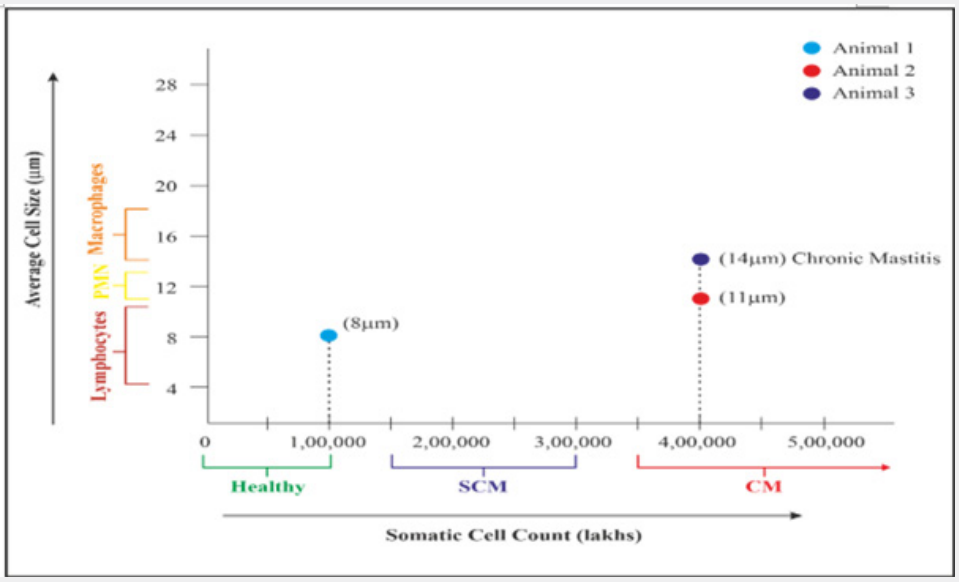

Figure 1: SCC and cells size role in detecting mastitis. Animal-1 is healthy with no apparent infection (SCC level 100,000) with an average cell size of 8 µm (due to predominance of lymphocytes). Animal -2 and Animal -3 have higher SCC level of 400,000 with an average cell size of 11 µm (due to predominance of PMN) and 14 µm (due to predominance of macrophages), respectively. Owing to predominance of immune cells and their sizes, Animal -2 and Animal -3 can be predicted to be the case of clinical mastitis and chronic mastitis, respectively.

The average diameter of viable cells in healthy cow milk was observed to be 5.94 µm, with a corresponding scatter plot distribution skewed towards the left (Figure 2A). In contrast, milk samples with elevated (SCC) exhibited an increase in both the average diameter of viable cells (7.52 µm) and a rightward shift in the scatter plot distribution (Figure 2B). Furthermore, milk from cows with mastitis demonstrated a higher density of viable cells with an even larger average diameter (9.53 µm) (Figure 2C).

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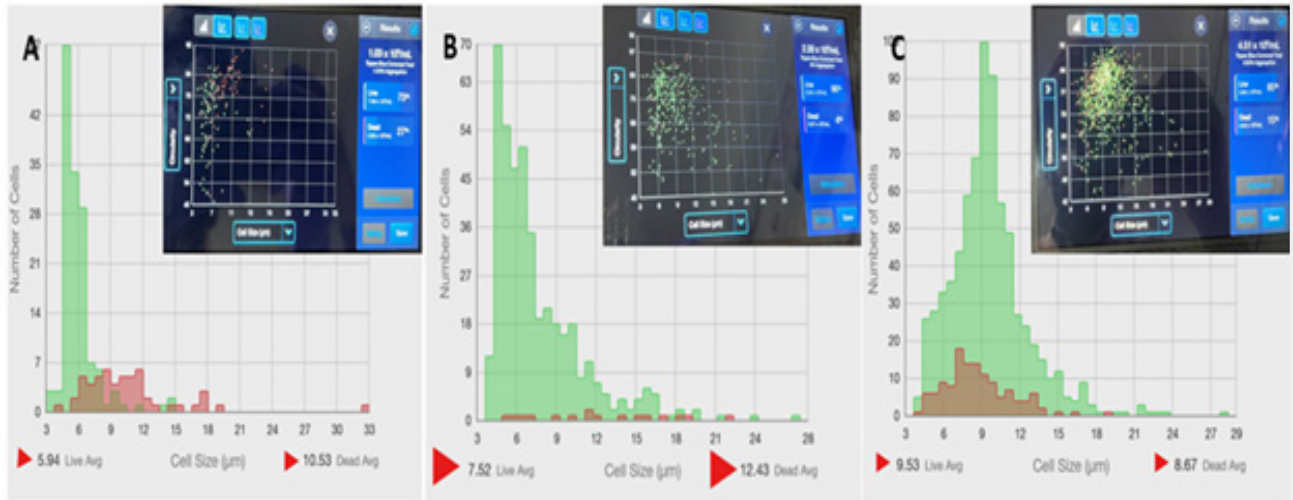

Figure 2: Representative distribution of SCC and cells size in milk of healthy (A), subclinical mastitis (B) and clinical mastitis (C) cow evaluated by automated cell counter using live and dead cell counting. Arrows indicating average size of live cells (left arrows in each panel) and dead cells (right arrow in each panel) in SCC. Cell size and dead cell percentage can be the parameters of detecting subclinical mastitis in dairy animals, in addition to differential SCC.

Comparing the percentage of dead cells within the SCC or DSCC could provide valuable insights into the severity of mastitis and the immune response dynamics. Dead cells in milk, such as apoptotic or necrotic immune cells (primarily neutrophils), indicate ongoing or severe inflammation. SCM might show a lower percentage of dead cells, with immune cells still functional and capable of clearing the infection. This could be early sign of mastitis. A higher percentage of dead cells could be indicative of advanced infection or a less efficient immune response, as seen in more severe forms of mastitis

The varying SCCs and immune cell proportions provide valuable insights into the health of the udder and the stage of infection in each animal. Monitoring SCC and identifying dominant cell types, such as lymphocytes, neutrophils, and macrophages, allow for more tailored treatment approaches, optimizing recovery while minimizing the use of antibiotics. Assessing the percentage of dead cells within SCC or DSCC will enhance understanding of infection severity and progression. It highlights the importance of early detection and continuous monitoring to manage mastitis more effectively and reduce its impact on milk production. Timely diagnosis can help timely treatment of the animal and thus prevent Farmer to suffer from economic losses due to milk production losses.

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