Management and technology solutions for improving milk quality

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MANAGEMENT AND TECHNOLOGY SOLUTIONS
FOR IMPROVING MILK QUALITY

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the Department of Animal and Food Sciences at the University of Kentucky

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2013

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ABSTRACT OF THESIS

MANAGEMENT AND TECHNOLOGY SOLUTIONS FOR IMPROVING MILK QUALITY

Mastitis is one of the most common and expensive dairy cattle diseases. Mastitis prevention and management are key factors in herd health and improved milk quality. One objective of this research was to evaluate management solutions to maintain a low somatic cell count, based on survey responses from Kentucky dairy producers. Because hyperkeratosis may increase mastitis incidence, another objective of this research was to examine changes in teat end hyperkeratosis in a herd transitioning from a standard pulsation milking system to an individual quarter pulsation milking system. The last objective of this research was to evaluate technologies that monitored ruminating time, neck activity, reticulorumen temperature, and milk yield as potential mastitis detection devices.

KEYWORDS: Mastitis, Somatic Cell Count, Dairy Management, Individual Quarter Pulsation, Precision Dairy Farming

Amanda Sterrett

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FREQUENTLY USED ABBREVIATIONS

CNS = Coagulase negative Staphylococci
HK = Teat end hyperkeratosis
SCC = Somatic cell count
SCS = Somatic cell score
NA = Neck activity
RT = Reticulorumen temperature
RU = Rumination time
MY = Milk yield
h = Hour
d = Day
kg = Kilogram
mL = Milliliter
µL = Microliter
CHAPTER ONE
Review of Literature

INTRODUCTION

Mastitis is an inflammatory reaction of udder tissue, usually caused by a bacterial infection in the mammary gland (Harmon, 1994, Oliver and Murinda, 2012, Sordillo et al., 1997). This disease alters udder secretory processes, lowers milk yield, changes milk composition (Beck et al., 1992, Harmon, 1994), and can be fatal. Mastitis destroys some to all secretory cells and epithelial tissue (Beck et al., 1992, Harmon and Heald, 1982). Additionally, mastitis may compromise animal welfare because of the resulting, yet often overlooked and underestimated, discomfort and pain (Fitzpatrick et al., 2013, Medrano-Galarza et al., 2012).

Mastitis is an important topic in the dairy industry, partly because milk cannot be sold from cows treated with antibiotics, which often occurs with mastitis infections. Milk from treated cows is usually discarded or fed to calves (Blosser, 1979, USDA, 2007). In 2006, 30.6% of producers surveyed by the National Animal Health Monitoring System fed calves unpasteurized waste milk and 2.8% fed pasteurized waste milk (USDA, 2007). In addition to milk losses from antibiotics, milk fat (Beck et al., 1992, Harmon, 1994) and milk yield decrease, which reduces revenue (Beck et al., 1992).

Mastitis is a concern even for well-managed herds throughout the world (Schukken et al., 2008). Dairy industry personnel generally accept that economic losses from mastitis are sizable (Beck et al., 1992, Blosser, 1979, Hogeveen et al., 2011) and are a limiting factor for profitability (Oliver and Murinda, 2012). Optimizing economic
results is important for farm survival (Hansson et al., 2011). Bar et al. (2008) claimed mastitis was “undoubtedly the disease with the highest economic importance” to the dairy industry. Economic losses from subclinical and clinical mastitis include: decreased milk production, treatment costs, increased labor, discarded milk, veterinary fees, reduced milk price because of high somatic cell count (SCC), increased risk of subsequent mastitis, increased risk of culling or death (Hansson et al., 2011, Nielen et al., 1992, Nielen et al., 1995b), increased risk of other diseases, and preventive care costs.

Decreased milk yield accounts for the largest economic loss (Bar et al., 2008, Blosser, 1979). In a New York study, the average clinical mastitis case cost totaled $71 per cow and year and $179 per case, but this result was admittedly an underestimate because fertility costs were excluded from the model. Milk loss accounted for $115 of the cost of a clinical case, followed by $50 for treatment, and $14 for increased mortality (Bar et al., 2008). Another study that modeled the cost of a mastitis case, including reproductive losses, reported a mean of $163.28 ± $18.16 per case using 1,000 simulation iterations. In this model, the cost of a mastitis case was sensitive to milk price, replacement price, unrealized milk, and feed costs for parity one cows and milk price, unrealized milk, feed costs, and replacement costs for parity ≥ 2 cows (Bewley, 2010b).

Total elimination of mastitis within a herd would increase the net return by €146 per cow per year (Østergaard et al., 2005). Milk price and cost of clinical mastitis are positively related. Bar et al. (2008) reported the cost per case of clinical mastitis was 18% higher when milk price was 20% higher and 17% lower with a 20% decrease in milk price. Unfortunately, producers often undervalue the cost of mastitis, likely because the losses are spread over the year and are not directly visible (Hogeveen et al., 2011).
Making sound economic decisions, particularly related to disease, allows for financial benefit to producers (Bar et al., 2008, Bewley, 2010a). Reducing mastitis incidence would be advantageous (Hansson et al., 2011). The economic benefits of preventive measures are determined by the implementation cost and the value in reducing mastitis incidence. Not all preventive measures are economical or provide the same benefits (Hansson et al., 2011, Hogeveen et al., 2011).

Major mastitis pathogens include *Staphylococcus aureus* (*Staph aureus*), Streptococci species, *Escherichia coli* (*E. coli*), and Klebsiella species (Hamann and Zecconi, 1998). Major pathogens cause the largest economic loss by producing substantial increases in SCC and other milk composition changes (Harmon, 1994). Minor pathogens include coagulase-negative staphylococcus (CNS) and *Corynebacterium bovis* (Hamann and Zecconi, 1998) and are rarely associated with clinical mastitis or extreme decreases in milk yield (Harmon, 1994).

Contagious pathogens, including *Staph aureus* and *Streptococcus agalactiae*, spread between cows during milking. Mastitis cases caused by contagious pathogens are usually subclinical and chronic (Harmon, 1994). Environmental bacteria include coliforms (Hogan and Smith, 2003, Smith et al., 1985b), or gram-negative pathogens that are present in most cow environments (Hogan and Smith, 2012). Seventy to 85% of intramammary infections caused by coliforms become clinical (Harmon, 1994, Hogan and Smith, 2012). *Escherichia coli* and *Klebsiella* species commonly inhabit the intestinal tract of warm-blooded animals (Hogan and Smith, 2012). Environmental pathogens are present in bedding, manure, and soil (Harmon, 1994, Hogan and Smith, 2003, Sant’Anna and Paranhos da Costa, 2011, Zdanowicz et al., 2004) and are always
present in the environment of dairy cows. Environmental streptococcal infections cause clinical mastitis 50% of the time they infect an animal (Hogan and Smith, 2012).

Coagulase-negative staphylococcus prevalence has varied between studies (Djabri et al., 2002). Coagulase-negative staphylococci accounted for 45% of infected quarters in one study (Fernando et al., 1985). In a University of Kentucky study comparing the prevalence of intramammary infection caused by Staphylococcus species in primiparous and multiparous cows in the periparturient period, quarter prevalence of CNS species pre-partum, at parturition, and weeks 1 to 5 in primiparous cows was 38.9, 27.8, 15.3, 14.6, 13.2, 15.3, and 14.6%, respectively. In multiparous cows, prevalence was 50.3, 12.3, 6.2, 8.1, 10.7, 7.1, and 8.1%, respectively (Matthews et al., 1992). Belgian researchers reported 6% CNS prevalence on a cohort of ten clinically healthy cows from each of six study herds (Piessens et al., 2011).

Although generally classified as an environmental pathogen, Streptococcus uberis (Strep uberis) can also behave like a contagious pathogen (Leigh, 1999, Neave et al., 1969, Zadoks et al., 2001). However, research complete understanding, of non-agalactiae streptococci pathogens acting as contagious pathogens is lacking. A Dutch study examined an outbreak of Strep uberis and reasoned that the transmission was contagious (Zadoks et al., 2001). Researchers came to this conclusion because infection prevalence within the herd was a significant predictor for the number of new intramammary infections and because of the decrease in predicted number of new IMI during periods that the producer employed post-dipping. Post-dipping reduces mastitis incidence by killing bacteria transmitted during the milking process, not the environmental pathogens transmitted by the lying surface of the cows. Interestingly, susceptibility of quarters that
recovered from a Strep uberis infection is higher than susceptibility of quarters that had never experienced a Strep uberis infection. This result implied that the immune response to the pathogen’s initial infiltration provides little protection from future infections (Zadoks et al., 2001).

Fernando et al. (1985) cited that cows infected with any one pathogen had mastitis in a mean of two quarters. Yet cows with an infection caused by two pathogens were infected in a mean of 2.8 quarters. Uninfected quarters within a cow increase in yield during a mastitis case, reducing the 30% reduction in yield from the infected quarter to 20% (Beck et al., 1992).

Today’s non-automated clinical mastitis detection approach involves observing inflammation through visual milk inspection and/or udder palpation. Producers can also monitor declines in milk yield because they can indicate a health problem (Leslie and Petersson-Wolfe, 2012, Lukas et al., 2009). However, these changes are not immediate, making “aggressive and early intervention” difficult. This type of intervention may set a cow up to produce more milk and remain healthier throughout her lactation (Aalseth, 2005).

Early diagnosis of clinical mastitis may reduce production losses and enhance prospects of recovery (Milner et al., 1996), making it very important to the dairy industry (Viguier et al., 2009). Proactive action may also decrease antibiotic use, which may decrease the chance of antibiotic residue in the bulk tank (Oliver and Murinda, 2012). In herds with a low frequency of clinical mastitis incidence, herd management and cow immunity is not fully understood (Erskine et al., 1988). While many mastitis management techniques exist, the labor and capital needed for these preventive measures
compete with other possible farm improvements (Hogeveen et al., 2011), making the decision on whether to implement suggested improvements difficult for producers. Unfortunately, subclinical mastitis detection is even more difficult for producers to detect in the absence of clinical signs.

**MASTITIS**

*Clinical Mastitis*

Producers or their employees often detect clinical mastitis during milking (Hogeveen et al., 2010, Nielen et al., 1995b). A sign of clinical mastitis is visually abnormal milk (clots and flakes from one or more quarters), which can only be detected when a cow is being milked (Beck et al., 1992, Harmon, 1994, Kelton et al., 1998). Abnormal milk may be accompanied by inflammation (signs include heat, swelling, or discoloration of the skin) (Kelton et al., 1998, Viguier et al., 2009) which can be detected by udder palpation (Beck et al., 1992). Intramammary infections can become severe and affect the cow systemically inducing anorexia, fever, dehydration, and diarrhea (Hogan and Smith, 2003).

In 1968, the average farm had 69 cows and 1.9 hired workers (Buffington and Reaves, 1968). As farm size increases, producers rely more on hired employees for daily activities (Russell and Bewley, 2011), increasing the variability in mastitis detection precision and chances for human error. Automated mastitis detection would reduce human detection error. In an automated milking system, the farmer may not be present during the milking process, making automated detection of clinical mastitis even more important (Mollenhorst et al., 2012).
In a Canadian study, mean clinical mastitis incidence rate was 23 cases per 100 cow-years (Olde Riekerink et al., 2008). Green et al. (2004) cited a clinical mastitis incidence rate ranging from 16.1% to 23.3%. Clinical mastitis incidence rate was also reported at 0.36 clinical mastitis cases per 365 cow-days at risk in another study (Kamphuis et al., 2010).

Erskine et al. (1988) cited that clinical mastitis incidence caused by coliforms was significantly higher in herds with a bulk tank SCC \( \leq 150,000 \) cells/mL versus herds with a bulk tank SCC \( > 700,000 \) cells/mL. Clinical mastitis incidence rate caused by \textit{E. coli} was higher in low SCC herds than medium or high \( (> 250,000 \) cells/mL) SCC herds. However, bulk tank SCC was not associated with the overall incidence rate of clinical mastitis. In contrast, Dutch researchers concluded that the mean clinical mastitis incidence rate was not different between low \( (\leq 150,000 \) cells/mL), medium \( (150,000 \) to \( 200,000 \) cells/mL), and high \( (250,000 \) to \( 400,000 \) cells/mL) bulk tank SCC groups \( (0.278, 0.257, \) and \( 0.252 \) cases per 365 cow-days at risk in herds, respectively) (Barkema et al., 1998b). Clinical mastitis incidence rate was positively associated with higher SCC in the previous month and the previous lactation (Steeneveld et al., 2008).

The increase in infection by environmental pathogens may be immunity-related; Cows exposed to pathogens and the resulting SCC increase may provide protection from future infections (Green et al., 1996). Still, a lower rate of contagious pathogens may be the cause. In a Dutch study, herds in the high and medium bulk tank SCC categories \( (250,000 \) to \( 400,000 \) and \( 150,000 \) to \( 250,000 \) cells/mL for high and medium, respectively) had a higher clinical mastitis incidence rate caused by \textit{Staph aureus} than did herds in the low bulk tank SCC category \( (< 150,000 \) cells/mL) (Barkema et al., 1999). In a Michigan
study, clinical mastitis incidence caused by *Streptococcus agalactiae* and *Staph aureus* was significantly higher in herds with a SCC > 700,000 cells/mL than herds with a SCC ≤ 150,000 cells/mL. In herds with a SCC > 700,000 cells/mL, clinical mastitis caused by *Streptococcus agalactiae* and *Staph aureus* attributed to 60% of total infections. *Streptococcus agalactiae* was never isolated from a clinical mastitis case and *Staph aureus* was rarely isolated in herds with a SCC ≤ 150,000 cells/mL (Erskine et al., 1988). Another study cited a lower clinical mastitis incidence rate caused by *Streptococcus agalactiae, Streptococcus dysgalactiae,* or *Staph aureus* in low SCC herds (≤ 150,000 cells/mL) than in medium to high SCC herds (150,000 to 400,000 cells/mL) (Barkema et al., 1998b). In contrast, Olde Riekerink et al. (2008) observed a higher clinical mastitis incidence rate caused by *Staph aureus* in low (< 150,000 cells/mL) SCC herds than medium (150,000 to 250,000 cells/mL) SCC herds.

Clinical mastitis incidence caused by coliforms in the herds with a SCC ≤ 150,000 cells/mL and by total percentage of cows with clinical mastitis caused by *Streptococcus agalactiae* was highest in July and August (Erskine et al., 1988). In contrast, Olde Riekerink et al. (2007) reported the highest clinical mastitis incidence in December. This difference could be a result of total confinement during winter months, but having pasture access in summer months. In the same study, a small peak in July was also observed, related to increased *Staph aureus* and *E. coli* clinical mastitis rates, particularly in high SCC herds.

*Escherichia coli* thrives in temperatures ranging from 19.3 to 44.5°C and *Klebsiella pneumonia* thrives in temperatures ranging from 22.7 to 41.0°C (Raghubeer and Matches, 1990). Because these environmental bacteria can survive in hot ambient
temperatures, but not in colder temperatures, it seems logical that more environmental pathogens would be present during the seasons with greater temperatures. An Ohio study examining intramammary infection rate from environmental pathogens explained that coliform numbers in bedding were lowest in winter and highest in summer in all three housing areas studied (freestalls, tiestalls, and maternity barns). Summer coliform counts in the freestalls were significantly greater (P < 0.05) than during all other seasons. The increased pathogen load in the bedding, and therefore at the teat ends of the cows lying on the bedding, during the summer was also associated with an increased rate of coliform intramammary infection during the summer (Smith et al., 1985a).

Clinical mastitis incidence, particularly caused by coliforms, streptococci other than Streptococcus agalactiae, non-isolated bacteria, and CNS, in herds with a SCC ≤ 150,000 cells/mL was higher (> 16%) during the first month of lactation (Erskine et al., 1988). Olde Riekerink et al. (2007) cited the highest incidence rate of clinical mastitis in the first 14 DIM. The beginning of lactation is when cows are immunosuppressed and exposed to new pathogens.

**Somatic Cell Count**

Mastitis management is most clearly related to SCC (Harmon, 1994, Nielen et al., 1992, Norman et al., 2011), which is a general indicator of udder health (Dong et al., 2012, Norman et al., 2011, Reneau, 1986) and milk quality (Wenz et al., 2007). However, no association between clinical mastitis incidence rate and bulk tank SCC was observed in a study by Olde Riekerink et al. (2008).

An uninfected udder usually maintains a composite, or the pooled milk from all four quarters of a cow, SCC of < 100,000 cells/mL (Dong et al., 2012) or < 200,000
cells/mL if foremilk is absent of clinical signs (Mein and Rasmussen, 2008). The odds of increased total bacteria count in the bulk tank increased by 2.4% for every 10,000 cells/mL increase in SCC (Pantoja et al., 2009). For every unit increase in the natural log SCC, yield decreased by 135 kg in the first lactation and 270 kg for all subsequent lactations. Loss was even greater for low SCC herds (Raubertas and Shook, 1982). Even after bacterial cures, high SCC continues until the udder heals (Harmon, 1994).

Somatic cells consist of leukocytes (white blood cells), including macrophages, lymphocytes, and polymorphonuclear neutrophil leukocytes (Harmon, 1994, Norman et al., 2011, Sordillo et al., 1997). The purpose of white blood cells is to combat infections, thus they increase upon bacterial invasion of the udder (Norman et al., 2011). Polymorphonuclear neutrophil leukocytes were present in mammary tissue of cows inoculated with *Staph aureus*, in contrast to their absence in tissue from healthy cows (Harmon and Heald, 1982). Figure 1 depicts the process of mastitis development in an infected udder. In this representation, neutrophils and somatic cells are shown engulfing bacteria in response to an invasion through teat cistern.

Intramammary infections can be eliminated if somatic cells move from the bloodstream to the udder quickly. If bacteria are eliminated quickly, SCC will return to normal levels speedily (Sordillo et al., 1997). However, scar tissue may form from damage to the epithelial cells, blocking the ducts and sometimes causing permanent loss of function of an infected quarter (Harmon, 1994, Viguier et al., 2009).

New Zealand researchers conducted a *Staph aureus* challenge, where bacterial isolates are infused into the mammary glands of the study animals to induce mastitis. In this study, infected quarters sustained a SCC nine times higher than uninfected quarters in
healthy cows. Infected quarters also maintained a SCC six times higher than that of uninfected quarters of cows where *Staph aureus* was isolated from another quarter of the same cow (Hillerton and Walton, 1991). *Staphylococcus aureus* cell walls interfere with the neutrophils’ ability to phagocytize the bacteria and can damage host tissues to promote bacterial growth (Sordillo et al., 1997), making this pathogen difficult to combat.

Unfortunately, producers often do not understand the importance of bulk tank SCC (Eberhart et al., 1982, Penry, 2011). High SCC indicates infection in the herd (Lievaart et al., 2009, Reneau, 1986) with a correlation coefficient of 0.77 between SCC and herd infection rate (Eberhart et al., 1982). Finding the sources of infection, resolving them, and preventing them in the future is imperative to minimize mastitis within a herd. Monitoring SCC enables a producer to examine the effectiveness of treatment and prevention strategies and provides evidence of chronic mastitis, which may affect treatment decisions. Nonetheless, cows should not be treated based on SCC data alone, but rather, high SCC cows should be cultured to determine the cause and evaluate treatment options (Reneau, 1986). Herds where treatment was administered based on SCC had lower milk yield and a lower percentage of cows in the low SCC group (SCC $\geq$283,000 cells/mL; $P < 0.05$) compared to cows that were not treated for high SCC or were cultured to determine the cause of the high SCC (Smith and Schmidt, 1987).

In recent years, producers have had more motivation to maintain low SCC because of consumer and processor demand (Dong et al., 2012). As SCC increases, shelf life decreases, making it an important concern for milk processors (Oliver and Murinda, 2012, Schukken et al., 2008). In 1993, the Food and Drug Administration implemented a reduction in the United States’ standard SCC from 1,000,000 cells/mL to 750,000
cells/mL (FDA, 1993) as recommended by the National Conference on Interstate Milk Shipments suggested a decrease in (FDA, 1991). Even with this reduction, the United States maintains higher regulatory cutoffs than other developed nations, posing a problem for exporting milk (Norman et al., 2011). To “remain competitive in the global market and maintain a positive image,” the United States dairy industry must work to lower bulk tank SCC (Wenz et al., 2007).

Premiums are paid for low SCC and have been effective in motivating producers to reduce SCC in the United States thus far (Norman et al., 2011), but may not be enough incentive to reduce it further. Schukken et al. (1992) explained that the Canadian penalty system, implemented in 1989, where $1/hectoliter was deducted from a producer’s milk check if the bulk tank SCC > 800,000 cells/mL and annual penalty limits would decrease by 50,000 cells/mL to 500,000 cells/mL in 1995. This motivated producers to decrease bulk tank SCC (P < 0.01).

Dairy Herd Improvement Association (DHIA) testing programs have helped motivate producers to decrease their herds’ SCC by giving them more information on their herds’ mastitis statuses. DHIA tests composite milk from each cow in the herd for SCC regularly in participating farms (Reneau, 1986). Because subclinical mastitis determination is based on SCC, detection is dependent on monthly SCC records. These records allow producers to see the mastitis patterns in their herds and, when coupled with a physical examination and medical history, can ensure that decisions made for individual animals are beneficial to the animal and the herd. Treatment outcomes can also be evaluated because reduced SCC often indicates bacteriologic cure (Rhoda and Pantoja, 2012). However, some limitations exist in DHIA testing. Because SCC data is only
recorded monthly, it is not a good indication of environmental mastitis problems in a herd, which are short-lived (Hogan and Smith, 2012). Mean test-day SCC may be higher than bulk tank SCC because producers may exclude milk from high SCC cows from the bulk tank to avoid regulatory issues and penalties (Lievaart et al., 2009, Norman et al., 2011). Also, basing management decisions on one test is inadequate because herds with high subclinical mastitis incidence usually have greater variation in monthly SCC than herds with low mastitis incidence (Reneau, 1986).

Herds enrolled in DHIA had lower bulk tank SCC than those not enrolled (Norman et al., 2011). In a Canadian survey study, herds with lower than average SCC for the year were enrolled in DHIA or other similar associations significantly more often than herds with SCC greater than average (Barnum and Meek, 1982). Managers who “had adequate time or made an effort to allocate more time” to analyzing their herd management records also maintained higher production within their herds (Smith and Schmidt, 1987).

Quarter samples for each individual cow are more accurate for determining infection status than a composite milk sample (Reneau, 1986). If a cow has a mild infection in one quarter, the elevated SCC from that quarter may be diluted by the healthy quarters in a composite sample. Likewise, in a herd with a low bulk tank SCC, a single cow with a period of extremely high SCC could cause an increase in the bulk tank SCC, even though the rest of the herd remains low (Nielen et al., 1995a). Identifying and preventing high SCC milk from entering the bulk tank could prevent sudden bulk tank SCC increases (Nielen et al., 1995a) and the associated financial penalties.
Variation exists in the literature for an accurate general SCC threshold to determine subclinical mastitis status. Uninfected quarters are generally below 200,000 cells/mL (Harmon, 1994, Schukken et al., 2008) while SCC in uninfected quarters in first parity cows is often below 100,000 cells/mL (Harmon, 1994). A composite udder sample threshold to determine subclinical mastitis status used by most DHIA processors is 200,000 cells/mL (Rhoda and Pantoja, 2012). A higher SCC may be an indication of more infected quarters (Harmon, 1994). In a field study, mean SCC in uninfected cows remained below 500,000 cells/mL while infected cells rose above 2,000,000 cells/mL (Fernando and Spahr, 1983).

Although SCC information is a useful tool for detecting subclinical mastitis within a herd, DHIA and laboratory results cost time and money to a commercial producer. In-line SCC systems do exist, but they are expensive, not yet sufficient (Díaz et al., 2012). An evaluation of 238 samples using an in-line SCC sensor correctly classified 95, 85, 76, 72, and 95% of samples in < 200,000, 200,000 to 500,000, 500,000 to 1,500,000, 1500,000 to 5,000,000, and > 5,000,000 cells/mL, respectively (Whyte et al., 2004). In addition, the systems are not available commercially in the United States. Examples of in-line SCC systems include Mastiline (Isogen Animal Care, The Netherlands), Herd Navigator™ (DeLaval, Sweden), and CellSense™ (Promar International, Cheshire).

**Bacteriologic Culturing**

Somatic cell count data does not provide a mastitis diagnosis, but can be especially useful when combined with bacteriologic culturing. Culturing milk from cows with mastitis is an effective diagnostic tool and an important step for understanding
mastitis-causing bacteria prevalence within a herd for both mastitis prevention and treatment.

Bacteriological cure, or cure of the intramammary infection, rate is often lower than clinical cure, or the cessation of clinical signs, rate. Clinical cure rate is pathogen-dependent and is rarely 100% (Milner et al., 1997). Roberson et al. (2004) examined clinical and bacteriological cures of clinical mastitis cases treated with intramammary amoxicillin and cited a 57% clinical cure rate and 67% bacteriological cure rate. Untreated clinical mastitis cases maintained cure rates of 67% and 55% for clinical and bacteriological cures, respectively. Clinical mastitis cases treated with frequent milk out resulted in clinical cure rates of 25% and bacteriological cure rates of 45%.

Nonetheless, bacteriological culture results can guide treatment decisions and may improve treatment outcomes because producers are better able to target treatments for the specific causative agent. A study of 9,007 subclinical mastitis cases in New York and Pennsylvania showed that overall bacteriological cure rate was 68%. Antibiotic treated cases had a greater bacteriological cure rate (75%) compared to untreated cases (65%) (Wilson et al., 1999). On-farm bacteriologic culturing systems provide results within 24 hours (Neeser, 2006), which is fast enough to wait on results for a treatment decision. But, likely more importantly, historical culture data provides information that producers can use to optimize the effectiveness of future mastitis treatments (Lam et al., 2009).

**MASTITIS PREVENTION**

**Teat End Hyperkeratosis**

The teat end is the first line of defense against bacteria (Gleeson et al., 2004, Sordillo et al., 1997) and is the route bacteria take from the environment into the udder.
The teat canal is lined with keratin, a wax-like material made from stratified squamous epithelium (Sordillo et al., 1997, Viguier et al., 2009). Teat canals contain cationic protein that can bind to and alter the cell wall of bacteria (Sordillo et al., 1997), providing protection from bacterial invasion of the udder.

The maintenance of healthy teat skin and teat ends is a key component of an effective mastitis prevention program (Mein, 2012, Mein et al., 2001). After repeated milkings, a callous ring around the teat orifice may develop, termed hyperkeratosis (HK) (Neijenhuis et al., 2001b). Hyperkeratosis refers to a histological response to chronic stimulation and is marked by an increase in the thickness of the stratum corneum, or the keratin layer of the teat end (Breen et al., 2009).

Overmilking occurs when the milk flow to the teat cistern is less than the flow out of the teat canal and can cause or exacerbate teat end HK. Rasmussen (2004) explained that breeding for high milk yield has produced cows with a higher percentage of cisternal capacity within the udder, reducing udder emptying functionality. Mechanical forces exerted by vacuum and the moving liner during machine milking impact teat end HK incidence (Neijenhuis et al., 2000, Neijenhuis et al., 2001b). Rasmussen (1999) proposed that the last 0.5 minutes of milking was the most sensitive period for developing HK because the teats were almost empty. Other factors that affect teat end HK include teat end shape (Gleeson et al., 2004, Neijenhuis et al., 2000), genetic predisposition (Gleeson et al., 2004), seasonal weather conditions (Mein et al., 2001), and long unit-on times (Zucali et al., 2008).

Teats with unit-on times < 4.30 minutes were 0.29 times less likely to develop HK scores of R (rough ring) or VR (very rough ring) than teats with unit-on times > 5.30
min \((P < 0.01)\), citing more liner collapses as a possible cause of high HK scores. In the same study, teats starting with a HK classification of N (no ring) were 0.38 times less likely to be scored R and VR than teats starting with a HK classification of S (smooth raised ring; \(P < 0.01\)). Conversely, teats that started with a HK classification of R or VR were 24.2 times more likely to end with a classification of R or VR than teats starting the experiment with a classification of S. The study results demonstrated the difficult recovery from severe HK (Zucali et al., 2008).

Dairy advisors associate more severe HK with increased clinical mastitis incidence (Neijenhuis et al., 2001a). In an 8-herd study, the odds of contracting *Streptococcus uberis* or *E. coli* clinical mastitis increased significantly with increased HK (Breen et al., 2009). O’Shea (1987) proposed that changes to teat end condition resulting from overmilking might increase the likelihood of bacterial penetration into the udder. Natzke et al. (1978) determined that more new infections occurred in cows when milking units were set at a fixed removal time of 12 minutes (40, 27, and 15 new infections for 3 experimental repetitions) compared to cows with milking units removed at the end of milk flow (19, 20, and 12 new infections for 3 experimental repetitions). Natzke et al. (1978) concluded that overmilking may have contributed to higher mastitis infection rates, likely due to teat end condition, but the differences in infection rates between the two groups were not significant \((P > 0.05)\). The reverse pressure gradient created when the vacuum in the teat cistern is higher than beneath the teat end may allow for bacterial invasion of the teat cistern, making overmilking a culprit for increased bacterial colonization at the teat end (Rasmussen, 1999, Rasmussen, 2004). Each increase of one teat end HK score increased the chances of intramammary infection by
30% in a study conducted by de Pinho Manzi et al. (2012). Hyperkeratosis scores were positively correlated with bacterial counts of the environmental pathogens *Streptococcus uberis*, *E. coli*, and other coliforms (*r* > 0.50, *P* < 0.01), but not with *Staph aureus* (Paduch et al., 2012). Rough teat ends may provide a site for bacterial colonization because they may be more difficult to clean during pre-milking preparation (Zucali et al., 2008). However, Shearn and Hillerton (1996) failed to identify a significant relationship between SCC and degree of HK at the herd level.

Individual quarter pulsation milking systems may prevent overmilking and improve teat end condition (Neijenhuis et al., 2000). Automated milking systems often incorporate individual quarter pulsation, but research on this feature in a conventional parlor is lacking. Automated milking systems have not been heavily adopted in the US because of the availability of inexpensive labor relative to other countries (Jacobs and Siegford, 2012).

**Cow Hygiene**

Mastitis incidence can be reduced by practicing preventive measures including: frequent stall cleaning, proper milking procedures, post-milking teat dipping, regularly scheduled milking equipment checks, dry cow therapy, and strict culling practices (Hansson et al., 2011). Mastitis management is mainly directed at reducing exposure of teats to bacteria (Beck et al., 1992, Hogan and Smith, 2012) because exposure to bacteria in manure can increase mastitis rates (Mein, 2012, Schreiner and Ruegg, 2003). Consequently, keeping cows clean and using a proper milking routine is the primary mastitis prevention strategy (Shook and Schutz, 1994). Managing consistently clean
cows requires intensive labor, but will benefit milk quality and work efficiency (Sant’Anna and Paranhos da Costa, 2011).

Cows with dirty udders require more time and effort to clean during the pre-milking routine. Teats of cows with dirty udders, therefore, may not be cleaned as well as already-clean animals if the extra time and effort is not taken (Sant’Anna and Paranhos da Costa, 2011). Cows with constantly dirty udders put them at a greater risk of mastitis.

Facility hygiene is an important focus of mastitis management because cows spend much of their day lying down and dirty lying areas can potentially cause dirty udders. In a Dutch study, cows spent the majority (54%) of their time lying down (Ipema et al., 2008). When animals lie down, they often seek manure-laden and wet places (Sant’Anna and Paranhos da Costa, 2011). In contrast, cows spent 1.1 hours/day less lying down on wet stalls than on dry stalls. Lying in wet areas, specifically during warm weather, could be a conductive cooling method, although no evidence has been shown on this theory (Reich et al., 2010).

Barkema et al. (1998a) cited that more attention was paid to hygiene in low SCC herds than in herds with medium to high SCC. Environmental factors are important for cow hygiene (Sant’Anna and Paranhos da Costa, 2011), but are difficult to control because environmental pathogens exist in all dairy cattle housing systems in manure, soil, and bedding (Zdanowicz et al., 2004). Sant’Anna and Paranhos da Costa (2011) observed higher percentages of very clean and clean cows in August (winter in Brazil) than in other months, at 32.15% and 52.96%, respectively. This study reported more very dirty and dirty cows in January (summer in Brazil) than in other months, at 23.39% and 30.33%, respectively. Rainfall was higher in the summer months and had negative

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effects on cow hygiene. When examined independently, cleanliness scores for each body area studied (leg, flank, abdomen, and udder) had significant effects on SCC (Sant’Anna and Paranhos da Costa, 2011). For each increase of 1 standard deviation in udder hygiene, leg hygiene, and the combination of udder and leg hygiene, linear somatic cell score increased by 0.13, 0.17, and 0.17.

Zdanowicz et al. (2004) were unable to find a clear relationship between udder cleanliness and bacterial counts on teat ends. However, a positive correlation ($r = 0.31; P < 0.01$) existed between coliform counts and sand bedding moisture content (Zdanowicz et al., 2004). Klebsiella counts were between 0.8 and 1 log units higher on teat ends of cows housed on sawdust versus sand, which may be a result of higher moisture and availability of nutrients. Conversely, teat ends of cows housed on sand had $\geq 1$ log unit more streptococci compared to cows housed on sawdust (Zdanowicz et al., 2004).

To maintain clean cows, facilities must also be clean. Bedding and stall management and manure handling impact SCC (Reneau, 1986) and bacterial counts (Hogan and Smith, 2012). Total confinement operations have been associated with an increased incidence rate of infections caused by $E. coli$ (Schukken, 1991). In another study, confined cows had 1.8 times more clinical mastitis and 8 times the culling rate for mastitis than pastured cows (Washburn et al., 2002). This may be because environmental bacteria (such as $E. coli$ and the environmental streptococci) are often present in organic bedding sources like wood shavings or straw (Hogan and Smith, 2003). Additionally, animals do not have alternative lying locations in total confinement operations, forcing them to lie in manure-laden areas. Manure also is more concentrated in a smaller space.
compared to a pasture where cows have the ability to defecate over a larger area and while walking, reducing the amount of manure in any one spot of the pasture.

In a study by Pomiès et al. (2000), turning cows out on pasture did not significantly increase SCC. Pastured herds displayed lower clinical mastitis rates compared with cows housed in confinement systems (Goldberg et al., 1992, Washburn et al., 2002), particularly clinical mastitis caused by E. coli when pastured at night (Barkema et al., 1999). Because mud, moisture, and manure are the primary sources of environmental pathogen exposure in the cow (Hogan and Smith, 2003, Reneau, 1986, Schreiner and Ruegg, 2003), ambient weather and maintenance of pastures likely affect SCC more than the act of pasturing animals itself. Sant’Anna and Paranhos da Costa (2011) cited more dirty cows on pasture during January to March when rainfall was increased, indicating that mud had a negative effect on cow hygiene. Hogan and Smith (2012) explained that mastitis outbreaks caused by coliform bacteria are common in rainy seasons when cows are exposed to dirt lots, but that pasturing animals generally reduces pathogen exposure.

**Milking Procedures**

While ensuring that udders are clean before entering the parlor makes pre-milking cleaning easier, employing a consistent and effective pre-milking routine to clean teats and udders is still an important mastitis prevention strategy. A pre-milking routine consists of components designed to improve milk quality, milk letdown, mammary health, and milking time efficiency (Watters et al., 2012). A written milking routine consists of milking procedures, listed in sequential order, that should be followed at each milking (Galton et al., 1988). Hispanic workers comprised a mean of 49% of all
employees in a Michigan survey, with one study herd employing 88% Hispanic (Mugera, 2008). Therefore, it is important to write and explain milking procedures in a manner understood by all employees.

Effective pre-milking udder preparations decrease the number of bacteria on teats and in milk (Galton et al., 1988, Galton et al., 1986), which can consequently decrease the incidence of intramammary infections (Galton et al., 1988). In a Canadian study, SCC in herds that used post milking teat dip were 70,300 cells/mL lower than herds that did not teat dip (Moxley et al., 1978). Teat-dipping and forestripping can be sequenced either way (Galton et al., 1988). Forestripping may increase mastitis detection because milk can be inspected for signs of clinical mastitis (Schukken et al., 2008).

After pre-dipping and forestripping the teats, drying the teats is key to an effective milking routine. Without adequate cleaning and drying, wetting the udder increases bacterial populations (Galton et al., 1986). In a New York study, the number of new intramammary infections was 66.3% less when pre-dip and drying was used compared to no pre-milking preparation (Galton et al., 1988). Manual teat drying was actually more important than the type of pre-dip used (Galton et al., 1986).

Single use paper towels have proven useful in an effective mastitis management program, resulting in a 43,000 cells/mL lower SCC per month compared to herds using multi-use towels (Barnum and Meek, 1982). Because producers can use cloth towels multiple times, keeping them clean is imperative to not spreading bacteria onto teats. Proper washing and drying technique can ensure cleanliness. Environmental pathogens were eliminated from washing towels using cold or hot wash water, drying or not drying after washing, and using bleach in wash. A Washington study recovered more pathogens
from cloth towels washed in cold water without bleach or hot air drying \((P < 0.05)\) than from other treatment combinations. Cold water without bleach or hot air drying was also the only treatment where \textit{Staph aureus} was recovered from the towels (Fox, 1997).

Post-dipping with a teat disinfectant after milking the last important mastitis prevention measure before a cow leaves the parlor (Norman et al., 2011). Post-dipping kills bacteria transmitted to teats during milking and kills environmental bacteria on the teat at the time of post-dipping, even though the teat will be exposed to these bacteria between milkings (Olde Riekerink et al., 2012). In a Washington study, post-dipping was associated with a lower herd prevalence of intramammary infection caused by coagulase-positive staphylococci (Hutton et al., 1991). Teat dipping can also effectively decrease the prevalence of other gram-positive intramammary infections (Brooks and Barnum, 1984a, b). Post-dipping reduces gram-negative bacteria on teat ends, but the effectiveness of the dip is short-lived (Hogan and Smith, 2003). In contrast, post-dipping can be a risk factor for clinical mastitis, especially in low SCC herds (Barkema et al., 1999, Elbers et al., 1998). Because post-dip may reduce the prevalence of minor pathogens, Rainard and Poutrel (1988) explained that the increased risk of infection may be because minor pathogens infecting the udder. Minor pathogens, particularly \textit{Corynebacterium bovis}, have an antagonistic effect on major pathogens and restrict them from infecting the mammary gland.

\textit{Coliform Vaccination}

Although proper milking procedures and good cow hygiene may prevent environmental mastitis, it is impossible to eliminate all risk of environmental mastitis infection. Vaccination with a gram-negative core antigen J5 vaccine may reduce the
duration and severity of infections caused by *E. coli* (Hogan and Smith, 2003). All cows possess innate immunity, or a nonspecific response that is the first line of defense in the early infection stages (Sordillo et al., 1997). Unlike innate responses, acquired, or specific, immune responses recognize pathogenic factors and eliminate them selectively. Specific immune responses are mediated by antibody molecules, macrophages, and lymphoid cells, which maintain a “memory” of the pathogens that they have been exposed to previously (Sordillo et al., 1997). Vaccinations expose animals to a unique and specific antigen so they develop specific antibody titers that can fight that infection in the future (Dosogne et al., 2002, Sordillo et al., 1997). Mastitis vaccines may reduce the frequency and severity of clinical mastitis (Sordillo et al., 1997) caused by coliforms. A New York study concluded that the incidence rate of clinical mastitis was estimated at 39.7 cases per 100 cow years for a herd using a J5 vaccine (Bar et al., 2008). In a study using dynamic simulation, clinical mastitis incidence without J5 vaccination was estimated at 42 cases per 100 cow years (Østergaard et al., 2005). Wenz et al. (2007) reported that herds not using a coliform mastitis vaccine were 1.65 times more likely to have a higher bulk tank SCC than those using one. In contrast, Wilson et al. (2007) observed 221 naturally occurring clinical mastitis cases, where J5-vaccinates (46.2%) were actually more likely to contract clinical mastitis overall, including from *E. coli*, than non-vaccinated control cows (34.3%; *P* = 0.03). A year later, Wilson et al. (2008) cited no difference between J5-vaccinates and control cows for all pathogens isolated from the 204 clinical mastitis cases cultured, including coliforms (*P* = 0.23).

Control of coliform mastitis is important because systemic illness, milk loss, and reproductive losses may occur because of coliform infections. Wilson et al. (2008) cited
that cows were significantly less likely to become pregnant if they had clinical mastitis caused by *E. coli* (42% pregnant) or *Streptococcus spp.* (38% pregnant) compared to 78% conception in cows with no mastitis.

Cows vaccinated with J5 maintained a lower mean daily milk loss from mastitis than for controls by 7.6 kg (P = 0.03). Another study explained that control cows with clinical mastitis caused by *Klebsiella* incurred a greater hazard of culling for mastitis than for J5 vaccinates (P = 0.001. Additionally, systemic illness or severe quarter swelling also occurred more often in coliform-infected controls than in coliform-infected J5 vaccinates (Wilson et al., 2007).

**Dry Cow Therapy**

Dry cow therapy may be one of the most important mastitis prevention methods (Barkema et al., 1998a, Norman et al., 2011, Rodrigues and Ruegg, 2005) and is recommended by most dairy advisors (Oliver and Murinda, 2012). Antibiotics from dry cow therapy may prevent infections in the early dry period, may enable regeneration of damaged udder tissue (Schmidt, 1969), and may cure existing infections at dry off (Oliver and Murinda, 2012). This prevention may be even more important in low SCC herds because new infections in these herds are likely to be caused by environmental bacteria (Bradley and Green, 2001). In a Canadian study, producers who dipped teats, used blanket dry cow therapy, and individual towels had a bulk tank SCC of 150,000 cells/mL less and 86.5 liters/cow more per month than producers not using these practices (Barnum and Meek, 1982). Ceftiofur hydrochloride as dry cow antibiotic therapy was associated with a lower incidence of clinical mastitis during the first 30 and 60 days post-partum, compared with penicillin dihydrostreptomycin (odds ratio = 0.38 and 0.27,
respectively). Ceftiofur hydrochloride also resulted in a lower incidence of subclinical mastitis during the first 30 and 60 days after calving compared with penicillin dihydrostreptomycin (odds ratio = 0.51 and 0.52, respectively) (Pinedo et al., 2012).

Selective dry cow treatment, or treating only the most susceptible cows in the herd, is difficult because producers are unable to identify cows in advance that would benefit from the therapy (Beck et al., 1992). Dutch researchers concluded that variation in costs were lowest with blanket dry cow therapy, followed by selective dry cow therapy, and no dry cow therapy, however, little variation occurred between the three groups. Treatment strategy is therefore dependent on the risk attitude of the producer and on the farm management conditions. Producers willing to take more risks may choose selective dry cow therapy to incur lower input costs, but risk higher costs overall (Huijps and Hogeveen, 2007). A Dutch study calculated a meta-analysis relative risk of new mastitis using selective, blanket, and no dry cow treatment based on 33 studies and observed that selective dry cow treatment protected cows from new intramammary infections better than no dry cow treatment. Selective dry cow treatment did not protect cows from new intramammary infection as well as blanket dry cow treatment (Halasa et al., 2009). However, when the selection was based on the whole cow instead of specific quarters (i.e. if milk from one quarter was high before dry off, only that quarter would be dry cow treated), no difference existed between selective dry cow treatment and blanket dry cow treatment.

**Other Mastitis Prevention Methods**

Regular milking machine maintenance is one of the most important parts of controlling mastitis (Rodrigues and Ruegg, 2005) and is a part of a good management
system (Beck et al., 1992). Milking machine maintenance by the producer includes evaluating vacuum pumps, cleaning regulator, receiver jars and weigh jars, and traps, and replacing milk hoses, receiver jar gasket, and all pulsator rubber parts, hoses, and air tubes. Maintenance conducted by trained service professional includes evaluating the vacuum pump, system leakage, regulatory performance, vacuum level and vacuum gauge, pulsator performance, air vents, condition of rubber and plastic parts, cleanliness, clean in place system function, and stray voltage (Jones, 2010).

Barkema et al. (1998a) associated clipping the udder hair of all cows every year with a lower herd SCC. Dirt and manure can adhere to udder hair, allowing bacteria close access to the teats. Removal of udder hair provides less area on the udder for bacteria to reside.

**Genetic Mastitis Resistance**

Genetic mastitis resistance is desirable, although it would not replace good management strategies. The potential exists to improve mastitis prevention with Predicted Transmitting Ability for Somatic Cell Score (PTASCS) as sire selection criteria for bulls chosen for artificial insemination. Somatic cell counts are converted using a base 2 logarithm to determine somatic cell score (SCS) by DHIA Dairy Records Processing Centers. Each unit increase in SCS equals a doubling of SCC. Genetic evaluations can use the SCS average over a cow’s lactation, which roughly characterizes the udder health. A low lactation average SCS indicates a cow has had few, if any, infections throughout her lactation (Shook and Schutz, 1994). Lineages of cows that are more susceptible to mastitis will be associated with a higher SCS (Nash et al., 2003, Shook and Schutz, 1994).
In a Pennsylvania study, herds that did not administer dry cow therapy before first calving had 36.9 times higher odds of a daughter having an intramammary infection at first parturition for just one unit increase in sire PTASCS. Daughters of sires that transmitted a higher SCS also had a higher proportion of quarters infected per cow at first parturition from intramammary infection caused by environmental organisms and CNS. The effect of selection for lower PTASCS on the incidence of intramammary infection at first parturition from other organisms was not examined because intramammary infection caused by these organisms were not prevalent (Nash et al., 2003).

Improved milk yield is important for genetic improvement, but disease is economically more important than some conformation traits commonly selected for (Shook, 1989). Genetic improvement of milk yield is proportional to genetic increase in mastitis incidence (Shook and Schutz, 1994). Increased milk yield will increase income by selling more milk. However, the cost of the increased mastitis incidence associated with the increased milk yield will cost the producer 5 to 10% of the gained income from the increased sale of milk.

Because genetic correlation between clinical mastitis and SCS is 60% to 80%, genetic selection of low SCS should minimize incidence of clinical mastitis and can do so rapidly (Shook and Schutz, 1994). Selection for lower PTASCS may reduce intramammary infection at first parturition, particularly from environmental organisms and CNS. Daughters of sires that transmitted a high SCS had a higher incidence of intramammary infection at first parturition. And daughters of sires with the lowest SCS transmittance had the lowest proportion of quarters infected at first parturition (Nash et al., 2003). Nash et al. (2000) cited that daughters of sires that transmitted the least
PTASCS maintained less incidence of clinical mastitis and the fewest clinical episodes during first and second lactations compared to daughters of sires that transmitted greater PTASCS. Genetic selection of SCS will not eliminate mastitis or the need for good sanitation and proper milking practices. Rather, it is another tool for mastitis prevention (Shook and Schutz, 1994).

**Nutrition**

Proper nutrition may be essential to prevent and control mastitis because deficiencies of any micronutrient can decrease immunity (Sordillo et al., 1997). Micronutrient feed supplements may help manage SCC by increasing a cow’s immune response to bacterial infections. Micronutrient supplementation has traditionally occurred with inorganic sources, or sources not arising from natural growth or relation to living things. Although organic, or naturally arising from living matter, forms may be more biologically available (Scaletti and Harmon, 2012). An inorganic selenium source is selenite and an organic selenium source is selenized yeast.

Scaletti and Harmon (2012) associated higher serum selenium concentrations with lower bulk tank SCC. This relationship is likely due to selenium’s positive effect on multiple measures of immune function (Gerloff, 1992). The antioxidant enzyme glutathione peroxidase needs selenium to function, making selenium important for immune function. Selenium should be supplemented into a ration because much of the U.S. soil is deficient in selenium (Heinrichs et al., 2009, Sordillo et al., 1997). However, the legal dairy cattle diet selenium supplementation limit in the U.S. is 0.3 mg / kg dry matter (The Merck Veterinary Manual, 2011).
Vitamin E supplementation during the dry period may have a positive effect on the udder health of early lactation cows (Bouwstra et al., 2010). Nevertheless, optimal mastitis prevention is achieved when both selenium and vitamin E are supplemented in the dry cow diet. Selenium and vitamin E boosts phagocytic activity (Heinrichs et al., 2009). The NRC (2001) cites vitamin E supplementation requirements at 80 IU/kg of dry matter and 15 to 20 IU/kg of dry matter for dry and milking cows, respectively. However, greater amounts of vitamin E supplementation than is recommended may have adverse effects on cows, which have also been shown in humans (Bouwstra et al., 2010).

Copper is essential to the enzyme superoxide dismutase and is present in the serum protein ceruloplasmin, an acute phase protein in cattle. Both proteins are important to immune function because they protect cells from oxidative products released by phagocytosis (Sordillo et al., 1997). In an *E. coli* intramammary challenge, Scaletti et al. (2003) reported that copper-supplemented cows had lower mean counts of *E. coli* in the milk at 12 ($P = 0.02$) and 18 h ($P = 0.05$) than the control animals (3.17 versus 4.44 log$_{10}$ colony forming units/ml and 2.51 versus 3.57 log$_{10}$ colony forming units/mL, respectively). Somatic cell counts in both copper-supplemented and control animals increased from hours 12 to 18 after the challenge, after which the SCC of supplemented animals were significantly lower ($P = 0.001$). The peak geometric mean SCC for control animals was four times higher than that of the copper supplemented-animals ($44.67 \times 10^6$ cells/mL and $10.96 \times 10^6$ cells/mL for the control and supplemented groups, respectively). Copper-supplemented animals also had significantly lower rectal temperatures at 18 hours post-challenge (40.0 vs. 40.8, respectively; $P = 0.001$).
Zinc may help to prevent and control mastitis (Sordillo et al., 1997, Spears and Weiss, 2008). Zinc is essential for skin integrity, the first line of defense against mastitis. Alongside copper, the antioxidant superoxide dismutase is dependent on zinc (Sordillo et al., 1997). Lactating cow rations should maintain zinc supplementation at 40 to 60 mg/kg (NRC, 2001).

**PRECISION DAIRY FARMING**

**Background**

Average herd size is increasing, making it more difficult for producers to devote adequate time to each animal (Bewley, 2010a, Brandt et al., 2010, Ipema et al., 2008, Schulze et al., 2007) and watching them all day every day is impossible (Berckmans, 2006). Dairy cow monitoring will increase in importance because of public concern of food safety and animal health and welfare (Ipema et al., 2008, Norberg, 2005, Schulze et al., 2007).

Precision agriculture refers to the use of technologies to increase efficiency and reduce environmental damage in crop farming. Precision livestock farming applies the precision agriculture principles to animals, focusing on individual animal production and environmental impact (Laca, 2009). Precision Dairy Farming (PDF) is the use of technologies to measure physiological, behavioral, and production indicators on individual animals to improve management strategies and farm performance (Bewley, 2010a).

Consumer pressure for control of zoonotic diseases, pathogens, and medical treatments, coupled with increased awareness of animal welfare issues has altered decision-making processes on farms (Bewley, 2010a, Schukken et al., 2008). Precision
Dairy Farming technologies have the potential to detect disease early, maximizing individual animal potential. Disease detection in the past has relied on producers observing clinical signs, but once clinical signs are displayed, it is often too late to act effectively. Clinical signs are often preceded by physiological changes that are undetectable with human senses, creating great potential to take preventive action (Bewley, 2010a).

Sensors that can monitor mastitis indicators (alterations in milk visual appearance or milk components) during or after milking and are implemented in research, but are rarely used on commercial dairy farms. Receiving daily information on individual animals provides a substantial support to management and an additional benefit compared with monthly records (Brandt et al., 2010).

Early mastitis detection is difficult because methods of continuous monitoring do not exist. Rather, producers must rely on point-in-time observations (Mein and Rasmussen, 2008, Sherlock et al., 2008). Sensors for mastitis detection became commercially available in the 1990’s, but until recently, were not applied on a large scale. Mastitis’ high economic value forced it to the forefront as the first disease of focus in sensor development (Hogeveen et al., 2010).

Producers can examine real time data and reports to identify abnormal deviations from a baseline (Bewley, 2010a) to identify clinical mastitis in its very early stages (Leslie and Petersson-Wolfe, 2012). However, the data itself is meaningless unless it is transformed into a good decision management program. Precision Dairy Farming technologies will never replace producer intuition and management, but rather, will
enhance it by providing extra information to make better-informed decisions (Bewley, 2010a).

**Sensitivity/Specificity**

Specificity is the probability that a negative sample is from a disease-negative cow. Sensitivity is the probability that a positive alert is a true indicator of a disease (Hamann and Zecconi, 1998, Hogeveen et al., 2010, Sherlock et al., 2008). Because sensitivity and specificity are interdependent, thresholds should be set to optimize both (Hogeveen et al., 2010).

Equations 1.1 and 1.2 explain sensitivity and specificity, where detected

\[
\text{Sensitivity} (\%) = 100 \times \frac{\text{detectedClinCount}}{\text{detectedClinCount} + \text{missedClinCount}} \quad \text{(Eq. 1.1)}
\]

\[
\text{Specificity} (\%) = 100 \times \frac{\text{detectednonClinCount}}{\text{detectednonClinCount} + \text{falsePosAutoCount}} \quad \text{(Eq. 1.2)} \quad \text{(Hogeveen et al., 2010, Sherlock et al., 2008)}.
\]

Positive predictive value is the proportion of true positives against the apparent positives (Hamann and Zecconi, 1998), also known as the success rate. A true positive occurs when the event occurs with an alert from the automated detection system (Hogeveen et al., 2010). Negative predictive value is the proportion of true negatives against the apparent negatives (Hamann and Zecconi, 1998). A true negative occurs when the event does not occur and an alert is not produced (Hogeveen et al., 2010). False
positives, or type I errors, can cause financial losses because healthy animals may be
treated (Burfeind et al., 2010). Conversely, false negatives, or type II errors, may leave
sick animals untreated causing animal welfare problems and decreased milk yield and
health throughout the lactation (Burfeind et al., 2010).

To be a valuable commercial management tool, cow performance should be
related to the potential improvement in management of subclinical mastitis (Nielen et al.,
1995a). While a 90% sensitivity may seem acceptable by most researchers, it would
likely be inadequate when applied in a herd setting (Sherlock et al., 2008). Sensitivity
and specificity of a subclinical mastitis detection tool depend on the disease definition
(Nielen et al., 1995a) and time window (Mollenhorst et al., 2012) in which alerts can be
given. Wider time windows will produce a higher sensitivity and specificity, but they
will also lose their practicality in a commercial setting (Kamphuis et al., 2010) because
producers given an alert before treatment or further diagnostic action can be taken are
unsure of how to handle the information. Figure 2 displays the relationship between time
windows and sensitivity and specificity where, with a longer time window, performance
(sensitivity and specificity) increases (Hogeveen et al., 2010).

Sensitivity and specificity will be lower if a new test disagrees with the
comparison to the gold standard. Disagreement between the gold standard and a new test
is often interpreted as the test lacking capability. However, the test could be better at
detecting negatives, causing true negatives to display as false negatives (Nielen et al.,
1992). However, neither the new nor the gold standard detection methods may be ideal
(Vickers et al., 2010). A universally accepted gold standard does not exist, though.
Another limitation of an automated disease detection method is that clinical infections are
infrequent, causing statistical analyses to be “weak” (Mein and Rasmussen, 2008) because of low degrees of freedom and power.

Unfortunately, researchers often forget producer preferences in the discussion of the ideal sensitivity and specificity. Steeneveld et al. (2010) explained that a general complaint of producers using robotic milking systems was the “relatively large” number of false alerts. The results of a survey of 139 Dutch producers that own an automated milking system revealed that farmers preferred a clinical mastitis detection system that produced few false alerts and provided alerts for severe cases with enough time to take effective treatment action. Producers preferred that time windows were set at a maximum of 24 hours before clinical symptoms appear. However, variation in responses to the survey varied greatly, suggesting that detection systems should be adaptable to match the conditions of each farm (Mollenhorst et al., 2012). Kamphuis et al. (2010) used an alert time window < 24 hours, but the authors were not confident that it was the correct window to use for other studies. The use of a decision tree and this narrow time window resulted in 40% sensitivity and 99% specificity. Rasmussen (2003) suggested that a clinical mastitis system should provide 80% sensitivity and 99% specificity and that time windows should be within 24 to 48 hours of a clinical mastitis event.

Reneau (1986) proposed that “the ideal clinical test would establish the presence or absence of disease in every case screened without any false positives or false negatives.” It also should “provide correct diagnosis, provide data to aid in prognosis, provide an indication of subclinical disease, provide for monitoring the effects of treatment, and provide data that may indicate possible reoccurrence of chronic disease.” When using SCC to group cows into infected and uninfected groups, mastitis detection
accuracy depends on the threshold used. This applies to all detection methods. Even the most sensitive and specific test still needs to be available and affordable (Reneau, 1986).

**Temperature Monitoring**

Body temperature is influenced by health, environment, ambient temperature, eating behavior, drinking behavior, estrus, and the pregnancy status of an animal (Bewley et al., 2008b). Fever, or a body temperature over a predefined threshold, is an indicator of disease (Burfeind et al., 2010, Leon, 2002). Fever is a complex physiological response to infection and inflammation. Once the body recognizes a pathogen invasion, macrophages and other immune cells release cytokines which signal the hypothalamus to increase the thermal set point. Although the mechanism of cytokine action remains unclear through studies in mice, this reaction causes body temperature to increase to match the increased thermal set point (Leon, 2002).

Producers often implement rectal temperature recording into their disease detection system (Burfeind et al., 2010, Schutz and Bewley, 2009, Vickers et al., 2010). The accuracy of commercially available electronic rectal thermometers is within 0.1°C (Vickers et al., 2010). However, several limitations to rectal temperature recording do exist. The first is that the presence of the recorder may affect temperature by making the animal nervous (Bewley and Schutz, 2010, Rogers Simmons et al., 1965). Other limitations include air in the rectum, failure to insert the probe deeply, and the creation of ulcers in the rectum from forceful insertion. Ambient temperature also has an effect and accuracy is related to the competency of the recorder (Aalseth, 2005).

Several temperature monitoring systems (known as passive systems) record temperatures when a cow walks past a reader (Small et al., 2008). Common placement of
readers is in the parlor entry or exit ways where temperatures are transmitted twice daily, before or after each milking. However, twice-daily recordings may not account enough for natural variation in body temperature (Bewley and Schutz, 2010) and reticular temperatures may be influenced by water intake if the readers are in a location where cows pass after drinking water (Bewley et al., 2008b). Siivonen et al. (2011) examined behavior around the time of an \(E.\ coli\) lipopolysaccharide challenge and discovered that drinking time was reduced from 4 to 6 hours post-challenge, but increased their drinking time 10 hours post-challenge. Because many producers milk cows twice daily at 12-hour intervals, increased drinking right before milking would make fever detection difficult for cows with mastitis.

Other temperature monitoring systems (known as active systems) use battery-operated transponders, which are scheduled to transmit at a set frequency, although these systems tend to be more expensive (Small et al., 2008). Frequent temperatures may allow removal of temperatures affected by water bouts while still allowing enough temperatures for analysis (Bewley et al., 2008b).

A commonly used threshold to define fever in lactating cows is 39.4°C (Aalseth, 2005). Healthy cows maintain a consistent rectal temperature of 38.6 ± 0.01°C temperature (Benzaquen et al., 2007). AlZahal et al. (2011) observed that cows in an intramammary challenge with lipopolysaccharide from \(E.\ coli\) consumed 23% less feed than cows not infected and did not eat when their rumen temperatures exceeded 40.0°C.

Researchers have described rectal temperature as diphasic. One study observed peaks between 5 and 7 pm and between 6 and 9 am, both related to feeding times, while another observed also observed peak temperatures in the early morning and late evening.
though exact times were not mentioned. Rectal temperatures increase when a cow stands and decreases when a cow lies (Rogers Simmons et al., 1965).

In a Canadian study evaluating rectal temperature measurements to determine intra- and inter-investigator variability and to determine the effects of penetration depth into the rectum and defecation on measured body temperature, repeated rectal temperatures by a single researcher were consistent (39.5 ± 0.1°C). Correlation between two researchers was high (r = 0.98; \( P < 0.001 \)). However, penetration depth within the rectum influenced results. Temperatures were 0.4°C ± 0.2°C higher when the probe was inserted deeper into the rectum (\( P < 0.001 \)). Temperature around defecation varied, with some cows having a difference of ≥ 3°C after defecation while others had a difference of ≥ 3°C before defecation and some had no difference before or after defecation (Burfeind et al., 2010). Conversely, rectal temperatures decreased by 0.3°C after drinking 22 liters of 1.1°C water (Cunningham et al., 1964).

In an *E. coli* lipopolysaccharide challenge study by Siivonen et al. (2011), rectal temperatures started to increase 4 to 6 hours post-infusion. Temperatures remained above 39.2°C from 6 to 10 hours post-infusion and returned to normal within 12 hours post-infusion (Benzaquen et al., 2007).

Some rectal thermometry limitations can be eliminated with a technique that does not require the recorder to be nearby and does not require the animal to be restrained or connected directly to a recording device (Rogers Simmons et al., 1965). Vaginal temperature monitoring is becoming more common in research and data loggers can collect continuous temperatures. Intra-vaginal thermometers can record core body
temperature every minute for up to six days, remaining with the cow as she moves throughout all areas of the dairy facility (Collier et al., 2006).

In a Canadian study, rectal and vaginal temperatures were highly correlated (r = 0.81; P < 0.001) in the 1,393 temperatures recorded for 29 fresh cows. However, rectal and vaginal temperatures were only moderately correlated (r = 0.46; P < 0.001) for the 556 temperatures recorded from the 13 peak lactation cows in this study. The correlation difference may have been because the fresh cows exhibited a larger temperature range (37.7 to 40.5°C) compared with peak-lactation cows (37.9 to 39.6°C) (Vickers et al., 2010).

A Texas study also noted that rectal and vaginal temperatures were highly correlated after a lipopolysaccharide challenge (r = 0.97; P = 0.0001) (Burdick et al., 2012). In a similar Canadian study, vaginal temperature increased for cows challenged with an intramammary injection of E. coli lipopolysaccharide two hours post-injection and returned to pre-injection temperatures 10 hours post-injection (AlZahal et al., 2011).

Cows with retained placentas averaged 0.1°C higher temperature than matched control cows (P < 0.001) (Vickers et al., 2010). Cows with puerperal metritis, a uterine infection, started a significant increase in rectal temperature 24 hours before clinical signs (reaching 39.2 ± 0.05°C on the day of clinical diagnosis) (Benzaquen et al., 2007).

Healthy cows and cows with retained placentas both showed diurnal rhythms in their vaginal and rectal temperatures, with increases in the afternoon and decreases during the morning (Vickers et al., 2010). Diurnal variations in temperatures may be attributed to individual cow or breed characteristics and ambient weather conditions (Bewley et al., 2008b). Some limitations to vaginal temperature monitoring are logger
movement (particularly around calving when the vaginal cavity was enlarged), influx of ambient air, expulsion from the vagina (Vickers et al., 2010), and potential infection from insertion of the temperature monitoring bolus.

Automated reticular temperature also has the potential to detect disease early (Bewley et al., 2008b). Schutz and Bewley (2009) created Eq. 1.3 and 1.4 for calculating rectal temperature from a measured reticular temperature from the data collected in Bewley et al. (2008a):

\[
\text{AM Milking: Rectal temperature} = 19.23 + 0.496 \text{ (reticular temperature)} \quad (\text{Eq. 1.3})
\]

\[
\text{PM Milking: Rectal temperature} = 15.88 + 0.587 \text{ (reticular temperature)} \quad (\text{Eq. 1.4})
\]

Reticular temperatures were lowest between noon and 4:00 PM (39.4°C) and between 8:00 AM and noon (39.5°C); temperatures were highest between 8:00 PM and midnight (40.2°C) and between midnight and 4:00 AM (40.3°C) (Ipema et al., 2008).

In an \textit{E. coli} mastitis challenge, ruminal temperature peaked between 40.5°C and 41.0°C and remained above 40.0°C for two hours (AlZahal et al., 2011). In a Purdue University study, reticular temperature of cows diagnosed with mastitis deviated more than 3 standard deviations from baseline temperature in 45.7% of cows (Bewley and Schutz, 2010).

Regurgitation of reticulorumen boluses and technology failure could pose problems for these temperature monitoring systems (Small et al., 2008). Although the effects are inconsistent in the literature (Bewley et al., 2008b), the main limitation for reticular temperature is the substantial effect of water intake (Schutz and Bewley, 2009). This effect is displayed in Figures 3 and 4. A Vermont study explained that both rectal and reticular temperatures decreased after a cow drank water (at 4 to 5°C) and did not
reach pre-drinking temperatures until 90 minutes later (Rogers Simmons et al., 1965). Bewley et al. (2008b) observed a decrease in reticular temperature after drinking water, but failed to see a relationship between water intake and rectal temperature. Reticular temperatures decreased by 0.4°C to 0.7°C, sometimes declining to a temperature lower than 37°C, in the hour of the drinking bout and the hour after (Ipema et al., 2008). Rumen temperature immediately decreased and remained 17.1°C below pre-watering temperatures 4.5 hours after the water bout (Cunningham et al., 1964). Colder water temperatures decreased reticular temperatures by a greater amount (Bewley et al., 2008b, Cunningham et al., 1964). The consumption of large amounts of cold water creates a sizable and sustained impact on reticular temperature (Bewley et al., 2008b). Because of the substantial and sustained temperature decreases around drinking, algorithms to smooth temperatures around these drinking bouts are imperative when technologies are used to monitor reticular temperature (Schutz and Bewley, 2009).

**Electrical Conductivity**

Electrical conductivity (EC) is a measure of the resistance of a chosen material to an electrical current (Hogeveen et al., 2010, Nielen et al., 1992, Norberg, 2005). Electrical conductivity is the reciprocal of resistance, which is defined as the cube of an electrolyte solution in 1 cm³ in volume. Resistance is equal to voltage divided by amperes. Electrical conductivity is calculated by dividing ampere by voltage and is measured in Siemens (Nielen et al., 1992).

The milk to udder barrier is impenetrable in a healthy animal (Hamann and Zecconi, 1998). However, the blood to udder barrier is semi-permeable through tight junctions for molecules up to 68 daltons (Hamann and Zecconi, 1998). Intramammary
infections damage tight junctions, allowing sodium and chloride ions to diffuse into the milk and potassium ions to diffuse out of the milk (Hamann and Zeconni, 1998, Janzkevovic et al., 2009). Secretory cells in the mammary gland actively pump sodium ions into the extracellular fluid while potassium is pumped into the cells (Kitchen, 1981). Therefore, sodium and chloride ion concentrations increase in milk from a cow with mastitis (Norberg et al., 2004). The sodium and chloride concentration is positively related to milk EC (Maatje et al., 1992). However, in an Illinois study, infections were more accurately predicted using milk EC than actual measurements of sodium, chloride, or potassium content (Fernando et al., 1985).

In-line EC systems would provide producers with a fast and simple means of obtaining mastitis information for the herd (Kitchen, 1981). In-line measurements are advantageous because the data can be collected and processed automatically by a computer and integrated with other data on-farm (Maatje et al., 1992) and can be stored from multiple milkings in contrast to SCC samples that are analyzed in a lab off-site (Nielen et al., 1993). Software could be developed or altered to fit each producer’s needs, for example, an alert could occur based on several EC deviations over a longer period instead of during one milking (Nielen et al., 1995a).

Milk EC from a healthy cow ranges from four to five milliSiemens at 25°C. Because milk temperature is close to 38°C when leaving the teat cistern, electrical conductivity is expected to be higher when measured at milking (Norberg et al., 2004). Figures 5 and 6 depict 4 quarters of a healthy cow and a cow with mastitis, respectively. The infected quarter shows a higher EC during most of the milking, especially in the beginning and end of the milking (Norberg et al., 2004).
Milk EC has been described as a mastitis indicator trait (Fernando and Spahr, 1983, Hamann and Zecconi, 1998, Jones et al., 1994) and has been used since the 1990’s for this purpose (Norberg, 2005). Jones et al. (1994) described EC as promising tool for detection of subclinical mastitis because it is reliable, inexpensive, and automatic. And researchers have studied whether or not this holds true (Díaz et al., 2012). However, published literature on EC’s effectiveness in detecting mastitis varies greatly (Hamann and Zecconi, 1998), likely due to different EC systems, different thresholds to determine what should be considered an alert, different time windows allowed for alerts, and different sensitivities and specificities between studies. Milk EC may be a practical tool for early detection of clinical or subclinical mastitis, depending on the predictive value of a positive test and the economics of treatment and mastitis losses of a specific herd (Nielen et al., 1992).

One EC limitation is that differences in gold standard definitions and time windows make comparisons between studies difficult (Mollenhorst et al., 2012). Other application limitations include training and validating models using only clear cases of healthy and infected quarters or cows. Unfortunately, all mastitis cases manifest themselves differently and one simple algorithm will not likely cover all cases. Detection performance will, therefore, be overestimated in studies lumping all cases into one model because mastitis is not always that clear-cut, rather, the “gray area” should be accounted for (Kamphuis et al., 2010).

Other factors that affect EC may cause confusion when EC shifts occur. An Illinois study concluded that an ambient temperature increase of 1°C caused a 0.01 increase in EC ($P = 0.02$). Additionally, for each increase in days in milk, EC increased
by 0.18 \((P < 0.01)\). Milk fever created the largest EC increase of 8.3\%, but ketosis, left displaced abomasum, retained placenta, and lameness also were associated with a significant EC increase. EC differences existed between morning and afternoon milkings (Lukas et al., 2009).

In a study looking at the effects of EC after a *Staph aureus* challenge, EC increased during the first milking after the challenge and exceeded the range for uninfected quarters by the third milking. However, SCC did not increase until the second milking after challenge. Throughout the study, milk from every subclinically infected quarter maintained a higher EC than uninfected quarters \((P < 0.01)\). In contrast, no EC differences were observed in cows subclinically infected with *Streptococcus uberis*. The subclinical *Streptococcus uberis* infections may have been too mild to detect because the mean SCC only reached 284,000 cells/mL. However, this displays a limitation because these quarters did not show signs that another method could have detected. Infected quarters from both bacterial challenges were detected by electrical conductivity when the infections presented clinical symptoms (Hillerton and Walton, 1991).

In a challenge study by Milner et al. (1996), 11 of the 12 (92\%) clinical cases caused by *Streptococcus uberis* experienced an EC increase on or before the milking where the clinical signs appeared. Only 33\% of the subclinical cases caused by *Streptococcus uberis* experienced an EC increase. The researchers recovered bacteria from infected quarters after a mean of 2.3 milkings post-infusion, SCC increased after 3.1 milkings, an EC change occurred after a mean of 3.5 milkings, and clots were observed at a mean of 5.2 milkings post-infusion. In the same study, 15 of the 17 (88\%) clinical cases caused by *Staph aureus* experienced an EC increase at the same time or in advance
of clots in the foremilk. Clots appeared before a significant EC change in only one quarter. Electrical conductivity changes were accompanied by a presence of bacteria, rise in SCC, and clots in milk (2 cases did not experience clinical signs of disease, but bacteria were recovered from the quarters). In an *E. coli* O55:B5 lipopolysaccharide challenge study by Siivonen et al. (2011), EC and SCC started to increase 4 hours post-infusion.

In goats, chloride levels increased significantly after the establishment of an intramammary infection unilaterally, whereas both chloride and the ratio of sodium to potassium increased in bilaterally infected glands. Healthy udders did not experience either increase. Bilaterally infected glands produced a greater EC increase than unilaterally infected glands, suggesting that the bilaterally infected glands experienced a greater effect of the blood-milk barrier change. Infected gland EC increased significantly after infection, whereas collateral healthy glands and glands free from infection did not. Bilaterally infected gland EC significantly increased one to four days post-infection whereas unilaterally infected did not experience an EC increase in EC until five to eight days post-infection. The authors also observed a tendency for the EC of a healthy quarter to increase when the collateral gland became infected (Díaz et al., 2012).

More accurate EC data may be obtained from foremilk, or the first milk taken from the udder at each milking, versus samples obtained after the stripped milk (16.5% false positives versus 42.7% false negatives for milk after foremilk and foremilk samples, respectively) (Fernando and Spahr, 1983, Fernando et al., 1985). In one study, foremilk EC changes indicated increased SCC, increased milk clotting, and establishment of bacteria in advance of visible changes in the milk (Milner et al., 1996). Jones et al.
(1994) explained that as milk flow increased, electrical conductivity also increased but was erratic. A possible source of the fluctuation was insufficient milk volume to cover the electrode, though the fluctuations in contact lasted only a few seconds (Jones et al., 1994).

The relationship between EC, SCC, and bacteriological presence is not straightforward (Nielen et al., 1995a). Nielen et al. (1992) presented a low positive correlation ($R = 0.37$) between SCC and EC, but prediction of SCC through EC was difficult (Nielen et al., 1995a). Longer periods of increased SCC would indicate deteriorated udder health and may be more closely related to an EC increase (Nielen et al., 1995a). Therefore, EC and SCC are both related to udder health, but may be less closely related to each other, which would account for the low sensitivity of EC detecting high SCC (Nielen et al., 1993).

A logistic regression model using EC correctly predicted 55% of 1,080 milkings from cows with subclinical mastitis and 90% of 4,614 cows without mastitis. Sensitivity and specificity for a period of 14 milkings were 54% and 92%, respectively. However, repeated measures within cows were not accounted for, though the authors acknowledged that they were present. In the same study, a neural network correctly classified 67% of 1,079 subclinical milkings and 78% of 4,572 healthy cow milkings. Sensitivity and specificity for a period of 14 milkings were 66% and 80%, respectively, using the neural network (Nielen et al., 1995a).

In a Dutch study, a 20% threshold was applied (the quarter was $\geq 20\%$ of the running average of the reference quarter during two successive milkings) with 100% of clinical cases and 50% of subclinical cases were identified from the quarter EC data.
However, the specificity was 4% (Maatje et al., 1992) so many false positives were identified. False positives are expensive to producers because producers check, and possibly treat, cows that are not sick.

Nielen et al. (1993) explained that EC was negatively related to daily milk production in a low SCC herd. Milk EC effects and the natural log of SCC appeared to be additive, suggesting that both could be used as indirect detection tools of subclinical mastitis.

Detection methods based on maximum values and time-series data analysis using historical information have shown promising results for mastitis detection (Norberg et al., 2004). Analysis within-cow may be the best way to evaluate changes in electrical conductivity (Hamann and Zecconi, 1998).

Regrettably, EC measurement errors can occur for three reasons: 1) fat or protein build-up on the sensor electrodes (Lake et al., 1992), 2) air entering the line because of liner slips (Jones et al., 1994), possibly caused by mastitic cows experiencing pain during the milking and moving (Norberg et al., 2004), and 3) clots in mastitic milk causing the sensors to measure air (Norberg et al., 2004).

Although cow-side and fast tests exist for mastitis detection, like EC, only the California Mastitis Test (CMT) has been widely implemented on commercial farms (Hillerton and Walton, 1991). Upon the addition of the detergent to a high SCC quarter milk sample, cells will lyse, releasing nucleic acids, leading to the formation of a gel (Viguier et al., 2009). The CMT is convenient and inexpensive, but it is also subjective (Lam et al., 2009, Mein and Rasmussen, 2008, Viguier et al., 2009) and not automated or quantitative.
Early mastitis detection may allow prompt treatment and may prevent high bulk tank SCC (Milner et al., 1997, Whyte et al., 2004). Early disease detection is only valuable when it can maintain low SCC and prevent udder damage (Milner et al., 1997). Early disease detection allows early administration of treatment to limit the severity of the disease and possibly prevent the disease from further developing (Lukas et al., 2009, Milner et al., 1997, Viguier et al., 2009) and decrease the culling rate (Lukas et al., 2009).

**Milk Yield**

Dairy cattle economic efficiency is closely related to milk production (Dohoo and Wayne Martin, 1984) because production losses decrease producer revenue. Unfortunately, mastitis has a long lasting effect on milk yield (Rajala-Schultz et al., 1999). Even after an infection is cured, milk yield remains depressed for two months (Bar et al., 2008). Additionally, cows may be unable to reach their pre-mastitis milk yield after a clinical mastitis case throughout their entire lactation (Rajala-Schultz et al., 1999).

Canadian researchers examined the effects of subclinical mastitis on milk yield and discovered that each unit increase in loge SCC was associated with a 6.2% milk yield loss (Dohoo and Wayne Martin, 1984). Cobo-Abreu et al. (1979) concluded that cows with mastitis produced significantly less milk in the lactation when the mastitis occurred compared with their lifetime average milk production ($P < 0.05$).

French researchers developed a mastitis simulation model using data from three herds and determined that overall losses amounted to 49,000 kg per 100 Holstein cows and 35,000 kg per 100 Friesian cows with clinical mastitis, which was 8 and 7% of total projected production. The model did not include discarded milk loss. The authors
concluded that one-third of cows experienced no significant response relative to control cows (a loss of 22 kg for cows with clinical mastitis). However, the other two-thirds of study cows experienced substantial milk losses between the week of mastitis occurrence and the five weeks following (144 kg) or experienced substantial milk loss extended throughout their lactation (911 kg) (Lescourret and Coulon, 1994).

A Finnish study examined milk yield changes around clinical mastitis and observed that milk yield began to decline four weeks before clinical mastitis detection. Milk yield of cows with clinical mastitis dropped below that of the healthy cows in the first two weeks after diagnosis. After this two-week period, yield gradually increased, but it did not reach the level it was at more than four weeks before the onset of mastitis during the rest of the lactation. However, the yield decrease of the cows with clinical mastitis was not significantly different from the healthy cows. Overall, total lactation milk yield loss caused by mastitis varied between 294 and 552 kg under the assumption of a 305-day lactation with clinical mastitis occurrence on day seven. Milk loss increased with increasing parity with older cows suffering greater losses. Milk loss among parity 1, 2, 3, and 4 or higher cows was 4.6, 4.1, 6.9, and 7.4% of the overall lactation yield, respectively (Rajala-Schultz et al., 1999).

Milk loss resulting from clinical mastitis may depend on the number of affected quarters and the number of clinical mastitis occurrences throughout the lactation. A Dutch study reported that first parity cows with clinical mastitis in only one quarter lost 40 kg as opposed to second parity cows that lost 140 kg. Milk loss in first and second parity cows infected in only one quarter did not change with increased months in lactation. In second parity cows, milk yield was more significantly reduced when three
or more cases of clinical mastitis were observed compared with two cases. Milk loss in
month eight of the second lactation was 527 kg (8.1%) and 214 kg (3.3%) for three or
two cases, respectively (Houben et al., 1993).

In a 6-month study on a 1700-cow Michigan Holstein dairy farm, total milk loss
over all clinical cases of mastitis was 341 kg. Of that loss, decreased production
accounted for 92 kg and milk withheld accounted for 249 kg. First lactation cows
maintained a significantly lower milk loss than ≥ 2 parity (177 kg versus 369 kg for first
and ≥ 2 parity cows, respectively; \( P < 0.01 \)). Milk withheld from first parity cows was
also significantly less than milk withheld from ≥ 2 parity cows (102 versus 269 for first
and ≥ 2 parity cows, respectively; \( P < 0.01 \)) (Bartlett et al., 1991).

**Rumination Time**

Rumination is defined as the regurgitation of fibrous ingesta from the rumen to the
mouth, re-mastication, followed by swallowing and returning of the material to the
rumen. Dairy cows normally ruminate for eight to nine hours a day (Welch, 1982).
Although eating is normally the predominant behavior in dairy cows, rumination can take
precedence when eating has been restricted (Grant and Albright, 2001). Researchers in a
Vermont study fitted steers with a facemask that restricted all jaw movement for ten
hours a day during the study period. When the facemask was removed, the steers were
offered hay, but the animals instead chose to ruminete (Welch, 1982).

In the past, researchers estimated rumination based on direct visual observations, but
systems now exist to automate this process (Schirmann et al., 2009). Automated
rumination-monitoring systems can provide a reasonable measure of rumination in
heifers older than nine months. Rumination recordings on animals of this age were
highly correlated (R = 0.88) with live observations. A similar study on cows validated an automated rumination logging device by comparing values with those from a human observer for 51 two-hour observation periods on 27 Holstein cows. Rumination times from the electronic system were highly correlated with those from human observation (R = 0.93), indicating that the automated system accurately monitored rumination in dairy cows (Schirmann et al., 2009).

Kansas researchers studied nine Angus-Hereford cows and observed that high cortisol levels (above 22 ng/mL, the mean of the group) were highly correlated with less time spent ruminating (r = −0.85, \( P < 0.01 \)). Cortisol is released when an animal is stressed, therefore an association between stress and decreased rumination may exist (Bristow and Holmes, 2007). However, decreases in rumination may not always occur around stress. A study examining behavioral changes related to increased stocking density reported that at 100% stocking density, 95.1% of rumination occurred within a stall, but as stocking density increased to 142%, only 87.3% of rumination occurred within a stall. However, overall rumination time did not decrease between any of the stocking densities (\( P > 0.05 \)) (Krawczel et al., 2012). Instead, decreases in rumination may be pain or disease related.

In an \textit{E. coli} challenge with 20 cows, rumination decreased (\( P < 0.05 \)) on the day of mastitis induction and gradually increased to levels before the induction during the following two days (Fogsgaard et al., 2012). Canadian researchers evaluated the effects of a non-steroidal anti-inflammatory drug on the pain mitigation of mastitis and discovered that an \textit{E. coli} lipopolysaccharide challenge did not affect daily rumination time, recorded by neck-mounted rumination loggers. However, when diurnal patterns were taken into account, an interaction between time and rumination recorded in two-
hour intervals was significant \( P < 0.01 \) where cows spent less time ruminating after the challenge, but made up for it later in the day (Fitzpatrick et al., 2013). Siivonen et al. (2011) also conducted an \textit{E. coli} challenge to evaluate rumination behavioral changes around mastitis and concluded that the mean time spent ruminating decreased between four and eight hours post-challenge compared to the control day \( (221.57 \pm 9.34 \text{ versus } 252.23 \pm 9.82 \text{ for control and induction days, respectively}) \).

\textit{Lying Time}

In dairy cattle, lying down is a high-priority behavior, which ensures that the necessary time to rest and ruminate is achieved. Danish researchers restricted time to feed access and explained that this restriction decreased time spent on all activities, but the proportion of time spent feeding and time spent on social contact remained constant. Yet the proportion of time spent lying increased. Therefore, the authors concluded that the priority for the behaviors studied were lying, followed by eating and social contact (Munksgaard et al., 2005). Ito et al. (2009) evaluated the lying time for 2,033 cows on 43 farms over five days using electronic data loggers to conclude the mean lying time of cows was 11 hours/day. Another study using electronic lying time data loggers on 77 cows for 408 days revealed a mean lying time of 10.5 hours/day (Bewley et al., 2010).

Changes in lying behavior may be related to a state of chronic stress (Ladewig and Smidt, 1989). Reduced mobility and increased rest may be strategy of energy conservation in order to allow more energy to be spent on fighting the infection and to allow the full development of a fever, which may help the animal recover (Aubert, 1999).

Cook et al. (2007) video recorded lying behavior of 14 dairy cows over all seasons and discovered that mean lying time decreased from 10.9 to 7.9 hours/day from the
coolest to the hottest session recorded because of heat stress ($P < 0.01$). Additionally, cows with greater locomotion scores (using a 1 to 4 scale where 1 represents non-lame and 4 represents severely lame) lied down more (2.9, 4.0, and 4.41 hours/day for locomotion scores 1, 2, and 3, respectively; $P < 0.01$ between 1 and 2; $P = 0.02$ between 1 and 3), indicating that pain may increase lying time.

While physical discomfort may decrease dairy cow lying time, lying on hard surfaces may also exacerbate pain caused by mastitis, causing lying time to decrease during mastitis (Cyples et al., 2012). Chapinal et al. (2013) explained that lying down at the time when the most severe signs of local inflammation occur causes pain, forcing cows to stand for longer periods during mastitis.

Canadian researchers challenged 19 cows with an $E. coli$ lipopolysaccharide and cited that baseline lying time (averaged from the two days before mastitis induction; 707.0 minutes/day) was higher than the day of induction (633.3 minutes/day; $P = 0.005$). Lying time increased on the two days after infusion (743.1 and 726.3 minutes/day for days one and two after infusion, respectively), but not significantly (Cyples et al., 2012). In a behavioral study of cows with naturally-occurring clinical mastitis, cows with clinical mastitis laid down more than control cows on the day after mastitis detection (707.5 versus 742.5 minutes/day, $P = 0.04$). However, no difference was observed in lying times of animals with mastitis that had been treated with antibiotics and control animals ($P > 0.10$) (Medrano-Galarza et al., 2012).

**CONCLUSIONS**

Mastitis, or udder inflammation usually caused by bacterial invasion, is an expensive disease that compromises cow well-being and milk production. Mastitis
prevention methods include maintaining healthy teat ends, clean cows, and consistent and hygienic milking procedures. Current mastitis detection methods rely on visual observation. However, early mastitis detection may allow producer intervention (i.e. antibiotic treatment), thus decreasing the negative economic and well-being implications of the disease. Precision dairy technologies, or technologies that reside in and on cows to monitor individual cow physiology and behavior, may be able to predict and detect mastitis and alert producers to cows with changes in the physiological or behavioral indicators monitored. Producers considering implementing precision dairy technologies into their mastitis management systems should consider sensitivity and specificity before investing.
Figure 1.1. Representation of mastitis development in an infected udder, caused by environmental and contagious pathogens (Viguier et al., 2009).
Figure 1.2. Sensitivities and specificities of previous studies plotted against the time windows used around a mastitis event (Hogeveen et al., 2010).
Figure 1.3. Reticular response patterns for a) cold water drench (7.6°C ± 0.4), b) warm water drench (18.2°C ± 0.4), or c) hot water drench (34.3°C ± 1.0) (Bewley et al., 2008b).
Figure 1.4. Reticular response patterns for a) no water drench, b) body-temperature water drench (38.9°C ± 0.2), or c) cold water drench (5.1°C ± 0.4) (Bewley et al., 2008b).
Figure 1.5. Electrical conductivity profiles (in milliSiemens) for all 4 quarters of a healthy cow (Norberg et al., 2004).
Figure 1.6. Electrical conductivity profiles (in milliSiemens) for all 4 quarters of a cow with clinical mastitis. The bold line indicates the electrical conductivity profile of the infected quarter (Norberg et al., 2004).
CHAPTER TWO

Characterization of management practices used on Kentucky dairy farms with low somatic cell counts

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INTRODUCTION

Mastitis costs $71 per cow annually and $179 per clinical mastitis (CM) case (Bar et al., 2008). Somatic cell count is a useful predictor of a herd’s udder health status. Maintaining a low SCC can be linked directly to a farmer’s management style (Barkema et al., 1999) and relates to the farm’s cleanliness (Haskell et al., 2009).

Shook (1989) explained, “where eradication is not possible, sanitation is an essential first step to reduce exposure of animals to disease.” Exposure to environmental pathogens may be reduced for clean animals (Sant’Anna and Paranhos da Costa, 2011). Excessive dirt or manure, particularly on the legs and udder, has been positively associated with increased mastitis (Reneau et al., 2005, Schreiner and Ruegg, 2003). Freestall cleanliness is often associated with reduced environmental bacteria exposure (Hogan et al., 1989). Management practices may help reduce cow exposure to environmental bacteria and minimize contagious bacteria spread. For example, pre-milking hygiene, including pre-dipping, can reduce the teat end and skin bacterial contamination before attaching the milking unit (Galton et al., 1986, Pankey, 1989).

In 2012, the average SCC for Southeast (Florida, Georgia, Kentucky, and Tennessee) dairy farms was 330,000 cells/mL, compared to 255,000 cells/mL in other regions of the United States (Dong et al., 2012). The objective of this research was to summarize management practices used by Kentucky dairy herds with low SCC. Results of this survey may be used to promote best management practices among other producers attempting to lower SCC.
MATERIALS AND METHODS

Kentucky herds with an average annual SCC of < 250,000 cells/mL were identified through data provided by DHIA and 4 participating cooperatives: Maryland and Virginia Milk Producers Cooperative Association, Inc. (Reston, VA), Organic Valley (La Farge, WI), and Mideast and Southeast Area Dairy Farmers of America, Inc. (Kansas City, MO). Although DHIA and cooperative SCC values were used to determine study eligibility, producers were also asked to report their most recent bulk tank SCC. The list of producers’ names and addresses was used only to distribute surveys and was not referred to after final survey distribution.

A 54-question survey (Appendix A) was mailed in late November 2010 to the 71 producers who met the SCC requirement. The same survey was mailed again in early January to those who had not responded by December 15, 2010. One incomplete survey was omitted, leaving 48 completed surveys to include in the analysis (68% return rate). Data were entered into and descriptive statistics were analyzed in Excel (Microsoft Corporation, Redmond, WA). The MIXED Procedure of SAS (SAS Institute, Cary, NC) was used to evaluate the fixed effects of breed group (Holstein, Jersey, Brown Swiss, Ayrshire, and mixed or combination herds) and milking frequency (2X or 3X daily) on DHIA- and cooperative-reported SCC.

RESULTS AND DISCUSSION

Mean (± SD) DHIA SCC and producer-reported SCC were 190,333 ± 36,281 (n = 27) and 223,475 ± 71,257 (n = 40) cells/mL, respectively. The same annual mean DHIA SCC was not updated between the first and second mailings to unresponsive survey recipients. However, because producers responded to the survey at different times, the
most recent bulk tank SCC reported by surveyed producers were obtained on different dates. Seasonal results on bulk tank SCC were unlikely because data was collected between the winter months of November and January.

Herd size ranged from 25 to 2,000 lactating cows with a mean (± SD) of 145 ± 297 (n = 48), almost twice as high as the Kentucky mean of 77 milking cows (from October to December 2010; NASS, 2012). Although the mean herd size is greater than the average Kentucky herd size, the largest herd in the state (2,000 milking cows) was included in the analysis, which skewed the mean. The median herd size in this study was 72 lactating cows, implying that the farms included in this survey were likely representative of the state’s dairy population. Herd size did significantly affect SCC in a study by Dong et al. (2012). Conversely, Ingham et al. (2011) observed a significantly higher mean SCC in small herds (≤ 118 cows) than in large herds (119 to 713 cows) and Confined Animal Feeding Operations (≥714 cows) of 369,000 cells/mL, 273,000 cells/mL, and 240,000 cells/mL, respectively. Norman et al. (2011) reported that large herds were less likely to break SCC quality limits.

Surveyed producers often owned more than one breed of cows. The most common breeds were Holstein (85%, n = 41) and Jersey (27%, n = 13). Other breeds represented included Brown Swiss (13%, n = 6), crossbred (8%, n = 4), Ayrshire (4%, n = 2), Guernsey (4%, n = 2), Dutch Belted (1%, n = 1), and Milking Shorthorn (1%, n = 1). Breed group did not have a significant effect on DHIA- or cooperative-reported SCC (P = 0.49).

Participating farms employed a mean (± SD) of 2 ± 2 milkers (n = 48) with a range of 0 to 9. Bartlett et al. (1992) associated the use of hired labor with lower SCC.
Hired labor may allow a producer to focus on management while designating milking tasks to the hired milkers. Ninety percent of farms milked twice daily (n = 43) and the other 10% milked three times daily (n = 5). Milking frequency did not have a significant effect on DHIA- or cooperative-reported SCC (P = 0.32). Dong et al. (2012) was also unable to identify an association between milking frequency and SCC.

Most producers managed partial confinement systems, providing cows ≥ 4 hours outside per day (54%, n = 26). Ten (21%) of the farms were total confinement with no daily pasture access and exclusively pasture or grazing systems (17%, n = 8). In a survey of 204 Kentucky farms by Russell and Bewley (2011), 48% of the farms were pasture or grazing systems, 38% were partial confinement, and 14% were total confinement. Producers surveyed in this study used more partial confinement systems and less pasture or grazing systems. Total confinement operations have been associated with an increased incidence rate of mastitis caused by *Escherichia coli* (Schukken, 1991). In a study by Pomiès et al. (2000), turning cows out to pasture did not significantly increase SCC. Sant’Anna and Paranhos da Costa (2011) cited a higher percentage of dirty cows on pasture during January to March when rainfall was increased, indicating that mud had a negative effect on cow hygiene. Because mud, moisture, and manure are the primary sources of teat end and skin environmental pathogen exposure (Schreiner and Ruegg, 2003), ambient weather and pasture maintenance likely affect SCC more than the act of pasturing animals itself.

Producers were asked to cite what they thought was the most important key to maintaining a low SCC (Table 2.1). The most commonly cited practice was keeping cows and facilities clean (n = 31). Barkema et al. (1998a) cited that more attention was
paid to hygiene in herds with a bulk tank SCC $\leq 150,000$ cells/mL. Cows with dirty udders require more care in pre-milking preparation and are a sign of facility and management problems (Sant’Anna and Paranhos da Costa, 2011). Cows with dirtier legs were 1.3 times more likely to have major pathogens isolated from milk samples (Schreiner and Ruegg, 2003). Using a 5-point hygiene scoring system, each increase of 1 standard deviation of udder, hind-legs, and the combination of udder and hind-leg hygiene scores was followed by an increase in somatic cell score ($\text{SCS}$) of 0.13, 0.17, and 0.17 for each category, respectively (Reneau et al., 2005).

The next most commonly cited practice for maintaining low SCC was providing cows with dry, clean bedding ($n = 14$). Hutton et al. (1991) associated dry, clean bedding with low SCC herds. Minimizing the bacterial content in bedding material is an important step of any mastitis control program. Mastitis rates are directly related to levels of environmental bacteria in the bedding and stall hygiene (Zdanowicz et al., 2004).

Ten producers (21%) cited employing a consistent milking routine as the most important key to maintaining a low SCC. Ensuring that milkers have proper training and follow a routine that keeps teats clean and dry may help prevent mastitis and high SCC because wet teats increase bacterial populations (Galton et al., 1986). This milking routine should include forestripping, pre-dipping, drying teats with an individual towel, and post-dipping. Posting a written milking routine, in the native language of the employees, can help ensure that desired milking procedures are understood and followed. Six of the producers surveyed (12%) posted a written milking procedure in the parlor whereas 88% ($n = 42$) of producers did not.
Fifteen percent of producers (n = 7) cited pre- and post-dipping as the most important practice related to low SCC. Pre- and post-dipping may improve teat cleanliness. Clean teats and udders have been associated with lower SCC (Bartlett et al., 1992b). Post-dipping has been associated with a lower herd prevalence of intramammary infection caused by coagulase-positive staphylococci (Hutton et al., 1991) and may control mastitis caused by all contagious pathogens (Blowey and Edmondson, 1996).

Forestripping was cited as the most important practice to maintain a low SCC by 7 producers (15%). Forestripping allows the milker to examine milk for early signs of CM (Bartlett et al., 1992b) and culture or treat with antibiotics when necessary.

Dry cow therapy, the intramammary infusion of antibiotics immediately after the last milking of a lactation, for all quarters of all cows was cited by 2 producers (4%) as the most important practice for maintaining a low SCC. Dry cow therapy may be one of the most important parts of controlling mastitis (Barkema et al., 1998a, Dufour et al., 2011) by curing existing infections at dry off and preventing new infections in the early dry period. Prevention of new intramammary infections during the dry period may be even more important in low SCC herds because new infections in low SCC herds are likely to be caused by gram-negative environmental bacteria (Barkema et al., 1998b, Bradley and Green, 2001).

Attention to detail was cited by 2 producers (4%) as an important part of maintaining a low SCC, which agrees with results from Hutton et al. (1990). Hutton et al. (1991) explained that keeping the regulator filter clean may be a reflection of a producer’s attention to detail. One producer also mentioned that focusing on preventing, rather than curing, mastitis is important.
Seventy-nine percent of surveyed producers received bulk tank SCC results from every pick-up (n = 37). Eighty-five percent (n = 40) of surveyed producers used bulk tank SCC to monitor mastitis.

Annual milking system maintenance checks were commonly employed among those surveyed (73%, n = 35). Dufour et al. (2011) recommended that systems should be checked at least annually to decrease mastitis incidence. Rodrigues and Ruegg (2005) cited regular milking machine maintenance as one of the most important parts of controlling mastitis.

Thirty-five percent of producers used the California Mastitis Test (CMT) for mastitis detection in the parlor (n = 17). The CMT is inexpensive and quick as a cow-side test (Sargeant et al., 2001). Results from the CMT enable producers to dump milk from a high SCC cow or to take further action to cure an existing infection.

Forty-four percent of producers (n = 21) removed udder hair. Barkema et al. (1998a) associated clipping the udder hair of all cows every year with lower herd SCC. Seventy-five percent of producers used a hoof trimmer at least once a year (n = 36). Though consistent hoof trimming has not yet been directly associated with SCC to the knowledge of the authors, this result may simply re-emphasize that the producers of low SCC herds pay close attention to detail and hygiene.

Fifty-seven percent (n = 27) of the surveyed producers fed waste milk to calves while the other 43% (n = 20) discarded waste milk. Thirty-five percent (n = 30) of producers discarded milk from non-treated high SCC cows. Barkema et al. (1998a) explained that calves in herds with a bulk tank SCC ≤ 150,000 cells/mL were fed high SCC milk from treated cows significantly less often than in herds with higher bulk tank.
SCC. Discarding or feeding milk from non-treated high SCC cows may help maintain a lower herd average SCC by keeping the high SCC milk out of the bulk tank.

Somatic cell count data does not provide a mastitis diagnosis, but can be especially useful when combined with other tools. Culturing milk from cows with mastitis is an effective diagnostic tool and an important step for understanding mastitis-causing bacteria prevalence within a herd for both mastitis prevention and treatment. Table 2.2 depicts how frequently the producers in this survey submitted milk samples for bacteriologic culture. Most producers rarely cultured (38%, n = 18). Bacteriologic culture results can guide treatment decisions and may improve treatment outcomes because producers are better able to target treatments for the specific causative agent.

**Milking Practices**

Ninety-two percent of producers practiced pre-dipping (n = 44) and 100% practiced post-dipping (n = 48). Non-return dip cups, which prevent dip contamination by mastitis pathogens, were the preferred dipping method among the producers surveyed (69%, n = 33). Nineteen percent used Thrifty Dippers (Mastitis Management Tools, Inc., Burley, ID; n = 9) and 17% used return dip cups (n = 8). The remaining 10% used spray hoses (n = 5). Spraying can be effective, but may result in partial teat coverage. Covering most of the teat is important to remove bacteria from the teat, rather than just the teat end. Dips lose their disinfecting ability when milk and feces are present in the cup (Blowey and Edmondson, 1996), making non-return dip cups a better choice than return dip cups.

The active ingredient in teat dips for both pre- and post-dip varied, but iodine was the most common ingredient (42%, n = 16 and 78%, n = 31 for pre- and post-dip,
respectively). Peroxide was the next most commonly used pre-dip, used by 24% of producers (n = 9), followed by bleach (n = 6, 16%), sodium dichloroisocyanurate (n = 4, 11%), sodium chloride (n = 2, 5%), and chlorhexadine (n = 1, 3%). Sodium chlorite was the second most commonly used post-dip (n = 7, 15%), followed by bleach (n = 1, 3%), and chlorhexadine (n = 1, 3%). Sixty-four percent of producers indicated that their milkers wore synthetic rubber gloves during milking (n = 30). In another study evaluating management practices incorporated by low SCC herds, Hutton et al. (1990) reported 86% of herds used synthetic rubber gloves during milking.

Ninety-eight percent of producers dried teats before attaching the milking unit (n = 46). Manual teat drying was a significant factor in the reduction of total bacteria counts on teat ends (Galton et al., 1986, Pankey, 1989) and had the second greatest effect in reducing SCC, behind teat dipping (Moxley et al., 1978). Galton et al. (1986) proposed that manual teat drying was more important than the pre-dip type. Paper towels were more commonly used than cloth towels for drying teats (61%, n = 27 for paper towels; 39%, n = 17 for cloth towels). Also, 82% of producers used individual towels to dry teats (n = 37). Use of individual cloth towels compared with sharing a towel between cows was associated with a lower mean bulk tank SCC (Bartlett et al., 1992b). Reuse of paper or cloth towels on multiple cows may spread contagious pathogens.

Five producers (10%) had a high SCC group separated from the rest of the herd while 42 producers (89%) did not. All of the producers who had a separate group milked the high SCC cows last (n = 5) to prevent the potential spread of mastitis-causing bacteria. Barnouin et al. (2004) cited that the probability for a herd to have a SCC of ≤ 150,000 cells/mL increased when high SCC and cows with mastitis were milked last.
Dry treating all quarters of all cows was a common practice among surveyed producers (85%, n = 41). Forty-four percent of producers (n = 21) also used a synthetic internal teat sealant such as Orbeseal® (Pfizer Animal Health, New York, NY) at dry-off. Both of these practices may help prevent infections originating in the dry period. Parker et al. (2008) cited that infusion of a teat sealant reduced the risk of new intramammary infection by 74%, reduced the prevalence of post-calving intramammary infection by 65%, and reduced the risk of new infection from *Streptococcus uberis* by 70% in quarters with an intramammary infection pre-calving.

**Parlor and Housing Systems**

Nineteen percent of producers (n = 9) described their milking system as a tie-stall or flat barn. The remaining producers (83%, n = 39) used a parlor as their milking system. Parlors were described as herringbone (47%, n = 22), parallel (15%, n = 7), side opening (11%, n = 5), parabone (9%, n = 4), and swing (2%, n = 1).

Table 2.3 shows the wide variety of lactating cow, dry cow, and springing heifer housing used by the surveyed producers. The most common housing system for lactating cows was an older (> 10 years old) freestall barn (27%, n = 14), but every possible housing system used by Kentucky dairy producers was represented. This result demonstrates that low SCC can be achieved in any type of dairy system and depends more on management. Dry cow and springing heifer housing types also varied greatly from farm to farm. No housing (cows are outside year round) was most common for both of these groups (44%, n = 21 for both). Smith and Ely (1997) reported that herds fed inside a freestall barn or under a covered roof had higher milk production and lower
somatic cell counts than herds fed outside. This result may not hold true for dry cow or springing heifers.

Freestall bed base and bedding type were variable. Dirt or clay was the most common bed base at 27% (n = 9), followed by solid rubber mats (21%, n = 7), mattresses (18%, n = 6), concrete (15%, n = 5), lime (9%, n = 3), and “other” (9%, n = 3). Wood shavings or sawdust was the most commonly used bedding at 54% (n = 19) followed by sand (34%, n = 12), straw (9%, n = 3), and lime (3%, n = 1).

Frequent and effective manure removal helps to keep cows clean (Bewley et al., 2001). Forty-two percent of producers removed manure from alleys at each milking (n = 20) and 42% did this once daily (n = 20). Magnusson et al. (2008) reported that freestall hygiene was improved with improved cleanliness of alleyways, using mechanical scrapers on rubber-slatted floors, citing less manure was tracked into stall from the cow claws. Less manure in the stalls would keep the udders and teats cleaner. Sixty-seven percent of producers groomed stalls more than once a day (n = 18) and added bedding to their freestalls weekly (56%, n = 14). Frequent grooming and bedding addition may reduce stall moisture content and keep stalls cleaner. Schukken et al. (1991) explained that freestalls groomed more frequently per day and those that had more bedding had lower cow exposure to *E. coli*. Herds with low bulk tank SCC had cleaner housing and cleaner cows than herds with higher bulk tank SCC (Barkema et al., 1998a).

Most producers cleaned their holding pens daily (44%, n = 21), followed by those who cleaned them with every milking (27%, n = 13), every 2 to 3 days (10%, n = 4), and once weekly (5%, n = 2). Lower CM incidence was associated with cleaning the holding pen at least twice per day (Peeler et al., 2000).
Records Management

Records management through National Dairy Herd Information Association, Inc. (Verona, WI; DHIA) and PCDart (Dairy Records Management Systems, Raleigh, NC) proved to be integral parts of SCC management. However, because DHIA records were partially used to identify survey herds, 83% (n = 40) of the producers surveyed used DHIA which may not represent all farms in Kentucky. Barnum and Meek (1982) explained that producers that employ a records management system were likely more progressive and willing to adopt mastitis prevention measures.

Of those who used DHIA, 74% examined each individual cow’s SCC and percentage contribution to the bulk tank as a mastitis monitoring tool (n = 29). Most producers in this study also examined SCC trends in PCDart (67%, n = 31). Seventy-one percent of producers used the SCC information gathered from both DHIA and PCDart to monitor mastitis and to then cull high SCC cows (n = 32). Moxley et al. (1978) explained that herds participating in monthly cell counting services had significantly lower SCC than those who did not, even after adjusting the data for hygiene and other mastitis control practices. Herd managers included in this study may have elected to subscribe to DHIA with an already-low SCC and were just proactive in obtaining all possible information to maintain a low SCC instead of DHIA subscription resulting in a lower SCC.

Vaccination

Coliform vaccines are often useful, particularly in low SCC herds, because they may minimize the effects of these infections. Gram-negative pathogens were more common in herds with a bulk tank SCC ≤ 150,000 cells/mL (Barkema et al., 1998b).
Twenty-seven producers (56%) in this study implemented a coliform vaccine into their management system. Of those who used these vaccines, J-Vac® (Merial Ltd., Duluth, Georgia) was the most commonly used (48%, n = 13), followed by J-5 Bacterin™ (Pfizer Inc., New York, NY) (30%, n = 8), and Endovac-Bovi® (Immvac Inc., Columbia, MO) (22%, n = 6). Lysigin® (Boehringer Ingelheim, St. Joseph, MO), a preventive measure against *Staph aureus*, was used by 10% of producers (n = 5).

**Cooling Systems**

Seventy-seven percent of producers used fans for cow cooling (n = 37). Producers who pastured their animals also commonly used shade trees (33%, n = 16). Cooling systems were placed in the holding pen (58%, n = 28) and the barn (54%, n = 26). Table 2.4 displays the types and locations of the various cooling systems used by the producers in this study. Kadzere et al. (2002) proposed that the influence of heat stress on the physiological functions of the high producing dairy cow may initiate clinical or subclinical disease, especially because disease-causing agents are better able to thrive in hot and humid environments.

**Reproduction and Genetics**

Over one-third of all the producers surveyed used both artificial insemination (AI) and a cleanup bull (40%, n = 19), followed by exclusively AI (35%, n = 17), and natural service, or herd bull, only (25%, n = 12). Thirty seven percent of producers (n = 13) used Predicted Transmitting Ability for Somatic Cell Score (PTASCS) as sire selection criteria for AI bulls.

Calving location varied among the herds surveyed and many producers selected more than one choice for this question. Fifty-six percent of producers (n = 27) provided
individual maternity pens for cows, 46% (n = 22) allowed cows to calve in a field, and 27% (n = 13) provided group housing. Use of a maternity area has been associated with lower mastitis incidence (Bartlett et al., 1992a). Generally, dairy professionals view individual calving pens as a healthier environment to calve. However, Bewley et al. (2001) cited no health differences between cows calving in individual maternity pens and those calving elsewhere. Parturition location does not seem to be as important as the management of the chosen location. Poor calving area hygiene has been associated with increased SCC (Bareille, 2000).

**Nutrition**

Of the herds included in this study, 14 producers (29%) fed organic selenium, 11 producers (23%) fed organic zinc, and 9 producers (19%) fed organic copper. Micronutrient feed supplements may help manage SCC by increasing a cow’s immune response to bacterial infections. Supplementation has traditionally occurred with inorganic sources, though recent research has indicated that organic forms may be more biologically available (Scaletti and Harmon, 2012). Scaletti and Harmon (2012) associated higher serum selenium concentrations with lower bulk tank SCC, likely due to its positive effect on multiple measures of immune function (Gerloff, 1992). Spears and Weiss (2008) explained that organic zinc may affect mammary gland health status. In an intramammary challenge with *E. coli* strain 727, Scaletti et al. (2003) reported that copper-supplemented animals had lower bacterial counts, lower SCC, lower clinical udder scores, and lower peak rectal temperatures than control animals.
CONCLUSIONS

Many factors contribute to maintaining a low SCC. Perhaps, the most interesting result from this study was that the Kentucky producers surveyed used many different management systems. This result implies that a low SCC is achievable in a wide variety of milking and housing systems as long as they are managed properly.

Kentucky producers who maintain low SCC prioritize cow cleanliness before cows ever enter the parlor. Producer responses to this survey emphasize the importance of milking clean, dry teats. In the parlor, most of these producers used pre-dip, post-dip, individual towels for drying teats, and milking gloves to minimize the spread of mastitis. Other preventive strategies implemented by many of these producers included dry cow treatment, mastitis vaccines, and regularly scheduled milking system maintenance checks. Dairy Herd Improvement Association, PCDart, and tracking bulk tank SCC proved to be important tools for frequent monitoring of SCC. These results may be used by other dairy producers and industry advisors to help dairy producers reduce SCC.

ACKNOWLEDGEMENTS

The authors would like to thank the dairy producers who completed this survey and Dairy Farmers of America (Mideast and Southeast Areas) for providing the financial support needed to complete the study. Also, DHIA (Dairy Herd Improvement Association) and the participating cooperatives played an integral role by providing the list of farms to survey. We appreciate the technical guidance and advice provided by Drs. Robert Harmon, Michelle Arnold, and Jack McAllister. Lastly, we would like to thank Randi Black, Karmella Dolecheck, Elizabeth Eckelkamp, Heather Mussel, and Barbara Wadsworth for their help in editing the manuscript.
Table 2.1. Reasons cited by producers of Kentucky herds with an average annual SCC of ≤ 250,000 cells/mL when asked to write “the single most important key to maintaining a low SCC.”

<table>
<thead>
<tr>
<th>Reason cited by producers for maintaining a low SCC</th>
<th>n</th>
<th>%1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keeping cows and facilities clean</td>
<td>31</td>
<td>65</td>
</tr>
<tr>
<td>Dry, clean bedding</td>
<td>14</td>
<td>29</td>
</tr>
<tr>
<td>Consistent milking routine</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>Forestripping</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Pre- and post-dipping</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Cow comfort</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Paying attention to detail</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Dry treating all quarters of all cows</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Preventing rather than curing</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

1Percent of Kentucky herds with an average annual SCC of ≤ 250,000 cells/mL that responded to the survey of management practices that maintain a low herd SCC.

2This question was open-ended and some producers listed multiple responses.
Table 2.2. Frequency of milk cultures collected by producers of Kentucky herds with an average annual SCC of ≤ 250,000 cells/mL.  

<table>
<thead>
<tr>
<th>Frequency of milk culturing</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rarely culture</td>
<td>18</td>
<td>38</td>
</tr>
<tr>
<td>Some cows with a high SCC</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>Some clinical cases</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>Most cows with a high SCC</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>All clinical cases</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Never culture</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Some fresh cows</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>All fresh cows</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

1Percent of Kentucky herds with an average annual SCC of ≤ 250,000 cells/mL that responded to the survey of management practices that maintain a low herd SCC.
Table 2.3. Lactating cow, dry cow, and springing heifer housing types used in herds with a SCC $\leq$ 250,000 cells/mL.

<table>
<thead>
<tr>
<th>Housing Type</th>
<th>Lactating cow</th>
<th>Dry cow</th>
<th>Springing heifer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>No housing</td>
<td>5</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>Bedded pack</td>
<td>3</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Winter housing only</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Older (10+ years old) freestall barn</td>
<td>14</td>
<td>27</td>
<td>4</td>
</tr>
<tr>
<td>Tie-stall or stanchion barn</td>
<td>10</td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td>New (&lt;10 years old) freestall barn</td>
<td>9</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>Compost bedded pack barn</td>
<td>7</td>
<td>13</td>
<td>2</td>
</tr>
</tbody>
</table>

$^1$Percent of Kentucky herds with an average annual SCC of $\leq$ 250,000 cells/mL that responded to the survey of management practices that maintain a low herd SCC.
Table 2.4. Types of cooling systems used and where they are located within each farm for Kentucky herds with an average annual SCC of $\leq 250,000$ cells/mL.$^{1}$

<table>
<thead>
<tr>
<th>Type of Cooling System</th>
<th>n</th>
<th>%$^{1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fans</td>
<td>37</td>
<td>77</td>
</tr>
<tr>
<td>Trees</td>
<td>16</td>
<td>33</td>
</tr>
<tr>
<td>Sprinklers</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>Misters</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>Shade cloths</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Tunnel ventilation</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Evaporative cooling pads</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Location of Cooling System</th>
<th>n</th>
<th>%$^{1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holding pen</td>
<td>28</td>
<td>58</td>
</tr>
<tr>
<td>Barn</td>
<td>26</td>
<td>54</td>
</tr>
<tr>
<td>Feed bunk</td>
<td>21</td>
<td>44</td>
</tr>
<tr>
<td>Alleyway</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Field</td>
<td>7</td>
<td>15</td>
</tr>
</tbody>
</table>

$^{1}$Percent of Kentucky herds with an average annual SCC of $\leq 250,000$ cells/mL that responded to the survey of management practices that maintain a low herd SCC.
CHAPTER THREE

Changes in teat end hyperkeratosis after installation of an individual quarter pulsation milking system

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INTRODUCTION

The teat end or orifice is an important first line of defense in protecting the udder from mastitis pathogen invasion (Gleeson et al., 2004). The maintenance of healthy teat skin and teat ends is a key component of an effective mastitis prevention program (Mein et al., 2001). After repeated milkings, a callous ring around the teat orifice may develop, termed hyperkeratosis (HK; Neijenhuis et al., 2001a). Hyperkeratosis refers to a histological response to chronic stimulation and is marked by an increase in the thickness of the stratum corneum, or the keratin layer of the teat end (Breen et al., 2009).

Overmilking occurs when the milk flow to the teat cistern is less than the flow out of the teat canal and can cause or exacerbate teat end HK. Rasmussen (2004) explained that breeding for high milk yield has produced cows with a higher percentage of cisternal capacity within the udder, reducing udder emptying functionality. Mechanical forces exerted by vacuum and the moving liner during machine milking impact teat end HK incidence (Neijenhuis et al., 2000; 2001a). Rasmussen (1999) proposed that the last 0.5 min of milking was the most sensitive period for developing HK because the teats were almost empty. Other factors not examined in this study that affect teat end HK include teat end shape (Gleeson et al., 2004; Neijenhuis et al., 2000), genetic predisposition (Gleeson et al., 2004), seasonal weather conditions (Mein et al., 2001), and long unit-on times (Zucali et al., 2008). Teats with unit-on times < 4.30 min were 0.29 times less likely to develop HK scores of R (rough ring) or VR (very rough ring) than teats with unit-on times > 5.30 min ($P < 0.01$), citing more liner collapses as a possible cause of high HK scores. In the same study, teats starting with a HK classification of N (no ring) were 0.38 times less likely to be scored R and VR than teats starting with a HK
classification of S (smooth raised ring; \( P < 0.01 \)). Conversely, teats that started with a HK classification of R or VR were 24.2 times more likely to end with a classification of R or VR than teats starting the experiment with a classification of S. The study results demonstrated the difficult recovery from severe HK (Zucali et al., 2008).

Dairy advisors associate more severe HK with increased clinical mastitis incidence (Neijenhuis et al., 2001b). In an 8-herd study, the odds of contracting *Streptococcus uberis* or *Escherichia coli* clinical mastitis increased significantly with increased HK (Breen et al., 2009). O’Shea (1987) proposed that changes to teat end condition resulting from overmilking might increase the likelihood of bacterial penetration into the udder. Natzke et al. (1978) determined that more new infections occurred in cows when milking units were set at a fixed removal time of 12 min (40, 27, and 15 new infections for 3 experimental repetitions) compared to cows with milking units removed at the end of milk flow (19, 20, and 12 new infections for 3 experimental repetitions). Natzke et al. (1978) concluded that overmilking may have contributed to higher mastitis infection rates, likely due to teat end condition, but the differences in infection rates between the two groups were not significant (\( P > 0.05 \)). Rasmussen (1999; 2004) explained that the reverse pressure gradient created when the vacuum in the teat cistern is higher than beneath the teat end may allow for bacterial invasion of the teat cistern, making overmilking a culprit for increased bacterial colonization at the teat end. Each increase of one teat end HK score increased the chances of intramammary infection by 30 percent in a study conducted by de Pinho Manzi et al. (2012). Hyperkeratosis scores were positively correlated with bacterial counts of the environmental pathogens *Strep. uberis, E. coli*, and other coliforms (\( r > 0.50, P < 0.01 \)), but not with
Staphylococcus aureus (Paduch et al., 2012). Rough teat ends may provide a site for bacterial colonization because they may be more difficult to clean during pre-milking preparation (Zucali et al., 2008). However, Shearn and Hillerton (1996) failed to identify a significant relationship between SCC and degree of HK at the herd level.

Individual quarter pulsation milking systems may prevent overmilking and improve teat end condition (Neijenhuis et al., 2000). Automated milking systems often incorporate individual quarter pulsation, but research on this feature in a conventional parlor is lacking. Automated milking systems have not been heavily adopted in the US because of the availability of inexpensive labor relative to other countries (Jacobs and Siegförd, 2012). The objective of this research was to examine changes in teat end HK in a herd transitioning from a standard pulsation system to an individual quarter pulsation milking system.

MATERIALS AND METHODS

This study was conducted at the University of Kentucky Coldstream Dairy from April to June 2011. Teat end HK was evaluated by the primary author immediately after cluster removal using the scoring system outlined by Mein et al. (2001) where N signifies no ring; S signifies a smooth, raised ring; R signifies a rough ring; and VR signifies a very rough ring. Scoring periods were classified relative to installation (April 28, 2011) of the Milpro P4C™ (Milkline, Gariga di Podenzano, Italy) system as follows: PRE1-April 7; PRE2-April 21; POST1-May 12; POST2-May 26; POST3-June 9.

Ambient weather conditions were recorded at 1 h intervals at the Lexington Bluegrass Airport (14.2 km from the study site). Temperature humidity index (THI) was
computed using the following formula (NOAA, 1976): \[ \text{THI} = \text{Temperature (°F)} - (0.55 - (0.55 \times \text{Relative Humidity}/100)) \times (\text{Temperature (°F)} - 58.8). \]

Cows were milked twice daily before and after installation of the new system. Milking routine pre- and post-installation included forestripping, predipping, drying teats with individual paper towels, unit attachment, automatic takeoff, and postdipping. No changes were made to the milking routine after installation. The herd was divided into two balanced groups based on parity, breed, and size for a separate research project throughout the entire study period. Each group was milked in one of two double-two bypass parlors, located in the same building. High lines were used before and after installation. Vacuum pressure was adjusted to manufacturer recommendations after installation of the new system.

Before installation, cows were milked using Surge\textsuperscript{TM} Eclipse\textsuperscript{TM} claws with 06 shells, 1st Choice\textsuperscript{TM} triangular inflations, and Surge\textsuperscript{TM} Omni\textsuperscript{TM} cylinder detachers with optical sensors (GEA Farm Technologies, Inc., Naperville, IL). The Milpro P4C\textsuperscript{TM} (Milkline, Gariga di Podenzano, Italy) system equipped with silicone liners, stops milking individual quarters based on flow rates, and has a unique individual quarter pulsation milking system with four pulsation channels instead of two. Pulsation ratio and frequency are variable and driven by the milk flow in the Milpro P4C\textsuperscript{TM} (Milkline, Gariga di Podenzano, Italy) system. As the flow increases, the length of the milking phase (A+B, where A is the opening phase and B is the milking phase) increases while the rest phase (C+D, where C is the closing phase and D is the massage phase) remains constant and the pulsation rate slows down. When milk flow is below 1.2 kg/min, the pulsation ratio is 60:40 and the pulsation rate is 60 pulses/min. The milking phase (A+B)
increases dynamically when milk flow is between 1.2 and 7.5 kg/min, while the rest phase remains at 400 milliseconds. Although the milking system used in this study detects individual quarter milk flow and stops milking individual quarters when low flow is detected, pulsation still occurs every 15 s and the liner remains in a massage phase between pulses. This process may reduce overmilking, acting on each quarter individually.

Teat end HK classifications were evaluated for 109 cows during the study. Only cows with classifications available for the entire study period were included in the final analysis to ensure that HK data was available before installation and that each cow had the full length of time to experience the effects of the individual quarter pulsation milking system. Forty cows were removed, leaving 69 cows (48 Holstein, 12 crossbred, and 9 Jersey) in the final analysis. Teat end HK classifications were converted to numerical values progressing from most desirable to least desirable as follows: \( N = 1; \ S = 2; \ R = 3; \ VR = 4. \) When a given HK score differed from preceding and subsequent scores by more than \( \pm 1, \) that score was removed from the data set to account for the subjectivity of the scoring system.

Score frequencies were calculated by scoring period using the FREQ Procedure of SAS® version 9.3 (SAS Institute, Inc., Cary, NC). The MIXED Procedure of SAS® (SAS Institute, Inc., Cary, NC) was used to evaluate fixed effects of breed, parity, teat position, and all interactions on teat end HK score. Variables were repeated by scoring with cow nested within breed as subject. All main effects were kept in each model regardless of significance level. Stepwise backward elimination was used to remove non-
RESULTS AND DISCUSSION

Mean daily milk production, parity, DIM, and DHIA-based somatic cell score for the herd were 34.31 ± 9.92 kg, 2.30 ± 1.24 lactations, 153.38 ± 90.10 days, and 1.66 ± 1.24, respectively. Average daily temperature humidity indexes were 63.66, 66.41, 60.83, 63.21, and 60.15 for PRE1, PRE2, POST1, POST2, POST3 scoring periods, respectively. Mean THI was 63.34 for the entire study period. Since ambient weather conditions were mild and stable during the study, the likelihood of weather influences on teat end HK were negligible. The timing of changes in teat end HK observed in this study was consistent with the 2 to 8 weeks necessary for a change in teat HK described by Mein et al. (2001) and Gleeson et al. (2004).

The frequency of the more desirable teat end condition classification N increased after the installation of the individual quarter pulsation milking system from 134 to 157 from PRE2 to POST3 (Table 1). The frequency of teat end HK classification R decreased from 3.6% at PRE2 to 1.4% at POST3 while the frequency of teat end HK classification of R remained constant throughout the study (3.2% at PRE2, POST1, and POST3). This may be a reflection of teat end shape, genetics, or the difficulty in recovering from severe teat end HK where teats classified as VR could only improve to a classification of R while classifications of R improved further.

Teat position and scoring period × breed had significant effects on teat end HK score (Table 3.1; \(P < 0.01\)). Scoring period, breed, and parity did not have significant effects on teat end HK score \((P > 0.05)\). The parity effect observed in this study
contrasted results from Mein et al. (2001) who concluded that HK scores increased with parity, possibly a result of udder and teat size changes with increasing parity. Seykora and McDaniel (1986) explained that teat length and diameter increased by approximately 10% from first to fourth lactations whereas udder depth increased by 20%.

Holstein HK scores improved from PRE2 to POST3 (Table 3.2; 1.63 ± 0.10 and 1.42 ± 0.10, respectively, \( P < 0.01 \) and POST2 to POST3 (1.53 ± 0.10 and 1.42 ± 0.10, respectively, \( P < 0.05 \)). These results indicate an improvement in teat end HK scores after the installation of an individual quarter pulsation milking system. However, a significant decrease in teat end HK scores for Holsteins did occur from PRE1 to PRE2 (1.75 ± 0.10 and 1.63 ± 0.10, respectively, \( P = 0.04 \)) and may represent biological variation in teat end HK over time unrelated to the installation of the individual quarter pulsation system.

Crossbred teat end HK scores did not differ among scoring periods (\( P > 0.05 \)). Jersey teat end HK scores increased from POST1 to POST3 (1.32 ± 0.21 and 1.63 ± 0.21, respectively, \( P = 0.05 \)). Teat end HK increased for Jersey cows and did not improve for crossbred cows, possibly reflecting differences in genetic predisposition or teat end shape, which were not evaluated in this study. Teat end shape was moderately to highly heritable in a study of 1740 Holstein cows in 9 herds with heritability estimates of 0.54, 0.44, and 0.56 for first, second, and all lactations, respectively (Chrystal et al., 1999). Seykora and McDaniel (1985) reported similar heritability estimates. The same study explained that lesion score (where 1 represented a smooth, raised ring and 7 represented an “ulcerated or raw appearing orifice”) decreased as teat end shape score (using a continuous scale of 1 to 50 where each increment of 10 represented pointed, round, flat,
disk, and inverted or cone-shaped teats, respectively) increased. However, teat end shape is likely influenced by the environment and multiple genes (Chrystal et al., 1999). Within the Holstein breed, teat end shape did not vary greatly with 90% of teats classified as cylindrical (Seykora and McDaniel, 1985). However, the authors are unaware of any studies comparing teat end shape between multiple breeds so a direct comparison to the differences in teat end HK found in this study is not possible. In this multiple breed herd, liners and equipment settings are set for Holstein cows and may not be ideal for Jersey and crossbred cows. Differences between breeds could also be a result of the flow-dependent pulsation ratio and frequency in the new system, which could affect unit on-times. However, data on pulsation is not recorded within the system and was beyond the focus of the hypothesis to evaluate independently.

For all cows over all scoring periods, right front HK scores were higher than right rear and left rear HK scores (1.58 ± 0.20, 1.37 ± 0.15, and 1.36 ± 0.15, respectively, \( P < 0.01 \)) and left front HK scores were higher than right rear and left rear HK scores (1.62 ± 0.38, 1.37 ± 0.15, and 1.36 ± 0.15, respectively, \( P < 0.01 \)). For Holstein cows only, teat end HK scores were higher for right front than left rear and right rear HK scores in PRE1 (Table 4; 1.83 ± 0.16, 1.52 ± 0.16, and 1.46 ± 0.16, respectively, \( P < 0.04 \)). Teat end HK scores for Holstein cows were higher for left front and right front than left rear in PRE2 (1.75 ± 0.14, 1.75 ± 0.14, and 1.40 ± 0.14, respectively, \( P < 0.01 \)). Neijenhuis et al. (2000) reported similar results. Only 40 percent of milk resides in the front quarters, making them more susceptible to overmilking. No significant change between front and rear teat end HK was observed after the installation of the individual quarter pulsation milking system and front teat HK scores improved more than rear teat HK scores over the
study period. This result may signify that individual quarter pulsation may stop milking front teats before rear teats when necessary, which would prevent the overmilking that results from the milk imbalance of the udder halves.

Although the improvement in HK shown in this study can be attributed to the individual quarter pulsation system, it is possible that some changes were a result of the different inflations and the flow-dependent pulsation ratio and frequency in the new system. It was not possible to differentiate the changes caused by the individual quarter pulsation and the new liners or differing pulsation ratio and frequency.

**CONCLUSIONS**

Holstein teat end HK scores decreased after installation of an individual quarter pulsation milking system, which may reflect decreased overmilking. The results of this study indicate that individual quarter pulsation milking systems may decrease overmilking and reduce teat end HK in Holstein cows, in agreement with Neijenhuis et al. (2000). Teat end HK did not improve in crossbred cows and actually increased in Jersey cows over the study period. Differences between breeds may be attributed to differing teat size and shape between breeds. This multi-breed herd may have included too few cow numbers for Jerseys (n = 9) and crossbreds (n = 12) to determine conclusions on the effects of individual quarter pulsation on teat end HK for these particular breeds. Additional research is needed to determine if these results are applicable to varying herd conditions and larger herd numbers, and to understand the breed differences noted in this study.

Changes in teat end HK are not immediate and should be monitored over months. Though there are multiple factors influencing teat end health, milking systems play an
integral part in maintaining smooth teat ends free of hyperkeratosis. Decreasing the incidence and severity of teat end hyperkeratosis may decrease mastitis incidence (Neave et al., 1969; Breen et al., 2009; de Pinho Manzi et al., 2012). Therefore, individual quarter pulsation milking systems may be beneficial to dairy producers and dairy cattle.

ACKNOWLEDGEMENTS

The authors extend our thanks to Milkline for donating the Milpro P4C milking system to the University of Kentucky. We appreciate the technical guidance and advice provided by Massimiliano Intini, Marco Cattaneo, and Pierandrea Conigliaro. We would also like to thank Barbara Wadsworth for her help calibrating the system. Finally, we express our extreme gratitude to Joey Clark, University of Kentucky Coldstream dairy manager, for maintaining the system.
Table 3.1. Teat end hyperkeratosis classification frequency by teat scoring period before and after the installation of an individual quarter pulsation milking system\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>Score</th>
<th>PRE1</th>
<th>PRE2</th>
<th>POST1</th>
<th>POST2</th>
<th>POST3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>60.9%</td>
<td>60.9%</td>
<td>65.9%</td>
<td>67.3%</td>
<td>71.4%</td>
</tr>
<tr>
<td></td>
<td>(n = 134)</td>
<td>(n = 134)</td>
<td>(n = 145)</td>
<td>(n = 148)</td>
<td>(n = 157)</td>
</tr>
<tr>
<td>S</td>
<td>28.6%</td>
<td>30.0%</td>
<td>27.7%</td>
<td>25.9%</td>
<td>24.1%</td>
</tr>
<tr>
<td></td>
<td>(n = 63)</td>
<td>(n = 66)</td>
<td>(n = 61)</td>
<td>(n = 57)</td>
<td>(n = 53)</td>
</tr>
<tr>
<td>R</td>
<td>3.6%</td>
<td>5.9%</td>
<td>3.2%</td>
<td>4.5%</td>
<td>1.4%</td>
</tr>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 13)</td>
<td>(n = 7)</td>
<td>(n = 10)</td>
<td>(n = 3)</td>
</tr>
<tr>
<td>VR</td>
<td>6.8%</td>
<td>3.2%</td>
<td>3.2%</td>
<td>2.3%</td>
<td>3.2%</td>
</tr>
<tr>
<td></td>
<td>(n = 15)</td>
<td>(n = 7)</td>
<td>(n = 7)</td>
<td>(n = 5)</td>
<td>(n = 7)</td>
</tr>
</tbody>
</table>

\textsuperscript{1}N signifies no ring; S signifies a smooth, raised ring; R signifies a rough ring; and VR signifies a very rough ring.

\textsuperscript{2}PRE1 and PRE2 refer to teat end condition observation periods using a standard pulsation milking system while POST1, POST2, and POST3 refer to teat end condition observation periods using an individual quarter pulsation milking system.
Table 3.2. Type III tests of fixed effects for teat end hyperkeratosis score mixed model relative to the installation of an individual quarter pulsation milking system\(^1\).  
\(^2\), \(^3\), \(^4\)

<table>
<thead>
<tr>
<th>Source</th>
<th>Numerator DF</th>
<th>Denominator DF</th>
<th>F value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teat position(^1)</td>
<td>3</td>
<td>171</td>
<td>7.84</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Scoring period(^2)</td>
<td>4</td>
<td>264</td>
<td>0.33</td>
<td>0.86</td>
</tr>
<tr>
<td>Breed(^3)</td>
<td>2</td>
<td>65</td>
<td>1.56</td>
<td>0.22</td>
</tr>
<tr>
<td>Parity(^4)</td>
<td>1</td>
<td>65</td>
<td>0.01</td>
<td>0.91</td>
</tr>
<tr>
<td>Scoring period  x breed</td>
<td>8</td>
<td>264</td>
<td>3.03</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

\(^1\)Teat position: left front, right front, left rear, right rear.

\(^2\)Scoring period: PRE1 and PRE2 refer to teat end condition observation periods using a standard milking system while POST1, POST2, and POST3 refer to teat end condition observation periods using an individual quarter pulsation milking system.

\(^3\)Breed: Holstein, Jersey, or crossbred.

\(^4\)Parity: 1 or ≥ 2.
Table 3.3. Least squares mean (± SE) HK scores within teat scoring period relative to the installation of an individual quarter pulsation milking system $^{1,2,3}$

<table>
<thead>
<tr>
<th>Breed</th>
<th>PRE1</th>
<th>PRE2</th>
<th>POST1</th>
<th>POST2</th>
<th>POST3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holstein</td>
<td>1.75 ± 0.10</td>
<td>a</td>
<td>1.63 ± 0.10</td>
<td>b</td>
<td>1.59 ± 0.10</td>
</tr>
<tr>
<td>Jersey</td>
<td>1.40 ± 0.21</td>
<td>ab</td>
<td>1.34 ± 0.21</td>
<td>ab</td>
<td>1.32 ± 0.21</td>
</tr>
<tr>
<td>Crossbred</td>
<td>1.35 ± 0.17</td>
<td>a</td>
<td>1.47 ± 0.17</td>
<td>a</td>
<td>1.40 ± 0.17</td>
</tr>
</tbody>
</table>

$^1$Least squares means within rows with different superscripts differ ($P < 0.05$).

$^2$1 (N) signifies no ring; 2 (S) signifies a smooth, raised ring; 3 (R) signifies a rough ring; and 4 (VR) signifies a very rough ring.

$^3$PRE1 and PRE2 refer to teat end condition observation periods using a standard pulsation milking system while POST1, POST2, and POST3 refer to teat end condition observation periods using an individual quarter pulsation milking system.
Table 3.4. Least squares mean (± SE) HK scores of Holstein teat end HK score by teat position relative to the installation of an individual quarter pulsation milking system.1,2

<table>
<thead>
<tr>
<th>Teat Position</th>
<th>Teat end HK scores (± SE)</th>
<th>Left Front</th>
<th>Left Rear</th>
<th>Right Front</th>
<th>Right Rear</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE1</td>
<td>1.80 ± 0.16&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.52 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.83 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.46 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>PRE2</td>
<td>1.75 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.40 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.75 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.51 ± 0.14&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>POST1</td>
<td>1.82 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.61 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.62 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.49 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>POST2</td>
<td>1.64 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.48 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.58 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.59 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>POST3</td>
<td>1.53 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.34 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.40 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.30 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>All scorings</td>
<td>1.71 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.45 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.63 ± 0.09&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.45 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

1Least squares means within rows with different superscripts differ ($P < 0.05$).

21 (N) signifies no ring; 2 (S) signifies a smooth, raised ring; 3 (R) signifies a rough ring; and 4 (VR) signifies a very rough ring.
CHAPTER FOUR

Milk yield, reticulorumen temperature, rumination time, and neck activity changes around clinical and subclinical mastitis events

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†Department of Statistics, University of Kentucky, Lexington 40546
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INTRODUCTION

Dairy industry personnel generally accept that economic losses resulting from mastitis are sizable (Beck et al., 1992, Blosser, 1979, Hogeveen et al., 2011). Recent clinical mastitis cost estimates range from $163 to $179 per case (Bar et al., 2008, Bewley, 2010). Economic losses from subclinical and clinical mastitis include decreased milk production, treatment, increased labor, discarded milk, veterinary fees, drug costs, reduced milk price through lost bonuses, increased risk of subsequent mastitis, and increased risk of culling or death (Hansson et al., 2011, Nielen et al., 1992, Nielen et al., 1995).

Dairy cattle economic efficiency is closely related to milk yield (Dohoo and Wayne Martin, 1984). Mastitis has a long lasting negative effect on milk yield (Rajala-Schultz et al., 1999). Even after an infection is cured, milk yield remains depressed (Bar et al., 2008) and cows may be unable to reach their pre-mastitis milk yield (Rajala-Schultz et al., 1999). However, because a milk yield decrease can indicate a health problem, producers can use milk yield recording as a mastitis detection aid (Leslie and Petersson-Wolfe, 2012, Lukas et al., 2009).

Today’s non-automated mastitis detection approach involves observing inflammation through visual milk observation and udder palpation. However, an earlier diagnosis of clinical mastitis may reduce production losses, enhance recovery prospects (Milner et al., 1996), and improve animal welfare (AlZahal et al., 2009). Proactive treatments may also decrease antibiotic use, which decreases the chances of antibiotic residues in the bulk tank (Oliver and Murinda, 2012). Automated dairy cattle behavioral and physiological monitoring systems may be useful for early mastitis detection.
Currently, behavioral and physiological mastitis monitoring systems include sensors that monitor body temperature (reticulorumen, vaginal, Eustachian tube, and ear skin, and image-based thermography temperature sensors), rumination time, milk parameters (yield, electrical conductivity, lactose, lactate dehydrogenase, blood, color, and SCC), and activity (movement and standing or lying time). Each of these parameters has mastitis detection potential because mastitis can affect dairy cattle behavior and physiology.

Fever, or a body temperature over a predefined threshold, is an indicator of disease (Burfeind et al., 2010, Leon, 2002). Passive reticulorumen temperature monitoring systems record temperatures when a cow walks past a reader, usually upon entry into or exit from the milking parlor. Technology failure could pose problems for reticulorumen temperature monitoring systems (Small et al., 2008), but the substantial and sustained decrease in reticulorumen temperature after drinking water is the main limitation (Bewley et al., 2008, Cunningham et al., 1964, Rogers Simmons et al., 1965). Other limitations include sensor drift, sensor placement, and missed readings. Nonetheless, reticulorumen temperature monitoring may hold potential for mastitis detection. In an *E. coli* mastitis challenge, reticulorumen temperature peaked between 40.5 and 41.0°C and remained above 40.0°C for 2 h post-challenge (AlZahal et al., 2011). Siivonen et al. (2011) also conducted a mastitis challenge study and discovered that rectal temperatures started to increase 4 to 6 h post-infusion and remained above 39.2°C from 6 to 10 h post-infusion, returning to pre-challenge temperatures within 12 h post-infusion.
AlZahal et al. (2011) observed that cows challenged with *E. coli* mastitis consumed 23% less feed than control cows. Additionally, infected cows stopped consuming feed when their rumen temperatures peaked (40.0°C) from 3 to 4 h post-challenge. Although feed intake is difficult to monitor, decreased feed intake or increased feeding time may negatively affect rumination time (Schirmann et al., 2012), which can be monitored.

Canadian researchers validated the Hi-Tag™ (SCR Engineers Ltd., Netanya, Israel) automated rumination monitoring system and showed that the recorded data was highly correlated with human-observed rumination time (R = 0.93) (Schirmann et al., 2009). Several researchers have conducted *E. coli* mastitis challenge studies that demonstrated a rumination time decrease post-challenge (Fitzpatrick et al., 2013, Fogsgaard et al., 2012, Siivonen et al., 2011). In an *E. coli* challenge with 20 cows, rumination time decreased ($P < 0.05$) on the day of the challenge and gradually increased to pre-challenge levels during the following 2 d (Fogsgaard et al., 2012). Neck activity has been monitored to predict lameness (Van Hertem et al., 2013) and estrus (Aungier et al., 2012, Kamphuis et al., 2012, Neves et al., 2012), but to the knowledge of the authors, has not been examined for mastitis detection.

Automated physiological and behavioral monitoring systems have been evaluated for estrus (Holman et al., 2011, Jónsson et al., 2011), lameness (Ito et al., 2010, Kramer et al., 2009, Van Hertem et al., 2013), and mastitis detection (de Mol and Ouweltjes, 2001, Fitzpatrick et al., 2013, Kramer et al., 2009). The objective of this study was to evaluate variation in reticulorumen temperature (**RT**), rumination time (**RU**), neck activity (**NA**), and milk yield (**MY**) around clinical and subclinical mastitis events.
MATERIALS AND METHODS

This study was conducted at the University of Kentucky Coldstream Dairy in Lexington, KY from September 15, 2011 to May 15, 2013. The lactating herd was divided into 2 groups balanced by parity, cow volume (length x width x height), and DIM for a separate research study. Both groups were housed in freestalls, one with sawdust-covered Dual Chamber Cow Waterbeds (Advanced Comfort Technology, Inc., Reedburg, WI) and the other with sawdust-covered rubber-filled PastureMat® (Promat, Ontario, Canada) mattresses. Cows shared a feed bunk, accessible from both pens. The lactating herd was fed a TMR of corn silage, alfalfa hay, alfalfa silage, whole cottonseed, and grain mix daily at 5:30 and 13:30. Cows were allowed pasture access for about 1 h/d at 10:00, weather permitting. Each group was milked 2X, at 04:30 and 15:30, in one of two double-two bypass parlors located in the same building. The milking routine included forestripping, pre-dipping with 0.5% iodine, drying teats with individual paper towels, unit attachment, automatic takeoff, and post-dipping with 1% iodine.

The herd manager determined all treatment decisions and was not asked to alter these decisions during the study. The herd manager was blind to all RT, RU, and NA data and to all bacteriological culture and SCC results. Milk yield data was available to the herd manager and milkers because this information was available pre-study.

Milk Sampling

To classify subclinical mastitis, a 90 mL composite milk sample was obtained in a clear polypropylene resin vial (Capitol Vial, Thermo Fisher Scientific, Hudson, New Hampshire) from every cow in the herd every 2 wk. Somatic cell count analysis (Fossomatic™ FC somatic cell counter, Foss, Hilleroed, Denmark) was conducted at the
Subclinical mastitis was defined as SCC > 200,000 cells/mL from the composite milk samples. All four quarters were sampled from cows with subclinical mastitis and the individual quarter SCC samples were taken 3 d later to determine which quarters were infected. An infected quarter was classified as a SCC > 50,000 cells/mL from the individual quarter SCC analysis. Uninfected quarters were removed from all further analyses.

Clinical mastitis was identified and recorded at each milking by visual and tactile assessment of milk (flakes, clots, or serous milk) and the udder (red, hard, swollen). If clinical signs were displayed in any quarter within 2 wk of the initial clinical diagnosis, the case was considered the same clinical case. Individual quarter milk samples were obtained for bacteriological and SCC evaluation from the affected quarter(s) at the first notice of clinical mastitis signs, before any antibiotic treatment was administered.

Individual quarter milk samples for bacteriological analysis were obtained following the procedure described by Hogan et al. (1999). After forestripping and pre-dipping, teat ends were cleaned with cotton balls soaked in 70% ethyl alcohol. About 10 mL of milk from each quarter was stripped into an individual sterile polypropylene test tube (Falcon®, Corning Life Sciences, Corning, NY). Individual quarter milk samples were frozen immediately after milking and delivered to a University of Kentucky laboratory for bacteriological analysis 5 d later. In the lab, individual quarter milk samples were thawed and 10 µL of each quarter sample were aseptically obtained from each tube and plated onto one quadrant of a Difco™ (BD Diagnostic Systems, Detroit, MI) Columbia blood esculin agar plate with 5% calf’s blood, which was collected aseptically from calves at the University of Kentucky Coldstream Dairy. Duplicates of
each individual quarter milk sample were plated to verify results. Plates were incubated at 37°C and bacterial growth was observed 48 h later. Bacteria on the primary culture medium were identified tentatively according to colony morphology and hemolytic characteristics. Isolates considered causative mastitis agents were placed in brain-heart-infusion broth and incubated at 37°C for 24 h. Ten µL of each broth was then heat-fixed to a microscope slide and Gram stained. Gram staining was conducted by drenching each slide in crystal violet for 1 min, Gram’s iodine for 1 min, alcohol for 30 s, and safranin for 30 s. Between drenches, slides were rinsed and blotted with bibulous paper. Slides were examined under a microscope and isolates identified as Gram-negative rods and streptococci were further evaluated by Vitek 2 Compact (bioMérieux, Durham, NC). Isolates identified presumptively as staphylococci were subsequently tested for coagulase activity (positive or negative) by the tube coagulase test using BBL™ coagulase rabbit plasma with ethylenediaminetetraacetic acid (BD Diagnostic Systems, Detroit, MI). Coagulase-positive staphylococci were considered Staphylococcus aureus. Samples with negative coagulase-status were considered coagulase negative staphylococci. Isolates identified as yeast or coryneform were not confirmed beyond microscopic identification.

**Precision Dairy Technologies**

The Milpro P4CTM (Milkline, Gariga di Podenzano, Italy) milking system measured MY, based on flow rate, per cow at each milking. This system was calibrated every 3 months by recording about 10 yields from both the automated system and the milk weigh jars at each milking stall for five milkings. After each milking where weights were recorded, the weigh jar weight was divided by the automated weight and multiplied by 1000, as per company recommendations. Using a Palm Pilot (Palm Inc., Sunnyvale,
California), each milking unit was updated with the calculated bias obtained from each milking. The DVM Systems, LLC (Boulder, CO) bolus system monitored RT using a passive RFID transponder (Phase IV Engineering, Inc., Boulder, CO) equipped with a temperature sensor queried twice daily by a panel reader placed in parlor entrances. HR Tags™ (SCR Engineers Ltd., Netanya, Israel) measured NA with a 3-axis accelerometer and RU with a microphone and microprocessor, summarized into 2 h time blocks. All heifers received a DVM bolus and HR Tag™ at least 2 wk before calving. HR Tag™s were only removed from cows if they quit reading in and needed to be replaced, if a cow was culled, or if a cow was ≥ 400 DIM. DVM boluses remained in the reticulorumen for the lifetime of each cow and were not recollected or reused after a cow was culled.

**Data Editing and Analysis**

General cow demographic information was obtained from PCDart (Dairy Records Management Systems, Raleigh, NC) records. All data were analyzed using SAS® version 9.3 (SAS Institute, Cary, NC). Daily cow mean RU, NA, and MY and per milking RT were used for all analyses.

Reticulorumen temperature, RU, NA, and MY were removed the d before, of, and after an estrus or breeding event, based on observations from the herd manager or farm staff. Reticulorumen temperature, RU, NA, and MY were removed the d of and 7 d before and after a metritis event, recorded by the researchers for a separate research study. Reticulorumen temperature, RU, NA, and MY were removed when DIM ≤ 14 or ≥ 400. A minimum of 7 d of RT, RU, NA, and MY data were required to establish a baseline, defined as a backward rolling mean of RT, RU, NA, and MY for each cow before a mastitis event.
Human and technology error resulted in the milking system not recognizing cows during 13% of cow milkings. Therefore, MY data were edited by removing all MY equal to 0. Milk yield data were also removed if the MY from that d divided by the cow’s 7 d backward moving rolling mean baseline was > 0.5 because this was likely the result of system misidentification. Raw RT data were edited to remove RT potentially influenced by water intake by removing RT with Z-scores < -3 from the cow’s 7 d backward rolling mean baseline. Raw RU and NA data were removed when equal to 0, when the reader did not recognize the cows. Rumination time and NA data were further edited to remove values < the 1st percentile or > the 99th percentile of the entire data set, determined by the UNIVARIATE procedure of SAS® version 9.3 (SAS Institute, Cary, NC).

Somatic cell score (SCS) per herd composite milk sampling period was calculated from SCC using the following equation:

\[ \text{SCS} = \log_2 \left( \frac{\text{SCC}}{100,000} \right) + 3 \] (Eq. 4.1)

A Z-score analysis was conducted using a backward rolling mean baseline from d -10 to d -4, where d 0 represented the day of clinical or subclinical mastitis detection, to examine the number of SD from which each respective RT, RU, NA, and MY varied from this baseline. Z-scores were calculated by subtracting each cow’s 7 d backward rolling mean baseline RT, RU, NA, and MY from the RT, RU, NA, and MY obtained each day and dividing by the standard deviation. Because RU, NA, and MY were expected to decrease around mastitis, -2 and -3 Z-score thresholds were used to signal alerts to calculate the percentage of data that breached the threshold over 3 alert windows (d -1, -2, and -3), or days relative to mastitis in which the decrease could occur. An example of a -2 RU Z-score threshold breach is displayed in Figure 1.
Reticulorumen temperature was expected to increase around mastitis so 2 and 3 Z-score thresholds were used to signal RT alerts to calculate the percentage of data that breached the threshold over 3 alert windows (2, 3, and 4 milkings). Dairy cow body temperature is higher during late night and early morning compared to morning and early afternoon because of diurnal variation (Bitman et al., 1984, Lefcourt and Adams, 1998), which can vary temperatures by 1°C (Lefcourt and Adams, 1998). To account for diurnal variation, alert windows included only AM or PM RT based on whether clinical mastitis was observed by the milkers at an AM or PM milking. Four RT thresholds (40.3, 40.0, 39.7, and 39.4°C) were also evaluated as alert levels to determine the percentage of data that breached the threshold of the RT monitoring system using the maximum RT over 2 and 3 milking (AM or PM) alert windows. The four RT thresholds were chosen because maximum RT in a healthy cow is 39.4°C (Benzaquen et al., 2007). Because RU, NA, and MY are not as well-understood as RT, strict thresholds were not evaluated.

The MEANS procedure of SAS was used to determine the mean Z-score of RT, RU, NA, and MY. The absolute value of each RU, RT, NA, and MY Z-score were calculated and the MEANS procedure of SAS was used to determine the mean Z-score of all variables combined combined.

**RESULTS AND DISCUSSION**

Mean DIM was 226.6 ± 142.1, 160.9 ± 141.3, and 215.1 ± 142.8 d for subclinical, clinical, and mastitis-free cows, respectively. Mean parity was 2.2 ± 1.3, 2.4 ± 1.6, and 2.2 ± 1.2 lactations for subclinical, clinical, and mastitis-free cows, respectively. Mean daily MY was 24.6 ± 9.6, 24.8 ± 9.2, and 27.8 ± 8.9 kg for subclinical, clinical, and
 mastitis-free cows, respectively. Mean daily NA was 316.6 ± 80.4, 339.4 ± 102.4, and 326.9 ± 86.5 for subclinical, clinical, and mastitis-free cows, respectively.

Mean daily RT was 38.7 ± 0.8, 38.8 ± 1.0, and 38.7 ± 0.8°C for subclinical, clinical, and cows without mastitis, respectively. This result was consistent with what has been described as average cow RT 38.6 °C to 39.2 °C reported by Piccione and Refinetti (2003). Rumen microorganisms produce heat, making RT about 0.5°C greater than core body temperatures (Bewley et al., 2008).

Mean daily RU in this study was 6.3 ± 1.7, 6.4 ± 1.7, and 6.4 ± 1.6 h for subclinical, clinical, and mastitis-free cows, respectively. Mean RU in this study was less than the 6.9 to 9.2 h cited by Fitzpatrick et al. (2013), Reith and Hoy (2012), and Gregorini et al. (2012), who also evaluated RU with HR Tags™ (SCR Engineers Ltd., Netanya, Israel). In contrast to Fitzpatrick et al. (2013), who explained that multiparous cows experienced longer RU than primiparous cows, no RU difference was observed between primiparous and multiparous cows (6.6 ± 1.6 and 6.2 ± 1.6 h/d for primiparous and multiparous cows, respectively; $P = 0.60$) in this study. Differences in diet (Beauchemin et al., 1994) may explain differences in mean daily RU between studies.

**Somatic Cell Count Trends**

Overall, 3,098 composite milk samples were collected throughout the study for SCC evaluation. Mean percentage of cows with SCC > 200,000 cells/mL (SCS >4) per sampling period are displayed in Table 4.1. Overall, mean percentage of cows with SCC > 200,000 cells/mL was 29.1% during the study period, comparable to the 23.1% prevalence cited by Barkema et al. (1997) using SCC ≥ 250,000 cells/mL.
LSMeans for SCS by herd composite milk sampling date are also displayed in Table 4.1. The highest LSMeans SCS occurred in mid-August (6.35 ± 0.39), likely related to increased ambient temperature humidity index (THI), which can increase oxidative stress and decrease immune function (Hammami et al., 2013). Not surprisingly, LSMeans SCS from January 30, 2012, February 14 and 27, 2012, March 12, 2012, and November 7, 2012 differed from August 13, 2012 (P < 0.05). The lowest SCS occurred in April 2012 (3.79 ± 0.54). Interestingly, LSMeans SCS on April 9, 2012 and May 7 and 21, 2012 differed from August 13, 2012 (P < 0.01, P = 0.02, and P = 0.01 for April 9, May 7, and May 21, respectively). This result was unexpected because THI is also high at those times, but SCC may not be affected by THI that quickly. A lesser percentage of cows with subclinical mastitis (15.1 and 22.9% for April and May, respectively) also occurred on the April 9 and May 7 sampling dates compared to all other sampling dates.

Pathogens Isolated

The percentage of bacteriological samples that resulted in no bacteria growth (Tables 4.2 and 4.3 for clinical and subclinical mastitis cases, respectively) was similar to results obtained by Schreiner and Ruegg (2003), but greater than Bartlett et al. (1992), Makovec and Ruegg (2003), and Sargeant et al. (1998). Reasons for no growth cultures also include pathogens not growing in standard culture media, milk contamination decreasing the viability of the mastitis-causing bacteria in culture (Taponen et al., 2009), and low presence or non-shedding bacteria in the sample (Sears et al., 1990). Still, inability to isolate a pathogen from the samples may be because the period with the largest milk bacteria load may have been missed. If physiological or behavioral changes
occur that can be detected by the technologies studied, they likely would occur during the acute phase of the infection. Unfortunately, this may not coincide with when the cow shows clinical signs. Because herd composite milk sampling for subclinical mastitis detection occurred every 2 wk, cows were likely at different stages of infection when sampled and may have been sampled at the end of the infection.

*Staph aureus* and coagulase negative staphylococci (CNS) were common isolates in subclinical mastitis cases (14% and 15% of total pathogens isolated in subclinical mastitis cases for *Staph aureus* and CNS, respectively, Table 4.3), consistent with the concept that mastitis caused by *Staph aureus* is usually subclinical and chronic (Harmon, 1994). Fernando et al. (1985) isolated coagulase-negative staphylococci from 45% of infected quarters and Belgian researchers reported 6% CNS prevalence on a cohort of ten clinically healthy cows from each of six study herds (Piessens et al., 2011). Although both *Staph aureus* and coagulase-negative staphylococci can affect SCC milk production, they may not affect the cow physiologically.

Surprisingly, environmental mastitis pathogens were not isolated from any clinical or subclinical quarters. Seventy to 85% of intramammary infections caused by coliforms become clinical (Harmon, 1994, Hogan and Smith, 2012). Scaletti and Harmon (2012) explained that *E. coli* counts in milk and clinical symptoms (using visual milk observation) were observed in challenged cows 12 h post-challenge, but then declined. Because coliform infections are short-lived, the most severe infection window may have been missed when clinical or subclinical samples were obtained, which may have resulted in no growth.
**Z-Score Results for Clinical and Subclinical Mastitis Cases**

The Z-score analysis included 776 subclinical cases (534 Holstein, 151 crossbred, and 91 Jersey cows) and 108 clinical mastitis cases (74 Holstein, 24 crossbred, and 10 Jersey cows). The mean Z-score of RU, RT, NA, and MY combined was greater for subclinical, clinical, and no mastitis cases compared to each variable alone (Table 4.4). The Z-scores using the mean of all variables combined were 0.88 ± 0.35, 0.94 ± 0.41, and 0.83 ± 0.38 for subclinical, clinical, and no mastitis, respectively. The next greatest mean Z-scores were obtained using MY with 0.83 ± 0.60, 0.93 ± 0.62, and 0.78 ± 0.55 for subclinical, clinical, and no mastitis, respectively. However, none of means of the variables evaluated reached the 2 or 3 Z-score thresholds evaluated to determine alerts, which helps explain why the thresholds were breached inconsistently and infrequently (Table 4.5).

The best threshold and observation window combination for RU in this study was obtained using a 1 d alert window and a -2 Z-score threshold for subclinical mastitis cases (48% breached the threshold). For clinical mastitis cases, the best threshold and observation window combinations for RU was achieved with a -2 or -3 Z-score threshold over a 3 d and 2 d alert window (49% breached the threshold). The best combinations for the clinical cases were also the best combination when no mastitis was detected (54% false positives). In a mastitis challenge study, Siivonen et al. (2011) observed a 180 min decrease from the control day to the *E. coli* induction day, based on human observation and observed that mean RU decreased between 4 and 8 h post-challenge compared to the control day (*P* ≤ 0.05). Fitzpatrick et al. (2013) observed a similar result, but explained that the cows made up for decreased RU immediately after the *E. coli* challenge by
increasing RU later in the day. Therefore, total daily RU was not different for cows with mastitis compared to cows without mastitis. Rumination time was evaluated as total daily means in this study so deviations per 2 h time block may have been present, but undetectable if the total daily RU did not decrease. The negative effects of delayed clinical mastitis detection were minimized by averaging over the day. Still, RU related to naturally occurring mastitis may not be affected in the same way as a challenge because the exact infection time is unknown, the severity of each case differs, and responses may differ between pathogens.

The best threshold and observation window combination for NA in this study was obtained using a 1 d alert window and a -2 Z-score threshold for subclinical mastitis cases (48% breached the threshold). For clinical mastitis cases, the best threshold and observation window combination for NA was achieved with both a 2 d and 3 d alert window with a -2 and-3 Z-score threshold (49% breached the threshold). The best combination for the clinical cases was also the best combination when no mastitis was detected (54% false positives). Although research is lacking on the relationship of mastitis to neck activity, mastitis may affect lying time similarly. Research varies on whether lying time increases or decreases around mastitis. During periods of infection, cows may increase resting time to allow for energy conservation to fight the infection (Aubert, 1999, Medrano-Galarza et al., 2012). Conversely, cows may increase standing time because of pain (Chapinal et al., 2013, Cook et al., 2007, Cyples et al., 2012), where lying on a hard surface may exacerbate that pain (Cyples et al., 2012). The relationship between lying time and neck activity is not clear and mastitis may not have greatly influenced NA because neck movement may not imply total body movement. Further
research is warranted to fully understand the relationship between general activity and lying time.

The best threshold and observation window combinations for MY in this study were obtained using a 1 d alert window and a -2 or -3 Z-score threshold for subclinical mastitis cases (15% breached the threshold). For clinical mastitis cases, the best threshold and observation window combination was achieved using a 1 d alert window and a -2 or -3 Z-score threshold (29% breached the threshold). Other researchers have cited a decrease in MY (Beck et al., 1992, Harmon, 1994, Rajala-Schultz et al., 1999), but the decrease may not be immediate. Although MY did not breach the threshold as often for all observation window and Z-score threshold combinations, it had the lowest false positive rate compared to RU and NA. This result implies that using MY to detect mastitis would result in fewer false positives than the RU and NA systems, but it would also only alert producers to fewer true positive cases.

The best threshold and observation window combination for RT was achieved using a 4 milking alert window and a 2 Z-score threshold (Table 4.6). However, RT breached the Z-score threshold in only 7% and 2% of clinical and subclinical mastitis cases, respectively (Table 4.5), making it the variable with the lowest detection value. Adams et al. (2013) used the DVM Systems, LLC (Boulder, CO) RT system used in this study and established that 67% of cows had a 0.08°C RT increase 4 d before clinical detection and 87% of cows had a 0.08°C RT increase within 2 d of clinical detection. However, Adams et al. (2013) did not remove cow days surrounding other diseases that could have affected RT and evaluated fresh cow mastitis, which may behave differently than mastitis occurring later in lactation.
Researchers have suggested that alert time windows be within 24 to 48 h of a clinical mastitis event (Mollenhorst et al., 2012, Rasmussen, 2003). As alert time windows increase, the true positive rate will also increase (Hogeveen et al., 2010, Kamphuis et al., 2010) because the detection method is given more time to detect a change in the variable monitored. However, wider time windows lose their practicality in a commercial setting (Kamphuis et al., 2010) because of the uncertainty of whether to treat the animal or wait until clinical signs are displayed. Many mastitis detection systems have the potential to detect other illnesses that may affect the same variable (i.e. lameness), making producers unsure of what disease to treat if an alert was given days ahead of any signs. However, if an alert occurs within the 24 to 48 h suggested, producers could pay closer attention to the alerted animals during that time and minimize the risk of missing the signs. Ideally, producers could culture milk from cows with alerts and determine treatment based on the results, before clinical signs are displayed.

**Reticulorumen Temperature Threshold**

The results from the RT thresholds (40.3, 40.0, 39.7, and 39.4°C) evaluated as alert levels are displayed in Table 4.6. The best threshold and observation window combination for clinical and subclinical mastitis cases was obtained with a RT threshold of 39.4°C over a 3 milking observation period (34% and 22% for clinical and subclinical cases, respectively). The lowest false positive rate was achieved with a 2 milking observation period with a RT threshold of 40.3°C (97%).

Using a strict RT threshold to detect mastitis is difficult because some cows naturally maintain high or low RT, negatively affecting sensitivity and specificity (Adams et al., 2013). Also, twice daily recordings may not account enough for natural
variation in body temperature (Bewley et al., 2008). In order to account for diurnal variation within cows in this study, RT recorded during AM milkings were not compared to RT recorded in PM milkings, and vice versa. However, this method greatly decreased the number of RT to compare by only using once daily RT.

Another limitation is that RT may be influenced by water intake if the readers are in a location where cows pass after drinking water (Bewley et al., 2008), meaning that an entire cow day could be removed from analysis since only the AM or PM milking RT was used to compare. In this study, readers were placed in the parlor entrance and water was not available in the holding pen, where cows stood for a maximum of 90 min per milking. However, because RT may take 3.5 h after a drink of water to return to baseline RT (Bewley et al., 2008), RT were likely still affected. Siivonen et al. (2011) examined behavior around the time of an E. coli mastitis challenge and discovered that drinking time increased 10 h post-challenge. Because many producers milk cows 2X at 12 h intervals, increased drinking 10 h after infection would make fever detection using reticulorumen temperature monitoring boluses difficult for cows with mastitis as this time may fall right before milking. More frequent RT may allow removal of RT affected by water bouts while still allowing enough temperatures for analysis (Bewley et al., 2008). More frequent RT may also allow for better fever detection since the RT spike may only last 2 h (AlZahal et al., 2011), which may be missed if RT is only recorded 2X.

Many commercially-available automated behavioral and physiological monitoring systems incorporate alerts based on strict thresholds. However, this approach is likely too simple. Many factors including ambient weather, DIM, parity, breed, and within-cow variation, can affect each variable that cannot be accounted for with a simple threshold.
**Study Limitations**

Because visual milk observation was the gold standard comparison to RT, RU, NA, and MY for clinical mastitis identification, limitations related to the milkers’ abilities to identify mastitis reduced the accuracy of mastitis identification timing. If the milkers missed clinical signs during a milking, that cow’s alert window would be shifted so that, if a change did occur within 24 to 48 h before clinical signs were displayed, they would have been missed with this analysis. The timing uncertainty in studies examining natural mastitis may explain why challenge studies have reported better automated mastitis detection results. However, natural mastitis may produce different RT, RU, NA, and MY responses compared to a mastitis challenge. In naturally-occurring mastitis, researchers cannot control the species or amount of bacteria entering the udder and cannot pick otherwise healthy animals to eliminate confounding factors. Because producers observe natural mastitis, testing natural conditions is important to the progress of automated mastitis detection systems.

Time and financial limitations limited the herd composite SCC frequency in this study. More frequent SCC sampling may have helped refine the timing of increased SCC. Each cow sampled with subclinical mastitis was at a different stage of infection, causing the alert window to be shifted for many cows. More advanced analyses like neural networks, fuzzy logic, and Bayesian models may provide higher sensitivities and specificities. Many commercial technology choices exist for RT, RU, NA, and MY monitoring. Although the parameters measured in this study were not effective predictors of mastitis by themselves, analyses considering the combined changes could lead to improved results.
Variation exists in how data is recorded, how often data errors occur, and how accurate the data is based on proprietary algorithms within each system. Additional research is needed to help dairy producers understand the value of mastitis detection technologies.

CONCLUSIONS

The results of this study demonstrated that the variables monitored in this study may have limited value for mastitis detection without additional algorithm refinements or a multivariate analysis. Reticulorumen temperatures were likely affected by water intake and more frequent RT recording may resolve this issue. The large number of missing data points likely affected MY negatively. The ability of these technologies to detect naturally occurring mastitis has not been as heavily studied as mastitis challenges and may produce different, or lessened, behavioral and physiological effects. Relationships between RT, RU, NA, and MY around the time of mastitis should be investigated further so algorithms can be improved to increase the usefulness of these technologies on-farm.

ACKNOWLEDGEMENTS

The authors would like to thank DVM Systems, LLC., IceRobotics, Micro Dairy Logic, Milkline, and SCR Engineers, for donating the technologies used and for their technical support. The authors greatly appreciate Kristin Brock and Bob Kiser from the University of Kentucky Regulatory Services for their technical and financial support of the SCC sampling. Also, Denise Ray’s help with technology assignments and downloads is appreciated. Thanks to Karmella Dolecheck (University of Kentucky) for editing this manuscript. Lastly, the authors would like to thank Carly Becker, Tamara Compton, Jessica Lowe, Emily Morabito, Heather Mussel, Samantha Smith, Alexis Thompson,
Candace Thompson, and Maegan Weatherly, and the University of Kentucky student milkers for their help with milk sampling.
Table 4.1. Somatic cell scores obtained from composite milk samples every 2 wk from every cow in the study herd, including the percentage of cows diagnosed with subclinical mastitis at each sampling.\(^1,2\)

<table>
<thead>
<tr>
<th>Herd Sampling Order</th>
<th>Sample day</th>
<th>N (cows)</th>
<th>LSMeans ± SE</th>
<th>% of cows with subclinical mastitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>September 19, 2011</td>
<td>91</td>
<td>4.14 ± 0.55(^{ab})</td>
<td>29.7</td>
</tr>
<tr>
<td>3</td>
<td>October 17, 2011</td>
<td>79</td>
<td>4.47 ± 0.58(^{ab})</td>
<td>29.1</td>
</tr>
<tr>
<td>4</td>
<td>October 31, 2011</td>
<td>77</td>
<td>4.33 ± 4.33(^{ab})</td>
<td>26.0</td>
</tr>
<tr>
<td>5</td>
<td>November 14, 2011</td>
<td>83</td>
<td>4.17 ± 0.57(^{ab})</td>
<td>27.7</td>
</tr>
<tr>
<td>8</td>
<td>December 23, 2011</td>
<td>93</td>
<td>4.93 ± 0.54(^{ab})</td>
<td>23.7</td>
</tr>
<tr>
<td>9</td>
<td>January 4, 2012</td>
<td>95</td>
<td>4.35 ± 0.52(^{ab})</td>
<td>23.2</td>
</tr>
<tr>
<td>10</td>
<td>January 30, 2012</td>
<td>98</td>
<td>4.06 ± 0.29(^b)</td>
<td>20.4</td>
</tr>
<tr>
<td>11</td>
<td>February 14, 2012</td>
<td>100</td>
<td>3.90 ± 0.52(^b)</td>
<td>22.0</td>
</tr>
<tr>
<td>12</td>
<td>February 27, 2012</td>
<td>101</td>
<td>4.24 ± 0.52(^b)</td>
<td>26.7</td>
</tr>
<tr>
<td>13</td>
<td>March 12, 2012</td>
<td>102</td>
<td>4.23 ± 0.52(^b)</td>
<td>21.6</td>
</tr>
<tr>
<td>14</td>
<td>March 26, 2012</td>
<td>100</td>
<td>4.52 ± 0.52(^{ab})</td>
<td>24.0</td>
</tr>
<tr>
<td>15</td>
<td>April 9, 2012</td>
<td>93</td>
<td>3.79 ± 0.54(^{ab})</td>
<td>15.1</td>
</tr>
<tr>
<td>16</td>
<td>April 23, 2012</td>
<td>91</td>
<td>4.70 ± 0.54(^{ab})</td>
<td>26.4</td>
</tr>
<tr>
<td>17</td>
<td>May 7, 2012</td>
<td>83</td>
<td>3.85 ± 0.56(^{b})</td>
<td>22.9</td>
</tr>
<tr>
<td>18</td>
<td>May 21, 2012</td>
<td>85</td>
<td>4.00 ± 0.57(^{b})</td>
<td>32.9</td>
</tr>
<tr>
<td>19</td>
<td>June 4, 2012</td>
<td>83</td>
<td>5.93 ± 0.56(^{ab})</td>
<td>34.9</td>
</tr>
<tr>
<td>20</td>
<td>June 18, 2012</td>
<td>83</td>
<td>4.66 ± 0.57(^{ab})</td>
<td>31.3</td>
</tr>
<tr>
<td>21</td>
<td>July 16, 2012</td>
<td>88</td>
<td>5.18 ± 0.55(^{ab})</td>
<td>27.3</td>
</tr>
<tr>
<td>22</td>
<td>July 30, 2012</td>
<td>93</td>
<td>4.39 ± 0.54(^{ab})</td>
<td>26.9</td>
</tr>
<tr>
<td>23</td>
<td>August 13, 2012</td>
<td>88</td>
<td>6.99 ± 0.55(^a)</td>
<td>34.1</td>
</tr>
<tr>
<td>24</td>
<td>August 27, 2012</td>
<td>88</td>
<td>5.71 ± 0.55(^{ab})</td>
<td>34.1</td>
</tr>
<tr>
<td>25</td>
<td>September 10, 2012</td>
<td>85</td>
<td>5.12 ± 0.56(^{ab})</td>
<td>37.6</td>
</tr>
<tr>
<td>26</td>
<td>September 24, 2012</td>
<td>80</td>
<td>5.51 ± 0.58(^{ab})</td>
<td>38.8</td>
</tr>
<tr>
<td>27</td>
<td>October 8, 2012</td>
<td>76</td>
<td>4.47 ± 0.58(^{ab})</td>
<td>34.2</td>
</tr>
<tr>
<td>28</td>
<td>October 22, 2012</td>
<td>78</td>
<td>5.04 ± 0.60(^{ab})</td>
<td>39.7</td>
</tr>
<tr>
<td>29</td>
<td>November 7, 2012</td>
<td>78</td>
<td>4.09 ± 0.52(^{b})</td>
<td>23.0</td>
</tr>
<tr>
<td>30</td>
<td>November 19, 2012</td>
<td>75</td>
<td>4.50 ± 0.60(^{ab})</td>
<td>37.3</td>
</tr>
<tr>
<td>31</td>
<td>November 23, 2012</td>
<td>78</td>
<td>4.93 ± 0.59(^{ab})</td>
<td>37.2</td>
</tr>
<tr>
<td>32</td>
<td>December 3, 2012</td>
<td>86</td>
<td>4.93 ± 0.39(^{ab})</td>
<td>25.6</td>
</tr>
<tr>
<td>33</td>
<td>December 19, 2012</td>
<td>79</td>
<td>4.75 ± 0.54(^{ab})</td>
<td>39.2</td>
</tr>
<tr>
<td>34</td>
<td>January 2, 2013</td>
<td>81</td>
<td>5.21 ± 0.59(^{ab})</td>
<td>38.3</td>
</tr>
<tr>
<td>35</td>
<td>January 14, 2013</td>
<td>80</td>
<td>5.61 ± 0.58(^{ab})</td>
<td>32.5</td>
</tr>
<tr>
<td>36</td>
<td>January 28, 2013</td>
<td>80</td>
<td>4.93 ± 0.55(^{ab})</td>
<td>32.9</td>
</tr>
<tr>
<td>37</td>
<td>February 11, 2013</td>
<td>81</td>
<td>5.24 ± 0.57(^{ab})</td>
<td>34.6</td>
</tr>
<tr>
<td>38</td>
<td>February 25, 2013</td>
<td>81</td>
<td>4.28 ± 0.55(^{ab})</td>
<td>32.1</td>
</tr>
<tr>
<td>39</td>
<td>March 11, 2013</td>
<td>86</td>
<td>4.97 ± 0.54(^{ab})</td>
<td>27.9</td>
</tr>
</tbody>
</table>

\(^1\)Subclinical mastitis was defined as a composite milk sample > 200,000 cells/mL (SCS 4).

\(^2\)LSMeans with different superscripts differ \((P < 0.05)\).
Table 4.2. Distribution of mastitis-causing pathogens from individual quarter milk samples of clinical mastitis cases detected by visual observation of clots, flakes, and serous milk by the milkers based on DIM, case number, and parity.¹²

<table>
<thead>
<tr>
<th>Pathogen isolated</th>
<th>DIM (n = 49)</th>
<th>Case number</th>
<th>Parity group</th>
<th>Primiparous (n = 35)</th>
<th>Multiparous (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No growth</td>
<td>27 (55%)</td>
<td>16 (42%)</td>
<td>27</td>
<td>11 (58%)</td>
<td>3 (50%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>2 (4%)</td>
<td>7 (18%)</td>
<td>5</td>
<td>3 (16%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>aureus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coagulase-</td>
<td>6 (12%)</td>
<td>7 (18%)</td>
<td>12</td>
<td>1 (5%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>negative staphylococcus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other pathogens</td>
<td>14 (29%)</td>
<td>8 (21%)</td>
<td>8</td>
<td>4 (21%)</td>
<td>6 (60%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Clinical mastitis was defined as abnormal milk (clots, flakes, or a serous secretion).¹²

¹ Other pathogens included coryneform and yeast.
Table 4.3. Distribution of mastitis-causing pathogens from individual quarter milk samples of subclinical mastitis cases based on individual quarter somatic cell count based on DIM, case number, and parity.\(^1\)\(^2\)

<table>
<thead>
<tr>
<th>Pathogen isolated</th>
<th>DIM 14 to 200 (n = 1272)</th>
<th>DIM 201 to 400 DIM (n = 773)</th>
<th>First case (n = 253)</th>
<th>Second case (n = 241)</th>
<th>Third case (n = 187)</th>
<th>Fourth case (n = 165)</th>
<th>≥ Fifth case(^3) (n = 1199)</th>
<th>Primiparous (n = 846)</th>
<th>Multiparous (n = 1199)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No growth</td>
<td>765 (60%)</td>
<td>503 (65%)</td>
<td>192 (76%)</td>
<td>161 (67%)</td>
<td>124 (66%)</td>
<td>97 (59%)</td>
<td>694 (58%)</td>
<td>439 (52%)</td>
<td>829 (69%)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>204 (16%)</td>
<td>72 (9%)</td>
<td>13 (5%)</td>
<td>17 (7%)</td>
<td>24 (13%)</td>
<td>19 (12%)</td>
<td>233 (19%)</td>
<td>211 (25%)</td>
<td>91 (8%)</td>
</tr>
<tr>
<td>Coagulase-negative staphylococcus</td>
<td>202 (16%)</td>
<td>98 (13%)</td>
<td>32 (13%)</td>
<td>28 (12%)</td>
<td>27 (14%)</td>
<td>21 (13%)</td>
<td>163 (14%)</td>
<td>131 (15%)</td>
<td>143 (12%)</td>
</tr>
<tr>
<td>Other pathogens(^1)</td>
<td>101 (8%)</td>
<td>100 (6%)</td>
<td>16 (6%)</td>
<td>35 (15%)</td>
<td>12 (6%)</td>
<td>28 (17%)</td>
<td>109 (9%)</td>
<td>65 (8%)</td>
<td>136 (11%)</td>
</tr>
</tbody>
</table>

\(^1\)Other pathogens included coryneform and yeast.

\(^2\)Subclinical mastitis was defined as a composite milk sample > 200,000 cells/mL.

\(^2\)n = 160, 136, 139, 113, 102, 84, 81, 75, 49, 41, 31, 32, 17, 24, 20, 15, 10, 8, 11, 11, 11, 9, 5, 5, and 2 for case 5 through 29, respectively.
Table 4.4. Mean Z scores for reticulorumen temperature, rumination time, neck activity, milk yield, and the mean of all variables combined for subclinical, clinical, and no mastitis cases.1,2,3

<table>
<thead>
<tr>
<th>Variable monitored</th>
<th>Subclinical mastitis (N = 115)</th>
<th>Clinical mastitis (N = 61)</th>
<th>No mastitis (N = 46,871)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticulorumen temperature (°C)</td>
<td>0.67 ± 0.59</td>
<td>0.68 ± 0.57</td>
<td>0.52 ± 0.55</td>
</tr>
<tr>
<td>Rumination time (h/d)</td>
<td>0.69 ± 0.65</td>
<td>0.70 ± 0.70</td>
<td>0.58 ± 0.61</td>
</tr>
<tr>
<td>Neck activity</td>
<td>0.58 ± 0.58</td>
<td>0.62 ± 0.58</td>
<td>0.59 ± 0.61</td>
</tr>
<tr>
<td>Milk yield (kg/d)</td>
<td>0.83 ± 0.60</td>
<td>0.93 ± 0.62</td>
<td>0.78 ± 0.55</td>
</tr>
<tr>
<td>Mean of all variables combined2</td>
<td>0.88 ± 0.35</td>
<td>0.94 ± 0.41</td>
<td>0.83 ± 0.38</td>
</tr>
</tbody>
</table>

1Z-scores were calculated by subtracting each cow’s 7 d backward rolling mean baseline reticulorumen temperature, rumination time, neck activity, and milk yield from the reticulorumen temperature, rumination time, neck activity, and milk yield obtained each day and dividing by the standard deviation.

2Subclinical mastitis was defined as a composite milk sample > 200,000 cells/mL and clinical mastitis was defined as abnormal milk (clots, flakes, or a serous secretion).

3Mean of all variables combined = mean of the absolute value of rumination time, neck activity, reticulorumen temperature, and milk yield combined.
Table 4.5. Percent of cows above and below Z-score thresholds and varying alert time windows from udder quarters from clinical, subclinical, and mastitis-free cows.1,2,3

<table>
<thead>
<tr>
<th>Z-score threshold</th>
<th>Observation window (d)</th>
<th>Variable monitored</th>
<th>Subclinical mastitis</th>
<th>Clinical mastitis</th>
<th>No mastitis detected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% Below</td>
<td>% Above</td>
<td>% Below</td>
</tr>
<tr>
<td>-2</td>
<td>1</td>
<td>RU</td>
<td>45</td>
<td>55</td>
<td>49</td>
</tr>
<tr>
<td>-3</td>
<td>1</td>
<td>RU</td>
<td>45</td>
<td>55</td>
<td>49</td>
</tr>
<tr>
<td>-3</td>
<td>2</td>
<td>RU</td>
<td>46</td>
<td>54</td>
<td>49</td>
</tr>
<tr>
<td>-3</td>
<td>2</td>
<td>RU</td>
<td>46</td>
<td>54</td>
<td>49</td>
</tr>
<tr>
<td>-2</td>
<td>3</td>
<td>RU</td>
<td>48</td>
<td>52</td>
<td>46</td>
</tr>
<tr>
<td>-3</td>
<td>3</td>
<td>RU</td>
<td>47</td>
<td>53</td>
<td>48</td>
</tr>
<tr>
<td>-2</td>
<td>1</td>
<td>NA</td>
<td>45</td>
<td>55</td>
<td>49</td>
</tr>
<tr>
<td>-3</td>
<td>1</td>
<td>NA</td>
<td>45</td>
<td>55</td>
<td>49</td>
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<td>3</td>
<td>NA</td>
<td>47</td>
<td>53</td>
<td>48</td>
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<td>4</td>
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<td>3</td>
<td>MY</td>
<td>15</td>
<td>85</td>
<td>29</td>
</tr>
</tbody>
</table>

1Variables monitored where RU = rumination time, NA = neck activity, and MY = milk yield.

2Subclinical mastitis was defined as a composite milk sample > 200,000 cells/mL and clinical mastitis was defined as abnormal milk (clots, flakes, or a serous secretion).

3Z-scores were calculated by subtracting each cow’s 7 d backward rolling mean baseline reticulorumen temperature, rumination time, neck activity, and milk yield from the reticulorumen temperature, rumination time, neck activity, and milk yield obtained each day and dividing by the standard deviation.
Table 4.6. Percent of cows above and below Z-score thresholds and varying alert time windows from udder quarters from clinical, subclinical, and mastitis-free cows using reticulorumen temperature.\(^1\)

<table>
<thead>
<tr>
<th>Reticulorumen temperature threshold (°C)</th>
<th>Clinical mastitis</th>
<th>Subclinical mastitis</th>
<th>No mastitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observation window (milkings)(^2)</td>
<td>% above threshold</td>
<td>% below threshold</td>
</tr>
<tr>
<td>39.4</td>
<td>3</td>
<td>34</td>
<td>66</td>
</tr>
<tr>
<td>39.7</td>
<td>3</td>
<td>23</td>
<td>76</td>
</tr>
<tr>
<td>40.0</td>
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<td>18</td>
<td>82</td>
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<td>40.3</td>
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<tr>
<td>40.3</td>
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<td>3</td>
<td>97</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Z-Score Threshold</th>
<th>Observation window (milkings)(^2)</th>
<th>% above threshold</th>
<th>% below threshold</th>
<th>% above threshold</th>
<th>% below threshold</th>
<th>% above threshold</th>
<th>% below threshold</th>
</tr>
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<tbody>
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<td>100</td>
</tr>
</tbody>
</table>

\(^1\)Subclinical mastitis was defined as a composite milk sample > 200,000 cells/mL

\(^2\)Observation windows were the number of AM or PM milkings before the AM or PM milking where clinical signs were displayed. If clinical signs were displayed during the AM milking, no PM milkings were included in analysis.
Figure 4.1. Example of an individual cow where a -2 rumination time Z-Score threshold was breached to signal an alert.\(^1\)

\(^1\)Lower confidence limit represents the rumination time -2 Z-scores.
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VITA

Amanda Sterrett was born in Greensburg, Pennsylvania. Amanda graduated from Greensburg Salem Senior High School in 2006. In 2009, she obtained Bachelor of Science degrees from the University of Findlay in Animal Science and Biology. In July 2010, Amanda began studying for her Master’s of Science under the direction of Dr. Jeffrey Bewley. Amanda’s research focused on mastitis management and mastitis detection using precision dairy technologies. Amanda presented her research findings at the 2011 and 2012 Joint Annual ADSA Meetings and the National Mastitis Council Annual meeting. Amanda was also involved in three additional projects; the first involved the use of a three-dimensional camera to monitor dairy cattle feed intake; the second examined development of an automated body condition scoring camera system; and the third compared two freestall bed bases for cow productivity and behavior using precision dairy technologies.

Outside of research, Amanda acted as a teaching assistant in the Animal Production Principles, Dairy Cattle Evaluation, and Livestock, People, and Their Interactions courses. She served as the Dairy Cattle Judging coach in 2012. Amanda was the ADSA Graduate Student Division Vice President in 2012 and President in 2013. She also served as the co-founder and President in the University of Kentucky Animal and Food Sciences Graduate Association in 2010 and 2011. Amanda received the National Milk Producers Federation National Dairy Leadership Scholarship in 2011, the National Mastitis Council Scholars Award in 2012, and the Farm Animal Integrated Research Graduate Student Recorder scholarship in 2012.
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