INVESTIGATING THE ROLE OF DIFFERENTIAL SOMATIC CELL COUNT IN GUIDING ANTIBIOTIC TREATMENT AND SELECTIVE DRY COW THERAPY FOR LACTATING DAIRY CATTLE

by

CARMEN MARIE BURNER

(Under the Direction of Valerie Ryman and Todd Callaway)

ABSTRACT

Mastitis remains a significant challenge for maximizing dairy cow health and well-being and reducing antibiotic usage. Therefore, investigation of novel mammary health parameters (**MHP**) for improving mastitis treatment plans is necessary. Our objectives were to: a) evaluate the relationship between differential somatic cell counts (**DSCC**) and antibiotic success during subclinical mastitis, b) determine DSCC thresholds associated with cure after antibiotic treatment, and b) evaluate how DSCC performs as a potential MHP in selective dry cow therapy (**SDCT**) programs modeled using machine learning. Results found differences in DSCC between quarters that cured an intramammary infection (**IMI**) compared to quarters that failed to cure following antibiotic administration. Total leukocyte counts were the optimal MHP to use in setting DSCC thresholds for maximizing IMI cure rates. Conversely, the integration of DSCC into machine learning models for detection of mastitis at the time of dry off did not improve the models' classification metrics. In addition, the combination of these MHP did not improve the models' classification metrics compared to each alone.

INDEX WORDS: Mastitis, Differential somatic cell count, Intramammary infection, Selective dry cow therapy, Antibiotic therapy

INVESTIGATING THE ROLE OF DIFFERENTIAL SOMATIC CELL COUNT IN GUIDING ANTIBIOTIC TREATMENT AND SELECTIVE DRY COW THERAPY FOR LACTATING DAIRY CATTLE

by

CARMEN MARIE BURNER

B.S., The University of Florida, 2023

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2025

INVESTIGATING THE ROLE OF DIFFERENTIAL SOMATIC CELL COUNT IN GUIDING ANTIBOTIC TREATMENT AND SELECTIVE DRY COW THERAPY FOR LACTATING DAIRY CATTLE

by

CARMEN MARIE BURNER

Major Professor:	Valerie Ryman
	Todd Callaway
Committee:	Anderson Alves
	Emmanuel Rollin

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
May 2025

DEDICATION

I would like to dedicate this to my mentors, friends and family who have guided me through my academic journey, as well as shape me into the person I am today.

ACKNOWLEDGEMENTS

I would like to acknowledge my master's committee for their continuous support these past two years and for always believing in me. I would like to thank Dr. Valerie Ryman for taking a last-minute chance on me and for all your mentorship. You have taught me so much both academically and personally and I have truly looked up to you these past two years. Thank you for believing in me and showing me how to advocate for myself.

To Dr. Todd Callaway, thank you for adopting me into your lab as one of your own. I greatly appreciated the writing opportunities you have provided for me as well as your continued academic support during a tough period of my life. To Dr. Anderson Alves, thank you for all of your hard work and dedication to my statistical design. Thank you for your patience in answering my endless questions and emails about coding and statistics in general. To Dr. Emmanuel Rollin, thank you for your continuous support on my committee and for providing valuable input on my projects and writing.

I would like to acknowledge my undergraduate researchers, Angela, Destyni, Sophie, and Yuli who came to me on my second ever sample collection and stuck with me for the past two years. I thoroughly enjoyed mentoring you all though in all aspects of school, research, and life. I look forward to seeing you all progress through this journey and to the next. I would also like to acknowledge the UGA Dairy Farm staff for all of their help and support throughout and after my projects. In particular I would like to shout-out Annie and Kayleigh who became my adopted undergraduate researchers and made long days pulling data and running tests much more enjoyable. I would also like to specially

thank Joey and Brittany for dealing with all my text messages and phone calls, for helping me move cows (especially 8028), and for being additional support for me throughout my masters. Thank you for everything you have done.

I would like to acknowledge the Staff throughout our building who make our lives so much easier, make up some of our biggest cheerleaders, and are wonderful people to rant with.

I would like to acknowledge all the graduate students who have been there for me through this journey and for always being a source of great conversations, laughs, memories and procrastination. Whether it's get togethers at Savannah's, picking up free Facebook furniture with Molly, Superbowl parties or Easter dinner with Jordan and Caitlyn, or endless conversations sitting in the bull pen. Thank you for your endless support.

Lastly, I would like to especially thank Hunter Perez for all of his unwavering support. I don't think there are enough words to convey everything you have done for me. From our endless nights of dinners, the way you continuously push me both academically and personally, for teaching me so much about locally sourced beef from Georgia, and quite honestly for just being my rock. Thank you for all your help with sample collections, especially pre-enrollments, and for letting me adopt some of your undergrads as well! I cannot imagine these past two years without you, and I look forward to seeing how you continue to push me while I continue on to my PhD. Thank you again for quite literally everything and for making Athens such a hard place to say goodbye to.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Mastitis remains one of the costliest diseases in the dairy industry with an estimated global impact of 22 billion dollars (Rasmussen et al., 2024). Mastitis is an inflammation of the mammary gland most commonly caused by an intramammary infection (**IMI**) with bacteria. Presentation of mastitis can be either clinical (i.e. with visual abnormalities to the milk or udder) or subclinical (i.e. lack of visual abnormalities, but changes in milk quality). Subclinical mastitis is typically described as an elevation in the somatic cell count (**SCC**) of ≥200,000 cells/mL. When an IMI occurs, the innate immune system is activated resulting in an influx of white blood cells to the mammary gland, in particular neutrophils, thus corresponding to the increased SCC characteristic of both clinical and subclinical mastitis. Though utilized for several decades and still fairly reliable, SCC could be altered as a result of various physiological and environmental factors in the absence of IMI.

Limitations to current diagnostic parameters have led to the exploration of differential somatic cell counts (**DSCC**) within the dairy industry for IMI detection. Differing from but related to SCC, DSCC focuses on the distribution of white blood cell types in milk which dramatically changes as a result of inflammation, particularly during IMI. An increase in specific cell types, i.e., neutrophils, in the milk could perhaps indicate an IMI with greater accuracy than SCC given the more refined snapshot of the mammary immune response. Previous research has established differences in cellular populations

between infected and uninfected quarters where the combination of neutrophils and lymphocytes were significantly elevated in infected quarters (Schwarz et al., 2011, Damm et al., 2017). As a diagnostic parameter, DSCC shows great promise, but the verdict is out on whether it can indeed outperform SCC universally. Even more important and perhaps more exciting, research on how DSCC could guide antibiotic treatment decisions is limited. Similar concepts related to differential white blood cell counts are commonly investigated in the human health field and have been used to better understand disease severity and prognosis for various diseases including bacterial infections and cancer (Yamanaka et al., 2007, Azab et al., 2012, Du et al., 2024, Garcia-Flores et al., 2024). Based on these reports, the utilization of DSCC could have the potential to better refine treatment decisions during lactation and for cows entering the dry cow period.

To address these gaps within literature the purpose of this research was to evaluate the relationship between DSCC and intramammary antibiotic success following an IMI. In addition, the performance of DSCC as a parameter in establishing a selective dry cow therapy program was investigated to determine its effectiveness compared to other common mammary health parameters (e.g. SCC) as well as inexpensive cow-side tests.

CHAPTER 2

LITERATURE REVIEW

In today's society, the dairy industry faces many challenges; however, mastitis remains one of the most economically significant diseases, and yet the ability to accurately define and diagnose mastitis also remains elusive. With fewer replacement heifers available for producers, herd demographics are shifting to an older population as they are retaining older cows to help maintain production levels. Unfortunately, these older cows are also more susceptible to mastitis. As the dairy industry prepares to face challenges associated with an aging herd, it is paramount to have methods for earlier detection of mastitis. Differential somatic cell count (**DSCC**) may offer a potential solution as it provides a more detailed assessment of the immune response occurring within the udder. This review will explore current parameters used to detect mastitis, as well as the current state of DSCC research and its role in improving antibiotic decision making.

What is mastitis?

Mastitis has become synonymous with intramammary infection in many areas of the dairy sector and in consumer populations. However, according to etymology of the word "mastitis"; this disease is an inflammation (*-itis*, English 19th century) of the breast or mammary gland (*mastos* meaning breast, Greek). In reality, mastitis can be caused by several different chemical, metabolic and physical factors, but is most commonly caused by an intramammary infection (**IMI**). However, the word "mastitis" is used throughout the studies but these studies typically also include microbiological culture indicating authors

are referring to an IMI specifically (Deluyker et al., 2005; Schwarz et al., 2011a; Pilla et al., 2012). Highlighting these distinctions in the use of the word "mastitis" only scratches the surface of yet to be determined agreements that must be made in the industry and will be a theme throughout this article. When described herein, mastitis and IMI can be considered synonymous.

When discussing mastitis as an IMI, it can be categorized in several ways depending on causative pathogen (environmental vs. contagious) and symptomatic presentation (subclinical vs clinical). Contagious pathogens commonly inhabit the skin of the teat or within the teat canal and cistern and spread from cow to cow particularly in the milking parlor via inflation lines, milker hands, or residual milk left in clusters between cows (Fox and Gay, 1993). Common contagious pathogens include *Staphylococcus aureus*, *Streptococcus agalactiae,* and *Mycoplasma bovis*. In contrast, environmental pathogens are typically opportunistic pathogens that enter the teat canal when directly exposed (e.g. laying in soiled bedding). Common environmental pathogens include Gram negative pathogens such as, *Escherichia coli* and *Klebsiella spp.*, as well as Gram positive pathogens including *Streptococcus uberis*. These infections are generally more robust but tend to have a shorter duration and resolve without antibiotic intervention particularly in the case of infections with Gram negative bacteria (Shuster et al., 1997; Suojala et al., 2013). Management of pathogens vary greatly based on this etiology. Given environmental pathogens spread outside of the milking parlor, even well-managed herds are still greatly impacted as Gram negative pathogens are ubiquitous in the cow's environment. Management of environmental pathogens typically involves reducing moisture and organic contamination within bedding, as well as keeping teat ends and mammary glands free of

organic matter (Hogan and Smith, 2012). On the other hand, contagious pathogens such as *Strep. agalactiae* and *Staph. aureus* are closely monitored in herds to prevent further spread, where the focus is typically on strong culling procedures or segregation of infected animals. To prevent the spread of contagious pathogens within a herd, optimal hygienic practices within the parlor are a necessity and include proper cleaning of milking equipment and following proper pre- and post-milking procedures (Farnsworth et al., 2011a; Farnsworth et al., 2011b).

The symptomatic presentation of mastitis (clinical vs. subclinical) continues to be a topic of interest, particularly as it relates to administration of antibiotic therapy. Clinical mastitis is characterized by visuals signs of inflammation in the milk or mammary gland. The mammary gland may appear red, swollen, hot, hardened, or painful, and milk may present with clots, flakes, ropiness, discoloration, or blood. An estimated 25% of cows in the US dairy industry will experience clinical mastitis over the course of a year (USDA, 2018). Subclinical mastitis, on the other hand, lacks visual signs in the milk or mammary gland, rather changes in milk quality not seen by the naked eye are altered. Typical diagnosis of subclinical mastitis depends on elevated milk somatic cell count (**SCC**), ideally coupled with microbial culturing to ensure accurate diagnosis (Smith, 1999; Williamson et al., 2022). Definitions of subclinical mastitis consist of a wide range of SCC coupled with confirmation via bacterial culture, while others depend on SCC alone. As a result, some authors present SCC thresholds that are much higher than the current industry standard for defining subclinical mastitis, which is ≥ 200,000 cells/mL according to the National Mastitis Council (**NMC**). Nielen et al. (1995) defined subclinical mastitis as > 500,000 cells/mL for at least a week when evaluating inline SCC systems, whereas

Deluyker et al. (2005) defined subclinical mastitis as SCC > 300,000 with confirmation of bacterial growth. Further contrasting, Schwarz et al. (2011) defined subclinical mastitis as a SCC of 100,000 cells/mL or greater (Schwarz et al., 2011b). In order to be able to properly monitor cows for mastitis, there is a need to be consistent for both on-farm application of tools but also for comparisons across published literature.

Historically the Dairy Herd Improvement Association (**DHIA**) has been a standard system to monitor SCC at the herd- and cow-level. Unfortunately, herd-testing programs such as DHIA have seen a 40% decline in herd enrollments from 2014 to 2022 (CDCB, 2022). The decline in enrollment could be attributed to the consolidation of farms or financial challenges of remaining with DHIA. Anecdotally, larger farms report hesitation to enroll in programs where they feel that cow and herd data is freely accessible to institutions and the public. However, given the cost of mastitis, investments in early detection and monitoring through systems like DHIA are invaluable to reduce costs attributed to mastitis.

Collectively, clinical and subclinical mastitis represent the costliest diseases of the global dairy industry, according to a recent 2024 publication, at an estimated cost of 22 billion dollars (Rasmussen et al., 2024). Associated losses consist of both direct and indirect cost including discarded milk, therapeutics, increased labor, premature culling, and decreases in milk production (Pinzón-Sánchez et al., 2011; Rollin et al., 2015; Leite de Campos et al., 2023). Financial models for assessing financial impact of clinical mastitis are easier to access as there are clear indications when a cow is clinically affected, and thus milk quality is compromised requiring milk discard. Subclinical mastitis it thought to have a greater financial impact as a result of decreased milk production potentially for a

prolonged period of time; however, accurate models are more difficult to establish with subclinical cows. Factors that can impact the models are differences in definitions for detection leading to the inability to properly differentiate between subclinical infected cows and healthy cows, point in time of diagnosis relevant to exposure, etc. (Zhao and Lacasse, 2008; Romero et al., 2018). If there was consensus in defining subclinical mastitis and factors economically impacted by it, there could be more targeted approaches to limit the financial impact. One proposed way to better understand if a cow is sub clinically infected is by understanding the role of the immune response to an infection.

Immune response during IMI

The immune system is responsible for protecting the mammary gland from microbial challenges. In the mammary gland and throughout most tissues in the body, there are three predominate populations of white blood cells (**WBC**): macrophages, neutrophils, and lymphocytes. Macrophages reside in the tissue and monitor for infection. Upon detection of a microorganism, macrophages initiate the innate immune response while also phagocytosing and killing microbes in advance of neutrophil influx (Sordillo et al., 1987). Neutrophils are primarily responsible for maximal phagocytosing and killing microbes once they are recruited. Lymphocytes, which include T and B cells, are mainly responsible for the production of memory cells and antibodies during the adaptive immune response, however their role in the mammary gland appears to be limited or not fully understood in the context of IMI (Sordillo and Streicher, 2002). These cell types, in addition to mammary epithelial cells (<7%), comprise SCC (Lee et al., 1980).

In the healthy mammary gland, 57 to 80% of the WBC population consists of macrophages and lymphocytes (Östensson, 1993; Kehrli and Shuster, 1994; Merle et al.,

2007; Pilla et al., 2012). When a pathogen breaches the physical and chemical barriers of the teat, macrophages recognize the infectious agent and initiate the innate immune response, and more specifically, inflammation (Figure 2.1). Robust and acute neutrophil recruitment and migration through a loosened vascular barrier are a key step in inflammation and responsible for robustly increasing SCC (Paape et al., 2003). Once at the site of infection, neutrophils have various defenses to contribute to microbial killing including phagocytosis, degranulation, and release of neutrophil extracellular traps (**NET**) by a process known as NETosis (Brinkmann et al., 2004). Neutrophils will eventually die by apoptosis, at which time macrophages will clean up dead and dying neutrophils by efferocytosis and promote tissue restoration.

The signs and symptoms detected during clinical mastitis are a result of this immune response. Inflammation is characterized by redness, pain, heat, swelling, and loss of function. Heat and redness are a result of increased blood flow to the infected region; swelling and pain due to loosened vascular barriers and movement of fluid and molecules from the blood into the infected tissue. Loss of function is very clearly seen when milk production rapidly declines during clinical mastitis. While these physiological events occur during subclinical mastitis, the severity is milder and thus not visible to the naked eye. However, with specific monitoring, increased SCC (as a result of neutrophil influx) can indicate an active or recent inflammatory event, typically a result of infection.

Parameters to detect subclinical mastitis

Producers can utilize either on-farm methods or laboratory testing (e.g. DHIA, private labs, Extension services, etc.) to detect subclinical mastitis or to monitor mammary health. Some of the most common parameters or tests include SCC, California Mastitis

Test (**CMT**), electric conductivity (**EC**), and microbiological culturing, though there are other methods including, but not limited to, polymerase chain reaction (**PCR**) assays and biomarkers such as lactate dehydrogenase or acute phase proteins (Chakraborty et al., 2019). Many of these methods can be used alone or in combination with each other, particularly as it relates to culturing for determination of causative pathogen. The following subsections discuss these parameters and existing literature as it relates to subclinical mastitis diagnoses.

Somatic cell count

As discussed previously, SCC is the measure of body cells within milk. The SCC primarily consists of WBC (lymphocytes, macrophages, and neutrophils) and a small percentage (<7%) of mammary epithelial cells. (Lee et al., 1980). When the mammary gland experiences an IMI, the SCC increases dramatically and rapidly as the immune response is activated to eliminate the invading pathogen and protect the mammary gland from long-term damage. The WBC, particularly neutrophils, within the SCC are responsible for this robust increase in SCC during clinical and subclinical mastitis. Historically, and currently still, SCC is widely used by producers (Smith, 1999).

Milk SCC can be enumerated either on-farm or in a laboratory. Common on-farm methods include cow-side options such as the Delval™ Direct Cell Counter (**DCC**) or in-line sensors within milking systems such as Afimilk™ (Afikim, Isreal) DeLaval™ (Tumba, Sweden), SomaDetect™ (Fredericton, New Brunswick, Canada), or robotic milking systems. The most common laboratory method for accessing SCC is flow cytometry via the Fossomatic™ 7 (FOSS, Hilleroed, Denmark).

Various studies have been conducted over the past several decades evaluating diagnostic test parameters (e.g. sensitivity, specificity, etc.) of several SCC detection methods compared to diagnosis by culturing. When Delaval™ DCC was compared to SCC via Fossomatic™ 7 DC (FOSS, Hilleroed, Denmark) as the gold standard, sensitivity was 75.8% and specificity was 97.5% (Hisira et al., 2023), demonstrating that DCC is reliable in ruling out non-infected cows. When subclinical mastitis was defined as a SCC of > 500,000 cells/mL, broader ranges of sensitivity (55 to 90%) and specificity (54 to 92%) were reported when evaluating inline systems (Nielen et al., 1995). The lower range of values seen with sensitivity is not ideal, as 45% of infected cows may go undetected and thus risk development of chronic infections. In contrast, a specificity as low as 54% could result in the overuse of antibiotics as 46% of cows could be identified as false positives. The wide variation seen in sensitivity and specificity of the inline system could be attributed to utilizing a SCC threshold of 500,000 cells/mL, which is not typical to the industry standard of ≥ 200,000 cells/mL.

The threshold of SCC indicative of subclinical mastitis has changed over time (Bhadwal et.at, 2011). Over the past 26 years the national SCC average for DHI test-day milk has decreased from 304,000 cells/mL in 1995 to 179,000 cells/mL in 2021 and is attributed to improved mastitis control and prevention strategies, genetic progress, and technological advancements for earlier mastitis detection (Norman et al., 2022). Along with the declining test-day milk SCC average, there has been consideration to reevaluate the threshold for subclinical mastitis diagnosis. Currently the National Mastitis Council states that a SCC of ≥ 200,000 cell/ml indicates an inflammatory response, and the quarter is likely infected or recovering from infection (NMC, 2001). Previous research has utilized

a cutoff of ≥ 300,000 cells/mL with confirmation of bacterial growth to define animals with mastitis (Deluyker et al., 2005). Though a higher threshold than industry normal Jadhav et al. (2018) found that a threshold of 310,000 cells/mL has a high sensitivity (92.6%) and specificity (91.5%). Schwarz et al. (2020) found a greater specificity (91%) when evaluating a SCC threshold of 300,000 cells/mL; however, authors reported much lower sensitivities (45 to 69%). The differences in sensitives of Schwarz et al. (2020) could be attributed to the wider range of SCC being enrolled within the study. A SCC of 300,000 cells/mL is higher than what is widely accepted, but the higher the cutoffs are set, the more animals within that population will be correctly captured as positive. If the goal of a farm is to accurately catch animals, a high threshold could be beneficial, but negative if trying to avoid overtreating animals. It is important to consider that discussed thresholds serve as a guideline as milk SCC in healthy mammary glands can fluctuate depending on physiological and environmental factors, a particular challenge when considering SCC as a diagnostic indicator (Mondini et al., 2024).

Environmental factors such as high humidity or temperatures can increase cow stress and the number of bacteria present within the environment (Morse et al., 1988; Vitali et al., 2020). As a result, the average SCC of a herd can vary based on geographical location. According to the USDA National Animal Health Monitoring System 2014 Dairy study, the eastern US had an average bulk tank SCC of 210,000 cells/mL compared to western regions that had an average of 171,000 cells/mL (USDA, 2014). Though there are many contributing factors to these differences, such as the number of cows and average herd sizes, environmental impact on SCC is recognized (Sharma et al., 2017). Additionally, physiological differences amongst cows, such as parity and stage of lactation, can also

impact SCC. As cows age and experience more lactations, the vacuum of the milking machine can weaken the teat sphincters increasing the risk of bacteria entering the teat canal during lactation (Seykora and McDaniel, 1985; Guarín et al., 2017). Older cows have also undergone more milk cycles, increasing opportunities to develop mastitis during the dry period, when most at risk (Nickerson, 2019). Moreover, previous infections may contribute to long-lasting tissue damage that increases SCC, regardless of infection status (Zhao and Lacasse, 2008). Factors such as these may result in multiparous cows having a higher baseline SCC in the absence of an IMI. For example, previous research demonstrated that multiparous cows had a baseline SCC around 95,000 cells/mL compared to primiparous cows at approximately 55,000 cells/mL (Hagnestam-Nielsen et al., 2009). Regarding lactation stage, SCC is at its highest shortly after calving and declines until approximately 45 days in milk (**DIM**), where it then slowly increases as the cow approaches the end of lactation (Kennedy et al., 1982). More specifically, when evaluating cows free of IMI, Sheldrake et al. (1983) reported a day 35 SCC of 80,000 cells/mL which increased up to 160,000 cells/mL on day 285 of lactation. Higher SCCs at calving are associated with colostrum production as the cow enters lactation, in the absence of an active infection, whereas elevation towards the end of lactation may be due to greater cellular concentration as milk volume decreases but could also be due to effects of infections on mammary tissue over the course of the lactation (Hallberg et al., 1995; Andrew et al., 2001).

Overall, the use of SCC for diagnosis of mastitis is widely accepted within the industry, although it is not without its challenges. As a result, more research is needed to

better understand the physiological changes in healthy cows and the mammary response to injury, trauma, and infection to help further guide treatment decisions.

California Mastitis Test

The California mastitis test (**CMT**) is a qualitative test that serves as an indirect estimate of SCC. To conduct the test, an equal amount of milk and reagent is mixed which lyses cell membranes, exposing DNA and proteins. The DNA and proteins react with the reagent to form a precipitate and increase the viscosity of the mixture (Schalm and Noorlander, 1957). As mentioned earlier, milk from a quarter experiencing mastitis has a high volume of cells (i.e. SCC), thus the more cells in a sample, the more gel-like the mixture becomes. Scoring of the CMT is based on a 5-point scale (Negative, Trace, 1, 2, 3), where each score equates to a range of SCC values (Table 2.1). A score of trace indicates a possible infection and a score of 1 and above is considered positive for infection.

Previous research suggested CMT to be an effective tool to diagnose IMI in cows due to its relatively good sensitivity 77.3 to 86.2% and specificity of 82.7 to 88.5% when compared to culture (Leslie et al., 2002; Fosgate et al., 2013). When evaluating the use of CMT in cows entering the dry period, Bhutto et al. (2012) and Sanford et al. (2006) reported sensitives of 70 to 86.5% and specificities of 48 to 53.1%. Physiological changes within the mammary gland during late lactation such as greater cellular turnover and concentration of cells in the milk due to decreased milk yield can increase false positives rates, impacting the test's specificity performance. On other hand, researchers have suggested the use of CMT to evaluate effectiveness of dry-off protocols (Sargeant et al., 2001; Dingwell et al., 2004). When evaluating the best time to use, Dingwell et al. (2003) found day 4 post calving to have the highest sensitivity and specificity (82.4 and 80.6%,

respectively); however, Sargent et al. (2001) reported a sensitivity of 56.7% when looking at 3 days post calving. Generally, CMT is not recommended for fresh cows or cows entering the dry period, as SCC maybe elevate due to physiological factors, not from an infection (Hagnestam-Nielsen et al., 2009).

Variation in CMT scores can be a result of errors in conducting the test and differences in interpretation, The primary challenge with CMT is that results are based on a subjective scale which can lead to great variation in sensitivity and specificity (e.g., gelling intensity; Marshall et al., 1993). For example, visual differences in a score of negative and trace may result in different conclusions based on the individual performing the test, contributing to the potential for increased false positives or false negatives. When using a cutoff of trace and above (1, 2, or 3) in early lactation cows, sensitivity and specificity for detecting an IMI was 68.8 and 71.5% respectively; however, raising the cutoff to a score of one or above decreased sensitivity to 55% while specificity increased to 86% (Dingwell et al., 2003). When considering cows across different stages of lactation, Kandeel et al. (2018) also saw a decrease in sensitivity (45 to 27%) and an increase in specificity (56 to 80%) when using trace versus a score of 1 as a cutoff. Utilizing a cutoff score of trace compared to 1 captures a broader range of cows, in turn catching more true positive cases and resulting in a higher sensitivity; however, it also means a wider range of cows could be classified as false positives which will bring specificity down.

Previous research highlighted the robust response in SCC when infected with a major mastitis pathogen compared to minor pathogens (Bannerman et al., 2004a; Bannerman et al., 2004b). Some researchers have suggested that this response to major pathogen can also be reflected within CMT, and at a minimum, aid producers in more

confidently finding quarters or cows infected with major pathogens. When evaluating the success of CMT in detecting IMI caused by major pathogens *(Staphylococcus aureus, Streptococcus spp*., and Gram-negative organisms) in early lactation, the sensitivity was 82.4% and specificity was 80.6% on day 4 of lactation (Dingwell et al., 2003). Meanwhile, when detecting minor pathogens (looking across all lactation stages) sensitivity decreased to 50%, and Middleton et al. (2004) deemed the sensitivity too low to be a useful screening tool. Furthermore, Sargent et al. (2006) found sensitivity for detection of IMI with a major pathogen to be 66.7%, whereas sensitivity for detecting minor pathogens to be 49.5%, suggesting that CMT is more accurate when detecting major pathogens than minor pathogens as it able to better detect the higher levels of SCC within the milk. An increased ability for CMT to detect quarters infected with a major pathogen vs. a minor pathogen makes sense as typically major pathogens cause a more robust SCC response with SCC exceeding 1 million cells per mL, whereas minor infections may only contribute to increases in SCC up to 400,000 or 500,000 cells per mL (Bannerman et al., 2004a; Bannerman et al., 2004b).

Given the varying ranges of sensitivity and specificity for CMT, Sargeant et al. (2001b) suggested CMT may not be ideal for making decisions about individual animals but rather could allow producers to establish the occurrence in a herd and estimate the frequency of IMI at herd level. Herd-level assessment could involve sampling a small population of cows at different production stages; however, this would not be feasible in larger herds as it would be time consuming and labor intensive to sample that many cows. Further, this would not aid producers in decision-making for antibiotic therapy.

Electrical Conductivity

Electrical conductivity (**EC**) is a measure of the conductance of milk to an electrical current and is representative of ions present within milk (Fahmid et al., 2016). During inflammation, blood vessels become more permeable and cellular damage results in ionic changes in milk (Kobayashi et al., 2013). Measurements of EC can occur at the quarter or cow level utilizing either in-line milking systems (Delaval™ or Afimilk™) or hand-held meters, such as Mas-D-Tec® (Wescor Inc., Logan, Utah). Reported values of EC in healthy quarters are highly variable due to factors such as stage of lactation (Mielke and Schulz, 1983), breed (Rothenbuhler, 1973), and milking interval (Fernando and Spahr, 1983).

The use of in-line sensors to monitor EC has existed within traditional parlors for close to 20 years and its adoption into robotic herds is more recent. In-line EC systems are more practical than handheld meters as they allow for continuous monitoring of EC with minimal labor. In many of these systems, if the sensor detects a cow with high EC, the producer is notified so that the producer can pull the individual cow for further diagnostic testing such as culturing. A survey performed in 2013, encompassing dairy producers from Michigan, Pennsylvania, and Florida reported that only 16% of herds use high EC to identify infected cows (Kayitsinga et al., 2017). In addition, only 16% of farms that use high SCC or high EC for detecting mastitis frequently or always culture milk samples to determine antimicrobial usage (Kayitsinga et al., 2017). Unfortunately, in-line EC systems can be expensive and thus inaccessible for producers to implement. Handheld conductivity meters are available (Mas-D-Tec®) and some literature suggests they are reliable in detecting subclinical mastitis (Mansell and Seguya, 2003) while others have concluded them to be unreliable (Musser et al., 1998). Unreliability of results for the handheld EC can be attributed to differences in cutoffs for defining subclinical mastitis. For example, based

off manufacturer guidelines for Mas-D-Tec®, the proposed cutoff is ≥ 5 (Okigbo et al., 1984), whereas other papers have suggested cutoffs for subclinical mastitis to be of ≥ 2 (Musser et al., 1998) or ≥ 7 (McDaniel et al,. 1994). Without a consensus on cutoffs for handheld meters, it is difficult to compare EC results between studies and disseminate out that information to producers.

In a meta-analysis by Nielen et al. (1992), an overall sensitivity of 66% and a specificity of 94% was reported for EC in mastitis detection. Although, it was noted that diagnostic capability of EC varied depending on the gold standard it was compared to (Nielen et al., 1992). When EC was compared to WBC measurements (e.g. SCC and CMT) for mastitis detection, sensitivity dropped to 57%; however, when compared to bacterial culture, sensitivity and specificity increased to 75 and 95%, respectively. The decrease in sensitivity when using WBC count measures could be due to the subjective nature of the CMT or Wisconsin Mastitis Test that contribute to this category, as they are more prone to misinterpretation (Okigbo et al., 1984; Musser et al., 1998).

Published literature suggests three common ways of utilizing EC to distinguish between healthy and mastitis quarters: absolute thresholds, inter-quartile ratios (**IQR**), or a combination of the two. The IQR is defined as the ratio between the quarter with the lowest conductivity and other quarters of the same cow. Absolute thresholds pertain to using a predetermined value regardless of individual cow variations. Literature suggests that using IQR improves sensitivity and specificity of mastitis detection compared to absolute thresholds (Jensen and Knudsen, 1991; Hamann and Zecconi, 1998; Norberg et al., 2004). When evaluating the combination of IQR and absolute thresholds, Nielen et al. (1992) reported the lowest sensitivity and specificity when absolute EC thresholds were

used (57 and 91% respectively) and highest sensitivity and specificity when a combination of absolute threshold and a difference between quarters was used (79 and 96%, respectively). The improvement in sensitivity with IQR is likely to be due to physiological factors not considered with absolute threshold. Generally, these principles are built into inline EC detection programs to account for herd and cow-level uniqueness as it relates to EC.

Physiological factors (e.g. estrus or systemic disease) are suggested to influence all quarters of a cow equally, thus by being able to compare EC between the quarters of an individual cow, mastitis detection for a specific quarter may improve (Nielen et al., 1992). In order to effectively utilize EC for mastitis detection, it is important to look at the mammary system as a whole instead of individually evaluating quarters. Variation in EC by farm and by cow makes setting thresholds difficult as trying to use a single threshold for every cow may not be an effective use of EC. If an absolute threshold is going to be used on a farm it would be most advantageous to understand the given EC dynamics of the herd or individual cow across lactation before establishing a threshold for mastitis detection.

Microbiological Culturing

Ideally, milk samples from cows or quarters demonstrating changes in SCC (direct or indirect with CMT) or EC should be cultured to determine if the changes are a result of an IMI before treating with antibiotics (Souza et al., 2016). Culturing milk samples allows for a visual diagnosis of bacteria causing mastitis enhancing the opportunity to make an informed decision with a veterinarian about a treatment regimen. Multiple options for establishing on-farm culturing currently exist on the market such as AccuMast® (Fera

Diagnostics and Biologicals Corp.; College Station, Texas), Minnesota Easy®Culture System (Saint Paul, Minnesota), and SSGN – SSGNC Quad Plate (Eurofins Scientific; Luxembourg). Generally, these plates consist of different selective media to guide a presumptive bacterial diagnosis, in particular to differentiate between Gram-positive and Gram-negative infections.

Farms that implement on-farm culturing are relatively successful, with proper training of farm staff to collect samples appropriately, conduct culturing, and read culture plates. However, collecting samples consistently utilizing aseptic techniques is challenging especially if multiple individuals are involved or composite samples are used (Royster et al., 2014). Additional concerns in culture plate reading arise when several emerging pathogens require special conditions (*Mycoplasma bovis*) or need a trained eye to identify (*Prototheca spp.*), or a less selective media to allow for growth of atypical microbes. In addition, it is possible to miss the active shedding of bacteria within a milk sample at the time of collection resulting in a false negative (Sears et al., 1990; Mues et al., 2025).

It is important to understand that on-farm culture is different from laboratory culture as generally on-farm culture provides limited information about the bacteria of interest. To more precisely diagnose pathogens, methods such as PCR assays or matrix-assisted laser desorption ionization-time of flight (**MALDI-TOF**) mass spectrometry would need to be utilized. Although PCR can detect pathogens, especially in small quantities due to DNA amplification, DNA from all pathogens live or dead will be detected. If the bacteria are already dead, treating with antibiotics would not be judicious use. Many producers who utilize PCR assay detection to identify animals infected with

contagious pathogens to segregate, and in many cases cull, expediently. Given this limitation to PCR, MALDI-TOF is considered the gold-standard as it based on bacterial culture, thus a live, dividing microbe. Bacterial identification via MALDI-TOF involves the ionization and separation of the proteins within the bacterial sample and then measured based on the time it takes ions to travel through a tube (Rychert, 2019). The use of MALDI-TOF for identifying mastitis pathogens has been evaluated and shown to have a high level of accuracy (Shell et al., 2017; Braga et al., 2018; Jahan et al., 2021). For example, Jahan et al. (2021) found an accuracy of 96% and 85% for identification of Gram-positive and Gram-negative isolates, respectively. Being able to correctly identify causative mastitis bacteria is crucial for appropriate use of antibiotics. Although MALDI-TOF is reliable, limitations to this method exist in that identification of an isolate is dependent on a fingerprint strain already being present in the comparative database, making identification of new bacteria or uncommon microbes difficult.

Overall, although the use of SCC (and CMT) and EC have been widely used to screen cows with possible IMI, they are not without their limitations. For example, SCC helps serve as a general indicator of inflammation but lacks the ability to tell exactly which WBC are increasing, a more specific aspect of active inflammation and infection. The subjective nature of CMT leaves room for increased rates of false negatives or false positives. Finally, EC can vary greatly and requires an understanding of what is normal for each cow and even for the herd. Given these limitations, the ability to have more specific insights into the health status of the mammary gland coupled with culturing for informed decision-making for antibiotic administration is crucial. Recent research suggests differential somatic cell counts (**DSCC)** could serve that need and improve detection of

IMI. The use of DSCC has the potential to improve early detection and refine treatment decisions, in turn helping to reduce antibiotic usage (Zecconi et al., 2023). Though much is still unknown about the application of DSCC, if greater research efforts are dedicated to understanding its full utility, it could pose a potential solution for one of the dairy industry's greatest challenges.

Differential somatic cell counts

As producers need better methods for mastitis detection and decision-making for antibiotic use, recent research has shifted to investigate the evaluation of DSCC. Unlike SCC, DSCC focuses on the proportions of WBC (i.e. neutrophils, macrophages, and lymphocytes) within a milk sample. Previous researchers highlighted the potential for DSCC to detect IMI across different stages of lactation (Bobbo et al., 2020) and to better understand the role of pathogens on the mammary immune response (Kirkeby 2021; Schwarz et al., 2020). Authors noted that DSCC is particularly useful in better understanding how WBC respond to an IMI.

Across literature, the percentage of cells present in a healthy mammary gland is variable, though there is a consensus that macrophages and lymphocytes predominate. Some data demonstrate macrophages as the predominate cell type (46.3 to 60%) in line with the immunological dynamics in the absence of an immune response (Kelly et al., 2000; Merle et al., 2007) while others report lymphocytes as the primary cell type ranging from 31 to 72% (Rivas et al., 2001; Schwarz et al., 2011a; Pilla et al., 2012). Reasons for these potential discrepancies could include the technique by which cells were enumerated. For example, Merle et al. (2007) evaluated cells using traditional cytology methods, whereas Schwarz et al. (2011) utilized flow cytometry. Although Rivas et al. (2001)

utilized both cytology and flow cytometry, lymphocytes were still reported as the predominant population. The differences between Rivas et al. (2001) and the other cytology papers could be a result of all three utilizing different cell isolation procedures. These technical approaches will be discussed in further detail in the following section.

There is a consensus, however, that neutrophils are present in lower percentages, around 5-12%, in the healthy mammary gland, but when a pathogen is present neutrophils increase and represent up to 90% the total milk cell population (Sordillo and Streicher, 2002). If mastitis is the result of an IMI, an influx in WBC—mainly neutrophils —would follow, and DSCC could provide producers with a clearer understanding of an active inflammatory response occurring within the mammary gland. Rather an elevated SCC could be the result of previous infections, stage of lactation, or other environmental, physiological, or metabolic factors. Moreover, after an IMI it could take up to 8 weeks for SCC to return to a baseline whereas the proportion of neutrophils would decrease far before then (Herry et al., 2017).

Strategies for DSCC Measurement

Methods to measure DSCC include light microscopy, fluorescent microscopy, and flow cytometry (Figure 2.2). Light microscopy involves staining cells within milk samples and individually counting the different WBC present. Staining procedures for milk somatic cells are similar to blood smear techniques (Schalm et al., 1975). In brief, the milk sample is centrifuged, a small sample is placed on a slide and then smeared. Following the smearing process, the sample will be stained to help improve visualization of the cells. Variations in stain type exist throughout literature but common examples include May or Wright Giemsa stain, as well as Methyl Green stain (Paape et al., 1963; Rivas et al., 2001;

Pilla et al., 2012). Wide scale application of manual cytology is not feasible as it is time-consuming and requires a trained individual to evaluate slides (Rivas et al., 2001). In addition, errors in obtaining accurate counts can be associated with missed cells from mechanically scanning the glass slide, or misidentification of similarly appearing cell types. For example, difficulty in distinguishing between macrophages and epithelial cells is a limitation to this method, requiring evaluation of more precise and practical methods (Pilla et al., 2012; Damm et al., 2017).

The two most common commercial or high-throughput methods for DSCC are fluorescent microscopy and flow cytometry and have been used in the development of two commercially available systems: QScout® Farm Lab (Advanced Animal Diagnostics Inc., Morrisville, NC) and Fossomatic™ 7 DC (FOSS, Hilleroed, Denmark). QScout® Farm Lab. QScout® Farm Lab utilizes a fluorescent microscope whereas Fossomatic™ 7 DC utilizes flow cytometry. The functionality of each method will be discussed in more detail in the context of these commercially available systems.

QScout® is an on-farm, benchtop machine that utilizes fluorescent microscopy to distinguish WBC types in milk (Figure 2.3). Fluorescent microscopy works to distinguish WBC types by measuring the fluorescent emission of the cell (Wardlaw et al., 2002). Specific to the QScout®, milk is collected and transferred to a milk-leukocyte differential (MLD) cassette which contains a dried fluorescent stain that mixes with the milk sample as it enters the slide (Wardlaw et al., 1999). Once loaded into the machine, fluorescent images are captured by the fluorescent microscope contained within the equipment housing and enumerated via the incorporated scanner, providing identification and quantification of individual WBC. Cell numbers are recorded and analyzed, stored on the machine, and

automatically uploaded to a cloud-based website. QScout® MLD reports the total leukocyte count, the individual cell counts for neutrophils, macrophages, and lymphocytes, as well as the proportion of each population relative to total amount of cells as a percentage. Unlike SCC, total leukocyte count excludes mammary epithelial cells from the total cell count (Joy Drach, Advanced Animal Diagnostics (AAD) Inc., Morrisville, NC, personal communication). The machine also features a user-friendly interface that provides positive or negative diagnostic results at both the cow and quarter level based on a proprietary algorithm. A unique feature of QScout® is its ability to run milk samples under different algorithm-based programs tailored to stage of lactation, clinical health status (including clinical mastitis), or other non-mastitis health concerns (e.g. lameness) (Godden et al., 2017), The diagnostic capability of QScout® to detect mastitis was tested against the gold standard of microbial culturing for validation (Godden et al., 2017) and found to have great repeatability and only minor differences were seen with sensitivity and specificity depending on the IMI definition. Robles et al. (2021) reported that QScout® had high precision ($R^2 = 0.94$) for SCC and TLC and an average difference between the QScout® and flow cytometry to be 0.96. Authors agreed with Godden et al. (2017) in that sensitivity varied based on the diagnostic threshold applied. More specifically, QScout® provides multiple programs and a range of thresholds to choose from which adjust the sensitivity and specificity of their diagnostic algorithm (Godden et al., 2017; Lozada-Soto et al., 2020). Flexibility in diagnostic accuracy allows individual farms to tailor detection to meet specific herd goals. Although the practicality of QScout® aids producers in making on-farm, real-time decisions, connects them to technical support through AAD, and potentially maximizes diagnostic success, the high initial investment cost can limit accessibility.

The Fossomatic™ 7 DC utilizes flow cytometry to differentiate cells based on light scatter through and around a cell and is referred to as forward angle scatter and side angle scatter. Flow cytometry can provide information about size and internal complexity of the cell, as well as quantify the different cells present based on laser light-detected morphometric characteristics. When the cell passes through the laser a detector on the other side receives fluorescence signals that are measured by two fluorescent channels (Süel, 2011). Cells are first identified as mammalian cells (SCC) or background "noise" (e.g. fat globules, bacteria, etc.). Cells determined to be SCC are then further differentiated into two groups: macrophages and the combination of neutrophils and lymphocytes. Results are reported as the combined percentage of neutrophils and lymphocytes. Damm et al. (2017) validated the capability of Fossomatic™ 7 DC to differentiate cell types against florescent microscopy and found high correlation (r = 0.84) between the two methods. In addition, the machine was found to have good repeatability when samples consisted of DSCC ranging from 33 to 96% with a standard deviation of 2%. Ultimately, Damm et al. (2017) concluded the Fossomatic™ 7 DC to be a reliable and repeatable method. Even more advantageous, the Fossomatic™ 7 DC can run 600 samples per hour, highlighting the rapid throughput benefits of flow cytometry, and is currently being used in some global DHIA programs. However, since milk samples must be shipped to a laboratory for testing, accessibility for producers is reduced. As mentioned, the manufacturer for the Fossomatic™ 7 DC, FOSS, is working to combat this challenge by researching how to implement their technology into monthly herd testing programs (i.e. DHIA) particularly in Demark. Though encouraging, based on the waning interest in DHIA enrollment as

discussed earlier in this article, the scope of impact may be limited in DHIA-based DSCC implementation.

Depending on if Qscout® or the Fossomatic™ is utilized, definitions of DSCC within literature will vary. Most literature refers to DSCC broadly; however, there are multiple definitions commonly seen throughout the literature. One definition frequently associated with use of the Fossomatic™ 7 DC, is the combined cellular proportions of neutrophils and lymphocytes (Schwarz et al., 2020; Zecconi et al., 2020). It can be speculated that the combination of neutrophils and lymphocytes when reporting DSCC can be attributed to the directionality of the cells. Given that lymphocytes play a role in regulating the initiation and suppression of the immune response, lymphocytes and neutrophils may move in the same direction whereas macrophages have the inverse direction. Another definition more commonly found when light microscopy is used to report DSCC is the log neutrophil: lymphocyte ratio (Pilla et al., 2012), whereas research utilizing QScout® will either report individual cell percentages (Mondini et al., 2024) or refer to the collective diagnostic outcome provided by an internal algorithm ® (Godden et al., 2017; Robles et al., 2021). As we move forward, a consensus on the definition and use of DSCC is needed to more accurately compare results across literature, in way that progresses the industry forward in teasing out the nuances of DSCC.

Comparison of DSCC and SCC in detecting intramammary infection

Arguably SCC is a widely accepted method for assessing mammary health, and literature has reported a significant correlation between SCC and DSCC (Zecconi et al., 2020; Dal Prà et al., 2022). In particular, numerous studies have found a high correlation between SCC, and the total leukocyte count ($r = 0.9$), and neutrophil proportions ($r = 0.89$)

(Pillai et al., 2001; Wall et al., 2018). Evidence of a correlation between DSCC and SCC suggests the potential for future use of DSCC in detecting IMI as it can provide additional insights to mammary health through additional data when compared SCC. The use of DSCC could help fill gaps in knowledge of the immune dynamics of the mammary gland, particularly where SCC may be limited.

Schwarz et al. (2011) analyzed DSCC in mammary quarters that had a SCC <100,000 cells/mL, thus considered to be healthy according to industry standards, and reported inflammatory responses in quarters with a SCC as low as 9,000 cells/mL due to elevated neutrophil and lymphocyte. In contrast, Zecconi et al. (2020) did not find DSCC to be a useful parameter for detection of IMI when compared to qPCR results in cows with a SCC ≤ 50,000 cells/mL. Authors also reported a correlation of 0.16 between DSCC and a somatic cell score (**SCS**) ≤ 50,000 cells/mL, and a correlation of 0.53 when assessing SCS > 50,000 cells/mL. Low correlation value was attributed to biological mechanisms that could impact proportions of WBC in healthy mammary quarters. As a result, cows considered to be healthy, but were actually infected could go undetected. In cases of IMI where an antibiotic regimen is needed to support curing, this situation may contribute to the development of chronic mastitis, further economic and production losses, and decreased animal health and well-being. However, the conversation on whether to treat infected cows with low SCC (e.g., as low as 9,000 cells/mL as reported in Schwarz et al. (2011) is debatable. A majority of pathogens from infected quarters reported in Schwarz et al. (2011) were coagulase-negative staphylococci (**CNS**) or non-aures staphylococci. These pathogens typically do not cause severe and chronic infections compared to many major pathogens and often do not require antibiotic therapy for successful by the cow's

own immune system (Condas et al., 2017; Freu et al., 2024). Previous research even suggests that the presence of these minor pathogens may be protective against infections by major pathogens (Yamanaka et al., 2007; De Vliegher et al., 2012; Schwarz et al., 2020). Therefore, many veterinarians deem it unnecessary to administer antibiotics; rather the recommendation is to monitor the animal over the course of days to weeks to ensure the infection clears or does not worsen (De Vligher et al., 2012; Vanderhagghen et al., 2015; Schwarz et al., 2020).

Regardless, the strong association between SCC and DSCC has led to current research efforts to assess the ability of DSCC to see if it could be a better parameter to access IMI alone, or if its combination with SCC can help improve an already widely accepted practice. According to previous literature, a mastitis diagnostic test would have an optimal sensitivity of at least 80% and a specificity of 99% (Hogeveen et al., 2010). When evaluating DSCC as a diagnostic tool for cows in early lactation, Godden et al. (2017) and Lozado-Soto et al. (2020) found a sensitivity ranging from 42.3 to 44.72% and a specificity of 90.5 to 92.69%. In addition, Godden et al. (2017) found the sensitivity and specificity of DSCC in late lactation cows to be 56.2 to 70% and 74.5 to 42.3% respectively. When looking across an entire lactation to evaluate multiple thresholds for DSCC in IMI detection of major mastitis pathogens, Schwarz et al. (2020) reported sensitivities and specificities across a series of thresholds ranging from 55 to 87% and 67 to 88%, respectively. When comparing the diagnostic capability of DSCC for subclinical mastitis compared to SCC, DSCC would diagnose mastitis with an accuracy of 75 to 81.6%. (Zecconi et al., 2019b; Dal Prà et al., 2022). Although most values reported within existing literature do not reach the ideal performance metrics as described in previous

publications, DSCC is still a relatively new parameter, and research is still working on the best ways to utilize DSCC, not to mention the differences in definitions or reported values across literature already discussed in this article.

As previously mentioned, there is wide variation in sensitivity and specificity of DSCC as a stand-alone parameter as we currently assess and report DSCC. Therefore, previous research suggested that the combination of DSCC and SCC could improve the sensitivity of IMI detection to greater than 75.8% (Hisira et al., 2023). In a study done by Pilla et al. (2013), two trials were performed; the first trial was to determine a cutoff point for DSCC with bacterial analysis for IMI detection as the method of confirmation, and the second trial was to field-test the cutoff point from the previous trial. In the second trial, the authors found that sensitivity and specificity of DSCC as a standalone parameter for detecting IMI were 75.7 and 92.3% respectively. However, when SCC and DSCC were combined, sensitivity increased to 97.2%, with no change to specificity. Several other studies suggest that combining DSCC and SCC could improve diagnostic sensitivity (Wall et al., 2018; Dal Prà et al., 2022). Increasing the sensitivity of a diagnostic metric would minimize the chance for false negatives helping to minimize the possible spread of mastitis through the herd.

However promising, there are conflicting reports in literature. Fonseca et al. (2024) reported an SCC of 150,000 cells/mL to have a sensitivity of 38% and a specificity of 77% and DSCC threshold of 65% to have a sensitivity and specificity of 80 and 56% respectively when diagnosing mastitis caused by any pathogen. However, when combining the two parameters using the same cut point, sensitivity dropped to 36% whereas specificity increased to 85%, concluding that the combination of SCC and DSCC did not improve the

sensitivity of IMI detection. Given the robust increase in SCC seen with major pathogens (Bannerman et al., 2004b), the additional nuanced information specific to the early immune response obtained from DSCC does not enhance the ability to detect the IMI. In contrast, Schwarz et al. (2020) found the combination of DSCC and SCC improved sensitivity from 87% (DSCC = 60%) and 70% (SCC = 200,000 cells/ml) to a combined sensitivity of 88%; however, there were differences in technical approaches. Schwarz et al. (2011) utilized composite milk samples whereas Fonseca et al. (2024) utilized quarter milk samples. Godden et al. (2017) reported that sensitivity was improved when tests were considered at the cow level versus the quarter level. As is evident, there is a wide variation in sensitivity and specificity for DSCC reported throughout literature. Though exploring all factors that contribute to spectrum of reported sensitivities and specificities are outside of the scope of this particular review, we aim to discuss the most predominant and investigated factors in the following section.

Factors that impact DSCC

After decades of research an industry-wide threshold for SCC as it relates to presumptive infectious status was established; a threshold which generally still holds to this day (Cornett 1998; Kelly, 2000). However, there are known factors that cause SCC to fluctuate (e.g. stage of lactation, milk yield, season, parity). Application of DSCC is relatively recent therefore the multitude of real-world factors that can impact DSCC have conflicting conclusions. The most reported factors that impact DSCC in the absence of an IMI include lactation number, days in milk, and breed. Additionally, few articles have also described distinct DSCC in the context of mastitis-causing pathogen.

Lactation number

Kirkeby et al. (2020) reported a tendency for DSCC to increase as lactation number increased. In addition, Schwarz et al. (2020) reported a lactation number > 4 to have a greater DSCC of 52 ± 3.13% compared to first (37.7 ± 2.99%), and second (35.3 ± 3.09%) lactation. Similarly, Stocco et al. (2023) reported that DSCC increased with each lactation number. Although the three studies found similar results, the authors used different definitions for DSCC. Schwarz et al. (2020) and Stocco et al. (2023) reported DSCC and the combined percentage of lymphocytes and neutrophils whereas Kirkeby et al. (2020) included TLC and the combined proportions of neutrophils and lymphocytes. However, Lozado-Soto et al. (2020) and Pilla et al. (2012) did not find any significant effect of lactation number on cellular percentages which might be attributed to the authors utilizing light microscopy whereas the earlier 3 papers utilized the Fossomatic^{TM} 7 DC.

Days in milk

Schwarz et al. (2020) reported DSCC had no association to DIM; however, Kirkeby et al. (2020) reported a tendency for DSCC to decrease over lactation. On the other hand, Stocco et al. (2023) reported an increase in DSCC during the first 60 days of lactation, stabilizing shortly after, then decreasing around 300 days in milk until dry off. Similar to concepts seen with SCC, different DSCC may be associated with different stages of lactation.

Breed

Literature investigating how breed alone impacts DSCC is extremely limited. Stocco et al. (2022) reported Holstein-Friesian cows have a greater SCC and DSCC compared to Italian Simmental. Lozado-Soto et al. (2020) found Jersey cows to have higher DSCC when infected compared to Holstein-Friesian. When evaluating differences between

Jersey and Holstein-Friesian cows challenged with *E. coli*, Bannerman et al. (2008) reported greater pre-infection SCC in Jersey cattle. There were no differences reported in peak SCC between the two breeds following infusion; however, there was an earlier increase in milk SCC of Holsteins compared to Jerseys postinfection. Differences in response times could be attributed to the early heightened induction of cytokines in Holsteins. Genetic differences between the breeds in regard to milk components as well as differences in among the innate immune response could play a role in the reposted differences in SCC or DSCC.

Pathogen

Most notably when discussing factors that impact DSCC, the effect of pathogens must be considered. Schwarz et al. (2020) found that cows infected with major pathogens had a significant increase of DSCC by approximately 33% compared to minor or other pathogens. The authors highlighted the increase in DSCC as a reflection of variation in the immune response to different pathogens. The type of pathogen can influence not only the magnitude of the immune response but the durations as well (Bannerman et al., 2004b; Bannerman, 2009). For example, major pathogens like *E. coli* have a robust spike in SCC before it decreases again, whereas minor pathogens like NAS may not have as robust of a spike but could mild elevations in SCC could persist. When looking at SCC in this context, especially after infection with a major pathogen, the effects on SCC can persist weeks to months after an infection (Henningsen et al., 2024; Haw et al., 2024). It is unknown if the same temporal phenomenon seen in SCC tracts the same way in DSCC. For example, after an infection resolves, there may be an increase in macrophages that "clean up" apoptotic neutrophils and aid in tissue restoration. While this would be reflected as an increase in

SCC, DSCC would be able to better capture the change in cell types occurring during this period. However, the cellular populations and how they change overtime still needs to be better understood.

Kirkeby et al. (2021) found that SCC increased after IMI with major pathogens to ~40 days, then declined, while it consistently increased for minor and other pathogens, most notably for the latter. In contrast, DSCC increased after IMI with major and minor pathogens until ~70 days, then declined, where it constantly increased for any pathogen. A limitation to the application of these findings across many different populations is that this study reported these findings from enrolling two small to mid-sized Danish dairy herds. Nonetheless, the difference in temporal changes between the two parameters further reiterates that SCC and DSCC may respond differently to IMI, and begs the question, is SCC alone sufficient or is there another parameter that could modernize our approach to mastitis detection, either as a replacement or in addition to? Another observation that could be investigated further from the Kirkeby et al. (2021) work is related to the sustained elevation in DSCC (70 days). Based on our discussion of increased neutrophils contributing to increased DSCC (and SCC), sustained elevation in DSCC would indicate the likelihood that neutrophils were present in the mammary gland for an extended period of time. Though neutrophils are critically important in protecting the mammary gland during the innate immune response and inflammation, the mechanisms by which they kill are nonspecific and damaging (degranulation, NETosis, etc.). One of the physiological reasons that neutrophils have such a short life once reaching the tissue is to prevent tissue damage as a result of neutrophil presence (Capuco et al., 1986). Though only a theory, could the sustained migration and influx of neutrophils into the mammary gland during IMI,

especially IMI caused by major contagious pathogens which are notoriously refractory to treatment like *Staph.aureus*, be contributing to mammary tissue damage and potentially even failure to cure with antibiotic treatment?

As a final point of discussion, it would be neglectful to not acknowledge the potential for a DSCC threshold for mastitis detection, similar to the widely accepted SCC threshold. As our understanding of factors that impact DSCC improves, algorithm-guided decision-making becomes more refined. The implementation of DSCC has the opportunity to enhance how the industry diagnoses mastitis and utilizes mammary health parameters for selection of cows and quarters for antibiotic therapy. In regard to selective dry cow therapy (SDCT), utilizing DSCC in the decision-making process could improve the ability to determine cows at risk for an IMI. The ability to refine decision making could help ensure more judicious use of antibiotics. Overall, leveraging DSCC-based thresholds could propel the industry forward in mastitis detection.

DSCC thresholds

As an industry, a threshold of ≥ 200,000 cells/mL for SCC is a long-standing standard in mammary health that has undergone decades of research to understand. With continued improvement to genetics and management strategies more refined thresholds for SCC have been discussed. For example, the establishment of a widely agreed-upon threshold (absolute or percentage) for DSCC useful for detecting IMI remains undetermined, perhaps related to differences in DSCC definition as described previously in this article, and other physiological and bacteriological factors also discussed. Nonetheless implementation of a threshold utilizing a more refined approach to assessing

mammary health status, i.e., DDSCC, may be beneficial in supporting the health and well-being of dairy cows, and reduction in antibiotic therapy.

Previous literature suggested that a DSCC between 65 – 72% could potentially distinguish between healthy and infected quarters(Damm et al., 2017; Zecconi et al., 2019a; Schwarz et al., 2020; Fonseca et al., 2024). In more detail, reported DSCC values can vary based on factors accounted for in the proposed threshold. For example, Zecconi et al. (2019) proposed a DSCC threshold of 66.3 to 69.3% depending on the range of days in milk. Whereas Schwarz et al. (2020) reported a DSCC threshold between 65 and 68% when evaluating cows infected with major pathogens including: *Staph. aureus, E. coli,* and *Strep agalactiae.* The range of thresholds that have been proposed across literature in combination with the various factors which appear to differentially contribute further highlights the need for research to better understand the application of DSCC for threshold setting. A central question is whether any one factor is more predictive or associated with IMI over the other.

An exciting area of investigation could be focused on the application of DSCC thresholds in predicting prognosis following antibiotic therapy. Such application would aid in reducing antibiotic use, maximizing cure rates, and providing additional information by which producers could make an informed decision to treat or not treat IMI in collaboration with their veterinarian. For example, previous literature has reported a relationship between SCC levels and success of antibiotic treatment. (Bradley and Green, 2009; Nickerson et al., 2018; Williamson et al., 2022).

While previous research has found associations of SCC and cure rates, the inability to differentiate the immune cells hinder its performance at the same potential as DSCC.

Given DSCC is more reflective of the actual immune system within the udder, the relationship between cell counts and antibiotic success could greatly benefit from more refined information.

Although the use of DSCC in this way is limited in the dairy industry as of yet, similar concepts have been explored or are commonly used in human health (Yamanaka et al., 2007; Azab et al., 2012). For example, researchers reported that neutrophils are effective in providing insight into disease severity and outcome (Yamanaka et al., 2007; Azab et al., 2012). Yamanaka et al. (2007) found that patients with a base line neutrophil to lymphocyte ratio above 2.5 had a poorer medical prognosis as the one-year survival rate was 30% compared to patients under 2.5 who had a one-year survival rate of 50%. Azab et al. (2012) also utilized the neutrophil-to-lymphocyte ratio but in breast cancer patients. Authors reported that a ratio above 3.3 yielded a 5-year mortality rate of 40% compared to patients with a ratio less than 1.8. Further, Garcia-Flores et al. (2024), found that patients that did not survive colorectal cancer had higher levels of neutrophils and specifically reported that neutrophils were the most important variable associated with survival rates. Du et al. (2024) also reported that increased mortality in patients infected with *Klebsiella pneumoniae* had significantly elevated neutrophil levels. Interestingly, but perhaps not surprisingly, Du et al. (2024) also noted that mortality was also increased in infected patients that had significantly low levels as well. This would physiologically make sense as neutrophils would be critically important in controlling *K. pneumoniae* during the innate immune response. Certainly, progress has been made in understanding cellular populations and disease outcomes creating the possibility to apply these concepts within the dairy industry.

Conclusions

Within the dairy industry there is great potential for DSSC to pave a new way to monitor udder health. However, arriving at a consensus for defining DSCC or even mastitis must occur in order to better make comparisons across studies. Given the growing interest in DSCC, it is imperative that future investigative efforts focus on understanding physiological and environmental factors that impact reported values in healthy mammary glands. In addition, the effect of pathogen-dependent immune responses must be further explored to better understand the role of DSCC in IMI detection. Advancements such as these can allow the use of DSCC to be better utilized for mastitis control and prevention plans to help with the more selective use of antibiotics and enhance the understanding of animal health.

Literature Cited

Azab, B., V. R. Bhatt, J. Phookan, S. Murukutla, N. Kohn, T. Terjanian, and W. D. Widmann. 2012. Usefulness of the neutrophil-to-lymphocyte ratio in predicting short- and long-term mortality in breast cancer patients. Ann Surg Oncol 19(1):217-224. doi: 10.1245/s10434-011-1814-0

Bannerman, D. D. 2009. Pathogen-dependent induction of cytokines and other soluble inflammatory mediators during intramammary infection of dairy cows. J Anim Sci 87(13 Suppl):10-25. doi: 10.2527/jas.2008-1187

Bannerman, D. D., M. J. Paape, J. P. Goff, K. Kimura, J. D. Lippolis, and J. C. Hope. 2004a. Innate immune response to intramammary infection with Serratia marcescens and Streptococcus uberis. Vet Res 35(6):681-700. doi: 10.1051/vetres:2004040

Bannerman, D. D., M. J. Paape, J. W. Lee, X. Zhao, J. C. Hope, and P. Rainard. 2004b. Escherichia coli and Staphylococcus aureus elicit differential innate immune responses following intramammary infection. Clin Diagn Lab Immunol 11(3):463-472. doi: 10.1128/cdli.11.3.463-472.2004

Bhutto, A. L., R. D. Murray, and Z. Woldehiwet. 2012. California mastitis test scores as indicators of subclinical intra-mammary infections at the end of lactation in dairy cows. Research in Veterinary Science 92(1):13-17. doi: https://doi.org/10.1016/j.rvsc.2010.10.006

Bobbo, T., M. Penasa, and M. Cassandro. 2020. Combining total and differential somatic cell count to better assess the association of udder health status with milk yield,

composition and coagulation properties in cattle. Italian Journal of Animal Science 19(1):697-703.

Braga, P. A., J. L. Gonçalves, J. R. Barreiro, C. R. Ferreira, T. Tomazi, M. N. Eberlin, and M. V. Santos. 2018. Rapid identification of bovine mastitis pathogens by MALDI-TOF Mass Spectrometry. Pesquisa Veterinária Brasileira 38:586-594.

Brinkmann, V., U. Reichard, C. Goosmann, B. Fauler, Y. Uhlemann, D. S. Weiss, Y. Weinrauch, and A. Zychlinsky. 2004. Neutrophil extracellular traps kill bacteria. Science 303(5663):1532-1535. doi: doi:10.1126/science.1092385

Capuco, A. V., M. J. Paape, and S. C. Nickerson. 1986. In vitro study of polymorphonuclear leukocyte damage to mammary tissues of lactating cows. Am J Vet Res 47(3):663-668.

Chakraborty, S., K. Dhama, R. Tiwari, M. Iqbal Yatoo, S. K. Khurana, R. Khandia, A. Munjal, P. Munuswamy, M. A. Kumar, M. Singh, R. Singh, V. K. Gupta, and W. Chaicumpa. 2019. Technological interventions and advances in the diagnosis of intramammary infections in animals with emphasis on bovine population-a review. Vet Q 39(1):76-94. doi: 10.1080/01652176.2019.1642546

Condas, L. A., J. De Buck, D. B. Nobrega, D. A. Carson, S. Naushad, S. De Vliegher, R. N. Zadoks, J. R. Middleton, S. Dufour, and J. P. Kastelic. 2017. Prevalence of non-aureus staphylococci species causing intramammary infections in Canadian dairy herds. J Dairy Sci. 100(7):5592-5612.

Dal Prà, A., F. Biscarini, G. L. Cavani, S. Bacchelli, A. Iotti, S. Borghi, M. Nocetti, and P. Moroni. 2022. Relationship between total and differential quarter somatic cell

counts at dry-off and early lactation. PLoS One 17(10):e0275755. doi: 10.1371/journal.pone.0275755

Damm, M., C. Holm, M. Blaabjerg, M. N. Bro, and D. Schwarz. 2017. Differential somatic cell count—A novel method for routine mastitis screening in the frame of Dairy Herd Improvement testing programs. J Dairy Sci. 100(6):4926-4940. doi: https://doi.org/10.3168/jds.2016-12409

De Vliegher, S., L. K. Fox, S. Piepers, S. McDougall, and H. W. Barkema. 2012. Invited review: Mastitis in dairy heifers: nature of the disease, potential impact, prevention, and control. J Dairy Sci 95(3):1025-1040. doi: 10.3168/jds.2010-4074

Deluyker, H., S. Van Oye, and J. Boucher. 2005. Factors affecting cure and somatic cell count after pirlimycin treatment of subclinical mastitis in lactating cows. J Dairy Sci. 88(2):604-614.

Dingwell, R. T., K. E. Leslie, Y. H. Schukken, J. M. Sargeant, and L. L. Timms. 2003. Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactation dairy cows. Can Vet J 44(5):413-415.

Dingwell, R. T., K. E. Leslie, Y. H. Schukken, J. M. Sargeant, L. L. Timms, T. F. Duffield, G. P. Keefe, D. F. Kelton, K. D. Lissemore, and J. Conklin. 2004. Association of cow and quarter-level factors at drying-off with new intramammary infections during the dry period. Prev Vet Med 63(1-2):75-89. doi: 10.1016/j.prevetmed.2004.01.012

Du, C., M. Lu, J. Zheng, C. Liu, P. Yang, J. Yi, L. Zhu, and N. Shen. 2024. Distinctive features and prognostic utility of neutrophil in severe patients with Klebsiella

pneumoniae infection. Front. Cell. Infect. Microbiol. 14:1406168. doi: 10.3389/fcimb.2024.1406168

Fonseca, M., D. Kurban, J.-P. Roy, D. E. Santschi, E. Molgat, and S. Dufour. 2024. Usefulness of differential somatic cell count for udder health monitoring: Diagnostic performance of somatic cell count, and differential somatic cell count for diagnosing of intramammary infections in dairy herds with automated milking systems. J Dairy Sci. doi: https://doi.org/10.3168/jds.2024-25404

Fosgate, G. T., I. M. Petzer, and J. Karzis. 2013. Sensitivity and specificity of a hand-held milk electrical conductivity meter compared to the California mastitis test for mastitis in dairy cattle. The Veterinary Journal 196(1):98-102. doi: https://doi.org/10.1016/j.tvjl.2012.07.026

Fox, L. K., and J. M. Gay. 1993. Contagious mastitis. Veterinary Clinics of North America: Food Animal Practice 9(3):475-487. doi: https://doi.org/10.1016/S0749-0720(15)30615-0

Freu, G., G. Gioia, B. Gross, F. Biscarini, P. Virkler, R. Watters, M. F. Addis, R. J. Franklin-Guild, J. Runyan, A. J. Masroure, V. Bronzo, M. V. dos Santos, and P. Moroni. 2024. Frequency of non-aureus staphylococci and mammaliicocci species isolated from quarter clinical mastitis: A 6-year retrospective study. J Dairy Sci. 107(6):3813-3823. doi: https://doi.org/10.3168/jds.2023-24086

Garcia-Flores, L. A., M. T. Dawid De Vera, J. Pilo, A. Rego, G. Gomez-Casado, I. Arranz-Salas, I. Hierro Martín, J. Alcaide, E. Torres, A. Ortega-Gomez, H. Boughanem, and M. Macias-Gonzalez. 2024. Increased neutrophil counts are associated with

poor overall survival in patients with colorectal cancer: a five-year retrospective analysis. Front. Immunol. 15:1415804. doi: 10.3389/fimmu.2024.1415804

Godden, S. M., E. Royster, J. Timmerman, P. Rapnicki, and H. Green. 2017. Evaluation of an automated milk leukocyte differential test and the California Mastitis Test for detecting intramammary infection in early- and late-lactation quarters and cows. J Dairy Sci. 100(8):6527-6544. doi: 10.3168/jds.2017-12548

Guarín, J. F., M. G. Paixão, and P. L. Ruegg. 2017. Association of anatomical characteristics of teats with quarter-level somatic cell count. J Dairy Sci. 100(1):643-652. doi: https://doi.org/10.3168/jds.2016-11459

Hagnestam-Nielsen, C., U. Emanuelson, B. Berglund, and E. Strandberg. 2009. Relationship between somatic cell count and milk yield in different stages of lactation. J Dairy Sci. 92(7):3124-3133. doi: https://doi.org/10.3168/jds.2008-1719

Hamann, J., and A. Zecconi. 1998. Evaluation of the electrical conductivity of milk as a mastitis indicator. Bulletin of the International Dairy Federation 334:5-22.

Hisira, V., F. Zigo, M. Kadaši, R. Klein, Z. Farkašová, M. Vargová, and P. Mudroň. 2023. Comparative analysis of methods for somatic cell counting in cow's milk and relationship between somatic cell count and occurrence of intramammary bacteria. Vet Sci 10(7)doi: 10.3390/vetsci10070468

Hogan, J., and K. L. Smith. 2012. Managing environmental mastitis. Vet Clin North Am Food Anim Pract 28(2):217-224. doi: 10.1016/j.cvfa.2012.03.009

Jadhav, P. V., D. N. Das, K. P. Suresh, and B. R. Shome. 2018. Threshold somatic cell count for delineation of subclinical mastitis cases. Vet World 11(6):789-793. doi: 10.14202/vetworld.2018.789-793

Jahan, N. A., S. M. Godden, E. Royster, T. C. Schoenfuss, C. Gebhart, J. Timmerman, and R. C. Fink. 2021. Evaluation of the matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) system in the detection of mastitis pathogens from bovine milk samples. J. Microbiol. Methods 182:106168. doi: https://doi.org/10.1016/j.mimet.2021.106168

Jensen, N. E., and K. Knudsen. 1991. Interquarter comparison of markers of subclinical mastitis: somatic cell count, electrical conductivity, N-acetyl-β-glucosaminidase and antitrypsin. Journal of Dairy Research 58(4):389-399. doi: 10.1017/S002202990002999X

Kandeel, S. A., D. E. Morin, C. D. Calloway, and P. D. Constable. 2018. Association of California Mastitis Test Scores with Intramammary Infection Status in Lactating Dairy Cows Admitted to a Veterinary Teaching Hospital. J Vet Intern Med 32(1):497-505. doi: 10.1111/jvim.14876

Kayitsinga, J., R. L. Schewe, G. A. Contreras, and R. J. Erskine. 2017. Antimicrobial treatment of clinical mastitis in the eastern United States: The influence of dairy farmers' mastitis management and treatment behavior and attitudes. J Dairy Sci. 100(2):1388-1407. doi: 10.3168/jds.2016-11708

Kehrli, M. E., and D. E. Shuster. 1994. Factors affecting milk somatic cells and their role in health of the bovine mammary gland. J Dairy Sci. 77(2):619-627. doi: https://doi.org/10.3168/jds.S0022-0302(94)76992-7

Kelly, A. L., D. Tiernan, C. O'Sullivan, and P. Joyce. 2000. Correlation between bovine milk somatic cell count and polymorphonuclear leukocyte level for samples of bulk milk and milk from individual cows. J Dairy Sci. 83(2):300-304. doi: 10.3168/jds.S0022-0302(00)74878-8

Kennedy, B., M. Sethar, A. Tong, J. Moxley, and B. Downey. 1982. Environmental factors influencing test-day somatic cell counts in Holsteins. J Dairy Sci. 65(2):275-280.

Kobayashi, K., S. Oyama, A. Numata, M. M. Rahman, and H. Kumura. 2013. Lipopolysaccharide disrupts the milk-blood barrier by modulating claudins in mammary alveolar tight junctions. PLoS One 8(4):e62187. doi: 10.1371/journal.pone.0062187

Lee, C.-S., F. P. Wooding, and P. Kemp. 1980. Identification, properties, and differential counts of cell populations using electron microscopy of dry cows secretions, colostrum and milk from normal cows. Journal of Dairy Research 47(1):39-50.

Leite de Campos, J., J. L. Gonçalves, A. Kates, A. Steinberger, A. Sethi, G. Suen, J. Shutske, N. Safdar, T. Goldberg, and P. L. Ruegg. 2023. Variation in partial direct costs of treating clinical mastitis among 37 Wisconsin dairy farms. J Dairy Sci. 106(12):9276-9286. doi: https://doi.org/10.3168/jds.2023-23388

Leslie, K., J. Jansen, and G. Lim. 2002. Opportunities and implications for improved on-farm cowside diagnostics. In: Proc. De Laval Hygiene Symp

Lozada-Soto, E., C. Maltecca, K. Anderson, and F. Tiezzi. 2020. Analysis of milk leukocyte differential measures for use in management practices for decreased

mastitis incidence. J Dairy Sci. 103(1):572-582. doi: https://doi.org/10.3168/jds.2019-16355

Mansell, P. D., and A. Seguya. 2003. The use of a hand-held conductivity meter for the diagnosis of subclinical mastitis in dairy cows during late lactation. New Zealand Veterinary Journal 51(1):21-25. doi: 10.1080/00480169.2003.36325

Merle, R., A. Schröder, and J. Hamann. 2007. Cell function in the bovine mammary gland: a preliminary study on interdependence of healthy and infected udder quarters. Journal of Dairy Research 74(2):174-179. doi: 10.1017/S002202990600238X

Middleton, J. R., D. Hardin, B. Steevens, R. Randle, and J. W. Tyler. 2004. Use of somatic cell counts and California mastitis test results from individual quarter milk samples to detect subclinical intramammary infection in dairy cattle from a herd with a high bulk tank somatic cell count. J Am Vet Med Assoc 224(3):419-423. doi: 10.2460/javma.2004.224.419

Mondini, S., G. Gislon, M. Zucali, A. Sandrucci, A. Tamburini, and L. Bava. 2024. Factors influencing somatic cell count and leukocyte composition in cow milk: a field study. J Dairy Sci. doi: https://doi.org/10.3168/jds.2024-25357

Morse, D., M. A. DeLorenzo, C. J. Wilcox, R. J. Collier, R. P. Natzke, and D. R. Bray. 1988. Climatic effects on occurrence of clinical mastitis. J Dairy Sci. 71(3):848-853. doi: https://doi.org/10.3168/jds.S0022-0302(88)79626-5

Mues, L., N. Kemper, and J. Blumenberg. 2025. Occurrence and diagnostic of intermittent shedding of Staphylococcus aureus in bovine mammary infection. Frontiers in Veterinary Science 12doi: 10.3389/fvets.2025.1523698

Musser, J. M., K. L. Anderson, M. Caballero, D. Amaya, and J. Maroto-Puga. 1998. Evaluation of a hand-held electrical conductivity meter for detection of subclinical mastitis in cattle. Am J Vet Res 59(9):1087-1091.

Nielen, M., H. Deluyker, Y. H. Schukken, and A. Brand. 1992. Electrical Conductivity of Milk: Measurement, Modifiers, and Meta Analysis of Mastitis Detection Performance. J Dairy Sci. 75(2):606-614. doi: https://doi.org/10.3168/jds.S0022-0302(92)77798-4

Nielen, M., Y. H. Schukken, A. Brand, H. A. Deluyker, and K. Maatje. 1995. Detection of Subclinical Mastitis from On-Line Milking Parlor Data. J Dairy Sci. 78(5):1039-1049. doi: https://doi.org/10.3168/jds.S0022-0302(95)76720-0

Norberg, E., H. Hogeveen, I. R. Korsgaard, N. C. Friggens, K. H. M. N. Sloth, and P. Løvendahl. 2004. Electrical Conductivity of Milk: Ability to Predict Mastitis Status. J Dairy Sci. 87(4):1099-1107. doi: https://doi.org/10.3168/jds.S0022-0302(04)73256-7

Norman, H., J. Megonigal, and J. Durr. 2022. Reasons that cows in Dairy Herd Improvement programs exited the milking herd (2021). Council on Dairy Cattle Breeding.

Okigbo, L. M., M. A. Sheliah, G. H. Richardson, C. A. Ernstrom, R. J. Brown, and E. L. Tippetts. 1984. Portable conductivity meter for detecting abnormal milk. J Dairy Sci. 67(7):1510-1516. doi: https://doi.org/10.3168/jds.S0022-0302(84)81468-X

Östensson, K. 1993. Total and differential leukocyte counts, N-acetyl-ß-D-glucosamtnidase activity, and serum albumin content in foremilk and residual milk during endotoxin-induced mastitis in cows. Am J Vet Res 54(2):231-238.

Paape, M. J., D. D. Bannerman, X. Zhao, and J. W. Lee. 2003. The bovine neutrophil: Structure and function in blood and milk. Vet Res 34(5):597-627. doi: 10.1051/vetres:2003024

Paape, M. J., H. D. Hafs, and W. W. Snyder. 1963. Variation of estimated numbers of milk somatic cells stained with wright's stain or pyronin y - methyl green dtain. J Dairy Sci. 46(11):1211-1216. doi: 10.3168/jds.S0022-0302(63)89248-6

Pilla, R., M. Malvisi, G. G. M. Snel, D. Schwarz, S. König, C. P. Czerny, and R. Piccinini. 2013. Differential cell count as an alternative method to diagnose dairy cow mastitis. J Dairy Sci. 96(3):1653-1660. doi: 10.3168/jds.2012-6298

Pilla, R., D. Schwarz, S. König, and R. Piccinini. 2012. Microscopic differential cell counting to identify inflammatory reactions in dairy cow quarter milk samples. J Dairy Sci. 95(8):4410-4420. doi: https://doi.org/10.3168/jds.2012-5331

Pillai, S. R., E. Kunze, L. M. Sordillo, and B. M. Jayarao. 2001. Application of differential inflammatory cell count as a tool to monitor udder health. J Dairy Sci 84(6):1413-1420. doi: 10.3168/jds.S0022-0302(01)70173-7

Pinzón-Sánchez, C., V. E. Cabrera, and P. L. Ruegg. 2011. Decision tree analysis of treatment strategies for mild and moderate cases of clinical mastitis occurring in early lactation. J Dairy Sci. 94(4):1873-1892. doi: https://doi.org/10.3168/jds.2010-3930

Rivas, A. L., F. W. Quimby, J. Blue, and O. Coksaygan. 2001. Longitudinal evaluation of bovine mammary gland health status by somatic cell counting, flow cytometry, and cytology. J Vet Diagn Invest 13(5):399-407. doi: 10.1177/104063870101300506

Robles, I., D. T. Nolan, C. A. Fendley, H. L. Stokley, T. L. France, J. L. Ferrell, and J. H. C. Costa. 2021. Technical note: Evaluation of a commercial on-farm milk leukocyte differential tester to identify subclinical mastitis cases in dairy cows. J Dairy Sci. 104(4):4942-4949. doi: https://doi.org/10.3168/jds.2020-19299

Rollin, E., K. C. Dhuyvetter, and M. W. Overton. 2015. The cost of clinical mastitis in the first 30 days of lactation: An economic modeling tool. Preventive Veterinary Medicine 122(3):257-264. doi: https://doi.org/10.1016/j.prevetmed.2015.11.006

Romero, J., E. Benavides, and C. Meza. 2018. Assessing financial impacts of subclinical mastitis on Colombian dairy farms. Frontiers in Veterinary Science 5:303723.

Royster, E., S. Godden, D. Goulart, A. Dahlke, P. Rapnicki, and J. Timmerman. 2014. Evaluation of the minnesota easy culture system II bi-plate and tri-plate for identification of common mastitis pathogens in milk. J Dairy Sci. 97(6):3648-3659. doi: https://doi.org/10.3168/jds.2013-7748

Sanford, C. J., G. P. Keefe, J. Sanchez, R. T. Dingwell, H. W. Barkema, K. E. Leslie, and I. R. Dohoo. 2006. Test characteristics from latent-class models of the California Mastitis Test. Preventive Veterinary Medicine 77(1):96-108. doi: https://doi.org/10.1016/j.prevetmed.2006.06.006

Sargeant, J. M., K. E. Leslie, J. E. Shirley, B. J. Pulkrabek, and G. H. Lim. 2001a. Sensitivity and specificity of somatic cell count and California Mastitis Test for identifying intramammary infection in early lactation. J Dairy Sci 84(9):2018-2024. doi: 10.3168/jds.S0022-0302(01)74645-0

Schalm, O., and D. Noorlander. 1957. Experiments and observations leading to development of the California mastitis test.

Schalm, O. W., N. C. Jain, and E. J. Carroll. 1975. Veterinary hematology.

Schwarz, D., U. S. Diesterbeck, S. König, K. Brügemann, K. Schlez, M. Zschöck, W. Wolter, and C. P. Czerny. 2011a. Flow cytometric differential cell counts in milk for the evaluation of inflammatory reactions in clinically healthy and subclinically infected bovine mammary glands. J Dairy Sci. 94(10):5033-5044. doi: https://doi.org/10.3168/jds.2011-4348

Schwarz, D., U. S. Diesterbeck, S. König, K. Brügemann, K. Schlez, M. Zschöck, W. Wolter, and C. P. Czerny. 2011b. Flow cytometric differential cell counts in milk for the evaluation of inflammatory reactions in clinically healthy and subclinically infected bovine mammary glands. J Dairy Sci 94(10):5033-5044. doi: 10.3168/jds.2011-4348

Schwarz, D., D. E. Santschi, J. Durocher, and D. M. Lefebvre. 2020. Evaluation of the new differential somatic cell count parameter as a rapid and inexpensive supplementary tool for udder health management through regular milk recording. Preventive Veterinary Medicine 181:105079. doi: https://doi.org/10.1016/j.prevetmed.2020.105079

Sears, P. M., B. S. Smith, P. B. English, P. S. Herer, and R. N. Gonzalez. 1990. Shedding pattern of Staphylococcus aureus from bovine intramammary infections. J Dairy Sci.73(10):2785-2789. doi: 10.3168/jds.S0022-0302(90)78964-3

Seykora, A., and B. McDaniel. 1985. Udder and teat morphology related to mastitis resistance: a review. J Dairy Sci. 68(8):2087-2093.

Sheldrake, R. F., R. J. Hoare, and G. D. McGregor. 1983. Lactation stage, parity, and infection affecting somatic cells, electrical conductivity, and serum albumin in milk. J Dairy Sci 66(3):542-547. doi: 10.3168/jds.S0022-0302(83)81823-2

Shell, W., M. Sayed, A. El-Gedawy, G. El Sadek, A. Samy, and A. Ali. 2017. Identification of Staphylococcus aureus causing bovine mastitis using MALDI-TOF fingerprinting. Int J Dairy Sci 12:105-113.

Shuster, D. E., M. E. Kehrli, P. Rainard, and M. Paape. 1997. Complement fragment C5a and inflammatory cytokines in neutrophil recruitment during intramammary infection with Escherichia coli. Infection and Immunity 65(8):3286-3292. doi: doi:10.1128/iai.65.8.3286-3292.1997

Smith, K. 1999. Suggested interpretation of mastitis terminology. Bulletin-International Dairy Federation (338):3-26.

Sordillo, L. M., S. C. Nickerson, R. M. Akers, and S. P. Oliver. 1987. Secretion composition during bovine mammary involution and the relationship with mastitis. Int J Biochem 19(12):1165-1172. doi: 10.1016/0020-711x(87)90098-x

Sordillo, L. M., and K. L. Streicher. 2002. Mammary Gland Immunity and Mastitis Susceptibility. Journal of Mammary Gland Biology and Neoplasia 7(2):135-146. doi: 10.1023/A:1020347818725

Souza, F. N., A. F. Cunha, D. L. Rosa, M. A. V. Brito, A. S. Guimarães, L. C. Mendonça, G. N. Souza, A. P. Lage, M. G. Blagitz, and A. M. D. Libera. 2016. Somatic cell count and mastitis pathogen detection in composite and single or duplicate quarter milk samples. Pesquisa Veterinária Brasileira 36(09):811-818.

Stocco, G., C. Cipolat-Gotet, B. Stefanon, A. Zecconi, M. Francescutti, M. Mountricha, and A. Summer. 2023. Herd and animal factors affect the variability of total and differential somatic cell count in bovine milk. J Animal Sci 101doi: 10.1093/jas/skac406

Süel, G. 2011. Chapter thirteen - Use of Fluorescence Microscopy to Analyze Genetic Circuit Dynamics. In: C. Voigt, editor, Methods in Enzymology No. 497. Academic Press. p. 275-293.

Suojala, L., L. Kaartinen, and S. Pyörälä. 2013. Treatment for bovine Escherichia coli mastitis - an evidence-based approach. J Vet Pharmacol Ther 36(6):521-531. doi: 10.1111/jvp.12057

USDA, A. 2018. Dairy 2014, Health and management practices on US dairy operations, 2014. USDA-APHIS-VS-CEAH-NAHMS Fort Collins (CO).

Vitali, A., A. Felici, A. M. Lees, G. Giacinti, C. Maresca, U. Bernabucci, J. B. Gaughan, A. Nardone, and N. Lacetera. 2020. Heat load increases the risk of clinical mastitis in dairy cattle. Journal of Dairy Science 103(9):8378-8387. doi: https://doi.org/10.3168/jds.2019-17748

Wall, S. K., O. Wellnitz, R. M. Bruckmaier, and D. Schwarz. 2018. Differential somatic cell count in milk before, during, and after lipopolysaccharide- and lipoteichoic-acid-induced mastitis in dairy cows. J Dairy Sci.101(6):5362-5373. doi: https://doi.org/10.3168/jds.2017-14152

Williamson, J., T. Callaway, E. Rollin, and V. Ryman. 2022. Association of milk somatic cell count with bacteriological cure of intramammary infection—a review. Agriculture 12(9):1437.

Yamanaka, T., S. Matsumoto, S. Teramukai, R. Ishiwata, Y. Nagai, and M. Fukushima. 2007. The baseline ratio of neutrophils to lymphocytes is associated with patient prognosis in advanced gastric cancer. Oncology 73(3-4):215-220. doi: 10.1159/000127412

Zecconi, A., F. Dell'Orco, D. Vairani, N. Rizzi, M. Cipolla, and L. Zanini. 2020. Differential somatic cell count as a marker for changes of milk composition in cows with very low somatic cell count. Animals 10(4):604.

Zecconi, A., G. Sesana, D. Vairani, M. Cipolla, N. Rizzi, and L. Zanini. 2019a. Somatic cell count as a decision tool for selective dry cow therapy in Italy. Italian Journal of Animal Science 18(1):435-440. doi: 10.1080/1828051X.2018.1532328

Zecconi, A., D. Vairani, M. Cipolla, N. Rizzi, and L. Zanini. 2019b. Assessment of subclinical mastitis diagnostic accuracy by differential cell count in individual cow milk. Italian Journal of Animal Science 18(1):460-465.

Zecconi, A., F. Zaghen, G. Meroni, V. Sora, P. A. Martino, G. Laterza, and L. Zanini. 2023. Early milk total and differential cell counts as a diagnostic tool to improve nntimicrobial therapy protocols. Animals 13(7)doi: 10.3390/ani13071143

Zhao, X., and P. Lacasse. 2008. Mammary tissue damage during bovine mastitis: Causes and control1. J Animal Sci 86(suppl_13):57-65. doi: 10.2527/jas.2007-0302

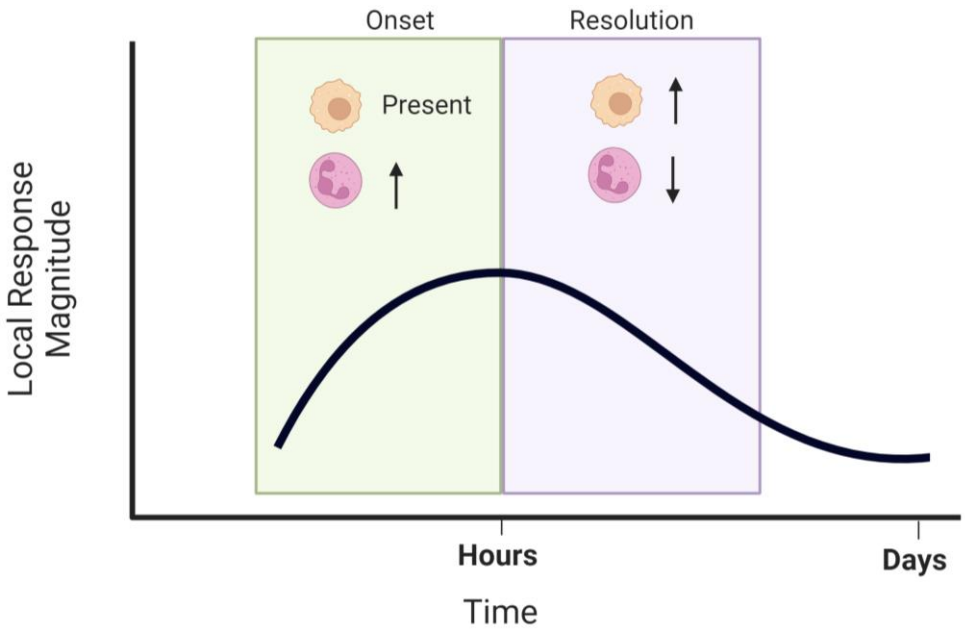


Figure 2.1. Changes in cellular populations of neutrophils and macrophages in the mammary during an infection. The green box represents the onset of the infection, and the light purple box represents the resolution of the infection. The yellow cell is representative of macrophages, the pink cells represent neutrophils. Adapted from Fullerton and Gilroy, 2016. Made with Biorender.com

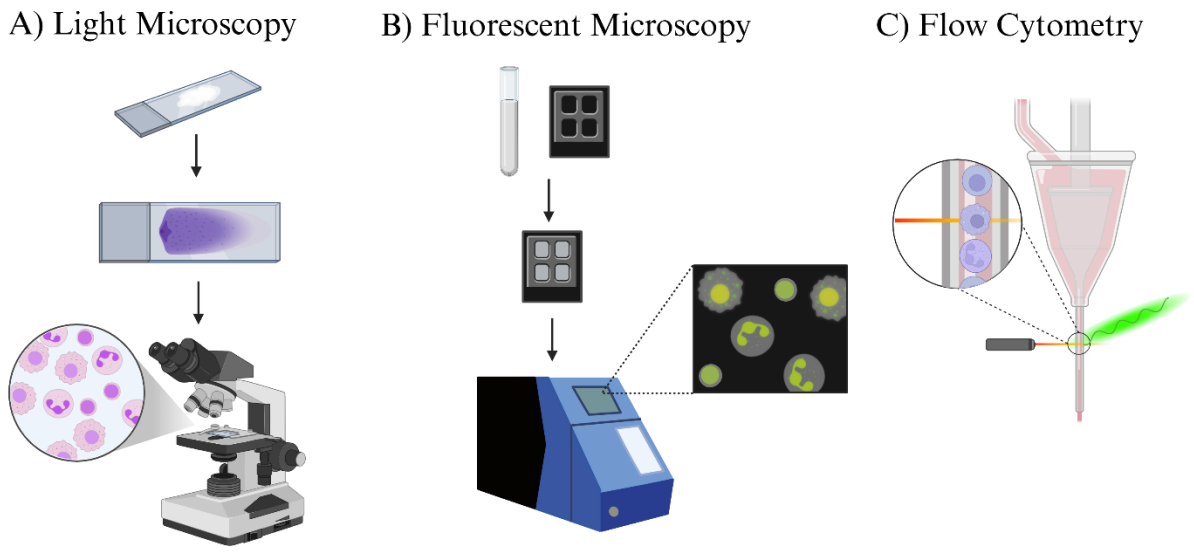


Figure 2.2. Common methods for obtaining differential somatic cell count (A) Light microscopy (B) Fluorescent Microscopy (C) Flow Cytometry. Made with Biorender.com

Figure 2.3. Image of the Scout® Milk Leukocyte Differentials (MLD) Machine (left) and the Q4 tray (right). Milk is expressed into the Q4 tray and then capped with an adapter and flipped upside down on to MLD slide to fill the coverslip.

Table 2.1. Interpretation of CMT scores and corresponding SCC range[1]

CMT Score	SCC Range (cells/mL)
Negative	0 to 200,000
Trace	150,000 to 500,000
1	400,000 to 1,500,000
2	800,000 to 5,000,000
3	> 5,000,000

[1]Adapted from Ruegg and Reinemann, 2002

Table 2.2 Sources that reference factor impacts on DSCC	
Factors That Impact DSCC in Absence of Infection	**Refereed Literature**
Lactation number	Kirkeby et al., 2019 Schwarz et al., 2020 Stocco et al., 2023 Lozado-Soto et al., 2020 Pilla et al., 2012 Fonseca et al., 2025
Days in milk	Schwarz et al., 2020 Kirkeby et al., 2019 Stocco et al., 2023
Breed	Stocco et al., 2023 Lozado-Soto et al., 2020
IMI-associated Factors that Impact DSCC	**Refereed Literature**
Pathogen	Schwarz et al., 2020 Kirkeby et al., 2020

CHAPTER 3

ASSOCIATION OF DIFFERENTIAL SOMATIC CELL COUNT WITH ANTIBITOIC SUCCESS FOLLWING AN INTRAMMARY INFECTION[1]

[1]Burner, C. M., A. A. C. Alves, E. Rollin, T. R. Callaway, V. E. Ryman. To be submitted for publication.

Abstract

Subclinical mastitis (SCM) is diagnosed by a milk somatic cell count (SCC) ≥200,000 cells/mL and typically confirmed by microbial growth. Other established methods of detection include California Mastitis Test (CMT) and electrical conductivity (EC). Differential SCC (DSCC) evaluates the milk leukocyte distribution and could serve as a better diagnostic for SCM according to previous literature. In particular, elevated % neutrophils (NEU) in humans have been correlated to negative medical outcomes. Our hypothesis is that elevated % NEU at time of SCM diagnosis is associated with failure to cure after intramammary (IMM) antibiotic therapy. Cows were pre-screened for enrollment according to monthly herd testing results of a SCC ≥ 200,000 cells/ml. Quarters were officially enrolled if there was positive bacterial growth of milk. At enrollment, milk samples were collected via aseptic techniques and DSCC determined by QScout® Farm Lab. Enrolled quarters were treated with 1 tube of Spectramast LC IMM antibiotic for 2 days. Post-antibiotic milk samples were collected and cultured once/week for 5 weeks. A bacteriological cure was defined as no growth in any post-treatment samples. Differences in total leukocyte count (TLC), % NEU, % macrophages (MAC), and % lymphocytes (LYM) between quarters that cured (CURE) and failed to cure (FTC) were analyzed in R 4.4.3 using a linear mixed-effects model. Sensitivity and other metrics to assess mastitis diagnosis were evaluated considering different thresholds for CMT, EC, SCC and DSCC variables. A bootstrap sampling approach was used to account for uncertainty. The overall cure rate was 51.5%. The SCC, TLC, MAC, CMT, and EC were lower in CURE quarters compared to FTC ($P < 0.03$). Importantly, % NEU was higher in FTC compared to CURE ($P < 0.03$). These data suggest that % NEU at time of SCM diagnosis could be associated

with treatment success following IMM antibiotic administration. When establishing thresholds, TLC had the greatest performance in classification models.

Introduction

Mastitis is defined as inflammation to the mammary gland and is commonly caused by an intramammary infection (**IMI**). Mastitis can be categorized into two categories: clinical and subclinical. Clinical mastitis is diagnosed by visual signs within the milk (e.g. clots or flakes) or udder (e.g., redness or heat, swelling, hardness, or pain) whereas subclinical mastitis lacks these visual abnormalities but instead is typically described as an elevation in the somatic cell count (**SCC**). Both subclinical mastitis and clinical mastitis impact milk production and milk components (e.g. casein) (Ogola et al., 2007). As a result, mastitis is reported to cost the global dairy industry 22 billion dollars annually, with antibiotic treatment accounting for 30% of direct costs per mastitis case (Rollin et al., 2015; Rasmussen et al., 2024).

Common methods used to detect subclinical mastitis include elevated SCC, California Mastitis Test (**CMT**), electrical conductivity (**EC**), and/or pathogen growth from cultured milk. Somatic cell count is the measure of body cells within milk and primarily consists of white blood cells and a small proportion of epithelial cells (Lee et al., 1980). The CMT is a qualitative, subjective test that serves as a proxy for SCC by measuring the gelling intensity of a milk sample as a result of the number of cells present. The EC is related to the change of ions within milk due to mastitis (Norberg et al., 2004). Historically, these common methods to detect mastitis have also been used to make treatment decisions, either because the test results are above industry-set thresholds, or culturing is performed in tandem.

The evaluation of CMT in detecting IMI is reported to have a specificity and sensitivity of 86.2 and 88.5%; however, given the subjective nature of CMT, errors in

conducting and interpreting the test are common, which can lead to misdiagnosis (Marshal et al., 1993; Leslie et al., 2002). A meta-analysis by Nielen et al. (1992) reported the overall sensitivity and specificity of EC in detecting IMI to be 66 and 94% respectively. In addition, studies utilizing bacterial culturing in conjunction with EC had increased sensitivity and specificity compared to those that used EC with SCC (Nielen et al., 1992). When evaluating a diagnostic test for mastitis detection an ideal sensitivity and specificity is 80 and 99% respectively (Hogeveen et al., 2010). Wide variation in EC between farms and cows makes an industry-wide threshold for IMI identification difficult to define (Nielen et al., 1992; Norberg et al., 2004).

The use of SCC has been a long-standing approach in subclinical mastitis detection (Smith, 1999). When an IMI occurs, there is an influx of white blood cells within the mammary gland, and based on a wealth of research decades ago, traditionally a SCC of ≥ 200,000 cells/mL is used for IMI detection (Sordillo et al., 1987). Importantly, previous studies have demonstrated a relationship between SCC levels and success in antibiotic treatment. (Bradley and Green, 2009; Nickerson et al., 2018). Williamson et al. (2022) reported that quarters with a SCC lower than 507,041 cells/mL had greater cure rates following antibiotic therapy compared to quarters with a SCC above that value; however, limitations to the utility of SCC exist. Elevations of SCC in the absence of an infection can occur due to physiological factors (i.e. parity, milk production, days in milk), stressors (i.e. nutrition), or trauma to the udder (Kennedy et al., 1982; Sheldrake et al., 1983). Therefore, the decision to treat a cow with antibiotics based on an elevated SCC alone could result in the overuse of antibiotics.

Recent research has evaluated the use of differential SCC (**DSCC**) for the detection of IMI. Differing from SCC, DSCC focuses on the distribution of white blood cells (i.e. neutrophils, macrophages, and lymphocytes) within the milk. The proportions of white blood cells change in response to an inflammatory response. In a healthy mammary gland, cellular populations consist mostly of macrophages or lymphocytes (Rivas et al., 2001; Dosogne et al., 2003; Pilla et al., 2012). On the other hand, an infected mammary quarter will be dominated by neutrophils, representing up to 90% of the total cell population (Sordillo and Streicher, 2002). A DSCC count between 65 to 72%, representing the sum of neutrophils and lymphocytes, is thought to distinguish unhealthy from healthy cows (Schwarz et al., 2011; Damm et al., 2017; Zecconi et al., 2020).

Although research evaluating the use of DSCC within the dairy industry is recent, similar concepts are routinely used in other industries such as human medicine (Yamanaka et al., 2007; Azab et al., 2012). In particular, neutrophils have been used to better understand disease outcomes and severity. Yamanaka et al. (2007) and Azab et al. (2012) reported that patients with higher ratio of neutrophils to lymphocytes had higher mortality rates and disease prognosis when evaluating patients with gastric cancer or breast cancer, suggesting that increased neutrophils could be associated with disease severity or progression. The success in understanding the dynamic changes in white blood cell populations within human medicine has the potential to translate to mastitis within the dairy industry. The utilization of DSCC has the possibility to allow for a more refined view of mammary health in areas where current assessment tools may be limited. As discussed earlier, DSCC has successfully differentiated between infected and uninfected quarters, yet there is no literature evaluating cure outcomes following intramammary antibiotics.

Therefore, the objective of this study was to evaluate the relationship between DSCC and antibiotic success in sub-clinically infected cows. A secondary objective of this study was to evaluate the use of cellular thresholds to predict cure outcomes following intramammary antibiotic treatment. We hypothesized that mammary quarters that cured their infection following antibiotic treatment would have lower neutrophil percentages than cows that failed to cure.

Materials and Methods

All procedures involving live animals were verified and approved by the University of Georgia Office of Animal Care and Use (AUP#: A2023 02-010-Y3-A1). The dairy cattle used in this study were located at the University of Georgia (**UGA**) Teaching Dairy in Winterville, GA (33°54'32.9"N 83°14'50.9"W).

Animal Selection

Holstein and Jersey cows were pre-screened for subclinical mastitis prior to enrollment based on a composite SCC of ≥ 200,000 cells/mL at the monthly Dairy Herd Improvement (**DHI**) test date. Quarter milk samples were collected using aseptic techniques and quarters were enrolled if there was the presence of pure bacterial growth for a single microbe as previously described (Williamson et al., 2022). Enrolled cows had average days in milk (**DIM**) of 132.5, an average parity of 2.18, and an average milk yield (**MY**) of 30.15 kg.

Sample Collections

On enrollment day (D0), enrolled quarter milk samples were collected using aseptic techniques from lactating Holstein and Jersey cows (N = 44 cows; N = 67 quarters) for microbiological culturing and SCC enumeration. The first few streams of foremilk were

expressed and not collected for any of the samples described. Additional quarter milk samples were collected for cow-side CMT and conductivity testing, as well as enumeration of DSCC on farm. CMT provides a qualitative measure of SCC (Hoque et al., 2015) and conductivity evaluates electroconductivity, a parameter widely used to identify abnormal milk properties (Norberg et al., 2004). Following collection, enrolled quarters were treated with SPECTRAMAST® LC (Zoetis, Parsippany-Troy Hills, NJ, USA) once/day for 2 days according to standard operating procedures at the UGA Teaching Dairy.

SCC, CMT, EC, and DSCC

SCC was determined using a DeLaval Direct Cell Counter (DeLaval; Tumba, Sweden) as described in Williamson et al. (2022) and reported in cells/µL. Milk from enrolled quarters was expressed into a handheld milk conductivity meter (Mas-D-Tec ®; Wescor Inc., Logan, UT, USA) and values were recorded. Readings were provided on a scale from 1-10 with a score ≥ 5 being considered abnormal according to manufacturer recommendations.

The DSCC was enumerated utilizing the QScout^{TM} Farm Lab Milk Leukocyte Differential test (Advanced Animal Diagnostics Inc., Morrisville, NC, USA) (Gonçalves et al., 2017). Milk samples were expressed into the Q4 milk collection kit and transferred to a milk leukocyte differential (**MLD**) slide. Samples were run under "Early Lactation" or "Mid-Lactation (30+ DIM)" program based on DIM at the time of enrollment. Tests were conducted in research mode following the manufacturer's instructions and algorithm threshold settings were at 10 for both "Early Lactation" and "Mid-Lactation (30+ DIM) (Godden et al., 2017). Results from the MLD test provided the total leukocyte count (**TLC**;

cells/mL) as well as the percentage of neutrophils (**NEU**), macrophages (**MAC**), and lymphocytes (**LYM**).

Microbial Culture

Milk samples were transported in a cooler to the UGA Mastitis Lab for culturing. Milk was plated using 10 microliter loops onto trypticase soy agar plates with 5% sheep blood and incubated at 37 °C. After 48 h, plates were examined for bacterial growth, pure colonies were streaked onto new plates and allowed to grow for another 48 h. Pure, isolated samples were sent to the UGA Veterinary Diagnostic Lab for genus and species identification using MALDI-TOF mass spectrometry. Samples were recorded as contaminated if the plate had three or more distinct bacterial colonies present (Dohoo et al., 2011). Samples that showed no bacterial growth on D0 after incubation, or exhibited growth of non-bacterial pathogens, were removed from the study and are not reported herein.

In addition to D0, samples were collected on D7, 14, 28, and 35 post-antibiotic treatment. Bacterial culture, SCC, DSCC, CMT, and conductivity were performed on each day as described above. Following D35 collection, a quarter was categorized as a "Cure" if the milk samples from D7 through D35 were free from bacterial growth of the original bacteria. A quarter was labeled "Fail to Cure" if the initial bacteria found on D0 was present on any subsequent sampling day.

Statistical Analysis

A Shapiro-Wilk test for normality was performed on collected variables, the values of TLC, SCC, NEU, MAC, LYM, and conductivity were then normalized using the bestNormalize package of R Studio. The response normalized variables were fitted for

Bayesian generalized linear mixed models using Markov Chain Monte Carlo methods. Fixed effects include treatment outcome (Cured or Failed), Day of collection, treatment by Day interaction, MY, DIM, breed (Holstein and Jersey), and parity, with the random effect of quarter nested within the cow. A generalized t-test was performed to compare the estimated marginal means of the normalized response variables across treatment outcomes (cure vs. fail to cure) within each day of collection. Degrees of freedom were approximated using the Kenward-Roger method as implemented in the emmeans R package (Kenward and Roger, 1997). Differences were considered significant at $P \leq 0.05$ after Tukey's adjustment for multiple comparisons.

Threshold Sensitivity Analysis

Threshold optimization was performed using a bootstrap-based approach to evaluate the classification performance of the different biomarkers (SCC, TLC, NEU%, MAC%, and LYM%) in predicting treatment outcomes (cure vs fail to cure). Candidate thresholds were defined based on the range of each biomarker, and classification metrics (sensitivity, specificity, accuracy, precision, and F1-score) were computed for each threshold. The best threshold for each biomarker was determined using three different criteria: (1) the threshold that maximized Youden's Index (Sensitivity + Specificity - 1), balancing sensitivity and specificity; (2) the threshold that maximized the F1-score, optimizing the trade-off between precision and sensitivity; and (3) the threshold that maximized overall accuracy. Threshold selection was performed using bootstrap resampling to account for variability in the data, with 100 bootstrap samples generated for each day of collection, and classification metrics recalculated for each resampled data.

Results and Discussion

For this study, the overall bacteriological cure rate was 51.5%. Bacterial cure rates of this study were similar to that reported by Sol et al. (2000) and Williamson et al. (2022) of 52 and 46.2%, respectively. Evaluation of microbial cultured showed 35% of infections were caused by Streptococci-like organisms (**SSLO**) consisting of *Lactococcus garvieae*, *Streptococcus dysgalactiae, Streptococcus uberis* and *Enterococcus cecorum* (Figure 3.1). Non-aureus staphylococci (**NAS**) accounted for 34% of infections and included *Staphylococcus chromogenes, Staphylococcus haemolyticus, Staphylococcus hyicus, and Staphylococcus xylosus*. *Staphylococcus aureus* caused 31% of infections, and 11% of infections consisted of dual infections or other infections such as *Nocardia, Mannheimia haemolytica, and* Coryneform spp.

Quarters that Cured had lower pre-treatment SCC and TLC on D0 compared to Failed groups ($P < 0.03$; Figure 3.2). Success of antibiotic treatment and pre-treatment SCC levels have been previously discussed in literature. Nickerson et al. (2018) and Willamson et al. (2022) reported Failed quarters to have 2- to 5-fold higher pre-treatment SCC compared to Cured quarters. Results from the current study further support that the success of antibiotic therapy is more likely when SCC is lower. Research on TLC has demonstrated its success in differentiation between infected and uninfected quarters; however, evaluation of TLC is limited within literature. Given the high correlation values (r = 0.88 to 0.9) reported between TLC and SCC (Pillai et al., 2001; Mondini et al., 2024) it is clear to see why TLC would follow a similar pattern to SCC. This data suggests that TLC could be an alternative to SCC for mastitis detection when implementing systems which utilize a specialized DSCC system like the QScout® MLD.

When evaluating DSCC, D0 NEU was lower ($P < 0.01$; Figure 3.3) in Cured quarters, than those that Failed. Research evaluating the relationship between antibiotic success and neutrophil levels within dairy cattle is limited; however, associations between medical prognosis and neutrophil levels within human research are quite common. Hannien et al. (1996) reported elevated neutrophils were an independent factor that reduced overall survival in kidney cancer patients treated with immunotherapy. Similarly, Yasuda et al. (2024) reported that elevated blood neutrophil-to-lymphocyte ratios were associated with failure of cancer eradication and higher mortality rates. The present data suggests the possibility of relating neutrophils to antibiotic treatment outcomes in the context of mastitis. Interestingly, an inverse effect to NEU was seen when evaluating MAC, as cellular percentages were greater ($P = 0.01$) in Cured quarters on D0 compared to Failed. Macrophages are suggested to be the dominating population in a healthy mammary gland (Merel et al., 2007). Although both treatment groups were experiencing an IMI on D0, greater MAC populations in Cured groups could be indicative of a less severe infection or a less intense immune response, perhaps because of infection with a minor pathogen vs. major pathogen. This study did not include an untreated, subclinical infected group, but there is a chance that some of these infections could spontaneously cure (Pinzón-Sánchez et al., 2011). Perhaps, greater MAC populations in the Cure group suggest that these infections were progressing towards a successful immune response and clearing the pathogen. Though we enrolled based on monthly DHIA testing given the applicability of this approach to the farm setting, we cannot be certain how long a quarter was infected prior to enrollment on D0. Lastly, there was no difference ($P = 0.37$) in LYM between

Cured and Failed quarters. These results were unsurprising as the role of lymphocytes in the mammary gland is believed to be limited, though much is still unknown.

When evaluating common cow-side tests such as CMT and milk EC, pre-treatment CMT scores were lower ($P < 0.01$; Figure 3.4) in Cured quarters compared to Failed. It is unsurprising that CMT follows a similar trend to SCC and TLC because, although subjective, it is a measure that is based on cellular levels within the milk (George et al., 2008). However, when evaluating pre-treatment conductivity levels between both groups, there was no difference ($P = 0.20$). The lack of differences between Cured and Failed when evaluating conductivity could be attributed to the collection method utilized. Based on our method of assessing conductivity, absolute threshold, regardless of herd or cow differences, was used for milk conductivity. Whereas the use of an interquartile ratio (IQR) has been widely supported as the most effective use of milk conductivity (Nielen et al., 1992; Norberg et al 2004), as it accounts for cow variation. In addition, handled milk EC meters are not calibrated so readings could vary more than in-line sensors. Milk EC scores can be more representative of the cow with an in-line system as the sensor is receiving a constant flow from the mammary gland, unlike the 4 to 5 streams with the handheld meter.

Threshold Analysis

A central piece of investigation in the current study was to assess the success of establishing a DSCC threshold, especially NEU percentage, that would enhance decision-making for antibiotic therapy during subclinical mastitis. The established thresholds would help optimize antibiotic success, or at a minimum, provide information to support culling decisions for infections not likely to cure. When evaluating thresholds, it is common to assess classification metrics such as sensitivity, specificity, precision, F1 score, and

accuracy (Ranio et al., 2024). Briefly, sensitivity is the proportion of true positives the test correctly identifies, while specificity is the proportion of true negatives the test correctly identifies. Precision is the proportion of positive predictions that are correct, unlike sensitivity, precision takes into account false positives. F1 score is the harmonic mean between precision and sensitivity. Accuracy is the overall proportion of correct predictions for both positive and negative cases. Choosing a threshold that solely maximizes one metric is met with a tradeoff to sacrifice the performance of other metrics. For example, in the current study, when evaluating the best threshold of NEU to maximize precision, sensitivity became 7%, whereas specificity became 100%. Therefore, it is best to choose thresholds that maximize multiple test characteristics. In practice, the threshold a farm chooses to implement will be dependent on the goals of the operation (e.g. minimizing the spread of mastitis, reducing antibiotic usage, etc.).

A farm wanting to reduce unnecessary treatment or to prevent the spread of mastitis throughout the herd should consider maximizing both sensitivity and specificity through the Youden index (**YI**; Table 3.1). Although YI is most commonly used to test the performance of machine-learning based diagnostic models, its use has also been reported to investigate the sensitivity of optimal biomarker thresholds for mastitis diagnosis in dairy cows. For example, when maximizing YI, Petzer et al., 2017 reported an optimal threshold for quarter SCC to be 200,000 cells/mL when detecting IMI. Using a threshold of 1,103,049 cells/mL for TLC resulted in the highest YI (0.46), meaning it had the best balance between sensitivity and specificity (77.74 and 69.24% respectively). Although TLC performed the greatest in terms of the YI, it is important to note that a score of 0.46 is less than ideal and indicates that there is still much room for improvement in the

development of thresholds. The optimal threshold for SCC to maximize YI was 635,000 cells/mL (YI = 0.42). The preset study is in line with Williamson et al. (2022) who reported differences in pretreatment SCC and cure rate. Authors reported that quarters with a pretreatment SCC of 507,041 cells/mL were more likely to cure the infection post antibiotic treatment. It makes sense that TLC would have an improved YI compared to SCC, as TLC reflects only the white blood cells, whereas SCC reflects white blood cells and any other body cells like mammary epithelial cells, though the concentration of this portion in our samples is unknown. The most optimal threshold associated with NEU percent to differentiate quarters as Cured or Failed was 67.33% (YI=0.31). Although literature evaluating the use of NEU percentage thresholds in this context is limited, the reported percentage for NEU thresholds is within previous ranges for IMI detection (Schwarz et al., 2020; Fonseca et al., 2024). When evaluating the percentage of MAC, the threshold that maximized YI was 23.44% (YI = 0.25). The inverse relationship reported earlier between NEU and MAC holds true to the directionality of thresholds, and again makes sense biologically given the importance of a robust neutrophil influx during infection. Values greater than the stated threshold for NEU, SCC, and TLC would indicate a greater likelihood of failure to cure, whereas a greater percentage of MAC than the threshold would be more likely to cure. There are also limitations to these thresholds in terms of applicability. Based on a TLC threshold of 1,103,000 cells/mL, of the 66 subclinically-infected quarters enrolled in this study, only 30 would be treated. Furthermore, if the MAC percentage threshold for maximizing sensitivity and precision was utilized, of the 66 subclinically-infected quarters enrolled in this study, 41 quarters would be treated. Although catching 41 is a much closer proportion, missing subclinical infections could

result in the establishment of chronic infections and spread to others in the herd (Roberson, 2012).

When screening an infected quarter, if the decision from the farm is to skip antibiotic treatment and cull the animal, the farm should consider a threshold that maximizes the F1 score (Table 3.1). To maximize both sensitivity and precision, the F1 score should be evaluated. The threshold with the greatest F1 score was TLC at 1,687,049 cells/mL (F1 = 0.77). The F1 score for SCC and NEU percentages was the same (F1 = 0.74) with thresholds being 937,000 cell/mL and 73.33% respectively. The use of MAC percentages yielded the lowest F1 score of 0.71 and a threshold of 15.44%. Using a TLC of 937,000 cells/mL consistently resulted in the highest F1, meaning it had the best balance between sensitivity and precision (84.70 and 70.80% respectively). Once again, it is unsurprising that TLC outperforms SCC given the composition of cellular populations in those parameters, but it is interesting that SCC and NEU percentages are equivalent in success for setting a threshold that maximizes sensitivity and precision. However, if we consider how these results apply back to the sample group, based on a TLC threshold of 1,687,049 cells/mL, of the 66 subclinically-infected quarters enrolled in this study, only 24 would be treated. If we utilized the MAC percent threshold for maximizing sensitivity and precision, of the 66 subclinically-infected quarters enrolled in this study, only 14 would be treated. When comparing detection for the YI threshold and the F1 score threshold it is clear to see fewer cows are being detected when utilizing F1 score. Given that F1 thresholds may be best utilized for culling decisions, thresholds are narrower to minimize the risk of culling a cow that may have cured an infection. Ultimately, additional investigation is

needed, and our results highlight the potential for refining mastitis treatment decisions utilizing elements of DSCC.

A farm whose goal is to minimize the number of missed cows, unnecessary antibiotic treatment, and maximize economic return should consider a threshold that maximizes accuracy (Table 3.1). Accuracy measures the proportion of correct predictions out of all predictions made. The threshold with the greatest accuracy was TLC at 1,687,049 cells/mL and an accuracy of 0.75. The accuracy of SCC when using the threshold 635,000 cells/mL was 0.71. When optimizing accuracy, NEU had a threshold of 63.33% with an accuracy of 0.66 and MAC had an accuracy of 0.62. When considering how the thresholds impact the sample herd, based on a TLC threshold of 1,687,049 cells/mL, of the 66 subclinically-infected quarters enrolled in this study, only 24 would be treated. If we utilized the MAC percent threshold for maximizing accuracy, of the 66 subclinically-infected quarters enrolled in this study, 33 would have been treated. Given a relatively balanced data set between Cured and Failed quarters, it is appropriate to evaluate accuracy of the thresholds as the data will not be skewed toward a specific outcome (Gu et al., 2009). For example, if the incidence rate was very low (~10%) the development of thresholds would favor a prediction of Cured given it makes up most of the population.

Previous research has utilized DSCC threshold to differentiate between infected and uninfected; however, the evaluation of its ability to predict cure outcomes is limited. Overall thresholds for TLC performed the best in all tests characteristic analyses; however, these results may be skewed given that the TLC thresholds are set at over one million cells per mL. Given such an elevation, it makes sense that quarters within that range would be more unlikely to cure the infection. On the other hand, a TLC elevated to this degree could

be indicative of the pathogen profile of the farm the thresholds were determined on, as the higher prevalence of *Staph. aureus* could be driving cell counts up. Regardless, cell shedding that high is a clear indicator of a robust inflammatory response and may require more interventions than just antibiotic treatment.

The gaps that exist with current cow side test could be filled with the implementation of DSCC. Where DSCC could be very valuable is within providing a more accessible way to understand the role of a pathogen-depending immune response elicited. Kirkeby et al. (2021) have utilized DSCC to report the temporal changes in DSCC based on pathogen classification. For example, when evaluating major and minor pathogens DSCC did not see a decrease in percentage levels until 70 days after an infection. Future research with a larger sample size should focus towards understanding the role of pathogens on the magnitude of the innate immune response as it pertains to DSCC.

The establishment of thresholds based on NEU percentage performed closely to that of SCC. Future research to better understand factors that impact NEU could help improve its ability to predict medical outcomes. It makes sense that quarters with elevated NEU would be less likely to cure an infection given that excess activity or prolonged presence of NEU would result in damage to the mammary tissue due to the release of proteolytic enzymes and other damaging compounds like reactive oxygen and nitrogen species. Understanding the cellular mechanisms behind the immune response is paramount to understanding how to better detect mastitis. Could it be possible to manipulate the immune response to decrease the level of NEU present in the mammary possibly supporting a cure rather than failure? Ultimately more research is needed to understand these immunological and physiological mechanisms.

Conclusion

Given the present data, DSCC has the potential to provide producers with a more detailed understanding of what is occurring within the mammary gland. This present study found that mammary quarters that cured their IMI following intramammary antibiotic treatment have lower SCC, TLC, NEU%, and CMT, while also having higher MAC%. It was also reported that TLC had the greatest performance in accuracy, the F1 score, and the Youden index when establishing a threshold. The threshold performance for SCC and NEU percentage were similar, the use of SCC may be better to use if wanting to maximize the YI or accuracy as it performed the best within those settings. However, the use of NEU percentages might be best suited for F1 thresholds where there is greater selection pressure and the more refined look at the mammary gland would be beneficial to decision making. By providing producers with more detailed information, treatment decisions for antibiotics can be made with a more thorough evaluation of probable success or targeting the appropriate desired outcome. Further research with a larger enrollment size is needed to better understand how these thresholds can be utilized.

Literature Cited

Azab, B., V. R. Bhatt, J. Phookan, S. Murukutla, N. Kohn, T. Terjanian, and W. D. Widmann. 2012. Usefulness of the neutrophil-to-lymphocyte ratio in predicting short- and long-term mortality in breast cancer patients. Ann Surg Oncol 19(1):217-224. doi: 10.1245/s10434-011-1814-0

Damm, M., C. Holm, M. Blaabjerg, M. N. Bro, and D. Schwarz. 2017. Differential somatic cell count—A novel method for routine mastitis screening in the frame of Dairy Herd Improvement testing programs. J Dairy Sci. 100(6):4926-4940. doi: https://doi.org/10.3168/jds.2016-12409

Dohoo, I. R., J. Smith, S. Andersen, D. F. Kelton, and S. Godden. 2011. Diagnosing intramammary infections: Evaluation of definitions based on a single milk sample. J Dairy Sci. 94(1):250-261. doi: https://doi.org/10.3168/jds.2010-3559

Fonseca, M., D. Kurban, J.-P. Roy, D. E. Santschi, E. Molgat, and S. Dufour. 2024. Usefulness of differential somatic cell count for udder health monitoring: Diagnostic performance of somatic cell count, and differential somatic cell count for diagnosing of intramammary infections in dairy herds with automated milking systems. J Dairy Sci. doi: https://doi.org/10.3168/jds.2024-25404

George, L. W., T. J. Divers, N. Ducharme, and F. L. Welcome. 2008. Chapter 8 - Diseases of the Teats and Udder. In: T. J. Divers and S. F. Peek, editors, Rebhun's Diseases of Dairy Cattle (Second Edition). W.B. Saunders, Saint Louis. p. 327-394.

Godden, S. M., E. Royster, J. Timmerman, P. Rapnicki, and H. Green. 2017. Evaluation of an automated milk leukocyte differential test and the California Mastitis Test

for detecting intramammary infection in early- and late-lactation quarters and cows. J Dairy Sci. 100(8):6527-6544. doi: 10.3168/jds.2017-12548

Gonçalves, J. L., R. L. Lyman, M. Hockett, R. Rodriguez, M. V. Dos Santos, and K. L. Anderson. 2017. Using milk leukocyte differentials for diagnosis of subclinical bovine mastitis. J Dairy Res 84(3):309-317. doi: 10.1017/s0022029917000267

Hoque, M. N., Z. C. Das, A. K. Talukder, M. S. Alam, and A. N. Rahman. 2015. Different screening tests and milk somatic cell count for the prevalence of subclinical bovine mastitis in Bangladesh. Trop Anim Health Prod 47(1):79-86. doi: 10.1007/s11250-014-0688-0

Kennedy, B., M. Sethar, A. Tong, J. Moxley, and B. Downey. 1982. Environmental factors influencing test-day somatic cell counts in Holsteins. J Dairy Sci. 65(2):275-280.

Kenward, M. G., and J. H. Roger. 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics:983-997.

Kirkeby, C., D. Schwarz, M. Denwood, M. Farre, S. S. Nielsen, M. Gussmann, N. Toft, and T. Halasa. 2021. Dynamics of somatic cell count (SCC) and differential SCC during and following intramammary infections. J Dairy Sci. 104(3):3427-3438. doi: https://doi.org/10.3168/jds.2020-19378

Lee, C.-S., F. P. Wooding, and P. Kemp. 1980. Identification, properties, and differential counts of cell populations using electron microscopy of dry cows secretions, colostrum and milk from normal cows. J Dairy Res 47(1):39-50.

Leslie, K., J. Jansen, and G. Lim. 2002. Opportunities and implications for improved on-farm cowside diagnostics. In: Proc. De Laval Hygiene Symp

Mondini, S., G. Gislon, M. Zucali, A. Sandrucci, A. Tamburini, and L. Bava. 2023. Risk factors of high somatic cell count and differential somatic cells in early lactation associated with selective dry cow therapy. Animal 17(10):100982. doi: 10.1016/j.animal.2023.100982

Nielen, M., H. Deluyker, Y. H. Schukken, and A. Brand. 1992. Electrical Conductivity of Milk: Measurement, Modifiers, and Meta Analysis of Mastitis Detection Performance. J Dairy Sci. 75(2):606-614. doi: https://doi.org/10.3168/jds.S0022-0302(92)77798-4

Norberg, E., H. Hogeveen, I. R. Korsgaard, N. C. Friggens, K. H. M. N. Sloth, and P. Løvendahl. 2004. Electrical Conductivity of Milk: Ability to Predict Mastitis Status. J Dairy Sci. 87(4):1099-1107. doi: https://doi.org/10.3168/jds.S0022-0302(04)73256-7

Ogola, H., A. Shitandi, and J. Nanua. 2007. Effect of mastitis on raw milk compositional quality. J Vet Sci 8(3):237-242. doi: 10.4142/jvs.2007.8.3.237

Pinzón-Sánchez, C., V. Cabrera, and P. Ruegg. 2011. Decision tree analysis of treatment strategies for mild and moderate cases of clinical mastitis occurring in early lactation. J Dairy Sci. 94(4):1873-1892.

Rasmussen, P., H. W. Barkema, P. P. Osei, J. Taylor, A. P. Shaw, B. Conrady, G. Chaters, V. Muñoz, D. C. Hall, O. O. Apenteng, J. Rushton, and P. R. Torgerson. 2024. Global losses due to dairy cattle diseases: A comorbidity-adjusted economic analysis. J Dairy Sci. 107(9):6945-6970. doi: https://doi.org/10.3168/jds.2023-24626

Roberson, J. R. 2012. Treatment of Clinical Mastitis. Veterinary Clinics: Food Animal Practice 28(2):271-288. doi: 10.1016/j.cvfa.2012.03.011

Rollin, E., K. C. Dhuyvetter, and M. W. Overton. 2015. The cost of clinical mastitis in the first 30 days of lactation: An economic modeling tool. Preventive Veterinary Medicine 122(3):257-264. doi: https://doi.org/10.1016/j.prevetmed.2015.11.006

Schwarz, D., U. S. Diesterbeck, S. König, K. Brügemann, K. Schlez, M. Zschöck, W. Wolter, and C. P. Czerny. 2011. Flow cytometric differential cell counts in milk for the evaluation of inflammatory reactions in clinically healthy and subclinically infected bovine mammary glands. J Dairy Sci. 94(10):5033-5044. doi: https://doi.org/10.3168/jds.2011-4348

Schwarz, D., D. E. Santschi, J. Durocher, and D. M. Lefebvre. 2020. Evaluation of the new differential somatic cell count parameter as a rapid and inexpensive supplementary tool for udder health management through regular milk recording. Preventive Veterinary Medicine 181:105079. doi: https://doi.org/10.1016/j.prevetmed.2020.105079

Sheldrake, R. F., R. J. Hoare, and G. D. McGregor. 1983. Lactation stage, parity, and infection affecting somatic cells, electrical conductivity, and serum albumin in milk. J Dairy Sci 66(3):542-547. doi: 10.3168/jds.S0022-0302(83)81823-2

Smith, K. 1999. Suggested interpretation of mastitis terminology. Bulletin-International Dairy Federation (338):3-26.

Sol, J., O. Sampimon, H. Barkema, and Y. Schukken. 2000. Factors associated with cure after therapy of clinical mastitis caused by Staphylococcus aureus. J Dairy Sci. 83(2):278-284.

Sordillo, L. M., S. C. Nickerson, R. M. Akers, and S. P. Oliver. 1987. Secretion composition during bovine mammary involution and the relationship with mastitis. Int J Biochem 19(12):1165-1172. doi: 10.1016/0020-711x(87)90098-x

Sordillo, L. M., and K. L. Streicher. 2002. Mammary Gland Immunity and Mastitis Susceptibility. Journal of Mammary Gland Biology and Neoplasia 7(2):135-146. doi: 10.1023/A:1020347818725

Williamson, J. R., T. R. Callaway, E. Rollin, and V. E. Ryman. 2022. Association of pre-treatment somatic cell counts with bacteriological cure following diagnosis of intramammary infection. Research in Veterinary Science 152:537-545. doi: https://doi.org/10.1016/j.rvsc.2022.09.015

Yamanaka, T., S. Matsumoto, S. Teramukai, R. Ishiwata, Y. Nagai, and M. Fukushima. 2007. The baseline ratio of neutrophils to lymphocytes is associated with patient prognosis in advanced gastric cancer. Oncology 73(3-4):215-220. doi: 10.1159/000127412

Yasuda, T., N. Yagi, T. Omatsu, H. Kitae, Y. Nakahata, Y. Yasuda, N. Sakamoto, A. Obora, Y. Murakami, and T. Kojima. 2024. High neutrophil-to-lymphocyte ratio at Helicobacter pylori eradication increases the risk of eradication failure and post-eradication gastric cancer. Scand J Gastroenterol 59(12):1277-1288. doi: 10.1080/00365521.2024.2428280

Zecconi, A., F. Dell'Orco, D. Vairani, N. Rizzi, M. Cipolla, and L. Zanini. 2020. Differential somatic cell count as a marker for changes of milk composition in cows with very low somatic cell count. Animals 10(4):604.

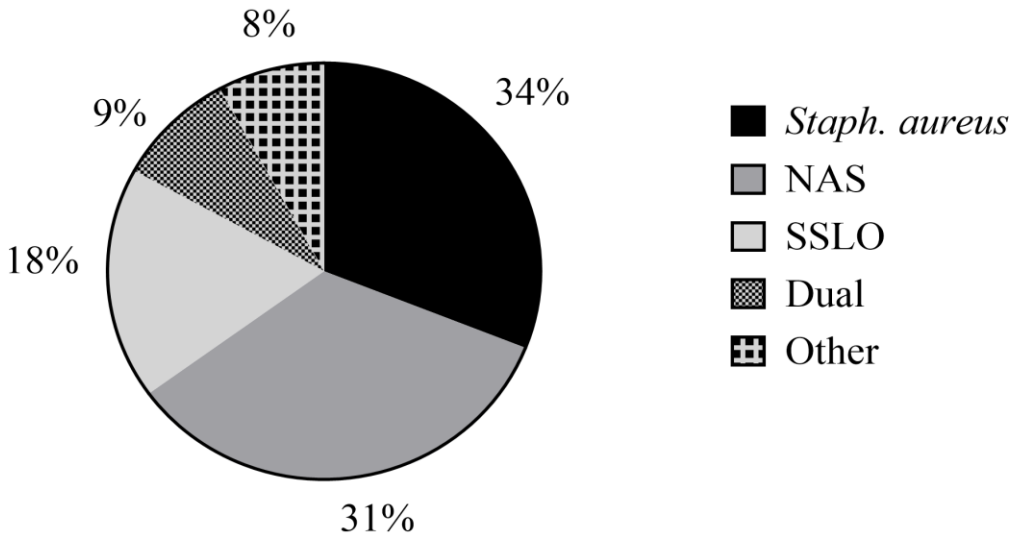


Figure 3.1. Pathogen distribution of subclinically-infected mammary quarters (*N* = 66) on enrollment day (D0). Milk samples were collected using aseptic techniques and plated on trypticase soy agar plates with 5% sheep blood. Positive growth samples were sent to the University of Georgia Veterinary Diagnostic laboratory for identification via MALDI-TOF. Streptococci-like organisms (SSLO) consisted of pathogen such as *Lactococcus garvieae, Streptococcus dysgalactiae, Streptococcus uberis* and *Enterococcus cecorum.* Non-aureus staphylococci (NAS) consisted of pathogen such as *Staphylococcus chromogenes, Staphylococcus haemolyticus, Staphylococcus hyicus, and Staphylococcus xylosus*. Dual infections consisted of quarters that had > 1 cultured pathogens. Other infections consisted of pathogens such as *Nocardia, Mannheimia haemolytica, and* Coryneform spp.

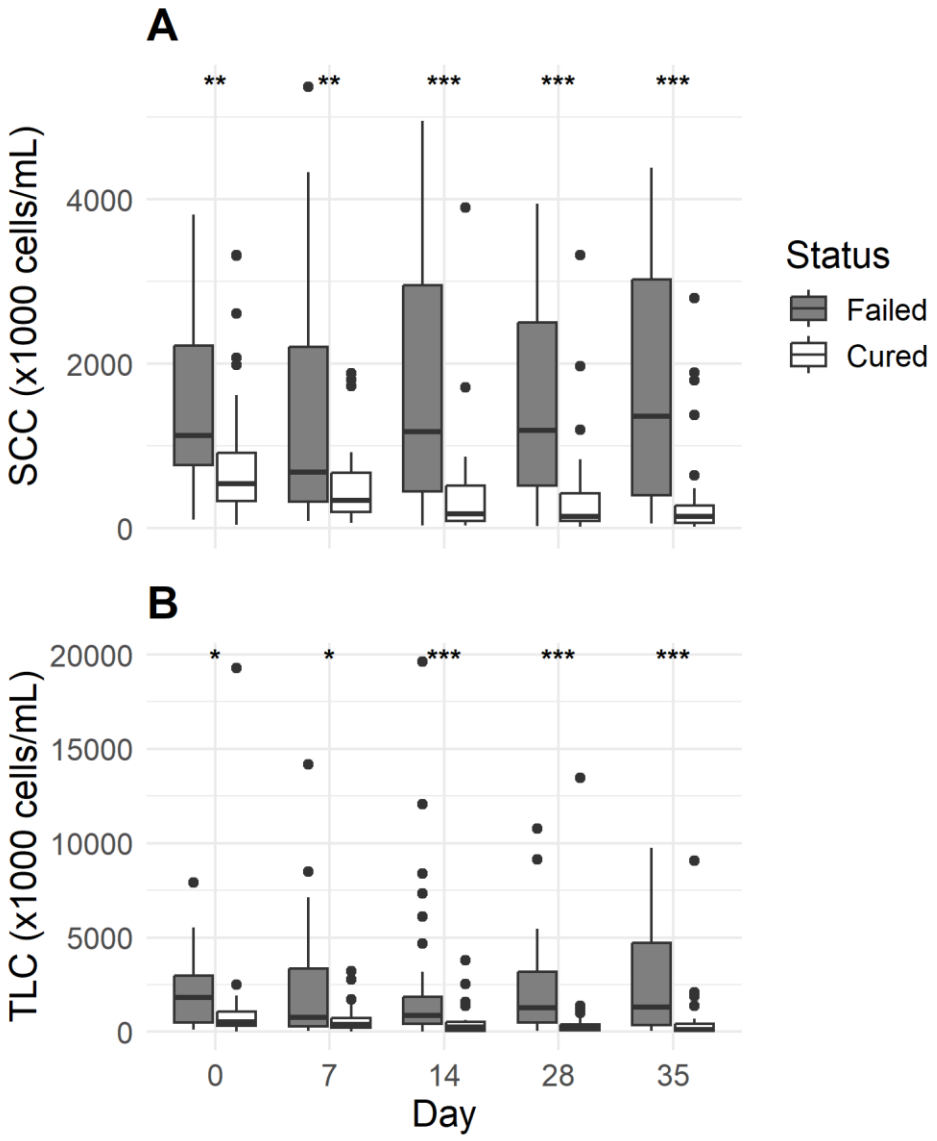


Figure 3.2. (A) Somatic cell count (**SCC;** cells/mL) and (B) Total Leukocyte (**TLC;** cells/mL) in mammary quarters that cured (white box plot) or failed to cure (gray box plot) an intramammary infection after receiving intramammary antibiotics. The boxplot displays the median and the range between the first and third quartile. The whiskers represent the spread of data within 1.5 times the first or third quartile. Outliers are indicated as dots either above or below the whiskers of the plot. Significance was declared at $P \leq 0.05$. (ns) $P > 0.10$; (*) $0.01 < P \leq 0.05$; (**) $0.001 < P \leq 0.01$; (***) $P \leq 0.001$.

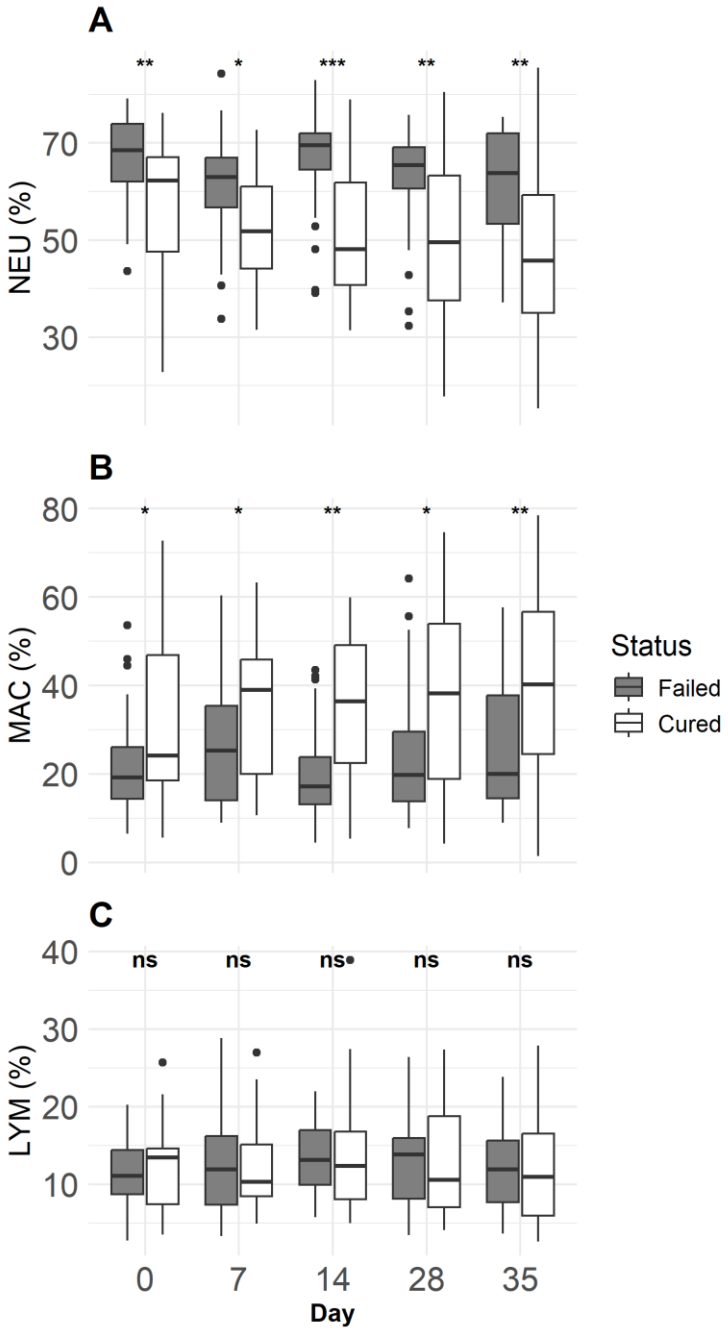


Figure 3.3. Percentage of (A) Neutrophils (**NEU**), (B) Macrophages (**MAC**), and (C) Lymphocyte (**LYM**) in mammary quarters that cured (white box plot) or failed to cure (gray box plot) an intramammary infection after receiving intramammary antibiotics. The boxplot displays the median and the range between the first and third quartile. The whiskers represent the spread of data within 1.5 times the first or third quartile. Outliers are indicated as dots either above or below the whiskers of the plot. Significance was declared at $P \leq 0.05$. (ns) $P > 0.10$; (*) $0.01 < P \leq 0.05$; (**) $0.001 < P \leq 0.01$; (***) $P \leq 0.001$.

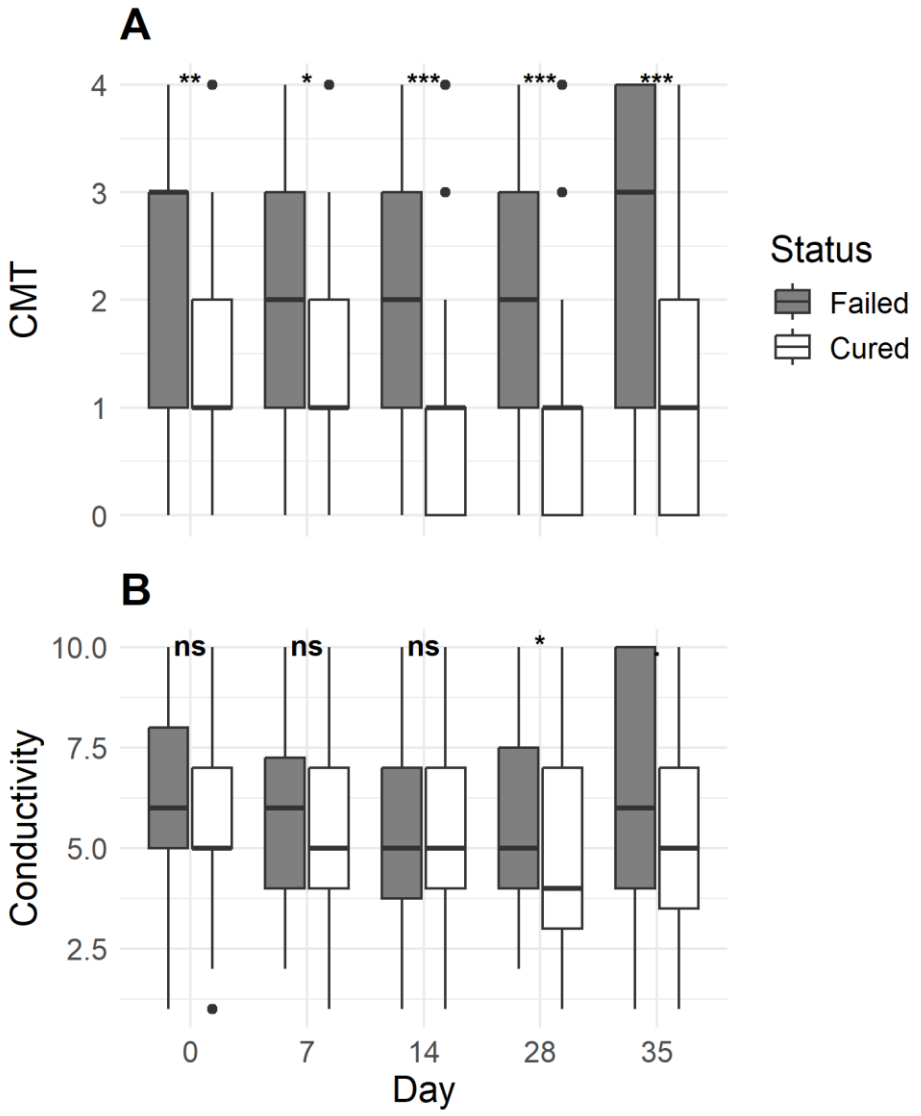


Figure 3.4. (A) California Mastitis Test (**CMT**) and (B) Milk conductivity in mammary quarters that cured (white box plot) or failed to cure (gray box plot) an intramammary infection after receiving intramammary antibiotics. The boxplot displays the median and the range between the first and third quartile. The whiskers represent the spread of data within 1.5 times the first or third quartile. Outliers are indicated as dots either above or below the whiskers of the plot. Significance was declared at Significance was declared at $P \leq 0.05$. (ns) $P > 0.10$; (*) $0.01 < P \leq 0.05$; (**) $0.001 < P \leq 0.01$; (***) $P \leq 0.001$.

Table 3.1. Optimal predictor thresholds for Youden index, F1 score, and Accuracy

Predictor	Threshold, %	Youden Index	Sensitivity	SD[1]	Specificity	SD	Accuracy	SD	F1 score	SD
Youden Index										
MAC[2], %	23.45	0.26	0.55	0.08	0.71	0.08	0.62	0.06	0.61	0.06
NEU[3], %	67.33	0.30	0.76	0.06	0.54	0.09	0.66	0.05	0.71	0.06
SCC[4]	635.00	0.42	0.64	0.08	0.78	0.08	0.70	0.05	0.70	0.06
TLC[5]	1103.05	0.44	0.77	0.14	0.68	0.11	0.73	0.11	0.75	0.12
F1 score										
MAC, %	15.45	0.22	0.89	0.09	0.33	0.09	0.64	0.06	0.73	0.08
NEU, %	73.33	0.27	0.95	0.04	0.32	0.08	0.67	0.05	0.76	0.05
SCC	937.00	0.39	0.77	0.07	0.62	0.09	0.70	0.05	0.74	0.05
TLC	1687.05	0.42	0.83	0.17	0.59	0.09	0.72	0.11	0.76	0.14
Accuracy										
MAC, %	17.45	0.24	0.80	0.09	0.44	0.09	0.64	0.06	0.71	0.06
NEU, %	73.33	0.27	0.95	0.04	0.32	0.08	0.67	0.05	0.76	0.05
SCC	635.00	0.42	0.64	0.08	0.78	0.08	0.70	0.05	0.70	0.06
TLC	1103.05	0.44	0.77	0.14	0.68	0.11	0.73	0.11	0.75	0.12

[1]SD = standard deviation.
[2]MAC = macrophages.
[3]NEU = neutrophils.
[4]SCC = somatic cell count, × 1000 cell/mL.
[5]TLC = total leukocyte count, × 1000 cell/mL.

CHAPTER 3

EVALUATING THE USE OF DIFFERNETIAL SOMATIC CELL COUNT IN DETECTING MASTITIS AT DRY OFF AND INTEGRATION INTO MACHINE LEARNING MODELS

---

[1]Burner, C. M., A. A. C. Alves, E. Rollin, T. R. Callaway, V. E. Ryman. To be submitted for publication.

Abstract

Blanket dry cow therapy accounts for approximately 1/3 of antibiotic use on dairies. Alternative management strategies such as selective dry cow therapy (SDCT) can be implemented which treat quarters or cows can with antibiotics based on herd-, cow-, or quarter-level criteria. Options for implementing SDCT include both cow-side tests (low-cost, rapid tests and higher cost culture-based SDCT) and historical data (algorithm-based SDCT), or a combination. Considerations made when exploring SDCT options include differences in diagnostic efficacy, cost, etc. The objective of this study is to evaluate the use of differential somatic cell count (DSCC) into culture-guided machine learning models as a standalone parameter and in combination with low-cost cow-side tests to identify infected quarters needing antibiotic administration. In particular, the California Mastitis Test (CMT) and milk conductivity as measured by Mas-D-Tec^{TM} were selected given their low input cost. Late-lactation dairy cows (N = 98) at the University of Georgia Teaching Dairy were enrolled 48 h prior to dry off. On enrollment day, quarter milk samples were aseptically collected for CMT, milk conductivity (EC), SCC, DSCC, and culturing. Milk samples were then plated on blood agar, following which plates were evaluated for microbial growth 48 h later. Quarters were identified as bacteriologically infected or uninfected based on microbial growth. This study utilized quarter-based SDCT and cow-based SDCT. Overall, 11.6% of quarters and 31.0% of cows were infected at collection. Differences between CMT, EC, somatic cell count (SCC), neutrophils (NEU) and macrophages (MAC) were seen between infected and uninfected quarters ($P < 0.05$). Relative to culture-based selection, only quarter-level CMT and cow-level SCC were found to be reliable diagnostic variables ($P < 0.03$). Different machine learning algorithms were

trained against culture results to classify IMI at the quarter- and cow-level across ten different variable combinations including DSCC as a standalone parameter and in conjecture with SCC. The performance metrics of the models were tested and included sensitivity, precision, F1 score, and Matthew's correlation coefficient. The use of DSCC did not improve performance metrics for mastitis detection, except for quarter-level sensitivity when used in combination with SCC. In addition, the combination of SCC and DSCC did not improve performance metrics of the models' ability to detect mastitis, however, overall performance metrics for sensitivity and precision improved when the machine learning models were trained on cow-level criteria vs quarter-level criteria. While additional data is needed to fully evaluate the applicability of DSCC in machine learning algorithms for mastitis detection, findings suggest that training on cow-level criteria improves model performance metrics.

Introduction

Approximately 80% of dairy herds in the United States utilize a long-standing practice, blanket dry cow therapy (**BDCT**), which involves treating all mammary quarters of every cow with antimicrobials at dry off (USDA, 2018). Cows entering the dry period have the greatest risk for developing an intramammary infection (**IMI**) compared to lactating cows due to several factors such as milk collection cessation, milk stasis, and environmental cleanliness (Dingwell et al., 2004; Green et al., 2007). The goal of BDCT is to prevent early dry period mastitis and its persistence into subsequent lactations. Within the dairy industry, BDCT accounts for 33% of total antimicrobial usage (Pol and Ruegg, 2007), given a cow is treated with antibiotics regardless of current infection status (Stevens et al., 2016). Due to growing public concerns about antimicrobial resistance, there is increasing pressure on producers to limit antimicrobial usage in livestock operations (Wemette et al., 2021). Additionally, as economic margins become tighter, reducing potentially unnecessary expenses is critical. One emerging management strategy is selective dry cow therapy (**SDCT**).

As the name suggests, SDCT involves selecting cows or mammary quarters for antibiotic treatment based on set cow- or quarter-level criteria. In general, existing approaches for implementing SDCT are algorithm-guided, culture-guided SDCT, or potentially a combination of the two (Cameron et al., 2014; Patel et al., 2017; Vasquez et al., 2018). Algorithm-guided SDCT utilizes health data (i.e., somatic cell counts [**SCC**] and clinical mastitis records) from dairy management systems to select cows at risk for dry cow mastitis. Other parameters that have been built into algorithm-guided SDCT models also include California Mastitis Test (**CMT**) results and milk electrical conductivity (**EC**),

though the use of CMT and EC is variable given previous investigation. Godden et al. (2017) reported that CMT sensitivity ranged from 19.1 to 86.4%, with specificity between 14.3 to 92.3% in late-lactation cows. However, the use of CMT in late lactation cows is not recommended as scores tend to be higher during this period due to physiological factors including the onset of mammary involution or a dilution effect on SCC as milk yield decreases (Green et al., 2006; Hagnestam-Nielsen et al., 2009). When evaluating EC for mastitis detection, Nielen et al. (1992) reported an overall sensitivity and specificity of 66% and 94% respectively. Although limited literature exists for EC performance in mastitis detection in late-lactation cows, the lactation stage has been reported to affect EC (Lu et al., 2024).

Ultimately, successful implementation of SDCT is dependent on continuous monitoring and accurate record keeping. Culture-guided SDCT uses milk samples collected prior to dry-off to determine microbial presence for SDCT selection. Drawbacks such as contaminated milk samples, cyclic bacterial shedding patterns, and low bacterial concentrations that fall below detection thresholds can impact program success (Sears et al., 1990; Mues et al., 2025). Achieving 80% sensitivity for mastitis detection is crucial for SDCT success; however, previous research has reported that either method has a 70% sensitivity (Patel et al., 2017; Vasquez et al., 2018).

To improve mastitis detection, research suggests differential SCC (**DSCC**) can increase sensitivity when combined with traditional SCC measurements (Godden et al., 2017; Schwarz et al., 2020; Zecconi et al., 2023). The DSCC focuses on white blood cell (**WBC**) proportions within milk, most predominately neutrophils, macrophages, and lymphocytes. Previous work surrounding DSCC has reported a strong correlation to SCC,

particularly with increasing neutrophils (Pillai et al., 2001; Wall et al., 2018). Pilla et al. (2013) reported sensitivity and specificity of 75.7 and 92.3% respectively when DSCC was tested as a stand-alone parameter in mastitis detection. Combining SCC and DSCC increased sensitivity to 97.2%, with no change to specificity. Many other studies agree that combining DSCC and SCC can improve diagnostic sensitivity (Wall et al., 2018; Dal Prà et al., 2022).

Research on DSCC evaluation in late-lactation cows and particularly its role in SDCT is limited. Therefore, the objective of this pilot study was to evaluate the incorporation of DSCC into a culture-guided machine learning model for IMI detection at the time of dry off as a standalone parameter and in combination with low-cost cow-side tests (CMT and EC) and SCC. We hypothesized that DSCC would improve the models' performance metrics better than low-cost cow-side test and SCC and in combination with SCC.

Materials and methods

All procedures involving live animals were verified and approved by the University of Georgia Office of Animal Care and Use Committee (AUP # A2023 02-010-Y2-A1).

Sample Collection

Late-lactation dairy cows (N = 93) at the University of Georgia Teaching Dairy were enrolled 48 h prior to dry off (**DO**). On enrollment day, foremilk was discarded and then approximately 15 mL of quarter milk samples were collected using aseptic techniques according to National Mastitis Council guidelines for culturing (Oliver et al., 2004). Additional quarter milk samples were collected for: SCC enumeration using a DeLaval Direct Cell Counter (DeLaval, Tumba, Sweden), California Mastitis Test (**CMT**) scoring,

milk electrical conductivity (**EC**), and DSCC enumeration. Forty-eight hours following collection, each quarter was dried off using SPECTRAMAST® DC (Zoetis, Parsippany-Troy Hills, NJ, USA) and ORBESEAL® internal teat sealant (Zoetis, Parsippany-Troy Hills, NJ, USA).

SCC, CMT, EC, and DSCC Collection

The DeLaval Direct Cell Counter (DeLaval; Tumba, Sweden) was used to determine SCC as described in Williamson et al. (2022) and reported in cells/μL and a SCC of ≥ 200,000 cells/mL was considered infected. To perform CMT testing, milk from each quarter was expressed into a divided paddle and mixed with equal amounts of CMT reagent, swirled and subjectively assigned a score of Negative, Trace, 1, 2, or 3 based on gel formation (Schalm and Noorlander, 1957). A score of trace or above was considered infected according to manufacturer specifications. For evaluation of EC, milk from enrolled mammary quarters was expressed 4 to 5 times into a handheld milk conductivity meter (Mas-D-Tec®; Wescor Inc., Logan, UT, USA) and values were recorded. Readings were provided on a scale from 1 to 10 with a score of 5 or higher being considered infected according to manufacturer specifications.

Differential somatic cell count was enumerated utilizing the QScout^{TM} Farm Lab Milk Leukocyte Differential (**MLD**) test (Advanced Animal Diagnostics Inc., Morrisville, NC, USA). Milk was expressed into the Q4 milk collection kit and capped with the provided plug. Milk samples were gently inverted 10 times and transferred to the MLD slide for testing. Tests were conducted in research mode under the "Dry Off" program according to manufacturer's instructions as described in (Godden et al., 2017), the program was set to a threshold of 6. Results from MLD test indicated a positive or negative mastitis

status at both the cow and quarter level. In addition, total leukocyte count (**TLC**; cells/mL) and percentage of neutrophils (**NEU**), macrophages (**MAC**), and lymphocytes (**LYM**) were provided.

Microbial Culture

Milk samples were transported back to the University of Georgia Mastitis Lab for culture. Milk samples were plated on 4Cast® plates from the Minnesota Easy® Culture system using sterile cotton swabs and incubated at 37ºC. After 48 hours, plates were examined and identified as infected or uninfected based on the presence of 3 or more pure microbial colonies. Samples with positive growth were sent to the University of Georgia Veterinary Diagnostic Laboratory for identification via MALDI-TOF. Plates with 3 or more distinct bacterial colonies were deemed contaminated.

Statistical Analysis

To compare standalone parameters to culture results 2 × 2 contingency tables were created at the quarter- and cow-level and analyzed with a Fisher's exact test to determine association between the variable and a positive IMI. For identification of mastitis at the cow-level, if ≥ 1 quarter of the cow was infected, the entire cow was classified as infected. A positive test for each of the variables are as follows: CMT with a score ≥ Trace; EC ≥ 5; SCC ≥ 200,000 cells/mL; NEU ≥ 55.88%; MAC ≤ 27.38%. Given the limited literature on defined cut-offs for NEU and MAC to diagnose IMI, the values used were based off the average value from this data for infected quarters.

For the machine learning analysis, three classification models (Logistic Regression, Gradient Boosting Machines (**GBM**), and Random Forest) were tested with 10 variable combinations:

Base: Days in Milk (**DIM**) + Parity + Milk Yield + Breed

CMT: Base + CMT

EC: Base + EC

SCC: Base + SCC

DSCC: Base + TLC + NEU% + LYM% + MAC%

EC+CMT: Base + EC + CMT

SCC+EC: Base + SCC + EC

SCC+EC+CMT: Base + SCC + EC + CMT

SCC+DSCC: Base + SCC + TLC + NEU% + LYM% + MAC%

A repeated 5-fold cross-validation scheme was run to test the models. Briefly, the dataset was divided into 5 folds of roughly equal size. The models were then trained using 4 folds and used to predict the observations for the held-out fold, this process was repeated 5 times until each fold was treated as a validation set once. The 5-fold cross-validation scheme was then repeated 5 times in total, with different observations randomly assigned to each fold. Differences across variable combinations were tested using a Wilcox test and significance was declared at $P \leq 0.05$.

Results and Discussion

Microbiological culture demonstrated that 46 out of 379 mammary quarters as infected yielding an 11.6% quarter infection rate and a 31% cow infection rate which represented cows with at least 1 quarter infected. Of the cows that were infected in at least 1 quarter, 31% had multiple quarters infected. Previous literature found quarter infection rates between 21 and 30% and a cow infection rate of 50.5% (Petzer et al., 2017; Rowe et al., 2019). Quarter infection rates were lower in this present study which could be attributed

to a smaller sample size as Petzer et al. (2017) had 162 cows and Rowe et al. (2019) had over 3000 cows enrolled. However, when compared to Pantoja et al. (2009) Pantoja et al. (2009) who had a similar sample size (N = 102) IMI prevalence at dry off was 12.8%. Further evaluation of microbiological culture showed that 50% of infections were caused by non-aureus staphylococci (NAS) including of *Staphylococcus chromogenes, Staphylococcus haemolyticus, and Staphylococcus xylosus* (Figure 4.1). *Staphylococcus aureus* caused 22% of infections and Streptococci-like organisms (**SLLO**), i.e., *Lactococcus garvieae,* represented 17% of infections. The remaining 11% of infections were caused by *Nocardia africana* and unidentified Gram-positive bacteria. High prevalence of NAS and CNS pathogens at the time of dry off has been seen in previous literature (Pantoja et al., 2009; Kiesner et al., 2016; Rowe et al., 2019). More specifically, Rowe et al. (2019) reported *Staphylococcus chromogenes* to have the highest prevalence which is in line with the present study. Although this study only evaluated one herd, similar pathogen profiles have been seen across literature.

When comparing the average value of the variables between infected and uninfected, EC, SCC, and NEU percentage were greater in infected quarters compared to uninfected quarters ($P < 0.05$). In contrast, MAC percentage and CMT were lower in infected quarters compared to uninfected quarters ($P < 0.05$); however, there was no difference in TLC and LYM percentage ($P < 0.99$; Table 4.1).

Fisher's exact from the 2 × 2 contingency table showed that with the exception of CMT ($P = 0.03$), all other variables assessed at the quarter level (SCC, EC, etc.) were not associated with infection status ($P > 0.11$; Table 4.2). For CMT, if trace was set to be indicative of infected, sensitivity and specificity were 64.4 and 53.5%, respectively,

compared to culture positive results. Reported sensitivities and specificities were similar to previous studies evaluating CMT in late lactation cows (Godden et al., 2017; Sanford et al., 2006); however, these values are not ideal for diagnostic test. A sensitivity of at least 80% and a specificity of 99% is preferred for diagnostic test (Hogeveen et al., 2010), therefore even though CMT was a reliable diagnostic parameter the application of it is not ideal for a successful SDCT program. It was surprising that SCC was not deemed reliable at the quarter level considering the CMT was significant and SCC is widely used in SDCT programs; however, a small sample size and low infection rates could be attributing to these results. Outside of study limitations, SCC is physiologically elevated in late lactation cows due to factors such as dilution effect which could also be contributing to the results (Green et al 2006). However, evaluation of these data using the Fisher's Exact test does not account for confounding factors which could be impacting the results.

The Fisher's exact analysis from the 2 × 2 contingency table showed that all other variables assessed at the cow-level (CMT, EC, etc.) except for SCC ($P = 0.01$), were not reliable diagnostic variables ($P > 0.06$). The sensitivity and specificity for cow-level SCC was 79.3 and 32.8% respectively. When evaluating SCC at dry off, Lipkens et al. (2019) and Kiesner et al. (2016) reported much lower sensitivity values of 34.1 to 57.6%; however, specificities were between 66.7 and 94.4%. The differences in sensitivity between literature and the current study could be attributed to how SCC was used to determine an infection at the cow-level. Lipkens et al. (2019) utilized composite milk SCC whereas in the present study, cow level determination for an infection was based on a SCC of ≥ 200,000 cells/mL in one or more quarter. Petzer et al. (2017) reported a decrease in sensitivity when utilizing composite SCC (57.8%) compared to quarterly SCC, and also

reported a slight decrease in specificity from composite SCC (77.5%) to quarterly SCC (63.6%). Although the reported specificity from the present study is still lower than previous literature, sensitivity values from the current study align more closely with quarter level analysis. The use of cow-level SCC has a more ideal sensitivity score than quarter-level CMT; however, a specificity of 32.8% for SCC would still impact the effectiveness of SDCT as 67% of cows could be identified as false positives resulting in the overuse of antibiotics and would be contradictory to an effective selective dry cow therapy.

Overall, it is not surprising that the use of milk EC was not deemed a reliable diagnostic variable at the quarter- or cow-level as our method of assessing milk EC was an absolute threshold. The method of milk EC assessment is a limitation to this study as an absolute threshold utilizes the same value for a positive mastitis diagnosis regardless of herd or cow differences. Rather, literature suggests the use of an interquartile ratio (IQR) to be the most effective use of assessment of milk EC as it accounts for cow variation (Nielen et al., 1992; Norberg et al., 2004). When considering DSCC, the absence of significance regarding the reliability of NEU percentage and MAC percentage for cows entering the dry period is anticipated, given the limited understanding of factors that influence these percentages. For example, DIM has been reported to impact DSCC, but how DSCC is impacted is undecided as Schwarz et al. (2020) reported NEU and LYM to increase as DIM increased; however, Kirkeby et al. (2020) reported a tendency for DSCC to decrease as DIM increased. The most common reporting of DSCC is the combined percentage of NEU and LYM, leaving literature on macrophage percentages in regard to sensitivity and specificity limited (Schwarz et al., 2020; Zecconi et al., 2020; Dal Prà et al., 2022) leaving minimal discussion on MAC. Further research evaluating impacting factors

for DSCC needs to be better understood before the true diagnostic capability can be evaluated.

Machine Learning Models

Results from the repeated 5-fold cross-validation scheme for quarter- and cow-level on the three classification models: Logistic Regression, GBM, and Random Forest are attached in Supplemental Figure 4.1 and 4.2. Evaluation of accuracy, area under the curve (**AUC**), and area under the precision-recall curve (**AUPRC**) were not reported herein due to low incidence rate of infection within the sample population, but can be found for both the quarter- and cow-level in Supplemental Figures 4.3 and 4.4.

Quarter-level analysis

The combination of SCC+DSCC had a greater sensitivity compared to BASE, EC, SCC and EC+CMT ($P < 0.03$; Figure 4.2), but did not differ from all other variables ($P = 1.00$). The use of BASE, EC, SCC and EC+CMT did not differ in sensitivity when compared to each other ($P = 1.00$). As a result, the combination of SCC+DSCC is more effective at identifying mastitis quarters compared to the use of SCC alone; however, the overall sensitivity values of around 55% demonstrate that there is room for improvement in the models. The use of SCC, DSCC, SCC+CMT, SCC+EC+CMT and SCC+DSCC had greater precision when compared to BASE and EC ($P < 0.04$) but did not differ from each other ($P = 1.00$). The combination of SCC+EC had greater ($P < 0.04$) precision than the use of EC alone. Therefore, the ability for the model to correctly predict an IMI, meaning fewer uninfected cows are being treated, is improved when SCC, DSCC, or any combination involving SCC compared to the use of EC alone. When compared to EC, the F1 score was greater in all variables ($P < 0.01$; Figure 4.3), except for CMT, DSCC, and

EC+CMT where it did not differ ($P > 0.11$). The remaining variables did not differ from each other ($P > 0.17$). The models using EC for prediction had a poorer performance at balanced detection for positive cases while minimizing the over treatment of cows. When evaluating Matthew's correlation coefficient (**MCC**) all variables had greater MCC when compared to BASE and EC ($P < 0.02$) but did not differ from each other ($P > 0.07$). The greater performance in MCC demonstrates that those variables have greater accuracy in detecting IMI. Overall, there was not a lone variable that outperformed the others across all of the different classification metrics; however, most variables outperformed the use of EC alone, suggesting it might not be successful for use as a standalone metric when developing a program for SDCT.

Cow-level analysis

The combination of SCC+DSCC yielded greater sensitivity when compared to BASE and EC ($P < 0.02$; Figure 4.4) but did not differ from all remaining variables ($P = 1.00$). In addition, BASE and EC did not differ ($P = 1.00$) from each other in sensitivity, further demonstrating that EC is not an ideal variable model for IMI detection. When evaluating precision, SCC+CMT and SCC+DSCC had greater precision when compared to BASE and EC ($P < 0.05$), but did not differ from all remaining variables ($P = 1.00$). Emphasizing that the combination of SCC with either CMT or DSCC for IMI detection improves the predictability of the model. There were no differences in F1 score between any of the variables ($P > 0.17$; Figure 4.5) demonstrating that at the cow-level the ability for the model to balance the sensitivity and specificity of detection is not improved when using a specific variable. The MCC for all variables, except for EC+CMT, was greater when compared to BASE ($P < 0.03$), but did not differ from each other ($P > 0.12$). The use

of SCC, DSCC, and all combining variables with the exception of EC+CMT has greater MCC compared to EC ($P < 0.01$); however, EC did not differ from BASE, CMT, and EC+CMT. Therefore, the use of SCC, DSCC or any combination involving these variables improves the model's ability to accurately predict an IMI.

All performance metrics are on a scale from 0 to 1, and it is most ideal to have a value as close to one when evaluating the performance of the machine learning model (Chicco and Jurman, 2020). However, when evaluating the machine learning model trained on quarter-level criteria, median values are all under 0.55. When the machine learning model is based on cow-level information, there was an improvement in diagnostic metrics when compared to the models trained to quarter-level. For example, quarter-level sensitivity for the majority of variables performed with a sensitivity around 55%; whereas cow-level sensitivity for the majority of variables performed at approximately 66%. Rowe et al. (2025) reported a sensitivity between 64 and 67% for the identification of IMI in late lactation cows depending on the machine model utilized. However, the variables used within the model consisted of traditional algorithm guided variables such as mastitis records, monthly SCC testing and milk components. The improvement to cow-level trained models is possibly due to a wider margin of error for catching mastitis compared to the quarter-level. This trend is also seen when evaluating the precision of the model but is more variable when evaluating F1 score and MCC.

It is important to note the differences between the sensitivities in the context of machine learning compared to what was done in the previous chapter and earlier in this chapter. Previously sensitivity was related to a particular metric, e.g. SCC, and its ability to predict mastitis against a "gold standard", in this instance, microbial culture; whereas

machine learning models are trained on a particular set of data and observe mastitis incidence-based on the now-trained algorithm. Following the observed values, the machine learning model then makes predictions on mastitis incidence based off the trained algorithm, and the difference between the observed and predicted values from the model is what makes up sensitivity. In the context of the present study, the increase in sensitivity and precision from quarter- to cow-level suggests that training machine learning modes off cow-level criteria improves the diagnostic metrics of the test.

Factors that could have impeded the performance of the models include the low incidence rate of mastitis within this herd resulting in an unbalanced data set. For example, the accuracy of the variable combinations was not reported because the unbalanced data set of infected versus not infected creates an overoptimistic estimation towards the uninfected category (Gu et al., 2009). In addition to unbalanced data, limited information on how to best apply machine learning to mastitis could also contribute to lower performance. Rowe et al. (2025) reported that machine learning models for detecting IMI in late-lactation cows had slightly better performance in AUC compared to conventional algorithm-based decisions, but the differences were not enough to consider widespread implementation. Machine learning has attempted to be used in other cow health aspects (e.g. lameness scoring, heat detection, etc.), but struggle to have sufficient test characteristics or do not translate well into commercial herds due to elaborate requirements for optimal accuracy (Mayo et al., 2019; Bonfatti et al., 2020; Taneja et al., 2020; Shahinfar et al., 2021).

In the context of our hypothesis, with the exception of combining DSCC and SCC at the quarter level, implementation of DSCC alone into a SDCT program showed no

differences when compared to SCC alone at quarter or cow-level. In addition, creating a machine learning model to assess the combination of SCC and DSCC showed no improvement over SCC or DSCC alone in the ability to detect mastitis for SDCT at either quarter or cow-level.

Conclusion

Given the present data, the implementation of DSCC into a SDCT program may not improve the ability to detect an IMI at the time of dry off. Further investigations should re-evaluate the use of DSCC with a larger sample size with a higher incidence rate of mastitis to confirm these data. In addition, further research needs to be directed towards better understanding the role of DIM, parity, breed, and other external factors on DSCC. While machine learning models have the potential to develop more sophisticated and precise detection methods; further research into accounting for factors within the models as ensuring easy implementation is crucial for improving performance.

Literature Cited

Bonfatti, V., P. Ho, and J. Pryce. 2020. Usefulness of milk mid-infrared spectroscopy for predicting lameness score in dairy cows. J Dairy Sci. 103(3):2534-2544.

Cameron, M., S. L. McKenna, K. A. MacDonald, I. R. Dohoo, J. P. Roy, and G. P. Keefe. 2014. Evaluation of selective dry cow treatment following on-farm culture: risk of postcalving intramammary infection and clinical mastitis in the subsequent lactation. J Dairy Sci 97(1):270-284. doi: 10.3168/jds.2013-7060

Chicco, D., and G. Jurman. 2020. The advantages of the Matthews correlation coefficient (MCC) over F1 score and accuracy in binary classification evaluation. BMC Genomics 21(1):6. doi: 10.1186/s12864-019-6413-7

Dal Prà, A., F. Biscarini, G. L. Cavani, S. Bacchelli, A. Iotti, S. Borghi, M. Nocetti, and P. Moroni. 2022. Relationship between total and differential quarter somatic cell counts at dry-off and early lactation. PLoS One 17(10):e0275755. doi: 10.1371/journal.pone.0275755

Dingwell, R. T., K. E. Leslie, Y. H. Schukken, J. M. Sargeant, L. L. Timms, T. F. Duffield, G. P. Keefe, D. F. Kelton, K. D. Lissemore, and J. Conklin. 2004. Association of cow and quarter-level factors at drying-off with new intramammary infections during the dry period. Prev Vet Med 63(1-2):75-89. doi: 10.1016/j.prevetmed.2004.01.012

Godden, S. M., E. Royster, J. Timmerman, P. Rapnicki, and H. Green. 2017. Evaluation of an automated milk leukocyte differential test and the California Mastitis Test for detecting intramammary infection in early- and late-lactation quarters and cows. J Dairy Sci. 100(8):6527-6544. doi: 10.3168/jds.2017-12548

Green, L. E., Y. H. Schukken, and M. J. Green. 2006. On distinguishing cause and consequence: Do high somatic cell counts lead to lower milk yield or does high milk yield lead to lower somatic cell count? Preventive Veterinary Medicine 76(1):74-89. doi: https://doi.org/10.1016/j.prevetmed.2006.04.012

Green, M. J., A. J. Bradley, G. F. Medley, and W. J. Browne. 2007. Cow, Farm, and Management Factors During the Dry Period that Determine the Rate of Clinical Mastitis After Calving. J Dairy Sci. 90(8):3764-3776. doi: https://doi.org/10.3168/jds.2007-0107

Gu, Q., L. Zhu, and Z. Cai. 2009. Evaluation measures of the classification performance of imbalanced data sets. In: Computational Intelligence and Intelligent Systems: 4th International Symposium, ISICA 2009, Huangshi, China, October 23-25, 2009. Proceedings 4. p 461-471.

Hagnestam-Nielsen, C., U. Emanuelson, B. Berglund, and E. Strandberg. 2009. Relationship between somatic cell count and milk yield in different stages of lactation. J Dairy Sci. 92(7):3124-3133. doi: https://doi.org/10.3168/jds.2008-1719

Hogeveen, H., C. Kamphuis, W. Steeneveld, and H. Mollenhorst. 2010. Sensors and clinical mastitis--the quest for the perfect alert. Sensors (Basel) 10(9):7991-8009. doi: 10.3390/s100907991

Kiesner, K., N. Wente, O. Volling, and V. Krömker. 2016. Selection of cows for treatment at dry-off on organic dairy farms. J Dairy Res 83(4):468-475. doi: 10.1017/s0022029916000662

Kirkeby, C., N. Toft, D. Schwarz, M. Farre, S. S. Nielsen, L. Zervens, S. Hechinger, and T. Halasa. 2020. Differential somatic cell count as an additional indicator for

intramammary infections in dairy cows. J Dairy Sci. 103(2):1759-1775. doi: https://doi.org/10.3168/jds.2019-16523

Lipkens, Z., S. Piepers, A. De Visscher, and S. De Vliegher. 2019. Evaluation of test-day milk somatic cell count information to predict intramammary infection with major pathogens in dairy cattle at drying off. J Dairy Sci. 102(5):4309-4321. doi: https://doi.org/10.3168/jds.2018-15642

Lu, X., M. Long, Z. Zhu, H. Zhang, F. Zhou, Z. Liu, Y. Mao, and Z. Yang. 2024. Comprehensive genetic analysis and predictive evaluation of milk electrical conductivity for subclinical mastitis in Chinese Holstein cows. BMC Genomics 25(1):1230. doi: 10.1186/s12864-024-11157-6

Mayo, L., W. Silvia, D. Ray, B. Jones, A. Stone, I. Tsai, J. Clark, J. Bewley, and G. Heersche Jr. 2019. Automated estrous detection using multiple commercial precision dairy monitoring technologies in synchronized dairy cows. J Dairy Sci. 102(3):2645-2656.

Mues, L., N. Kemper, and J. Blumenberg. 2025. Occurrence and diagnostic of intermittent shedding of Staphylococcus aureus in bovine mammary infection. Frontiers in Veterinary Science 12doi: 10.3389/fvets.2025.1523698

Nielen, M., H. Deluyker, Y. H. Schukken, and A. Brand. 1992. Electrical Conductivity of Milk: Measurement, Modifiers, and Meta Analysis of Mastitis Detection Performance. J Dairy Sci. 75(2):606-614. doi: https://doi.org/10.3168/jds.S0022-0302(92)77798-4

Norberg, E., H. Hogeveen, I. R. Korsgaard, N. C. Friggens, K. H. M. N. Sloth, and P. Løvendahl. 2004. Electrical Conductivity of Milk: Ability to Predict Mastitis

Status. J Dairy Sci. 87(4):1099-1107. doi: https://doi.org/10.3168/jds.S0022-0302(04)73256-7

Pantoja, J. C. F., C. Hulland, and P. L. Ruegg. 2009. Somatic cell count status across the dry period as a risk factor for the development of clinical mastitis in the subsequent lactation. J Dairy Sci. 92(1):139-148. doi: https://doi.org/10.3168/jds.2008-1477

Patel, K. E., S. M. Godden, E. Royster, J. Timmerman, B. Crooker, and N. E. McDonald. 2017. Pilot study: Impact of using a culture-guided selective dry cow therapy program targeting quarter-level treatment on udder health and antibiotic use. The Bovine Practitioner 51(1):48-57. doi: 10.21423/bovine-vol51no1p48-57

Petzer, I. M., J. Karzis, E. F. Donkin, E. C. Webb, and E. M. Etter. 2017. Somatic cell count thresholds in composite and quarter milk samples as indicator of bovine intramammary infection status. Onderstepoort J Vet Res 84(1):e1-e10. doi: 10.4102/ojvr.v84i1.1269

Pilla, R., M. Malvisi, G. G. M. Snel, D. Schwarz, S. König, C. P. Czerny, and R. Piccinini. 2013. Differential cell count as an alternative method to diagnose dairy cow mastitis. J Dairy Sci. 96(3):1653-1660. doi: 10.3168/jds.2012-6298

Pillai, S. R., E. Kunze, L. M. Sordillo, and B. M. Jayarao. 2001. Application of differential inflammatory cell count as a tool to monitor udder health. J Dairy Sci 84(6):1413-1420. doi: 10.3168/jds.S0022-0302(01)70173-7

Pol, M., and P. L. Ruegg. 2007. Treatment Practices and Quantification of Antimicrobial Drug Usage in Conventional and Organic Dairy Farms in Wisconsin. J Dairy Sci. 90(1):249-261. doi: https://doi.org/10.3168/jds.S0022-0302(07)72626-7

Rowe, S. M., S. M. Godden, E. Royster, J. Timmerman, B. A. Crooker, and M. Boyle. 2019. Cross-sectional study of the relationships among bedding materials, bedding bacteria counts, and intramammary infection in late-lactation dairy cows. J Dairy Sci. 102(12):11384-11400. doi: https://doi.org/10.3168/jds.2019-17074

Rowe, S. M., E. Zhang, S. M. Godden, A. K. Vasquez, and D. V. Nydam. 2025. Comparison of a machine learning model with a conventional rule-based selective dry cow therapy algorithm for detection of intramammary infections. J Dairy Sci 108(1):760-772. doi: 10.3168/jds.2024-25418

Schalm, O., and D. Noorlander. 1957. Experiments and observations leading to development of the California mastitis test.

Schwarz, D., D. E. Santschi, J. Durocher, and D. M. Lefebvre. 2020. Evaluation of the new differential somatic cell count parameter as a rapid and inexpensive supplementary tool for udder health management through regular milk recording. Preventive Veterinary Medicine 181:105079. doi: https://doi.org/10.1016/j.prevetmed.2020.105079

Sears, P. M., B. S. Smith, P. B. English, P. S. Herer, and R. N. Gonzalez. 1990. Shedding pattern of Staphylococcus aureus from bovine intramammary infections. J Dairy Sci 73(10):2785-2789. doi: 10.3168/jds.S0022-0302(90)78964-3

Shahinfar, S., M. Khansefid, M. Haile-Mariam, and J. Pryce. 2021. Machine learning approaches for the prediction of lameness in dairy cows. Animal 15(11):100391.

Stevens, M., S. Piepers, K. Supré, J. Dewulf, and S. De Vliegher. 2016. Quantification of antimicrobial consumption in adult cattle on dairy herds in Flanders, Belgium, and

associations with udder health, milk quality, and production performance. J Dairy Sci 99(3):2118-2130. doi: 10.3168/jds.2015-10199

Taneja, M., J. Byabazaire, N. Jalodia, A. Davy, C. Olariu, and P. Malone. 2020. Machine learning based fog computing assisted data-driven approach for early lameness detection in dairy cattle. Computers and Electronics in Agriculture 171:105286.

USDA, A. 2018. Dairy 2014, Health and management practices on US dairy operations, 2014. USDA-APHIS-VS-CEAH-NAHMS Fort Collins (CO).

Vasquez, A. K., D. V. Nydam, C. Foditsch, M. Wieland, R. Lynch, S. Eicker, and P. D. Virkler. 2018. Use of a culture-independent on-farm algorithm to guide the use of selective dry-cow antibiotic therapy. J Dairy Sci. 101(6):5345-5361. doi: https://doi.org/10.3168/jds.2017-13807

Wall, S. K., O. Wellnitz, R. M. Bruckmaier, and D. Schwarz. 2018. Differential somatic cell count in milk before, during, and after lipopolysaccharide- and lipoteichoic-acid-induced mastitis in dairy cows. J Dairy Sci. 101(6):5362-5373. doi: https://doi.org/10.3168/jds.2017-14152

Wemette, M., A. Greiner Safi, A. K. Wolverton, W. Beauvais, M. Shapiro, P. Moroni, F. L. Welcome, and R. Ivanek. 2021. Public perceptions of antibiotic use on dairy farms in the United States. J Dairy Sci. 104(3):2807-2821. doi: https://doi.org/10.3168/jds.2019-17673

Williamson, J. R., T. R. Callaway, E. Rollin, and V. E. Ryman. 2022. Association of pre-treatment somatic cell counts with bacteriological cure following diagnosis of intramammary infection. Research in Veterinary Science 152:537-545. doi: https://doi.org/10.1016/j.rvsc.2022.09.015

Zecconi, A., F. Dell'Orco, D. Vairani, N. Rizzi, M. Cipolla, and L. Zanini. 2020. Differential somatic cell count as a marker for changes of milk composition in cows with very low somatic cell count. Animals 10(4):604.

Zecconi, A., F. Zaghen, G. Meroni, V. Sora, P. A. Martino, G. Laterza, and L. Zanini. 2023. Early milk total and differential cell counts as a diagnostic tool to improve antimicrobial therapy protocols. Animals 13(7)doi: 10.3390/ani13071143

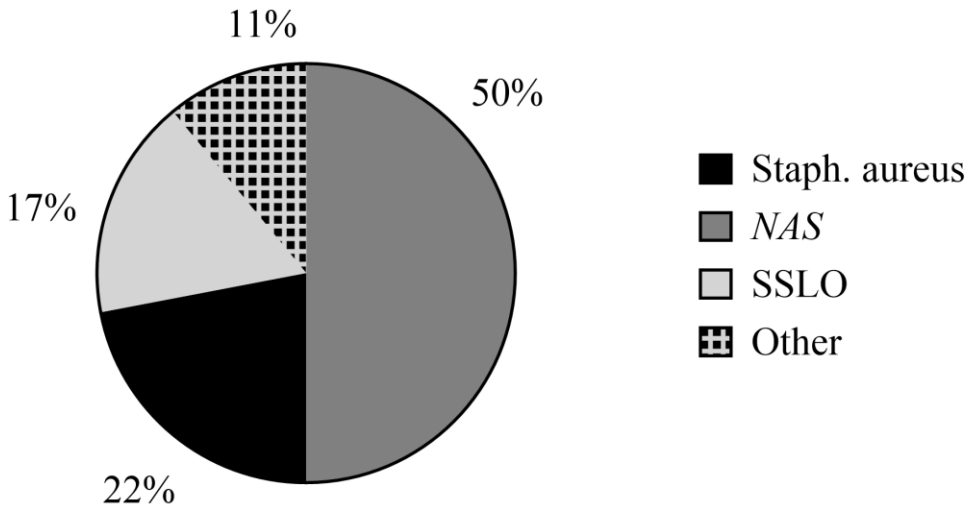


Figure 4.1. Distribution of pathogens in infected quarters 48 h prior to dry off (*N* = 46) Milk samples were collected using aseptic techniques and plated on trypticase soy agar plates with 5% sheep blood. Positive growth samples were sent to the University of Georgia Veterinary Diagnostic laboratory for identification via MALDI-TOF. Non-aureus staph. (NAS) includes *Staphylococcus chromogenes, Staphylococcus haemolyticus,* and *Staphylococcus xylosus*; Streptococci-like organisms (SSLO) includes *Lactococcus garviea*; Other includes *Nocardia africana* and unidentified Gram-positive bacteria.

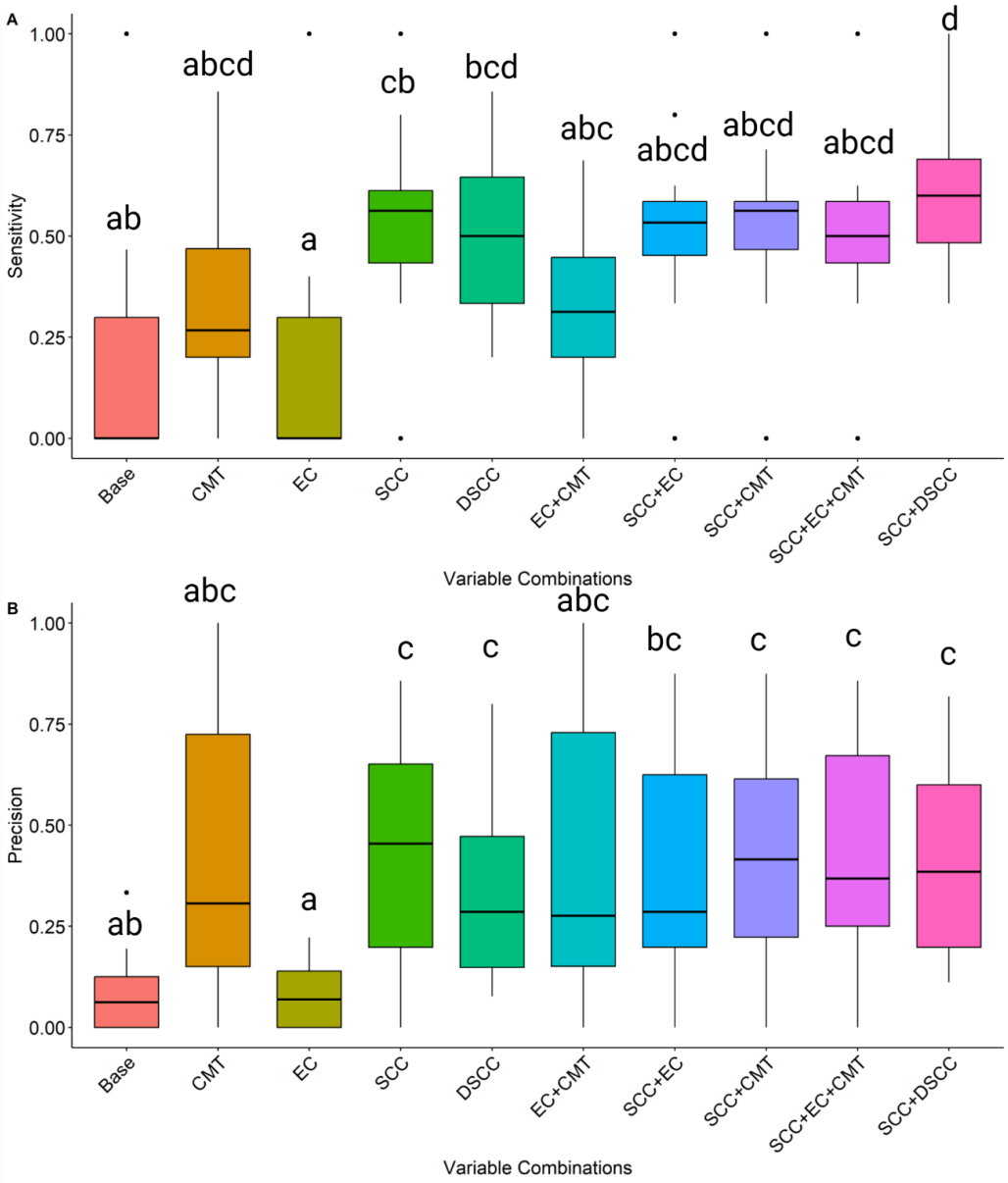


Figure 4.2. (A) Sensitivity and (B) Precision of the following 8 variable combinations for detecting mastitis at dry off at the quarter level: **Base** = (Days in milk [**DIM**], Parity, Milk Yield [**MY**], Breed); **CMT** = (California Mastitis Test [**CMT**], Base); **EC** = (Electrical conductivity [**EC**], Base); **SCC** = (Somatic cell count [**SCC**], Base); Differential SCC (**DSCC**) = (Total Leukocyte Count [**TLC**]. Neutrophils [**NEU**], Lymphocytes [**LYM**], Macrophages [**MAC**], Base); **EC+CMT** = (EC, CMT, Base); **SCC+EC** = (SCC, Cond, Base); **SCC+CMT**: (SCC, CMT, Base); **SCC+EC+CMT** = (SCC, EC, CMT, Base); **SCC+DSCC** = (SCC, DSCC, Base). Difference in letter represents significance. Significance was declared at $P \leq 0.05$.

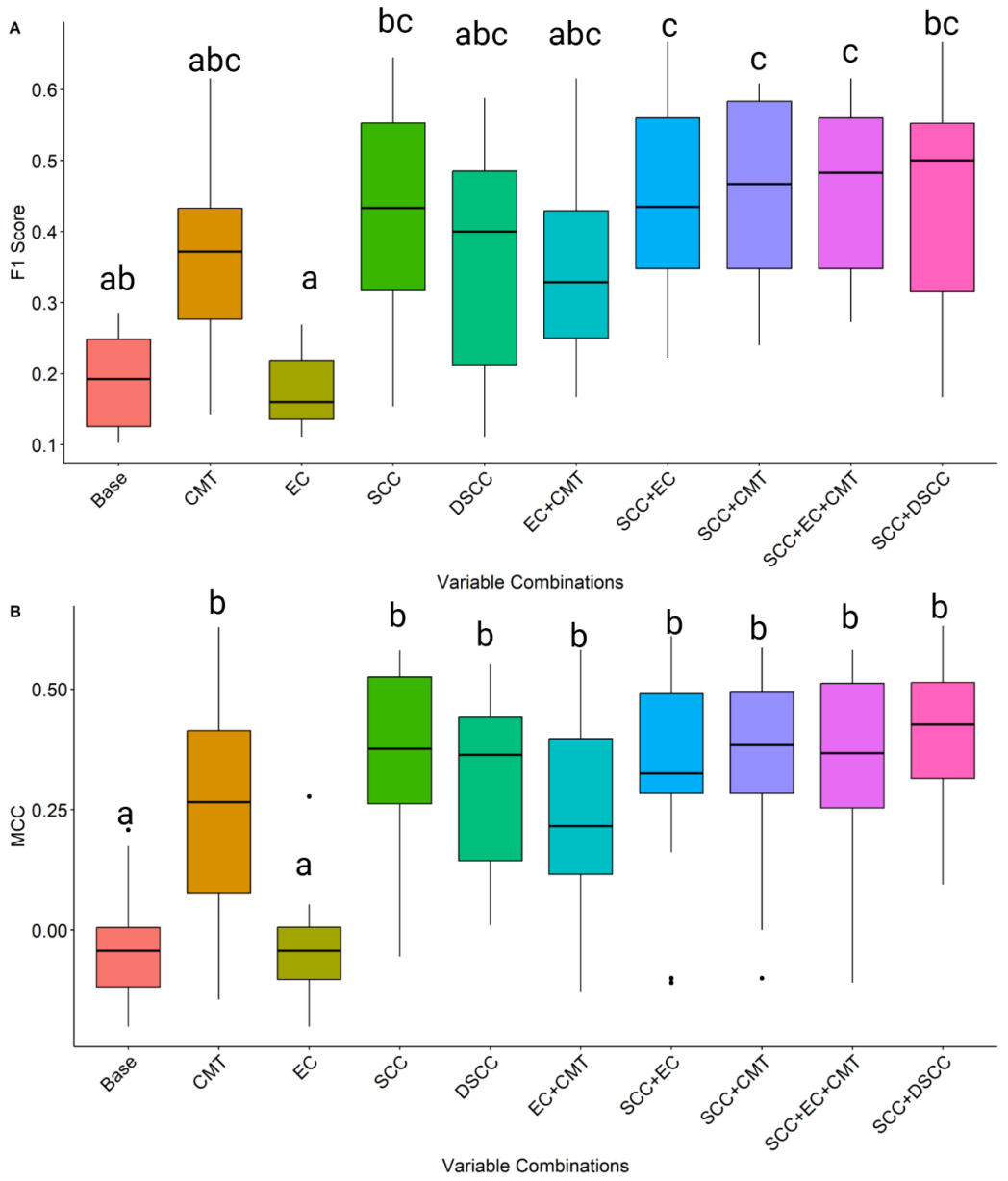


Figure 4.3. (A) F1 score and (B) Matthews correlation coefficient of the following 8 variable combinations for detecting mastitis at dry off at the quarter level: : **Base** = (Days in milk [**DIM**], Parity, Milk Yield [**MY**], Breed); **CMT** = (California Mastitis Test [**CMT**], Base); **EC** = (Electrical conductivity [**EC**], Base); **SCC** = (Somatic cell count [**SCC**], Base); Differential SCC (**DSCC**) = (Total Leukocyte Count [**TLC**]. Neutrophils [**NEU**], Lymphocytes [**LYM**], Macrophages [**MAC**], Base); **EC+CMT** = (EC, CMT, Base); **SCC+EC** = (SCC, Cond, Base); **SCC+CMT**: (SCC, CMT, Base); **SCC+EC+CMT** = (SCC, EC, CMT, Base); **SCC+DSCC** = (SCC, DSCC, Base). Difference in letter represents significance. Significance was declared at $P \leq 0.05$.

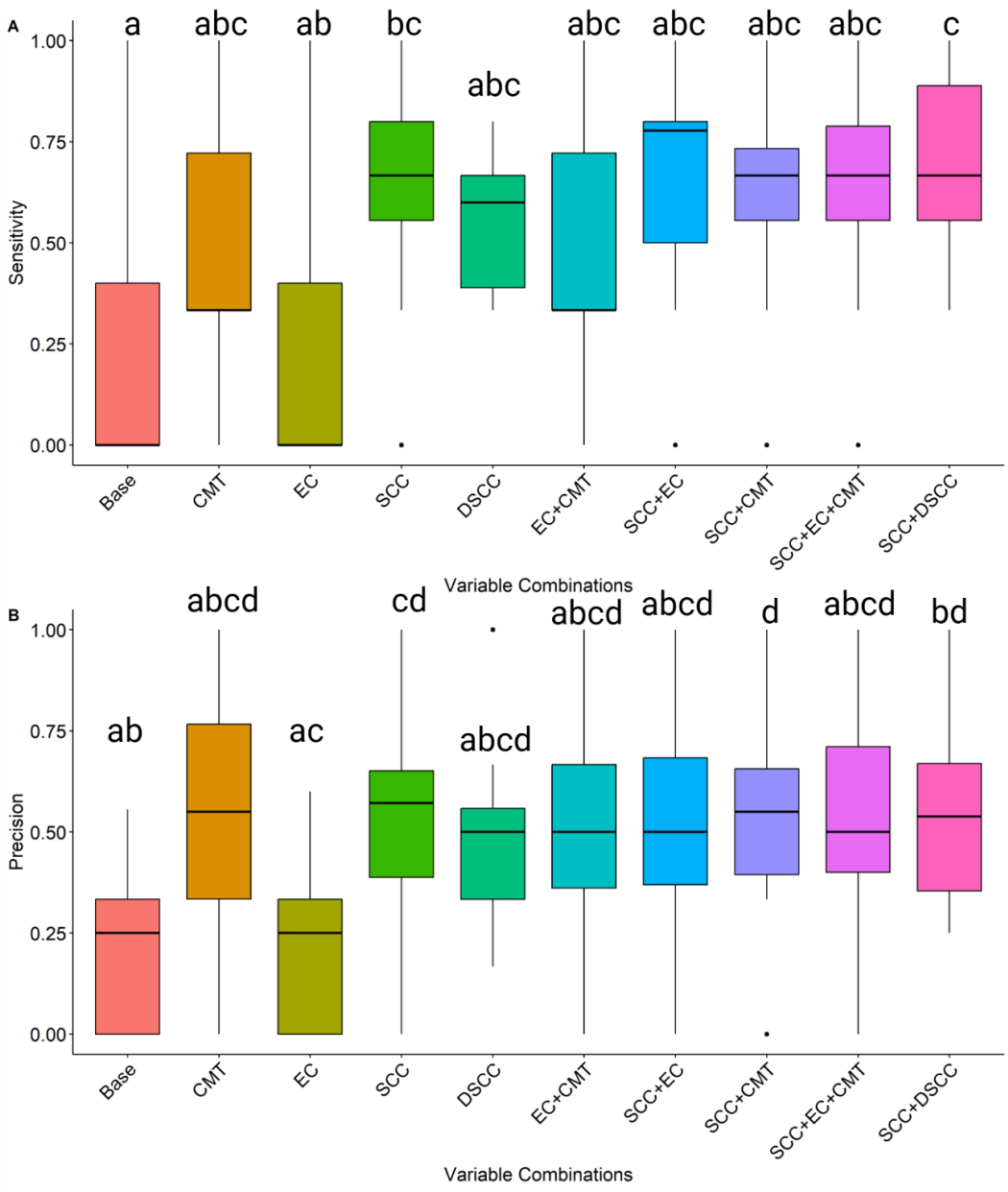


Figure 4.4. (A) Sensitivity and (B) Precision of the following 8 variable combinations for detecting mastitis at dry off at the cow level: : **Base** = (Days in milk [**DIM**], Parity, Milk Yield [**MY**], Breed); **CMT** = (California Mastitis Test [**CMT**], Base); **EC** = (Electrical conductivity [**EC**], Base); **SCC** = (Somatic cell count [**SCC**], Base); Differential SCC (**DSCC**) = (Total Leukocyte Count [**TLC**]. Neutrophils [**NEU**], Lymphocytes [**LYM**], Macrophages [**MAC**], Base); **EC+CMT** = (EC, CMT, Base); **SCC+EC** = (SCC, Cond, Base); **SCC+CMT**: (SCC, CMT, Base); **SCC+EC+CMT** = (SCC, EC, CMT, Base); **SCC+DSCC** = (SCC, DSCC, Base). Difference in letter represents significance. Significance was declared at $P \leq 0.05$.

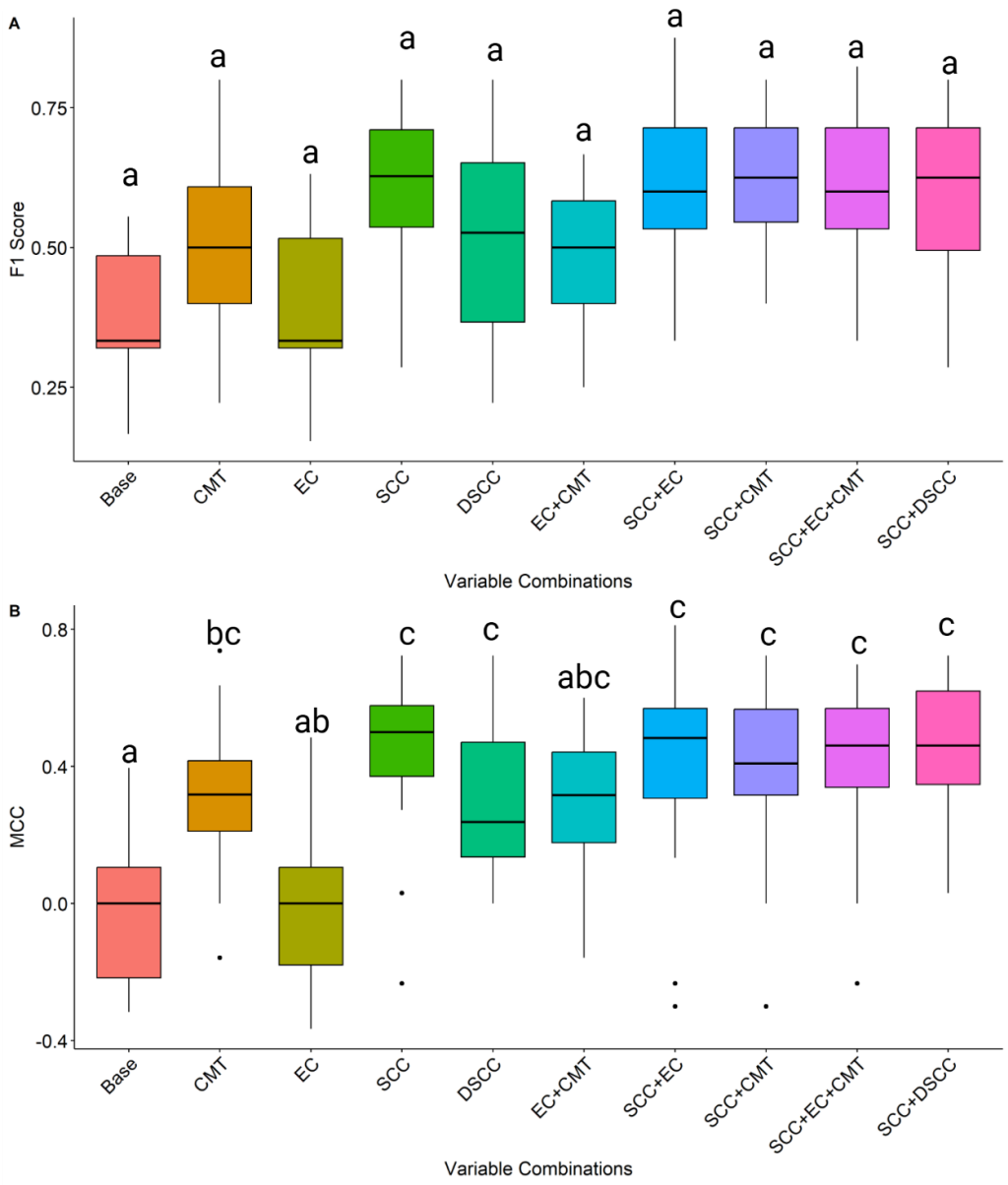
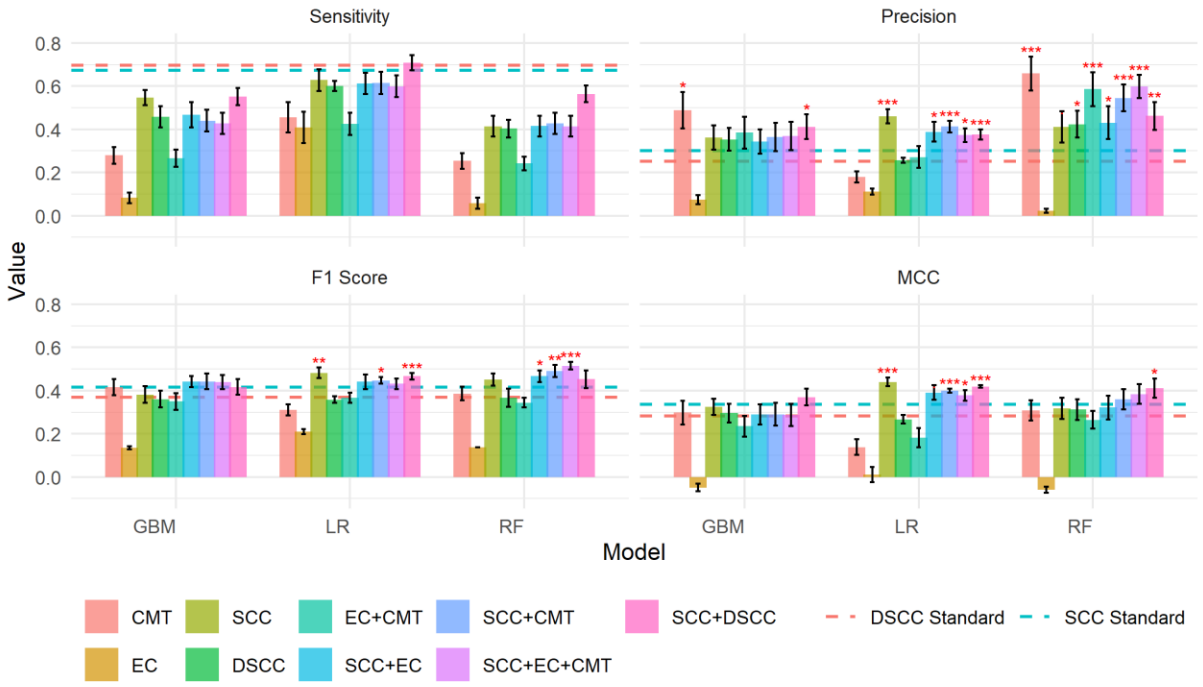
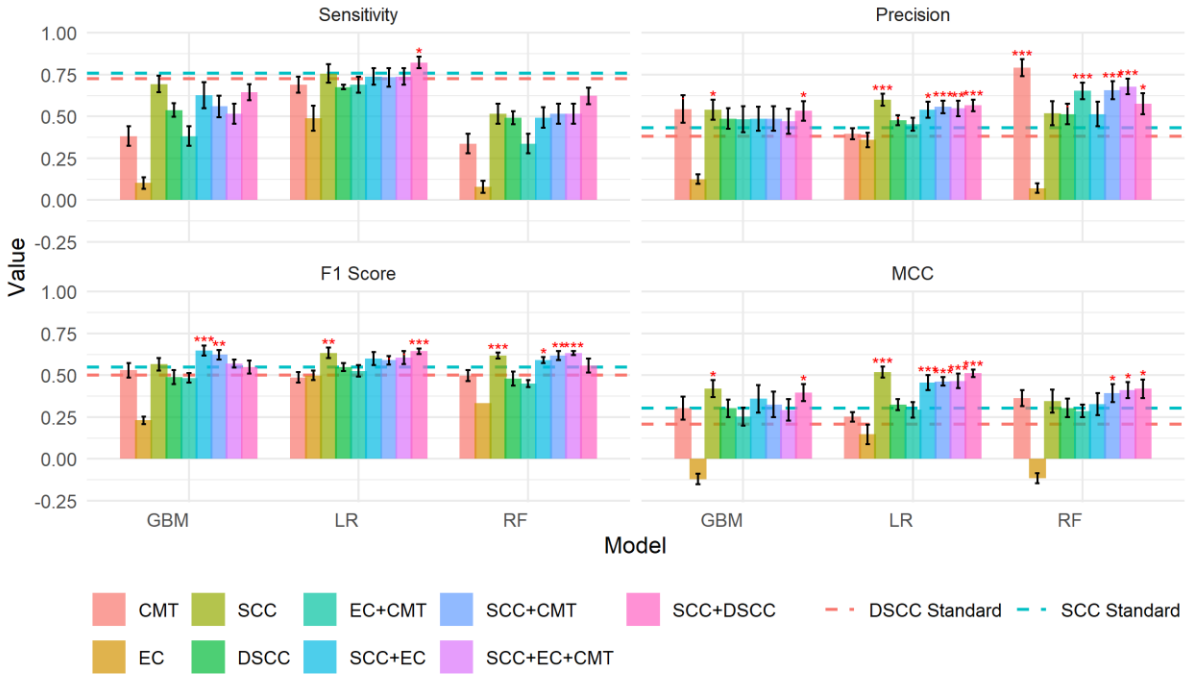


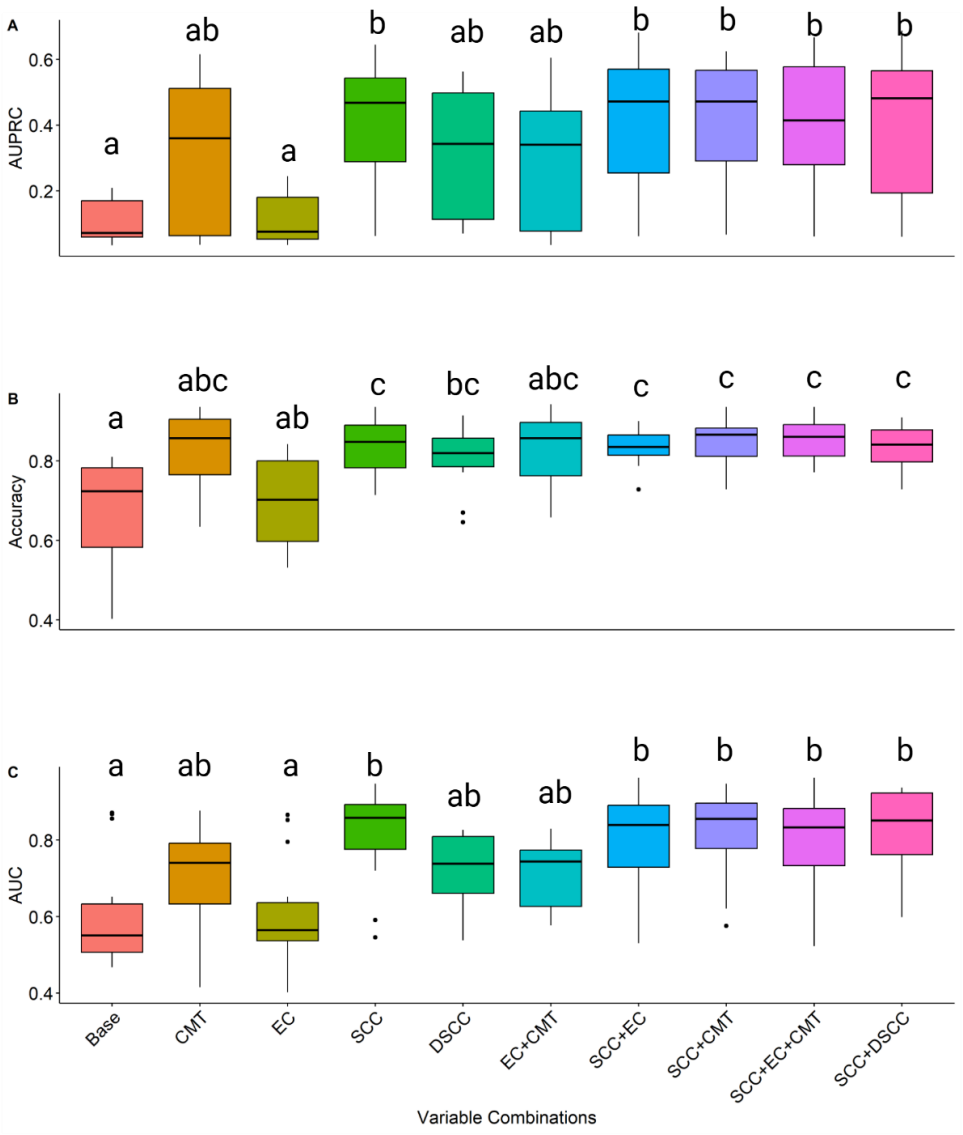
Figure 4.5. (A) F1 score and (B) Matthews correlation coefficient of the following 8 variable combinations for detecting mastitis at dry off at the cow level: : **Base** = (Days in milk [**DIM**], Parity, Milk Yield [**MY**], Breed); **CMT** = (California Mastitis Test [**CMT**], Base); **EC** = (Electrical conductivity [**EC**], Base); **SCC** = (Somatic cell count [**SCC**], Base); Differential SCC (**DSCC**) = (Total Leukocyte Count [**TLC**]. Neutrophils [**NEU**], Lymphocytes [**LYM**], Macrophages [**MAC**], Base); **EC+CMT** = (EC, CMT, Base); **SCC+EC** = (SCC, Cond, Base); **SCC+CMT**: (SCC, CMT, Base); **SCC+EC+CMT** = (SCC, EC, CMT, Base); **SCC+DSCC** = (SCC, DSCC, Base). Difference in letter represents significance. Significance was declared at $P \leq 0.05$.



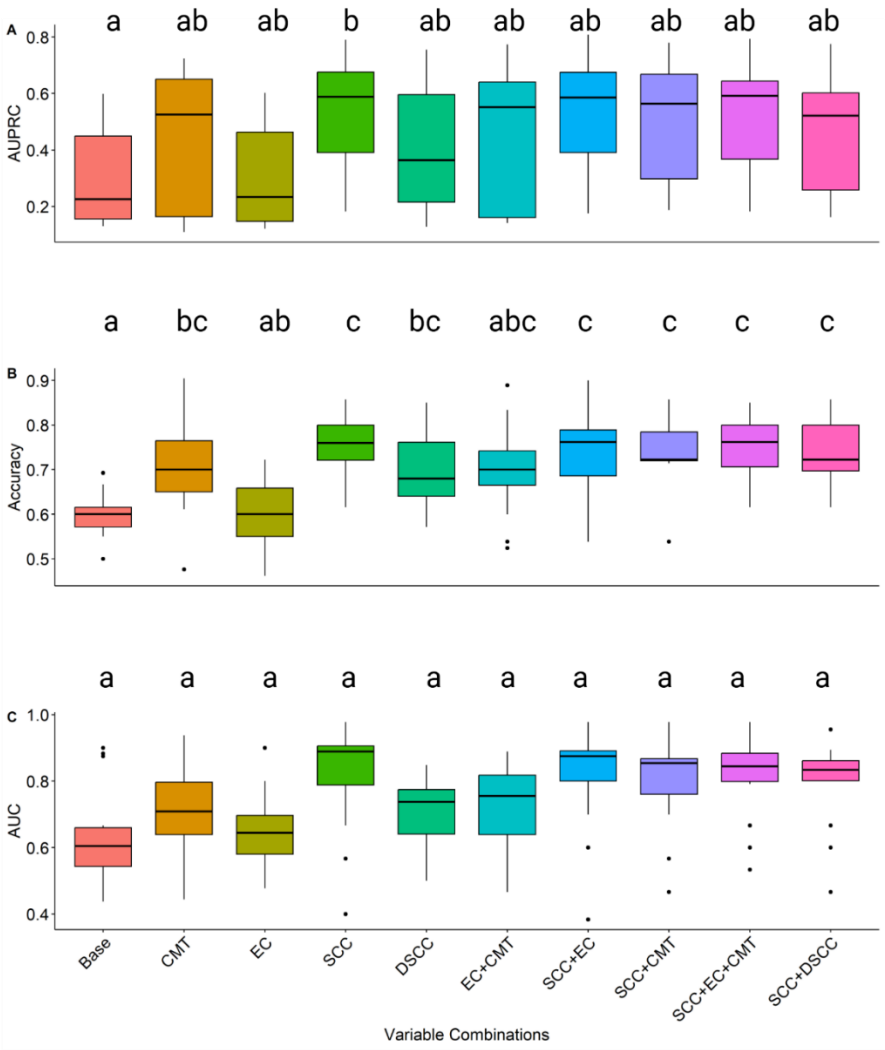
Supplemental Figure 4.1. Model classifications for each observation at the quarter level. Milk samples were taken 48hs prior to dry off and mastitis was determined at the cow level based on positive culture results. Three classification models (Logistic Regression [**LR**], Gradient Boosting Machines [**GBM**], Random Forest [**RF**]) were tested with 8 variable combinations: **Base** = (Days in milk [**DIM**], Parity, Milk Yield [**MY**], Breed); **CMT** = (California Mastitis Test [**CMT**], Base); **EC** = (Electrical conductivity [**EC**], Base); **SCC** = (Somatic cell count [**SCC**], Base); Differential SCC (**DSCC**) = (Total Leukocyte Count [**TLC**]. Neutrophils [**NEU**], Lymphocytes [**LYM**], Macrophages [**MAC**], Base); **EC+CMT** = (EC, CMT, Base); **SCC+EC** = (SCC, Cond, Base); **SCC+CMT**: (SCC, CMT, Base); **SCC+EC+CMT** = (SCC, EC, CMT, Base); **SCC+DSCC** = (SCC, DSCC, Base). Significance was declared at $P \leq 0.05$.



Supplemental Figure 4.2. Model classifications for each observation at the cow level. Milk samples were taken 48hs prior to dry off and mastitis was determined at the cow level based on positive culture results. Three classification models (Logistic Regression [**LR**], Gradient Boosting Machines [**GBM**], Random Forest [**RF**]) were tested with 8 variable combinations: **Base** = (Days in milk [**DIM**], Parity, Milk Yield [**MY**], Breed); **CMT** = (California Mastitis Test [**CMT**], Base); **EC** = (Electrical conductivity [**EC**], Base); **SCC** = (Somatic cell count [**SCC**], Base); Differential SCC (**DSCC**) = (Total Leukocyte Count [**TLC**]. Neutrophils [**NEU**], Lymphocytes [**LYM**], Macrophages [**MAC**], Base); **EC+CMT** = (EC, CMT, Base); **SCC+EC** = (SCC, Cond, Base); **SCC+CMT**: (SCC, CMT, Base); **SCC+EC+CMT** = (SCC, EC, CMT, Base); **SCC+DSCC** = (SCC, DSCC, Base). Significance was declared at $P \leq 0.05$.



Supplemental Figure 4.3. (A) AUPRC, (B) accuracy, and (C) area under the curve of the following 8 variable combinations for detecting mastitis at dry off at the quarter level: : **Base** = (Days in milk [**DIM**], Parity, Milk Yield [**MY**], Breed); **CMT** = (California Mastitis Test [**CMT**], Base); **EC** = (Electrical conductivity [**EC**], Base); **SCC** = (Somatic cell count [**SCC**], Base); Differential SCC (**DSCC**) = (Total Leukocyte Count [**TLC**]. Neutrophils [**NEU**], Lymphocytes [**LYM**], Macrophages [**MAC**], Base); **EC+CMT** = (EC, CMT, Base); **SCC+EC** = (SCC, Cond, Base); **SCC+CMT**: (SCC, CMT, Base); **SCC+EC+CMT** = (SCC, EC, CMT, Base); **SCC+DSCC** = (SCC, DSCC, Base). Difference in letter represents significance. Significance was declared at $P \leq 0.05$.



Supplemental Figure 4.4. (A) AUPRC, (B) accuracy, and (C) area under the curve of the following 8 variable combinations for detecting mastitis at dry off at the cow level: : **Base** = (Days in milk [**DIM**], Parity, Milk Yield [**MY**], Breed); **CMT** = (California Mastitis Test [**CMT**], Base); **EC** = (Electrical conductivity [**EC**], Base); **SCC** = (Somatic cell count [**SCC**], Base); Differential SCC (**DSCC**) = (Total Leukocyte Count [**TLC**]. Neutrophils [**NEU**], Lymphocytes [**LYM**], Macrophages [**MAC**], Base); **EC+CMT** = (EC, CMT, Base); **SCC+EC** = (SCC, Cond, Base); **SCC+CMT**: (SCC, CMT, Base); **SCC+EC+CMT** = (SCC, EC, CMT, Base); **SCC+DSCC** = (SCC, DSCC, Base). Difference in letter represents significance. Significance was declared at $P \leq 0.05$.

Table 4.1. Uninfected and infected quarter milk profiles 48h prior to dry-off

	Quarters			
Item	Uninfected	Infected[1]	SEM	*P*-value
CMT[2]	0.66	0.54	0.042	0.05
EC[3]	4.9	4.2	0.18	0.05
SCC[4] (×1000 cells/mL)	141.7	413.2	42.83	< 0.01
TLC[5] (cells/mL)	265.4	663.1	88.29	> 0.99
NEU[6] (%)	50.04	52.88	0.894	0.05
MAC[7] (%)	31.36	27.38	1.166	0.04
LYM[8] (%)	18.60	19.74	0.650	> 0.99

[1]Infected quarter determined from positive cultured milk.

[2]California Mastitis Test; score scale was adjusted for negative = 0, Trace = 1, 1 = 2, 2 = 3, 3 = 4.

[3]Electrical conductivity.

[4]Somatic cell count.

[5]Total leukocyte count.

[6]Neutrophils.

[7]Macrophages.

[8]Lymphocytes.

Table 4.2. Test characteristics at the quarter and cow level for detecting IMI[1] in late-lactation cows [estimate (95% confidence limit)]

	Sensitivity	Specificity	PPV	NPV	*P*- Value
Quarter-level					
CMT[2]	64.4 (49.8, 76.7)	53.4 (48.1, 58.7)	15.8 (11.2, 21.7)	91.7 (87.0, 94.9)	0.03
EC[3]	38.6 (25.7, 53.3)	54.7 (49.2, 60.0)	10.1 (6.4, 15.6)	87.1 (81.9, 91.0)	0.42
SCC[4] (x 1000 cells/mL)	28.4 (20.1, 37.8)	38.1 (73.3, 82.2)	28.4 (20.1, 37.8)	38.1 (73.3, 82.2)	0.18
NEU[5] (%)	56.8 (42.2, 70.3)	56.1 (50.8, 20.1)	14.6 (10.1, 20.6)	90.8 (86.0, 94.0)	0.11
MAC[6] (%)	52.2 (37.9, 66.2)	54.4 (49.0, 59.6)	13.1 (8.9, 19.0)	89.6 (84.6, 93.1)	0.42
Cow-level[7]					
CMT	82.8 (65.5, 92.4)	28.4 (23.5, 44.8)	33.3 (23.5, 44.9)	79.2 (59.5, 90.8)	0.31
EC	69.0 (50.8, 83.7)	41.8 (30.7, 53.7)	33.9 (23.1, 46.6)	75.7 (59.9, 86.6)	0.37
SCC (x 1000 cells/mL)	79.3 (61.6, 90.1)	56.7 (44.9, 67.9)	44.2 (31.6, 57.6)	86.4 (73.2, 93.6)	0.01
NEU (%)	79.3 (61.6, 90.1)	32.8 (22.8, 44.7)	33.8 (23.7, 45.7)	78.6 (60.5, 89.8)	0.33
MAC (%)	82.8 (65.5, 92.4)	38. (28.1, 50.8)	36.9 (26.2, 49.1)	83.9 (67.4, 92.9)	0.06

[1]Infected cows are determined from positive cultured milk.
[2]California Mastitis Test (**CMT**); score scale was adjusted for negative = 0, Trace = 1, 1 = 2, 2 = 3, 3 = 4.
[3]Electrical conductivity.
[4]Somatic cell count.
[5]Neutrophils.
[6]Macrophages.
[7]Lymphocytes.
[8]Cows were determined positive for a metric if ≥ 1 quarter was positive based on metric definition

CHAPTER 5

CONCLUSIONS

Thought the present studies we sought to evaluate if differential somatic cell count (DSCC), in particular if neutrophils, could improve antibiotic cure rates for subclinical mastitis. The current data supports those quarters with lower neutrophil percentages are more likely to cure a subclinical intramammary infection (IMI). In addition to neutrophils, similar results were seen with somatic cell count (SCC), total leukocyte count (TLC), and an inverse relationship was seen with macrophage percentages. When examining the development of thresholds for SCC, TLC, neutrophils, and macrophages, we suggested different thresholds-based on the goals of the producers. Across the board TLC had the greatest model performance, followed by SCC, neutrophils, then macrophages. At the present time, factors that need to be accounted for in models utilizing DSCC is limited, future research should be focused on understanding psychological and environmental factors to account for in order to improve threshold performance for DSCC.

When evaluating the performance of DSCC in cows entering the dry cow period, the present data suggest that DSCC was not a reliable diagnostic measure to detect IMI at dry off at the quarter or cow level. When evaluating the use of machine learning models in predicting infected quarters, the use of DSCC did not differ from SCC. In addition, the combination of the two parameters did not improve the model's ability to predict mastitis. The lack of differences seen with DSCC could be attributed to a very low incidence rate

of mastitis which could affect the models ability to perform Although we did see that training a model on cow level criteria performed the overall performance of al the models. There is still a lot to be learned about DSCC as well as the implementation of machine learning algorithms into mastitis detection.

In conclusion, the utilization of DSCC in predicting cure rates could be a promising addition to mammary health, although further research with larger sample sizes will be needed to confirm these findings.