The use of animal activity data and milk components as indicators of clinical mastitis

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ABSTRACT

A study was conducted to examine the correlation between a novel behavior monitoring system and a validated data logger. We concluded that the behavior monitoring system was valid for tracking daily rest time in dairy cows (R=0.96); however the correlation values for rest bouts and rest duration were relatively low, (R=0.64) (R=0.47), respectively. Daily monitoring of animal activity and milk components can be used to detect mastitis prior to clinical onset. Data from 268 cases with clinical mastitis and respective controls (n=268) from Virginia Tech and the University of Florida dairy herds were examined. Variables collected included daily milk yield, electrical conductivity, milk fat, protein, and lactose percent, as well as activity measurements including daily rest time, daily rest duration, daily rest bouts, and daily steps taken. Variables were collected for case and control cows in the 14 d prior to and after clinical diagnosis, for a total 29 d monitoring period. A milk sample was aseptically collected upon detection of clinical signs as observed by milker's at both farms. A statistical method (candisc discriminant analysis) was used to combine all measurements and sensitivity and specificity was calculated. Virginia Tech cows on d -1 (sensitivity=95%, specificity=95%), Virginia Tech and University of Florida cows on d -1 (sensitivity=88%, specificity=90). Overall, daily monitoring of animal activity and milk components can detect mastitis prior to onset of clinical signs of disease. This may allow producers to intervene and make proactive management decisions regarding herd health prior to clinical diagnosis.

Keywords: animal activity, milk component, mastitis, detection

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"Being a graduate student is like becoming all of the Seven Dwarves. In the beginning you're Dopey and Bashful. In the middle, you are usually sick (Sneezy), tired (Sleepy), and irritable (Grumpy). But at the end, they call you Doc, and then you're Happy."
-Ronald T. Azuma

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"All our dreams can come true-if we have the courage to pursue them" ~ Walt Disney

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1.1 Introduction

Management practices are a primary focus on most North American dairy farms. Today, cow numbers are increasing, while the actual number of farms is decreasing (Taure, 2006, USDA, 2011). As a result, management practices are changing because of a larger cow to worker ratio. Farm personnel must manage larger groups of cows resulting in less attention per individual cow and this increases the risk of diseases going undetected. Disease is a major component of management and a priority on all dairy farms. With the current trends in the industry, novel detection options continue to be a focus to prevent and control disease. Developments in on-farm monitoring technologies have allowed producers to enter the realm of "Precision Dairy Farming". The data generalized from Precision Dairy Farming can allow for the early detection of disease.

Precision Dairy Farming

Precision Dairy Farming (PDF) is defined as a collection of technological advances that can measure physiological, behavioral, and production indicators on individual animals (Bewley, 2010). These advancements provide management tools to identify problem areas related to reproduction, nutrition, dairy calf management and feeding, dairy cattle health, mastitis and milk quality. Technologies currently existing monitor a variety of outcomes including daily milk yield, milk components (e.g. fat, protein, and SCC), animal activity, rumen and milk temperatures, milk conductivity, milk replacer intake, estrus detection, and daily body weight measurements (Bewley, 2010).

One study utilizing PDF examined the ability of milk yield and electrical conductivity (EC) to detect disease prior to clinical signs. Health and reproductive records were recorded for 587 cows. Daily milk yield and EC were observed for significant changes up to 10 days prior to a clinical health event (Reneau et al., 2010). This study indicated that daily monitoring of milk components can be useful in clinical disease detection prior to onset of signs, which provides the opportunity to change management strategies. Early detection could prevent clinical signs or lessen the severity, as well as reduce costs associated with disease.

One of the most important diseases affecting the dairy industry today is mastitis. Recent estimates suggest each case of clinical mastitis is associated with a $231-$289 loss (Hogeveen, 2010). PDF technologies such as milk component monitoring, biosensors, and thermography are all different methods that can be incorporated into dairy farm management systems. Utilization of PDF could identify changes in individual animals sooner and allow farmers to intervene before the full onset of disease, thus reducing costs associated with mastitis.

The objectives of this review are to 1) provide background knowledge on mastitis and current detection methods, 2) provide information on the use of milk component and animal activity data monitoring on dairy farms, 3) discuss detection of clinical mastitis through utilization of Precision Dairy Farming, 4) discuss studies that have examined early detection of disease through activity and milk component data.

Overview of mastitis

Mastitis is defined as an inflammation of the mammary gland and is prevalent in dairy herds around the world (Kehrli and Shuster, 1994). This disease can be caused by a wide range of bacterial pathogens or from a physical force to the mammary gland and/or teat end. This disease can be divided into chronic, subclinical, acute, or clinical forms based on its severity and

symptoms (Brandt et al., 2010, Viguier et al., 2009). Chronic mastitis is characterized by persistent inflammation of the mammary gland (Viguier et al., 2009). Subclinical mastitis is not visibly detectable but causes reduced milk production. Acute mastitis affects a cow for a short period of time and has a rapid onset, whereas clinical mastitis is defined by visual changes in the milk (flakes and/or clots). An increase in the rate of clinical cases was seen with increasing cow parity (Sargeant et al., 1998). Producers suffer economic losses as a result of discarded milk, replacement of animals, treatment costs, veterinarian costs, and reduced milk production. However, subclinical mastitis poses the most financial hardships to dairy operators because it is most often undetected.

Common mastitis causing pathogens

Mastitis-causing bacterial pathogens come in multiple forms and can be categorized into contagious, environmental, or opportunistic pathogens. Common contagious pathogens include *Streptococcus agalactiae* and *Staphylococcus aureus*, both Gram-positive organisms, and *Mycoplasma* spp. Characterized by having no cell wall. Contagious pathogens spread predominantly at milking time within a herd (Barkema et al., 2009). Environmental mastitis pathogens included Gram-negative organisms including the subgroup called coliforms, and also some Gram-positive organisms. Coliform mastitis is commonly misclassified as containing all Gram-negative pathogens, however, coliform mastitis causing pathogens only consist of *Klebsiella* spp., *Escherichia coli* and *Enterobacte*r spp. (Hogan and Larry Smith, 2003). Gram-negative pathogens not classified as coliforms that commonly cause environmental mastitis include *Serratia* spp., *Pseudomonas* spp., and *Proteus* spp. (Hogan and Larry Smith, 2003). Gram-positive environmental pathogens include *Streptococcus dysgalactiae*, *Streptococcus*

uberis, *Enterococcus* spp. and the broadest class called environmental *Streptococcus* spp. (Van Eenennaam et al., 1995). Opportunistic pathogens are usually considered minor pathogens, although they are frequently isolated from herds and most often are coagulase negative *Staphylococcus* spp. Opportunistic pathogens can be found on healthy teat skin and milkers hands.

Mastitis Detection

Early diagnosis is important due to the costs associated with mastitis. Detection of mastitis can be achieved in many different ways, but current detection methods focus on checking the quality of milk. The most common detection methods include observation for clinical signs, somatic cell count (SCC) commonly measured through the Dairy Herd Improvement Association (DHIA) testing, bacteriological culturing, the California Mastitis Test (CMT), and through automatic milking systems which contain biosensors.

During a normal milking session forestripping is standard protocol to prepare for milking. When a cow is stripped the milkers observe this milk to look for clinical mastitis by visual inspection i.e. off color, flakes, clots, etc. However, subclinical mastitis can not be detected by forestripping. Another method to detect mastitis is through SCC measuring, commonly performed by DHIA (Laevens et al., 1997). Knowledge of SCC can be useful for identifying subclinical mastitis. An increase in the SCC serves as a positive indicator of infection. Consequently, monitoring of SCC is a useful tool in mastitis detection (Barkema et al., 2009).

Another useful tool in the detection of mastitis is bacteriologic culturing. Culturing allows for specific pathogen recognition and is useful to determine control and treatment options.

Treatment options are based on what type of pathogen is causing the infection. However, this detection method is labor intensive and laboratory results can take several days to obtain (Viguier et al., 2009). Furthermore, costs associated with culturing may be high depending upon if cultures are run anaerobically or aerobically and where the samples are processed.

The CMT test can give rapid results and be a useful cow-side test for farmers and veterinarians. A small amount of milk is added to a small amount of bromocresol-purple-containing detergent that breaks down the cell membrane of somatic cells to create a viscosity proportional to leukocyte number (Viguier et al., 2009). Advantages of this test include cost, as this test averages $12 for 350 tests, results are produced rapidly, and the test can be used cow-side (Viguier et al., 2009). Although this test has many advantages, the results can often be difficult to interpret and represent false results.

Perhaps automatic milking systems hold the most difficult challenge in detection of mastitis due to its hands-off approach. The farm crew's presence in the parlor will be minimal and detection of clinical mastitis by forestripping is non-existent. The majority of automatic milking systems have built in sensors to measure electrical conductivity and producers rely on these sensors to detect mastitis cases in the herd (Norberg, 2004, Norberg et al., 2004). Color sensors can measure presence of off colors such as yellow or red indicating blood in the milk are present, if these colors are present, that cow is likely to have mastitis. Benefits of automatic milking systems include reduced labor, increased milk production, and many argue increased cow comfort. However, sensors associated with the machines are not always accurate at detecting disease and milk fat has also been known to effect the sensitivity of milk color monitoring (Viguier et al., 2009).

Despite available detection and treatment options, mastitis is still the most costly disease affecting the dairy industry (Harmon, 1994). Detection of mastitis prior to the onset of clinical signs could be a very useful tool to help reduce costs associated with mastitis and improve animal well-being. Milk components and SCC monitoring may be a useful tool in early mastitis detection. Little day to day variation is observed in milk component and SCC measurements of healthy lactating cows and therefore daily monitoring of these components could serve as an early indicator of disease (Forsback et al., 2010).

Somatic Cell Count

One of the most common mastitis detection methods is the use of SCC data. This can be measured on an individual cow or for a bulk tank and can indicate the extent of a herd's infection status. SCC is a measure of the cellular immune defense intensity (Urech et al., 1999). Somatic cells include neutrophils, macrophages, lymphocytes, eosinophils, and various other epithelial cell types of the mammary gland (Kehrli and Shuster, 1994). The normal healthy mammary gland of a cow has a low SCC in milk, which help to ward off bacteria from establishing inside the mammary gland (Suriyasathaporn et al., 2000). The SCC of healthy mammary glands will range from 50,000 to 200,000 cells/mL of milk depending on the age of the cow, thus a SCC >200,00 cells/mL could indicate an intramammary infection (IMI) (Smith, 1995).

The ability of the immune system to fight an IMI and the causative pathogen both play an important part in SCC. If bacteria establish in the mammary gland, chemoattractants are released which signal white blood cells (WBC) to migrate to the site of infection and help reduce bacterial numbers (Kehrli and Shuster, 1994). Neutrophils, a critical WBC in IMI, will then migrate through the blood and into the infected quarter of the cow. If the neutrophils succeed

and the bacteria are destroyed, then migration of the neutrophils will conclude and only a mild inflammatory response will be seen (Kehrli and Shuster, 1994). If the neutrophils are unable to eliminate the bacteria the SCC will rise and a heightened inflammatory response will occur as evidenced by presence of more neutrophils and other immunes cells such as leukocytes and macrophages to fight off the infection.

One study examined bulk tank SCC from 274 different herds with low, medium, or high readings to determine the relationship between the incidence rate of clinical mastitis and SCC (Barkema et al., 1998). They found that bulk tank SCC was significantly impacted by type of bacterial pathogen found in the herd. Gram-negative pathogens such as *E. coli* and *Klebsiella* spp. were found in bulk tanks with low SCC while contagious pathogens such as *S. aureus* and *Strep. agalactiae* were found more in herds with high SCC (Barkema et al., 1998). This study indicates the importance SCC plays not only determining the presence of IMI but the role specific pathogens play in bulk tank SCC.

In a different study by de Hass et al. (2002) they observed the effects of clinical mastitis on SCC throughout lactation. Over the lactation of a healthy cows, SCC followed a pattern slightly increasing following parturition, decreasing around 50 DIM, and increasing slightly at the end of the lactation (de Haas et al., 2002). Before a case of clinical mastitis in multiparous animals caused by *S. aureus*, *Strep. uberis*, or *Strep. dysgalactiae* was detected the SCC was already well above the average SCC of healthy animals (356,000 ± 34,000 cells/mL, 212,000 ± 46,000 cells/mL, and 226,000 ± 46,000 cells/mL), respectively. This indicates the pathogen was already subclinically present in the mammary gland for some time before clinical signs were observed (de Haas et al., 2002). After clinical signs were observed for *S. aureus* mastitis, the

SCC remained high 460,000 ± 30,000 cells/mL whereas with cases of *E. coli* mastitis the SCC retreated to normal levels after the observation of clinical signs. Close observation of SCC may not only indicate presence of an IMI but may also be indicative of the causative pathogen.

The SCC measured prior to and during the dry period can also be a good indicator to determine whether or not a cow will develop clinical mastitis. One study examined whether previous SCC information could be used as a risk factor for clinical mastitis in the next lactation. Duplicate quarter samples were taken at dry off, in the next lactation between 2-9 DIM, and before the treatment of all first cases of clinical mastitis that occurred within 120 days of the lactation (Pantoja et al., 2009). Cows with a SCC ≥200,000 cells/mL at either dry off or 2-9 days post-calving in the subsequent lactation were at an increased risk for clinical mastitis (Pantoja et al., 2009). Those quarters with SCC ≥200,000 cells/mL at both dry off and 2-9 days post-calving were 2.7 times more likely to develop a case of clinical mastitis compared to quarters with a SCC<200,000 cells/mL (Pantoja et al., 2009). The role of SCC monitoring in mastitis detection will continue to be a focus and may be beneficial to determine what pathogen is causing the IMI.

Electrical conductivity

Electrical conductivity (EC) is another useful milk characteristic, first introduced as an indicator of mastitis in the 1940's. Since then, numerous studies have examined its ability to detect mastitis. This measurement determines the ability of a solution to conduct an electric current between two electrodes, or the resistance of a material to an electric current (Hogeveen, 2010, Norberg, 2004). Measured in milliSiemens (mS), cations and anions are the main components of EC (Hogeveen, 2010). The elements most important in determining the electrical

conductivity of milk include Na^+, K^+, and Cl^-. During a mastitis infection tight junctions become leaky and allow Na^+ and Cl^- to pass through the junctions and into the lumen of the alveolus, while K^+ moves out of milk. Therefore, Na^+ and Cl^- concentrations are increased while K^+ concentrations are decreased during mastitis. If EC readings deviate outside the normal range of 4.0 to 5.0 mS, there is a greater probability for a mastitis infection (Norberg et al., 2004).

Two different systems can measure EC. The first system allows EC of whole milk to be read and combines the EC reading from all four quarters. This system is located in the milk meter. The second system measures the EC of each individual quarter and is located in the claw of the milking cluster in traditional milking systems (Hogeveen, 2010). In automatic milking systems, EC is measured on individual quarters and is located in the long milk tube. Measuring EC of individual quarters could increase probability of detecting mastitis cases because mastitis affects a cow at the quarter (or individual mammary gland) level. In a normal healthy cow, EC ranges between 4.0 to 5.0 mS at 25 °C (Norberg et al., 2004), deviations from this range could indicate IMI. Sloth et al., 2003 found healthy cows to average 4.61 mS/cm (n=520), whereas cows in their subset defined as different from healthy cows had an average EC of 4.98 mS/cm.

Milner et al., (1996) infused *S. aureus* or *Strep. uberis* into the mammary gland of 20 lactating dairy cows. Detection of clinical mastitis by changes in EC before visible changes in milk was determined. In 12 cases of *Strep. uberis* infusion, 11 (92%) were detected by increases in EC before or on the milking at which clots in the milk first appeared. The EC in 55% of clinical mastitis cases changed 2 milking's before the onset of clinical signs, and in 34% of the cases EC changed coincidently with appearance of clots (Milner et al., 1996). This study demonstrated the ability of EC to identify clinical mastitis as early as or earlier than milkers (Milner et al., 1996). Norberg et al, (2004) examined EC in 322 lactating cows at 2-s intervals

from each quarter at every milking. Milking machines were structured to keep milk from each quarter separate until after EC measurements were recorded. The 20 highest EC readings were averaged for each healthy, clinically infected, and subclinically infected cows. Healthy cows averaged a 4.87 ± 0.01 EC reading, subclinical cows averaged 5.37 ± 0.02, and clinical cows averaged 6.44 ± 1.53, all significantly different from each other ($P<0.01$). A ratio of milk EC was calculated from the four quarters and identified as the inter quarter ratio (IQR). IQR identified 80.1% of clinical cases of mastitis correctly and 74.8% of healthy cases correctly (Norberg et al., 2004).

EC differences between clinical and healthy cows can partly be explained by the physical changes of milk that occurs during a clinical episode. Clinical mastitis clots can slow milk flow from the teat, cause cow discomfort during milking thus the cow may fidget and cause air slippage through the teat cup liner, and clots in the milk can stick to the EC sensors affecting measurements (Norberg et al., 2004). Although EC can be a predictor of an IMI, EC combined with other milk components could increase the sensitivity and specificity of detecting clinical mastitis before clinical signs are seen.

Lactose

Lactose is the major carbohydrate found in milk. Certain types of bacteria, such as coliforms including *E. coli* and *Klebsiella* spp., may utilize this sugar to thrive in milk, thus decreasing overall concentration. Lactose concentrations may also drop during a mastitis infection due to tissue damage. During an infection, enzyme systems of the secretory cells in the mammary gland, will not be fully functioning and the biosynthesis of lactose will be decreased (Pyorala, 2003) During a healthy lactation, day-to-day variation of milk lactose at the udder-quarter level remained consistent at 4.7% ± 0.9% , and therefore deviations could serve as an

indicator of disease (Forsback et al., 2010, Pyorala, 2003). Other studies have reported average lactose concentrations in healthy Danish-reds (n=108), Danish-Holsteins (131) and Jerseys (n=83) lactose percent at 4.73% ± 0.22% (Park et al., 2007, Sloth et al., 2003).

In another study, researchers examined milk samples from cows infected with major pathogens, defined as all streptococci, *Staphylococcus aureus, Escherichia coli,* and *Klebsiella,* and from cows with minor pathogens, defined as coagulase-negative staphylococci, micrococci, and other isolates (e.g., *Corynebacterium bovis*). The mean lactose percentages for cows isolated with major pathogens was 4.75% ± 0.42% and cows that isolated minor pathogens 4.88% ± 0.35% (Berning and Shook, 1992). These researchers concluded that lactose could not be used as a predictor of mastitis, however lactose levels in healthy cows from their study (4.92% ± 0.25%) were well above the average (4.7% ± 0.9%) reported in most cows (Berning and Shook, 1992). Park et al. (2007) measured 30,019 milk samples from healthy and mastitic cows on 390 farms in Korea. Mastitis-causing pathogens were classified as environmental (*Streptococcus* spp., *Enterococcus* spp., CNS, Yeast, *E. coli,* and *Pseudomonas* spp.) and contagious (*Staphylococcus aureus)*. Cows isolated with environmental pathogens had mean lactose concentrations of 4.63% ± 0.03%, contagious pathogens mean lactose concentrations were 4.59% ± 0.04%, compared to healthy cows 4.85% ± 0.1% (Park et al., 2007).

Silanikove et al., (2011) examined the changes in key metabolites, including lactose, following challenge with lipopolysaccharide (LPS). Twelve Holsteins were infused in one front and one rear quarter with 10 μg of LPS dissolved in 10 mL of sterile nonpyrogenic saline, the opposite quarters served as controls. These researchers also measured the ability of *E. coli* to grow in broth depleted of lactose. When lactose concentration in media was decreased to 0%, 3%, and 5%, growth of a pathogenic strain of *E. coli* was impaired (Silanikove et al., 2011).

Researchers suggested that *E. coli* could not grow in broth depleted of lactose, indicating a bacterial dependence on this sugar (Silanikove et al., 2011).

Nielsen et al., (2005) analyzed lactose concentrations throughout milking in cows with healthy and unhealthy quarters. Results suggest that cows with unhealthy quarters had significantly ($P<0.001$) lower lactose concentrations ($4.37\% \pm 0.06\%$) compared to healthy quarters ($4.70\% \pm 0.05\%$) throughout the milking process (Nielsen et al., 2005).

Lactose concentrations in milk have also been linked to cull rate and SCC. Miglior et al (2007) found that Holstein cows with a lactose concentration threshold <4.49% and Ayrshire cows <4.45% were at a higher risk of being culled. Whereas Holstein cows with a lactose concentration threshold (>4.91% and Ayrshire cows >4.83% had a lower cull rate. Cows with lower levels of lactose may indicate IMI thus may explain a higher cull rate. Park et al (2007) noted as the SCC increased the percentage of lactose present in the milk significantly decreased $P < 0.001$ suggesting an inverse relationship between SCC and milk lactose (Park et al., 2007). These results are also in agreement with a study that found lactose percentage in milk to be negatively correlated -0.202 with somatic cell score (Miglior et al., 2007). The previous studies indicate a relationship between lactose, SCC, and culling and indicate that consistent lactose concentrations of 4.7% or higher are related to a reduced SCC, reduced culling, and thus reduced risk of IMI.

Protein

It has been suggested that milk protein percent might also be useful in the early detection of clinical mastitis. Milk contains numerous proteins, the primary group being caseins and the secondary group being whey. During a mastitis infection there is an increase in the amount of plasmin, a proteolytic enzyme that can causes damage to casein, thus reducing its concentration

in milk (Uallah et al., 2005). However, it has also been reported that milk protein concentrations increase during a mastitis infection, primarily due to an increase in whey proteins. A number of serum proteins, a part of the whey protein group, include serum albumin, immunoglobulins, and transferrin, these proteins pass into milk because of leaky tight junctions and as a result may increase protein concentrations in milk (Harmon, 1994).

Very few reported studies have examined changes in total protein percentage during a mastitis infection (Hortet and Seegers, 1998). Within the studies that have been conducted, changes in milk protein have contradictory results (Hortet and Seegers, 1998, Houben et al., 1993). Forsback et al (2010) monitored nine Swedish Red cows for day-to-day variation in milk components found average protein concentrations in milk to be 3.47% ± 0.24% (Forsback et al., 2010). In contrast, Sloth et al (2003) determined that healthy Danish-red, Danish-Holstein, and Jersey cows averaged 3.75% ±0.50% protein in milk.

Toni et al., (2011) examined milk protein and fat percent from test day records on three different farms for a total of 1,498 primiparous and multiparous Holstein cows. Protein concentrations differed by location and by farm, but on all farms, multiparous cows had decreased or had no change in protein percent. Primiparous cows from herd A averaged 3.2% ± 0.3% whereas multiparous cows averaged 3.1% ± 0.3%, in Herd B primiparous cows averaged 3.1% ± 0.2% whereas multiparous cows averaged 3.1% ± 0.3%, and finally primiparous cows in Herd C averaged 3.0% ± 0.3% whereas multiparous cows averaged 3.0 ± 0.3% (Toni et al., 2011).

Changes in milk protein concentration have been examined during subclinical mastitis. In one study, the composition of total protein in healthy quarters SCC ≤100,000 cells/mL and

unhealthy quarters SCC >100,000 cells/mL were examined (Urech et al., 1999). Animals with a SCC >100,000 cells/ml were defined as having a subclinical infection. Of the animals chosen for the study, none exhibited clinical signs. Milk samples were collected from the foremilk (milk from the machine at the beginning of milking), bucket milk (sample from the milk receiving vessel), stripping milk (the last milk obtained after the milking had ended), residual milk (milk collected after an oxytocin injection) and young milk (milk sampled 1.5 h after a second oxytocin injection) (Urech et al., 1999). Healthy quarters had an average protein content of 36.1 g/L ± 0.28 g/L recorded for foremilk while unhealthy quarters had a reading of 36.9 g/L ± 0.28 g/L. Bucket milk readings were 36.9 g/L ± 0.28 g/L for healthy quarters while unhealthy quarters read 37.7 g/L ± 0.28 g/L. Stripping milk protein was 35.5 g/L ± 0.28 g/L for healthy quarters while unhealthy quarters were 37.4 g/L ± 0.28 g/L. Residual milk readers were 33.9 g/L ± 0.28 g/L for healthy quarters while unhealthy quarters ready 36.5 g/L ± 0.28 g/L. Young milk readers were 35.8 g/L ± 0.28 g/L while unhealthy quarters were 36.9 g/L ± 0.28 g/L. Bucket milk protein percentage was 3.6% for unhealthy quarters and 3.5% for healthy quarters as calculated from milk yield. Stripping milk, residual milk, and young milk percentages were not calculated as milk yields were not recorded for these milking fractions. Of the quarters examined from animals as defined by subclinical mastitis, total protein percentage increased for all milking fractions (Urech et al., 1999).

Nielsen et al., (2005) analyzed milk protein concentrations on 11 cows with healthy and unhealthy quarters at 2 different time intervals on the same day. The cows were milked in the morning at a 12-h interval, and then again 6-h later. Milk was collected every 45 s from each quarter. Researchers found that cows with unhealthy quarters had significantly higher ($P<0.01$) protein concentrations (3.49% ± 0.14%) compared to healthy quarters (3.18% ± 0.13%) (Nielsen

et al., 2005). The difference between protein percent of healthy and unhealthy quarters was largest towards the end of milking. No significant differences were found between cows milked at 6 h, (3.31% ± 0.13%) and 12 h, (3.35% ± 0.13%) (Nielsen et al., 2005). Researchers concluded higher concentrations of protein in unhealthy quarters may be a result of reduced milk production in those quarters.

Milk protein concentration has also been recorded in buffalo experiencing mastitis. Uallah (2005) collected milk samples from 150 buffalo. Mastitis was graded on severity; P1=mild clumping, P2=rapid/moderate clumping, and P3=rapid/heavy clumping. The mean milk protein concentration for buffalo not experiencing mastitis was 3.85% ± 0.07 % whereas in samples from quarters experiencing mastitis, protein concentration decreased in P1= 3.56% ± 0.10%, P2=3.26% ± 0.06%, and P3=3.14% ± 0.10% (Uallah et al., 2005). Total protein percentage may be beneficial in the early detection of mastitis when examined in combination with other components. The few studies that have been conducted to examine milk protein concentration indicate the need for more research in this area, as study results disagree.

Fat

Fat percent is a major component of dairy cow milk. Few studies have examined milk fat concentrations in cows with clinical mastitis and results disagree on whether or not milk fat concentrations decrease or increase during mastitis (Hortet and Seegers, 1998). The predominant type of fat in milk is in the form of triglycerides. Lipase is an enzyme that increases in concentration during mastitis and causes breakdown of triglycerides which releases free fatty acids, as a result this can cause off flavors in milk and decrease fat concentrations in milk (Harmon, 1994).

One study examined 9 Swedish Red cows for day-to-day variation in milk components and found average fat concentrations in morning milking to be 3.76% ± 0.70% while night milking was 5.73% ±1.01% indicating a day-to-day variation of 7.2% (Forsback et al., 2010). In this study milk fat concentrations were the parameter with the greatest day-to-day variation. A different study examining mastitis in primiparous heifers found that fat percent in healthy animals was constant at 4.4% (Myllys and Rautala, 1995). Toni et al., (2011) examined milk protein and fat percent from test day records on three different farms for a total of 1,498 primiparous and multiparous Holstein cows. Fat concentrations differed by lactation and by farm, but on all farms multiparous cows had higher fat yields. Primiparous cows from herd A averaged 3.4% ± 0.8% whereas multiparous cows averaged 3.5% ± 0.8%, in Herd B primiparous cows averaged 3.4% ± 0.7% whereas multiparous cows averaged 3.8% ± 1.1%, and finally primiparous cows in Herd C averaged 3.4% ± 0.7% whereas cows 2+ averaged 3.5% ± 0.9% (Toni et al., 2011).

In a study that examined 1,192 lactations, first lactation cows without clinical mastitis produced 330 kg of fat and 7,675 kg of milk throughout a 305-d lactation. First lactation cows with clinical mastitis were compared to nonmastitic cows. Clinical cows experienced a reduced fat yield as compared to nonmastitic cows of 0-27 kg which was 0-11% of total milk yield less than their non-mastitic herdmates (Hagnestam et al., 2007). Multiparous cows without clinical mastitis produced 7,862 kg of milk and 337 kg of fat throughout a 305-d lactation. In multiparous cows with clinical mastitis, fat yield was reduced 0-41 kg which was 0-21% of total milk yield compared to cows not affected with clinical mastitis. Researchers concluded a reduction in fat yield could be attributed to a drop in milk yield due to clinical mastitis and not by changes in fat and protein content of the actual milk (Hagnestam et al., 2007). Nielsen et al.,

(2005) analyzed milk fat concentrations on 11 cows with healthy and unhealthy quarters at 2 different time intervals. The cows were milked in the morning at a 12-h interval, and then again 6-h later. Milk was collected every 45 s from each quarter. Cows were milked at 6 h and then again at 12 h. These researchers found that cows with unhealthy quarters had significantly lower ($P<0.01$) fat concentrations (4.40% ± 0.33%) compared to healthy quarters (4.72% ± 0.31%) however, this difference was only significant during the last half of milking (Nielsen et al., 2005). Researchers also determined milk fat was affected by milking interval, cows milked at a 6 h interval had increased milk fat percent (4.86% ± 0.35%) compared to cows at a 12 h interval (4.25% ± 0.35%). However, at the 6 h interval, milk was only significantly higher compared to the 12 h interval during the last half of milking (Nielsen et al., 2005). Unhealthy quarters may display a decreased concentration of milk fat because cows with mastitis infections may have impaired milk fat synthesis due to epithelial cell damage.

Sloth et al (2003) analyzed 108 Danish Red, 131 Danish Holstein, and 83 Jersey cows for parameters including milk yield, fat percent, protein percent, lactose percent, citrate percent, composite SCC (1000 cells/ml), and two conductivity parameters. Milk samples were collected every eighth week throughout the lactation. Cows were either assigned to the healthly data set or a data set defined as different from the healthy data set. Cows in the healthy data set were required to have a negative bacteriological quarter foremilk sample on two consecutive samplings, show no clinical symptoms, and no symptoms of other diseases throughout the sampling interval (Sloth et al., 2003). The mean fat concentration for cows in the healthy subset (n=520) 5.08% ± 1.07% and cows included in the subset defined different from healthy (n=301) had a fat percent of 4.97% ± 1.02%, however these data were only numerically different. In buffalo, fat concentrations decreased as a result of mastitis and quarters were graded; P1=mild

clumping, P2=rapid/moderate clumping, and P3=rapid/heavy clumping (Uallah et al., 2005). Buffalo with healthy quarters had a mean fat concentration of 5.01% ± 0.19% whereas unhealthy quarters concentrations were 4.91% ± 0.17%, 4.46% ± 0.19%, and 4.39% ± 0.15% in P1, P2, P3 grade mastitic quarters respectively (Uallah et al., 2005).

The majority of the literature suggests fat concentrations are depressed during mastitis infections, however some research suggests otherwise. In a study examining milk components of cows infected with subclinical mastitis, cows were placed into group I (n=8) if the log SCC/ml was >6.0 in the infected quarter or into group II (n=8) if the log SCC/ml was 5.6-6.0 in the infected quarter. Infected quarters of group I had significantly greater milk fat 54.6 g/L ± 4.6 g/L when compared to unaffected contralateral quarter 44.1 g/L ± 1.9 g/L. In group II, no significant differences were seen (Bruckmaier et al., 2004) . These results disagree with many studies that show fat concentration to be reduced during mastitis (Bruckmaier et al., 2004). Another study that reviewed several papers on milk fat and protein concentrations as well as yield during mastitis suggested fat concentrations may increase during mastitis, however it also suggested papers that found trends in fat reduction as well (Hortet and Seegers, 1998).

The previous studies indicate changes in fat concentrations during clinical mastitis infection vary. Given the day-to-day variation of 7.2% in fat concentrations found by Forsback et al., 2010, examination of fat percent and other milk components together may be beneficial in predicting IMI before the onset of clinical signs. The use of milk fat as a predictor of clinical mastitis prior to the onset of signs is an unreliable predictor based on the studies listed above.

Milk Temperature

The body temperature of a dairy cow averages 38.6°C, whereas the temperature of milk is 0.09°C lower than the normal body temperature (Rossing, 1976). In quarters defined as

healthy, a consistent milk temperature indicates regular milk flow from the udder to the cistern (Gil, 1988). Deviations in milk temperature may illustrate problems associated with milk flow from alveoli and fine ducts to the cistern which could be caused by epithelial damage as a result of mastitis (Gil, 1988). Monitoring milk temperature could serve as a useful tool in the detection of mastitis.

Milk temperature can be measured through an NTC thermistor which is attached to the short milk tube of a milking machine allowing milk temperature from each quarter to be read (Gil, 1988, Maatje et al., 1992). Maatje et al., (1992) monitored milk temperature, milk yield, and electrical conductivity through a parlor which milked 65 cows daily. Data was collected for 12 months and quarter milk samples were collected monthly for bacteriologic analysis and SCC. A total of 19 of 25 cows with clinical mastitis had a significant increase in milk temperature and a decrease in milk yield, or a rise in milk temperature alone before clinical signs appeared (Maatje et al., 1992).

Gill (2008) utilized 114 cows to evaluate changes in milk temperature, electrical conductivity, and SCC. In healthy quarters (n=324) milk temperature averaged 0.2°C lower than the average body temperature. Milk temperature of subclinical quarters (n=132) averaged 1.5°C below the average body temperature 38.6°C (Gil, 1988). Cow's with a subclinical infection had milk temperatures that were highly correlated with electrical conductivity (R=0.91) and chloride content (R=0.87) (Gil, 1988). These milk temperature fluctuations found in cows with subclinical mastitis may indicate irregular milk flow from the teat. Subclinical mastitis infections may damage epithelial cells in the mammary gland to cause irregular milk flow.

Furthermore, of the quarters isolated with *Staph aureus*, 43/51 showed unexplained temperature fluctuations compared to 38/324 of all healthy quarters representing 11.8% false-positives.

Another form of detecting clinical mastitis before the onset of signs is through infrared thermography (IRT) which utilizes generation of heat captured in images. An infrared camera measures the amount of radiation emitted from an object and that radiation is a function of surface temperature making it possible for the camera to calculate and display the temperature (Kunc P., 2007). Infections can cause a localized increase in temperature due to the inflammatory response (R. J. Berry, 2003). This process has been used for many years in human medicine helping doctors to diagnose various cancers (Colak et al., 2008).

Colak et al (2008) used 94 cows (n=49 Brown Swiss and n=45 Holsteins) to compare IRT with CMT scores.. CMT score was highly correlated with IRT (R=0.85) Further analysis showed that udder skin surface temperature for healthy quarters (SCC ≤400,000 cells/mL; n = 94) averaged 33.45°C ± 0.09°C which was lower than for subclinical quarters (35.80°C ± 0.08°C; SCC >400,000 cells/mL; n = 135) (Polat et al., 2010). In a similar study (Hovinen eta l 2008) IRT was examined as a way to detect clinical mastitis. Healthy cows (n=6) were infused in the left front quarter with 10 µg of *E. coli 055:B5* LPS diluted in 5 mL of NaCL while the right front quarter served as a control. Images of the udder were taken from the lateral and medial angles of the quarters. Mean udder skin temperature measured through IRT was increased in experimental and control quarters 4 h post challenge. The correlation between rectal temperature and udder skin temperature of the lateral angle was R=0.92 ($p<0.001$) indicating as rectal temperature increased, udder skin temperature simultaneously rose (Hovinen et al., 2008).

This may be a valuable tool for early detection of mastitis; however research should be conducted in determining IRT's ability to detect naturally occurring mastitis.

Although milk temperature has been evaluated since the 1970's, progress towards implementing it as a tool for early detection of mastitis on farms is still in the research stage. New technology such as IRT could prove to be a more beneficial rapid method for detecting clinical mastitis, but more research needs to be done to examine these new technologies.

Milk Yield

Mastitis causes a reduction in MY. During an IMI, an influx of neutrophils will pass between the milk producing cells of the mammary gland and into the lumen of the alveoli to help destroy bacteria (Akers and Nickerson, 2011). As a result, secretory cells are damaged. Once leukocytes reach the lumen they will aggregate and form clots which can block milk ducts and result in incomplete milk removal (Khan, 2006). If milk ducts remain clogged, secretory cells revert to a non-producing state, and alveoli begin to shrink being replaced by scar tissue, thus causing a reduction in MY (Pyorala, 2003).

Clinical mastitis can be affected by stage of lactation. Researchers examined 24,276 Finnish Ayrshire dairy cows and collected monthly test day MY. When mastitis occurred during late lactation (>120 DIM), MY was reduced 2-4 wk prior to clinical symptoms suggesting the presence of subclinical mastitis (Rajala-Schultz et al., 1999). In parity 1 cows, daily yield loss was -0.5 kg ± 0.3 kg, parity 2 cows showed a loss of -1.1 kg ± 0.2 kg, parity 3 cows had a reduced yield of -1.3 kg ± 0.3 kg, and parity 4 cows showed a loss of -0.7 kg ± 0.3 kg (Rajala-Schultz et al., 1999). Overall daily losses between 1.0 to 2.5 kg were observed following

mastitis diagnosis and over the lactation 110 to 552 kg of milk were lost depending upon parity and timing of mastitis (Rajala-Schultz et al., 1999).

Another study examined the impact of lactation on milk loss due to mastitis by reviewing 1,192 lactation records over a 16.5 yr period. Researchers found that cows that developed clinical mastitis around peak milk, showed a reduction in MY 4 wk before signs of clinical mastitis, while cows in mid lactation showed a reduction in MY 3 wk prior to clinical signs, and finally cows in late lactation displayed a decreased MY 2 wk prior to clinical signs (Hagnestam et al., 2007). Although a depression in MY was observed 2-4 wk before clinical signs were present, the change was only significant 1 wk prior to clinical mastitis diagnosis. Furthermore, cows that developed clinical mastitis originally produced more milk compared to non-mastitis cows and were never able to achieve the baseline level of milk production (Hagnestam et al., 2007). One hypothesis for the reduced MY several wk before the onset of clinical signs could potentially be attributed to a subclinical infection (Hagnestam et al., 2007, Rajala-Schultz et al., 1999).

Mastitis-causing pathogens have a significant effect on MY (Schukken et al., 2009). Schukken et al. (2009) examined milk loss following repeated cases of clinical mastitis with Gram-positive and Gram-negative pathogens. These researchers discovered that Gram-negative cases caused 304 kg of milk loss in the 50 d following clinical mastitis diagnosis in multiparous cows, while 128 kg was lost due to Gram-positive pathogens (Schukken et al., 2009). Cows in first lactation that eventually developed clinical mastitis produced significantly more milk before diagnosis compared to cows that never developed clinical mastitis. A similar trend was found with multiparous cows who developed clinical mastitis, they produced significantly ($P<0.0004$)

more milk then their herdmates who never developed clinical mastitis. These results agree with another study where researchers found multiparous cows that eventually developed clinical mastitis produced 1.7 kg/d more milk than their healthy herdmates before clinical mastitis developed (Bar et al., 2007). Study results indicate higher producing cows may be more at risk for mastitis. Furthermore, cows that experienced at least 1 case of clinical mastitis in a previous lactation produced 1.2 kg/d less milk over the current lactation compared to cows who did not experience clinical mastitis in the previous lactation (Bar et al., 2007).

Another study examined milk loss in relation to the specific pathogen isolated from the clinical quarter (Grohn et al., 2004). Second parity cows infected with *Streptococcus* spp. produced more milk (3.1 kg/d) 3 to 4 wk before mastitis diagnosis compared to herdmates who never developed mastitis (Grohn et al., 2004). Furthermore, 1 wk prior to mastitis diagnosis, those cows infected with *Streptococcus* spp. produced less milk compared to non-infected herdmates. However, *E. coli* mastitis had the biggest impact on milk production throughout lactation. Parity 1 cows lost 6.7 kg milk/d for the first week following mastitis diagnosis, and 5 kg milk/d in the following 3 wk (Grohn et al., 2004). Multiparous cows lost 13.1 kg of milk/d in the week following mastitis diagnosis, in the second week cows lost 7.2 kg of milk/d (Grohn et al., 2004). Although no significant milk loss occurred prior to clinical onset, cows produced significantly more milk prior to diagnosis compared to non-mastitic cows. *Staph aureus*, *E. coli*, and *Klebsiella* spp. caused the greatest loss of milk yield among first lactation cows (Grohn et al., 2004). *Streptococcus* spp., *Staph aureus*, *E. coli*, *Klebsiella* spp., and *Arcanobacterium pyogenes* (*A. pyogenes)* caused the most milk loss in multiparous cows (Grohn et al., 2004). Milk yield for both primiparous and multiparous cows began to decline before the onset of clinical mastitis signs, however this drop was not significant. This study demonstrated how

different mastitis pathogens can affect milk production and contribute to economic losses farmers may experience as a result of clinical mastitis.

Milk production loss as a result of clinical mastitis occurs predominantly after signs of clinical mastitis. Milk yield loss based on current findings suggests that this variable alone may not be sufficient enough to predict clinical cases of mastitis. However, when milk yield is combined with other milk component data may serve as a valuable method for early detection of clinical mastitis in dairy cows.

Using animal activity data to predict disease

Several studies have examined early detection of clinical and subclinical mastitis by monitoring one or multiple milk components. However, monitoring milk components alone may not be sufficient to detect all cases of mastitis. However, daily monitoring of milk components and activity measures together may improve the ability to detect more cases of mastitis. Very few studies have looked at animal activity data as a means to detect disease.

Traditionally, monitoring of animal activity data has been used to detect estrus but activity data may also be used to detect disease. Edwards and Tozer (2004) examined milk yield and daily walking activity together as a way to predict different digestive and metabolic disorders. Metabolic disorders examined included ketosis, retained placenta, and milk fever. Digestive diseases included left displaced abomasums (LDA), indigestion, reduced feed intake, traumatic gastritis (hardware disease), acidosis, and bloating. Three different farms were studied for a total of 1,445 cows and activity and MY measures were collected through Special Agricultural Equipment Afikim computerized dairy management system (Kibbutz Afikim,

Israel). Ketosis was diagnosed on 10.0 ± 8.2 DIM, LDA on 14 ± 11.9 DIM, and digestive disorders on 23 ± 21.5 DIM (Edwards and Tozer, 2004). Cows that experienced ketosis, LDA, or digestive disorders had significantly ($P<0.01$) increased activity compared to healthy cows 8 to 9 d prior to diagnosis with disease (Edwards and Tozer, 2004). Activity data for cows with ketosis, LDA, and digestive disorders began to decrease from d -8 to d -1 relative to day of diagnosis. Activity data for cows with ketosis remained different ($P<0.01$) until 1 d prior to diagnosis, digestive disorders until d 0 ($P<0.01$), whereas cows with LDA had variable data from d-8 to d-1 (Edwards and Tozer, 2004). Overall, cows diagnosed as unhealthy walked on average 8-14 steps/h less than healthy cows ($P<0.001$) (Edwards and Tozer, 2004). Milk production began to decline -6 d before diagnosis for ketotic cows, -7 d for those with LDA, and -5 d for those with digestive disorders (Edwards and Tozer, 2004). These researchers concluded monitoring of daily walking activity and MY together increases the ability to detect transition cow disorders. Perhaps activity data variables such as lying time and standing time can further increase detection methods.

DeVries et al (2010) examined changes in lying and standing time relative to IMI. It has generally been recommended that cows be provided fresh feed following milking to prevent the cow from lying down in dirty stalls. This allows the teat canal to close and is thought to help prevent IMI. In their study, lying behavior was recorded using data loggers (HOBO Pendant, G Data Logger, Onset Computer Corporation, Pocasset, MA) to determine if lying behavior effected frequency of IMI post-milking. They reported that if cows laid down 40-60 minutes following milking they were 1.4 times less likely to get an IMI compared to cows who laiddown within 40 minutes of milking, however differences were not statistically significant (DeVries et al., 2010).

Yeiser (2011) collected activity measures such as rest bouts, rest duration, rest time, and average daily steps and summed for daily measurements of these activities on 216 dry cows from 21 d prepartum to 30 d postpartum. Activity measures were recorded to determine the ability to detect diseases before clinical diagnosis. Rest time increased (398 ± 43 min) 5 d prior to onset of signs as compared to non-mastitic cows (307 ± 17 min). Cows later diagnosed with mastitis showed decreased rest time on d -2 and d-1 (349 ± 43 min and 391 ± 43 min, respectively) as compared to non-mastitic cows (481 ± 17 min and 488 ± 16 min), respectively. On d -5 and d -4 steps/h was significantly decreased for cows later diagnosed with mastitis (3267 ± 465 steps and 3003 ± 460 steps, respectively) compared to herdmates who were never diagnosed with mastitis (5553 ± 183 steps and 4573 ± 175 steps, respectively). Cows diagnosed with milk fever walked on average (4242 ± 529 steps and 2549 ± 514 steps, respectively) on d -1 and d 1 relative to clinical diagnosis. Cows that were later diagnosed with subclinical ketosis had increased rest bouts on d -1 (15.6 ± 1.5 bouts) as compared to animals without detectable disease (12.2 ± 0.6 bouts) (Yeiser, 2011). They concluded that activity parameters could be used as a valid method for proactively monitoring herd health on dairy operations.

The use of activity monitoring on dairy farms to detect disease prior to the onset of clinical signs is not currently a widely accepted concept. Dairy operations that utilize such systems have the opportunity to implement proactive management strategies to better prevent disease. Activity monitoring may allow producers to introduce early intervention strategies, thus minimizing the negative effects and reducing the severity of disease.

Non-traditional novel detection methods

Several studies have examined milk component deviations around mastitis, while few have examined activity measurements, and none have examined milk component and activity data together. Electrical conductivity and SCC are currently primary aids for the the early detection of mastitis. Even so, many cases of clinical mastitis still go undetected. Several new technologies and application models are being researched to aid producers in early detection. One new technology being tested is the use of a neural network.

A neural network can help detect patterns within the data collected at milking. For instance a cow with clinical mastitis will have a different electrical conductivity pattern compared to her non-diseased counterpart (Nielen et al., 1995). If several EC measurements are missing, the network will still be able to run, whereas some models will not be able to perform an analysis with missing data. A neural network is based on connections between different units and is defined by specifying these connections among units. Different variables can be entered into the neural network such as electrical conductivity and milk yield, and these units are weighted to produce an output signal, such as clinical or healthy (Nielen et al., 1995). Nielen et al., (1995) used EC as the sole input measure into a neural network to identify clinical and healthy quarters (Nielen et al., 1995). The network was trained with 17 healthy and 13 clinical mastitic quarters. The trained neural network predicted 34 of 38 healthy quarters and 21 of 38 mastitic quarters correctly in the predictive data set. Researchers concluded that a neural network including more input variables such as milk temperature and milk yield may be more beneficial for differentiating clinical and healthy quarters.

A different application that could be used to identify clinical and healthy cows is through a fuzzy logic analysis. Fuzzy logic is used for classification and controlling processes that have no easy mathematical approach, and has been used in oestrus detection (Cavero et al., 2006).

The fuzzy logic is a three step process. The first step is fuzzification which turns each input value such as electrical conductivity into a membership function and a membership grade between (0-1). For example if an electrical conductivity measure of 6.0 mS/cm was recorded its membership function would be middle or high and its grade would be 0.35 or 0.65 (Cavero et al., 2006). The second step is the fuzzy inference and is based on the following procedure: if condition, then rule, and whether or not each condition is met depends on membership grade. The last step is defuzzification in which fuzzy values are transformed into a single number which represents whether or not the cow has clinical mastitis or not. An alert signal is activated if values from defuzzification exceed a certain threshold determined by the mastitis definition. The alerts were compared with the actual number of mastitis cases seen.

Cavero etal., (2006) utilized fuzzy logic as a detection method and accumulated a dataset over a 4 year period including 403,537 milking's from 478 Holstein Friesian cows, and 645 lactations. The dataset was split into 2/3 of the original dataset and this was used to create a fuzzy logic model, this dataset was called the training set. The remaining data was used as the test data, and the fuzzy logic model calculated from the training set was used to analyze the test set to determine predictability. If the sensitivity was set to be at least 80%, the specificities ranged between 93.9% and 75.8% and the error rate varied between 95.5% and 41.9% depending on mastitis definition. The average number of true positive cows per day ranged from 0.1 to 7.2, and the average number of false negative cows per day ranged from 2.4 to 5.2 (Cavero et al., 2006).

The use of a neural network and a fuzzy logic model may be beneficial to producers in the future. Novel detection models and applications such as the neural network and fuzzy logic

may be more beneficial if more milk component variables are added. Furthermore, detection may be enhanced if activity measurements are added to these applications.

Conclusions

As the dairy industry moves from an era of treatment and antibiotics to an era of prevention, novel detection techniques will have to become a focus in order to improve disease detection. As mastitis is currently the most costly disease for the dairy industry, attention to new and improved detection methods should be emphasized. Monitoring of milk components and activity together may allow for early detection of mastitis before actual disease diagnosis. This would allow farmers to intervene before the onset of clinical disease, thus reducing costs associated with mastitis. As technology continues to growand human contact plays less of a role in disease detection, novel methods for detecting disease will be increasingly important.

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Chapter 2: An evaluation of a novel behavior monitoring system compared to a validated data logger

ABSTRACT

Many studies have used tracking devices such as the HOBO© data logger (HOBO Pendant G Data Logger, Onset; Pocasset, MA) to record cow activity and behavior changes, but very few have examined the AfiPedometerPlus© system (S.A.E. Afikim, Israel), and no studies have documented on device equivalence. Therefore, the objective of this study was to examine the ability of a novel behavior monitoring system to track activity data when compared to the validated data loggers, as well as to quantify its accuracy in different dairy breeds. The sum of total daily lying time variable collected by the data loggers was compared to daily rest time in the behavior monitoring system, which is the equivalent variable. Daily rest time from the behavior monitoring systems was highly correlated (R=0.96) to the sum of total daily lying time from the data loggers. Daily rest bout from the behavior monitoring system was compared with the sum of lying bouts from the data loggers (R=0.64). Daily rest duration from the behavior monitoring system was compared with lying duration from the data loggers (R=0.47). When individual breeds were examined daily rest duration (R=0.79, R=0.83) and daily rest bouts (R=0.93, R=0.91) from the behavior monitoring system were both validated variables for Jersey and Crossbred cows compared to the data loggers. Daily rest duration and daily rest bout could not be validated for Holstein cows at this time. The novel behavior monitoring system was concluded as a reliable tracking device for daily rest bouts and daily rest duration in Jersey and Crossbred cows, and for daily rest time in Jersey, Crossbred, and Holstein cows. Future studies should be conducted on Holstein cows to confirm or reject the validation of daily rest bouts and daily rest duration in this breed.

Key Words: Animal activity, validation, data logger, behavior monitoring system

INTRODUCTION

Management practices are a primary focus on most North American dairy farms and disease detection is a major component in dairy operation management strategies. All medium and large sized dairy operations have reported at least one case of clinical mastitis, lameness, retained placenta, reproductive problems, or milk fever with an even higher percentage having at least one cow with a health problem (USDA, 2007). The costs associated with these diseases can be anywhere from $200 for a case of clinical ketosis or mastitis, to more than $300 for an identified case of lameness (Kelton et al., 1998). As the mentality of dairy producers is changing from an era of treatment to an era of proactive management, novel detection techniques have become a major focus to prevent disease. Identifying clinical signs prior to the onset of disease may allow farmers to intervene early, thus reducing costs associated with disease. Traditional activity data has been used to detect estrus, however animal activity and behavior have proven to be a valuable way of identifying animals that are at risk for periparturient diseases.

Many research studies have recorded animal activity data using cameras which transfer to a time-lapse videocassette recorder (PanasonicTime-Lapse VCR, AG-6540) and record the video on VHS tapes for later use. More recently activity data is being used to detect diseases and discomfort associated with dairy cows. Using this videotape method, cows in one study that had greater than 30 bouts in the 24 h before calving were at a greater risk for dystocia (Proudfoot et al., 2009). Although video tapes are considered the gold standard for monitoring animal activity, this method is not applicable to commercial dairy herds and is very time consuming for research herds. Recent advancements in on-farm monitoring technologies provide the tracking of animal activity and behavior. One advanced tracking method, the IceTag® (IceRobotics, Edinburg, UK), is a system that has been used extensively to characterize behavior in dairy cattle

(Munksgaard, 2006, Trenel et al., 2009). The device records lying and standing time in addition to step time and step counts through the use of intensity measures as percentages of those specific activities. Researchers placed this device on the same leg as an AfiPedometerPlus© (S.A.E. Afikim, Israel) to measure the correlations between the systems. The step correlation (R=0.82), lying bout correlation (R=0.98) and lying time correlation (R=0.90) were all very high (Higginson, 2009). The high correlations between the behavior monitoring system and previously validated IceTags® provide support for the use of the pedometers in commercial dairy production (Higginson, 2009). Edwards and Tozer (2004) used this tracking device and found animals that experienced a metabolic or digestive disorder in the prebreeding stage of lactation, walked an average of 8 to 14 steps/h less than healthy cows following calving. While the overall activity was less, there was an increase in steps taken 8 to 9 d prior to clinical diagnosis with a gradual decline until the day of diagnosis (Edwards and Tozer, 2004).

Another commonly used system is the HOBO© data loggers device (HOBO Pendant G Data Logger, Onset; Pocasset, MA). In addition to the lying and standing behavior, these data loggers, through recording of the *g* force on the x, y and z-axes can also determine the laterality of the animal using the degree of tilt of the axes. Through the use of 30-s recording intervals and some data filtering to remove ambiguous, short-lasting behaviors, a 99% predictability, sensitivity, and specificity was validated with this system. The logger has been shown to accurately measure lying and standing behavior (Ledgerwood et al., 2010) by comparison with video recording. Predictability, sensitivity, and specificity were all >99%.

While many studies have used the behavior monitoring system and data loggers to record cow activity and behavior changes, none have documented if activity variables from both devices are comparable. Furthermore, most studies have been conducted on Holstein dairy cows or beef

cattle. Therefore the objective of this study was to examine the ability of the behavior monitoring system to track activity data when compared to data loggers, as well as to quantify its accuracy in different dairy breeds.

MATERIALS & METHODS

Behavior monitoring system

Study animals included a total of 14 lactating dairy cows (5 Holstein, 5 Jersey, and 4 Crossbred) housed at the Virginia Tech Dairy Center (Blacksburg, Virginia). Cows were housed in a freestall barn containing sawdust bedded stalls with rubber mattresses. Stalls were bedded with sawdust twice weekly. Animals had a behavior monitoring system affixed to a rear leg fetlock, which collected activity data on a daily basis. Cow activity was collected between milking sessions and stored in the memory of the pedometer. The activity information collected was then transmitted at each milking through a reader box to the AfiFarm© (S.A.E. Afikim, Israel) Herd Management software program. Activity variables collected by the behavior monitoring system included daily steps, rest bouts, and rest time between milking sessions for each and summed daily measurements of these activities. A rest bout was defined as the number of events the animal changed from a standing position to a lying position. A bout was only counted as such if the animal was lying for greater than 3 min. Rest time is the amount of time, in minutes, that the cow was lying down in a day. The rest duration is the average duration, in minutes, of the rest bouts. Average daily steps were calculated by averaging the steps per hour for the two collection sessions and multiplying that average by 24.

Data Loggers

Data loggers were attached to the same rear leg as the behavior monitoring system. This device measured acceleration and angle displacement on an x, y, and z axis. Through these

measurements, activity was classified. Cow activity variables associated with the acceleration and angle displacement included lying bouts and average lying times for both left and right sides in addition to total lying and standing time and average daily steps. The data loggers were set to start recording acceleration and angle displacement data upon the start of the 1-wk study period with data collected every minute throughout the study. On the last day of the study, the data loggers were removed and the collection of data ceased. To assess daily activity, the minute data were summed together to obtain daily activity.

Linear Regression

The purpose of the behavior monitoring system data collection was to validate daily rest time, daily rest bouts, and daily rest duration when compared to data loggers (considered the 'gold' standard). Data from both systems was stored in a database (Microsoft Excel 2007 for Windows©; Microsoft Corporation, Redmond, Washington) until analysis. The relationship between total activity data collected from the data loggers and the behavior monitoring system were analyzed using a linear regression procedure in GraphPad Prism version 4.0 for Windows, GraphPad Software, La Jolla California USA. Correlation values were calculated for daily rest time and sum of total lying time, daily rest bouts and sum lying bouts, daily rest duration and sum of lying duration.

RESULTS

Predictability of Activity Monitors

Daily rest time from the behavior monitoring system was highly correlated to the sum of total daily lying time from the data loggers for all breeds Jersey ($R=0.97$, $P<0.001$, Table 2.1 A), Crossbred ($R=1.00$, $P<0.001$, Table 2.1 B), and Holstein ($R=0.92$, $P<0.001$, Table 2.1 C). Daily

rest time and total daily lying time were highly correlated for all breeds (R=0.96, *P*<0.001, Figure 2.1). Daily rest bout from the behavior monitoring system was compared with the sum of lying bouts from the data loggers and correlation values varied among breeds. Jersey (R=0.93, P<0.001, Table 2.1 A) and Crossbred (R=0.91, P<0.001, Table 2.1 B) correlations were both very high compared to a low correlation found in Holsteins (R=0.21, P<0.01, Table 2.1 C). When daily rest bout and sum of lying bouts were combined for all breeds correlation was low (R=0.64, P<0.001, Table 2.1 C). Daily rest duration from the behavior monitoring system was compared with lying duration from the data loggers and results varied among breeds. Jersey (R=0.79, P<0.001, Table 2.1 A) and Crossbred cows(R=0.84, P<0.001, Table 2.1 B) were correlated but Holsteins had a low correlation (R=0.10, P=0.08, Table 2.1 C). When daily rest duration and sum of lying duration were combined for all breeds, the correlation value was low (R=0.47, P<0.001, Table 2.1 C).

DISCUSSION

The behavior monitoring systems daily rest time, daily rest duration, and daily rest bouts were expected to be highly correlated to the data loggers sum total lying time, lying duration, and lying bouts. Lying bouts and lying duration correlation values to rest duration and rest bouts were lower than expected when all breeds (Jersey, Crossbred, Holstein) were examined together (Table 2.1 C), however when individual breeds were examined Jersey and Crossbred animals had high correlations for all variables measured. The difference between breed correlation values is unknown. The behavior monitoring system recorded a rest bout only if a cow was lying down for longer than 3 min, whereas the data loggers recorded a rest bout if the cow was lying down for at least 1 min. Correlation values may have been lower than expected in Holsteins due to difference in bout recording definitions. Differences in bout recording would also make rest

duration correlations low as this is a measurement of average bout time in minutes, however this does not explain lower correlation values in Holsteins compared to high correlations seen in Jersey and Crossbreds. Holstein cow correlation values may have been lower than expected due to these animals enrollment in a challenge trial. Frequent sampling periods of study animals were required during this challenge trial, which required people entering and exiting animal pens more frequently. As a result, this could have caused cows to change positions more frequently and caused differences in data. Furthermore, the behavior monitoring system may have malfunctioned during the time period of Holstein data collection, as this data was collected a year previous to Jersey and Crossbred cows. Future studies should be conducted that set data loggers to record bouts >3 minutes to better access compatibility with the behavior monitoring system.

Data loggers have previously been validated (Ledgerwood et al., 2010) and are considered a 'gold' standard tracking device in measuring animal activity. A different tracking device, the IceTag data logger (IceTag Sensor, IceRoboticsc Ltd, Edinburgh, UK) was examined to see effects of lying behavior and the extent of lying laterality in Holstein dairy cows (n=40). The IceTag was compared to the data logger as the data logger had been previously validated (Ledgerwood et al., 2010). Total lying time, frequency of lying time, duration of lying bouts, and percentage of time lying on each side did not differ between the two devices (Jenny Gibbons et al., 2012). Researchers concluded the IceTag data logger to be a reliable tool to measure animal activity. The IceTag data logger was also compatible to the behavior monitoring system. Lying bouts ($R=0.98$, $p<0.0001$) and lying duration ($R=0.90$, $p<0.0001$) were both highly correlated between devices for Holstein cows (n=16), however correlation values were only high when both devices were placed on the same leg (Higginson, 2009).

In the present study Holstein cows had lower correlation values compared to Jersey and Crossbred animals when the data logger was compared to the behavior monitoring system. Devices in the present study were both attached to the same leg, ruling out any possibility of differences between leg activities. Both the behavior monitoring system and the data loggers have been validated to the IceTag, however in this study the behavior monitoring system's daily rest bouts and daily rest duration could not be validated to the data loggers for Holstein cows.

The high correlation value between daily rest time and lying time confirms the behavior monitoring system to be a valid tracking device in dairy cows for this variable. Furthermore, the behavior monitoring system is a valid method to track activity and behavior data from different breeds of dairy cows for this variable. Daily rest time, daily rest duration, and daily rest bout from the behavior monitoring system were all validated variables for Jersey and Crossbred cows compared to the data loggers. Daily rest duration and daily rest bout could not be validated for Holstein cows at this time, and the reason for this is unclear.

Study results conclude the novel behavior monitoring system is a reliable device to predict daily rest bouts and daily rest duration in Jersey and Crossbred cows, as well as daily rest time in Jersey, Crossbred, and Holstein cows. The novel behavior monitoring system cannot be validated to track daily rest bouts and daily rest duration in Holstein cows at this time. Future studies should be conducted that examine daily rest bout and rest duration in Holstein cows to further confirm or reject whether or not this device is valid in Holsteins.

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A

Cow (breed)	Day	Daily rest time[1]	Total lying time[2]	Daily rest bouts[1]	Total lying bouts[2]	Daily rest duration[1]	Total lying duration[2]
4269(Jersey)	1	767	.	17	.	93	.
4269(Jersey)	2	902	914	20	20	101	46
4269(Jersey)	3	839	891	22	21	74	42
4269(Jersey)	4	854	916	20	20	97	46
4269(Jersey)	5	821	848	16	16	107	53
4269(Jersey)	6	662	675	20	20	65	34
4407(Jersey)	1	410	.	14	.	61	.
4407(Jersey)	2	419	424	13	13	66	33
4407(Jersey)	3	425	430	16	16	52	27
4407(Jersey)	4	428	438	14	14	68	31
4407(Jersey)	5	494	493	13	13	78	38
4407(Jersey)	6	428	433	13	13	85	33
4553(Jersey)	1	467	.	26	.	38	.
4553(Jersey)	2	521	574	28	25	39	23
4553(Jersey)	3	668	699	25	25	54	28
4553(Jersey)	4	473	522	22	22	42	24
4553(Jersey)	5	449	508	24	23	38	22
4553(Jersey)	6	458	559	21	19	43	29
4572(Jersey)	1	695	.	8	.	340	.
4572(Jersey)	2	653	661	10	10	148	66
4572(Jersey)	3	737	747	14	18	106	42
4572(Jersey)	4	587	609	21	20	56	30
4572(Jersey)	5	614	631	13	12	100	53
4572(Jersey)	6	551	567	17	15	75	38
4581(Jersey)	1	578	.	16	.	71	.
4581(Jersey)	2	542	571	10	10	107	57
4581(Jersey)	3	626	681	12	10	103	68
4581(Jersey)	4	539	580	12	10	104	58
4581(Jersey)	5	638	646	12	11	101	59
4581(Jersey)	6	578	578	13	10	83	58
Jersey		Rest time correlation r = 0.97		Bout correlation r = 0.93		Duration correlation r = 0.79	

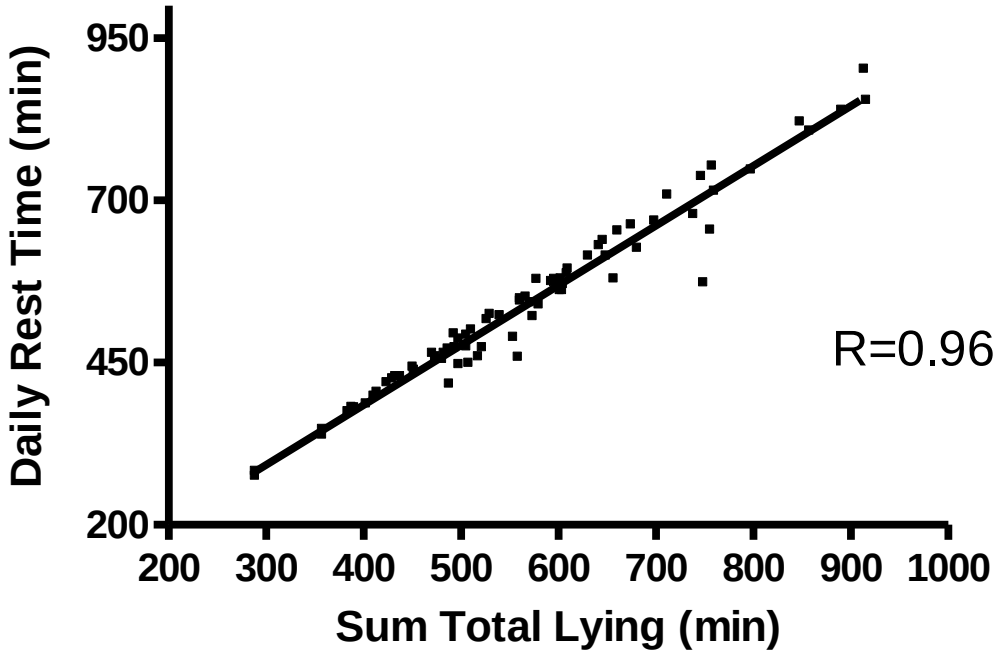
B

Cow (breed)	Day	Daily rest time[1]	Total lying time[2]	Daily rest bouts[1]	Total lying bouts[2]	Daily rest duration[1]	Total lying duration[2]
4298(Crossbred)	1	404	.	8	.	102	.
4298(Crossbred)	2	548	561	10	12	124	47
4298(Crossbred)	3	374	384	6	6	124	64
4298(Crossbred)	4	614	649	11	13	119	50
4298(Crossbred)	5	380	391	7	7	107	56
4298(Crossbred)	6	347	358	8	7	86	51
4400(Crossbred)	1	302	.	10	.	58	.
4400(Crossbred)	2	398	411	12	11	66	37
4400(Crossbred)	3	473	494	18	17	51	29
4400(Crossbred)	4	338	358	16	16	43	22
4401(Crossbred)	5	386	403	16	15	47	27
4400(Crossbred)	6	275	289	14	12	40	24
4563(Crossbred)	1	578	.	15	.	79	.
4563(Crossbred)	2	524	530	13	11	96	48
4563(Crossbred)	3	575	593	16	16	70	37
4563(Crossbred)	4	578	596	17	16	70	37
4563(Crossbred)	5	545	561	13	13	98	43
4563(Crossbred)	6	464	471	11	11	82	43
4610(Crossbred)	1	494	.	15	.	68	.
4610(Crossbred)	2	500	511	12	12	76	43
4610(Crossbred)	3	443	451	10	10	88	45
4610(Crossbred)	4	455	481	12	12	81	40
4610(Crossbred)	5	404	414	7	7	111	59
4610(Crossbred)	6	464	483	11	11	83	44
Crossbred		Rest time correlation r = 1.00		Bout correlation r = 0.91		Duration correlation r = 0.83	

C

Cow (breed)	Day	Daily rest time[1]	Total lying time[2]	Daily rest bouts[1]	Total lying bouts[2]	Daily rest duration[1]	Total lying duration[2]
4073(Holstein)	1	717	.	23	.	72	.
4073(Holstein)	2	678	739	18	16	64	47
4073(Holstein)	3	747	798	20	16	84	37
4073(Holstein)	4	807	858	17	11	78	53
4073(Holstein)	5	573	749	15	14	77	49
4073(Holstein)	6	654	756	23	14	107	45
4073(Holstein	7	579	657	20	24	59	37
4248(Holstein)	1	555	.	14	.	89	.
4248(Holstein)	2	561	602	12	17	86	67
4248(Holstein)	3	753	758	11	13	164	71
4248(Holstein)	4	522	540	11	14	113	45
4248(Holstein)	5	561	604	14	18	95	53
4248(Holstein)	6	573	596	12	16	94	51
4248(Holstein)	7	714	760	13	15	91	47
4402(Holstein)	1	723	.	13	.	140	.
4402(Holstein)	2	618	659	11	19	94	65
4402(Holstein)	3	792	820	17	19	137	46
4402(Holstein)	4	720	770	13	17	100	55
4402(Holstein)	5	723	751	14	18	132	50
4402(Holstein)	6	651	718	11	16	111	44
4402(Holstein)	7	729	767	11	18	137	64
4437(Holstein)	1	345	.	11	.	84	.
4437(Holstein)	2	315	328	7	14	72	33
4437(Holstein)	3	588	630	14	15	152	35
4437(Holstein)	4	363	454	18	17	40	39
4437(Holstein)	5	519	552	15	14	73	31
4437(Holstein)	6	483	591	19	21	51	43
4437(Holstein)	7	552	603	22	24	69	34
4464(Holstein)	1	576	.	12	.	109	.
4464(Holstein)	2	598	509	8	15	73	58
4464(Holstein)	3	783	822	13	14	120	46
4464(Holstein)	4	768	783	10	14	117	63
4464(Holstein)	5	555	590	15	19	133	33
4464(Holstein)	6	621	633	11	12	146	53
4464(Holstein)	7	573	613	11	13	106	48
Holstein		Rest time correlation r = 0.92		Bout correlation r = 0.21		Duration correlation r = 0.10	
All Breeds		Rest time correlation r = 0.96		Bout correlation r = 0.64		Duration correlation r = 0.47	

Table 2.1 An evaluation of a novel behavior monitoring system[1] was compared to a validated data logger[2]. Daily activities were recorded in Jerseys (n=5) (A), Crossbred (n=4) (B), and Holsteins (n=5) (C). Correlation values were calculated for individual breeds and all breeds combined. Daily rest time from the behavior monitoring system[1] was highly correlated with the sum of total daily lying time from the data loggers[2] (R=0.96). Daily rest bout from the behavior monitoring system[1] compared with the sum of lying bouts from the data loggers[2] (R=0.64). Daily rest duration from the behavior monitoring system[1] compared with lying duration from the data loggers[2] (R=0.47).


Daily Rest Time (min)
950
700
450
200
200
300
400
500
600
700
800
900
1000
Sum Total Lying (min)
R=0.96

P<0.001

Figure 2.1 The behavior monitoring system and data loggers were used to quantify daily activities of Holsteins (n=5), Jerseys (n=5), and Crossbred (n=4). Daily rest time from the behavior monitoring system was highly correlated with the sum of total daily lying time from the data loggers ($R=0.96$) ($P<0.001$).

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Chapter 3: The use of animal activity data and milk components as indicators of clinical mastitis in dairy cows

ABSTRACT

Daily monitoring of animal activity and milk components around the onset of clinical mastitis can be used to detect disease prior to clinical onset. A total of 268 clinical mastitis cases were examined from Virginia Tech and the University of Florida dairy herds for daily changes in milk yield, electrical conductivity, milk fat, protein, and lactose percent, as well as activity measurements including daily rest time, daily rest duration, daily rest bouts, and daily steps taken. A milk sample was aseptically collected following onset of clinical signs observed by milkers at both farms. During a clinical mastitis infection milk fat and protein percent, and electrical conductivity increased, while milk yield and lactose percent decreased, and no change was seen in activity measurements prior to clinical onset. Sensitivity and specificity values were determined for the ability of each individual milk component and activity measurement to accurately predict case and control cows. MY (sensitivity=88%, specificity=89%) and EC (sensitivity=71%, specificity=90%) were determined to be reliable predictors. Activity measurements were not able to predict case and control cows accurately when examined individually. The candisc discriminant analysis combined all measurements and sensitivity and specificity values were calculated. Virginia Tech cows on d -1 (sensitivity=95%, specificity=95%), Virginia Tech and University of Florida cows on d -1 (sensitivity=88%, specificity=90). Overall, daily monitoring of animal activity and milk components can detect disease prior to clinical diagnosis and allow producers to make proactive management decisions regarding herd health.

Keywords: animal activity, milk component, mastitis

INTRODUCTION

Management practices within the dairy industry are a common focus on most farms throughout North America. Good management includes prevention and detection of different diseases that can affect the cow. However, management practices are changing because of a larger cow to worker ratio. Farm personnel must manage a larger group of cows resulting in less attention per individual cow and this increases the risk of diseases going undetected. With the current trend in the industry, novel detection and prevention options continue to be a focus to prevent disease.

Recent advancements in on-farm monitoring technology may allow for the detection of disease before the onset of clinical signs. Clinical mastitis is the most costly disease affecting the dairy industry with recent estimates suggesting each case produces a loss of $231-$289 (Hogeveen, 2010). Measurements of milk components in healthy lactating cows show minimal daily variation, however mastitis can dramatically alter concentrations of various milk components (Forsback et al., 2010). Daily monitoring of these components through advanced technologies could serve as an early indicator of disease

The concentration of milk protein is known to increase during subclinical mastitis in dairy cows and in buffalo (Nielsen et al., 2005, Uallah et al., 2005). Similarly, changes in electrical conductivity was able to predict 92% of mastitis cases prior to or on the day clinical signs were present (Milner et al., 1996). When daily milk fat concentrations were compared in healthy and mastitic cows, fat concentrations decreased in those cows with mastitis (Nielsen et al., 2005). This change may be due to lipase, which is an enzyme that breaks down triglycerides,

the main fat found in milk. During a mastitis infection this enzyme increases, and as a result fat concentrations are thought to decrease, though results have varied (Uallah et al., 2005). Lactose concentrations may decrease during mastitis due to tissue damage and/ bacteria utilization of this sugar. Multiple studies have shown that cows experiencing mastitis will have decreased milk lactose concentrations compared to healthy cows (Berning and Shook, 1992, Nielsen et al., 2005). While several studies have examined changes in milk components during mastitis, results have varied, and few have reported on the ability to use components to effectively detect mastitis. Daily milk component monitoring alone may not be sufficient enough to detect all cases of mastitis. However, daily monitoring of milk components and activity measures together may improve the ability to detect more cases of mastitis.

Daily monitoring of animal activity has proven to be a successful method to identify animals at risk for periparturient disease. Cows that experienced a metabolic or digestive disease had an increase in the number of steps/h taken on d 9 or d 8 prior to clinical diagnosis. This activity slowly decreased following d 8 until clinical diagnosis. Overall, cows diagnosed as unhealthy walked on average 8-14 steps/h less than healthy cows (Edwards and Tozer, 2004). In another study cows later diagnosed with mastitis showed a decreased resting time on d-2 and d-1 (349 ± 435 min and 391 ± 43 min, respectively) as compared to non-mastitic cows (481 ± 17 min and 488 ± 16 min, respectively) (Yeiser, 2011). Furthermore, cows that were later diagnosed with subclinical ketosis displayed more rest bouts on d -1 (16 ± 2bouts) relative to diagnosis as compared to animals without detectable disease (12 ± 1 bouts) (Yeiser, 2011). They concluded that activity parameters could be used as a valid method for proactively monitoring herd health on dairy operations.

While several studies have examined milk component changes in relation to mastitis, none have documented the effects of milk component and activity data together (Hortet and Seegers, 1998, Sloth et al., 2003, Toni et al., 2011). Therefore, the objective of this study was to examine the changes in daily milk components and animal activity around the onset of clinical signs of mastitis. The early detection of clinical mastitis provides the opportunity to change management strategies before the onset of signs and could lessen the severity as well as reduce costs associated with disease.

MATERIALS AND METHODS

Farms

All lactating dairy cows from the Virginia Tech Dairy Center (Blacksburg, Virginia) and the University of Florida (Gainesville, Florida) were used in the study. Cows from the Virginia Tech herd were housed in a freestall barn with sawdust bedding. Cows from the University of Florida were housed in a freestall barn with sand bedding. At both farms bedding material was changed twice weekly. Animals at the Virginia Tech Dairy were milked in a double-eight herringbone parlor while animals at the University of Florida were milked in a double-twelve herringbone rapid exit parlor. Cows in both herds were milked twice daily.

Animals

All lactating animals were eligible for enrollment in this study as a case or control, provided study requirements were met. A case animal had to be greater than or equal to 15 DIM and less than or equal to 291 DIM at the onset clinical mastitis, and animals had to be free of clinical signs for at least 14 d prior to enrollment. Only a cow's first clinical episode was included in the study. A control animal was matched to each case based on breed, lactation number, and DIM. A cow was eligible to serve as a control for multiple case cows and every case cow was assigned one control cow. If a cow became clinical she was no longer eligible to be a control cow. A control cow could be eligible as a case cow provided one month of time passed since she was a control.

Lactating cows from the Virginia Tech dairy herd were monitored for signs of clinical mastitis from March 2011 until March 2012 and lactating cows from the University of Florida

dairy herd were monitored from June 2011 to March 2012. General milking routines at both parlors included forestrip and observation of milk from each quarter for signs of clinical mastitis (flakes and/or clots in the milk), predipping, drying each teat with a single use towel, attaching milking unit, removing milking unit, and applying a teat dip. Upon examination of a quarter with clinical mastitis, milkers from both farms collected a milk sample aseptically and placed it into the freezer at the respective farm. Samples were collected from the Virginia Tech farm on a weekly basis and transported to the Virginia Tech Mastitis and Immunology Lab (Blacksburg, VA). Frozen milk samples from the University of Florida were sent to the Virginia Tech Mastitis and Immunology Lab on a monthly basis. For shipment, samples were packaged on ice and in a Styrofoam cooler and sent via overnight shipment to ensure they were cold on delivery. Milk samples from both herds were submitted for standard aerobic bacteriologic culture using previously published guidelines (NMC, 1999). A subset of milk samples collected from the University of Florida was also analyzed anaerobically for the identification of *Mycoplasma* spp.

Milk culture diagnosis

In brief, 10 µl of each clinical milk sample was plated onto a blood agar plate containing 5% blood. Additionally, 100 µl of each clinical milk sample was plated on MacConkey agar. Plates were incubated at 37°C for a total of 48 h. Plates were observed at 24 h and 48 h for bacterial growth. Biochemical tests were performed based on bacterial growth and included CAMP test, esculin hydrolysis, catalase test, coagulase test, reactions in the Triple Sugar Iron agar (TSIA), Simmons Citrate agar, motility, growth on 6.5% NaCl agar, hemolysis patterns, and Gram-staining. Bacterial isolates were presumptively identified based on hemolytic patterns and colony morphology.

Biochemical tests completed for isolates resembling staphylococci included Gram-staining, production of catalase, and coagulase incubated for 24 h and read at 4 h and 18 h. Biochemical tests done for isolates resembling all streptococci included Gram-staining, reaction to the production of catalase, esculin hydrolysis, CAMP reaction, and growth in 6.5% NaCl. Gram-negative colonies were distinguished based on results of lactose fermentation, reaction in the triple sugar iron test, Simmons citrate agar reaction, motility, and Gram-staining. All other colony types were identified using Gram-staining, colony morphology, hemolytic patterns, and the production of catalase. Once culturing was complete, each sample was assigned a bacterial group or placed into the "other" category. The Gram-positive bacterial group consisted of *Staphylococcus aureus*, all other *Staphylococcus* spp., and all *Streptococcus* spp. The Gram-negative group contained *Klebsiella* spp., *Escherichia coli*., *Citrobacter* spp., *Enterobacte*r spp., *Serratia* spp., *Pseudomonas* spp., and *Proteus* spp., whereas the "other" group contained *Prototheca*, yeast, and unknown microorganisms. Samples with no bacterial growth remained a separate category.

Afifarm herd management software

The Virginia Tech and University of Florida dairy operations were both equipped with the AfiFarm© (S.A.E. Afikim, Israel) Herd Management software program. Each lactating animal at the Virginia Tech Dairy Complex had an Afi PedometerPlus© (S.A.E. Afikim, Israel) affixed to a rear leg fetlock, while the University of Florida cows had an AfiPedometer© affixed to a rear leg fetlock (S.A.E. Afikim, Israel). Both systems were used to collect activity data on a daily basis. Activity variables were recorded between milking sessions in the memory of the pedometer. The University of Florida collected step activity on all lactating cows, while Virginia Tech collected step activity, rest bouts, rest duration, and rest time. The activity information

stored in the memory of the pedometer was then transmitted at milking time through a reader box to the AfiFarm© (S.A.E. Afikim, Israel) Herd Management software program and was summed for daily measurements of these activities.

Daily steps were defined as the number of steps taken per day. Daily rest bouts were defined as the number of times the cow laid down in a 24 h period. A rest bout was only counted if the cow was lying down for more than 3 minutes. Rest time was defined as the amount of time, measured in minutes; the cow was lying down in a 24 h period. Rest duration was defined as the average length (min) of each rest bout Additionally, the milking parlors at both herds were equipped with the milk component monitoring technology AfiLab© (S.A.E. Afikim, Israel). Milk fat, protein, and lactose percent, as well as electrical conductivity were recorded for each cow at each milking and transferred to the herd software program. Other variables collected at Virginia Tech included mastitis grade, whether or not the animal was culled, and treatment, which was available through PC Dart (DRMS, Ames, Iowa). A reason for any cull was noted, as was length and type of treatment.

Statistical analyses

Milk component and activity data from the herd software program were transferred into SAS V. 9.2 (SAS Institute Inc., Cary, North Carolina) for statistical analysis. Data from PC Dart (DRMS, Ames, Iowa) and bacteriological data were stored in a database (Microsoft Excel 2007 for Windows©; Microsoft Corporation, Redmond, Washington) until analysis.

Statistical models to analyze milk component and activity were created to examine differences between case and control cows from d -14 through d 14, relative to diagnosis of clinical mastitis (d 0). Models were applied using the PROC GLIMMIX procedure of SAS V.

9.2 (SAS Institute Inc., Cary, NC). Outcome variables for Virginia Tech and the University of Florida included milk lactose, fat and protein percent, electrical conductivity, milk yield, and daily steps, while only daily rest time, daily rest bouts, and daily rest duration were available for Virginia Tech. Variables entered into the models included lactation number (1, >1), farm (VT, FL), day (-14 through 14), bacterial group (Gram-negative, Gram-positive, no growth, "other", and clean), cow within lactation and farm, a covariate for MY and protein, as well as the associated interactions. Significance was declared at $P<0.05$. Bonferroni adjusted slice differences were used to identify significance within day for each interactions.

Cumulative Sum Analysis

A cumulative sum analysis was used to determine the probability of acquiring mastitis based on changes in milk composition and activity in the 3 d prior to clinical mastitis. Change was measured by taking the outcome variables (milk fat, protein, and lactose percent, electrical conductivity, daily milk yield, daily rest bouts, daily rest duration, daily rest time, and daily steps) values on d -3, d -2, and d -1 and subtracting it from the previous wk average to get a sum of difference for each day. A 7 d running average of d -10 to d -4 was calculated for d -3, d 9 to d -3 for d -2, and d- 8 to d -2 for d -1. Day -3, -2, and -1 were subtracted from each 7 d running average to obtain a numerical difference. These 3 numerical differences were summed to create a cumulative sum difference. A large cumulative sum in the 3 days prior to clinical mastitis diagnosis was hypothesized to be highly related to the onset of clinical mastitis. The variables offered into the analysis included breed (Holstein, Jersey, Mixed), lactation number (1, >1), and mastitis status (mstatus) defined as case or control. Outcome variables included milk lactose, fat, and protein percent, electrical conductivity, milk yield, daily rest time, daily rest bouts, daily rest duration, and daily steps. Significance was declared at $P<0.05$.

Slope Analysis

Using data from d -3 to d 0 relative to mastitis diagnosis, a slope analysis was conducted for milk lactose, protein, and fat percent, electrical conductivity, milk yield, daily rest time, daily rest bouts, daily rest duration, and average activity. The variables offered to the analysis included breed, lactation number, and mstatus. The slope was calculated for each described variable using the defined 4-d time period, and the slope was then converted to a percent of average day. Case animals were compared to controls using GLIMMIX procedure in SAS V. 9.2. The model included mstatus (control or case), breed (Jersey, Holstein, Mixed), lactation number (1, >1). Significance was determined at $P<0.05$. Threshold levels were evaluated for each variable. A threshold was selected based on high positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity.

Pattern Analysis

A pattern analysis was used to examine day to day pattern variation in the 3 d prior to onset of clinical signs. Standard deviation for each variable measured (milk lactose, fat, and protein percent, electrical conductivity, milk yield, daily rest time, daily rest duration, daily rest bouts and daily steps) was calculated from d-10 to d-4 and that numerical value was multiplied by 2. In SAS V. 9.2 a code was created that examined 3 changes (d -3 to d -2, d -2 to d -1, d -1 to d 0) to obtain a 3-letter pattern. If the change on any of the given days were greater than 2 standard deviations for that cow, as calculated above, that change was denoted with the letter A. If the change stayed the same it was denoted with a B and if the change decreased by more than 2 standard deviations it was denoted with a C. All 3 changes were concatenated to give each cow a 3 letter pattern for each variable. For example, CAB indicate a 2 standard deviation decline from d -3 to d -2, a 2 standard deviation increase from d -2 to d -1, and little change from

d -1 to d 0. A BBB would be little change in any day. The GLIMMIX procedure was different from this analysis. Variables offered to this model included breed (Holstein, Jersey, Crossbred), lactation number (1, >1), and farm (VT, FL). For each variable measured a binomial analysis was performed on whether the cow was a case (1) or a control (0), resulting in a probability of clinical mastitis for each pattern (and its significance). Significance was determined at $P<0.05$.

Discriminant Analysis

A discriminant analysis examined the feasibility of combining independent variables (milk lactose, protein, and fat percent, lactation number, DIM, weight, electrical conductivity, milk yield, daily rest time, daily rest bouts, daily rest duration, and daily steps) to predict groups of cows with or without clinical mastitis. A multivariate one-way analysis of variance (one-way MANOVA) was run using the PROC CANDISC procedure in SAS V.9.2 and statistical measures produced from the analysis included *Wilks' lambda*, eigenvalue, canonical correlation, and raw canonical coefficients for each variable provided to the model. The *Wilks' lambda* tested the hypothesis that the population means were equal, the eigenvalue provided an estimate of the models effectiveness, and higher canonical correlation coefficients indicated better overall model fit. The raw canonical coefficients were the linear combination of the variables that provided the greatest difference between the class means (case versus control). Variables offered into the model for data originating from Virginia Tech included milk lactose, protein and fat percent, lactation number, DIM, weight, electrical conductivity, milk yield, daily rest time, daily rest bouts, daily rest duration, and daily steps. Variables offered to the Virginia Tech and University of Florida combined model excluded daily rest time, daily rest duration, and daily rest bouts as these variable were not recorded at the University of Florida. Normal distribution of

independent variables was required and was tested using PROC UNIVARIATE in SAS V. 9.2. Significance was determined at $P<0.05$.

Thirty percent of the original dataset was removed at random and reserved as a test dataset, leaving a dataset with 70% of the original observations and defined as a training dataset. Data from the Virginia Tech herd alone and the entire data set were run on the training dataset to obtain raw canonical coefficients for each variable. Canonical coefficients for each variable were applied to the test dataset in an equation that calculated a canonical coefficient for each cow. Cow canonical coefficients were used to determine the effectiveness of the models to place cows accurately into case or control cow groups. Thresholds of the canonical sums were determined based on PPV, NPV, sensitivity, and specificity.

RESULTS

A total of 426 Holstein (90 primiparous, 336 multiparous), 34 Jersey (18 primiparous, 16 multiparous), and 76 Crossbred (20 primiparous, 56 multiparous) were enrolled in the study. Cow's with clinical mastitis infectionsisolated a wide range of bacterial pathogens (Table 3.1). Bacterial pathogens were placed into bacterial categories including Gram-positive (n=59), Gram-negative (57), and no growth (n=136), or placed into the other category (16) (Table 3.2). A total of 536 case (n=268) and control (n=268) cows were used in the study including 93 case cows from Virginia Tech and 175 case cows from the University of Florida with respective controls (Table 3.3).

Lactose

There was a significant bacterial group by day by farm interaction ($P<0.0001$). Significant differences between the Virginia Tech and University of Florida herds may be due to breed or nutritional differences, or both. There was also a significant bacterial group by day interaction ($P<0.0001$). Milk lactose concentration in cows isolated with Gram-positive bacteria was significantly decreased on d -8, -6, -5, -4, -3, -2, -1, 0, 1, 2, 3, 4, 5, 7, 9, 11, 13, and 14 compared to control cows as shown in Table 3.4A. Milk lactose concentration was significantly reduced on d -1 relative to onset of signs for animals from which Gram-negative pathogens were isolated (4.6% ± 0.0%) compared to controls (4.8% ± 0.0%). On the day of clinical diagnosis all case cows, regardless of bacterial group had significantly ($P<0.05$) lower mean lactose concentrations compared to control cows (Table 3.4A). Mean lactose concentrations were (4.50% ± 0.0%), (4.60% ± 0.0%), (4.70% ± 0.0%) and (4.60% ± 0.0%) for Gram-negative, Gram-positive, no growth, and other, respectively, compared to control cows (4.80% ± 0.0%).

Cows with no bacterial growth had significantly lower milk lactose concentrations 5 d following clinical diagnosis compared to controls while cows isolated in the other category had significantly lower concentrations on the day of diagnosis only (Table 3.4A). Lactose concentrations were significantly reduced in case animals with Gram-negative bacteria isolated from d -1 through d 14 compared to controls (Figure 3.1).

Fat

Lactation number was removed from the model because it was not significant. There was a significant bacterial group by day by farm interaction ($P<0.0001$). Significant differences between the Virginia Tech and University of Florida herds may be due to breed or nutritional differences, or both. There was a significant bacterial group by day interaction ($P<0.0001$), however only those cows with Gram-positive infections showed significant deviations in fat prior to clinical signs. On d -6, -4, and -1 cows isolated with Gram-positive bacteria had significantly greater fat concentrations compared to control cows (Figure 3.2). No other significant deviations in fat concentrations were found prior to the onset of clinical mastitis with any bacterial group compared to control cows. On d0 cows in the Gram-positive group had significantly increased fat concentrations (4.1% ± 0.1%) compared to control cows (3.7% ± 0.0%). Cows isolated with Gram-negative bacteria had significantly greater fat concentrations from d 1 to d 10 compared to control cows (Table 3.4B). While cows isolated with Gram-positive bacteria had significantly higher fat concentrations on d 1, 2, 3, 6, 8, 9, and 10 compared to control cows (Figure 3.2). Fat concentration for case animals in the no growth and other categories did not differ from controls during the 29 d monitoring period.

Protein

There was a significant bacterial group by day by farm interaction (P<0.0001). Significant differences between the Virginia Tech and University of Florida herds may be due to breed or nutritional differences, or both. There was a significant bacterial group by day interaction (P<.0001). Clinical cows that isolated Gram-positive pathogens had a significantly higher protein concentration compared to control cows on d -14. A covariate was included in the model to account for differences in protein concentrations on d -14 for all bacterial groups. Cows isolated with Gram-positive pathogens had significantly greater protein concentrations compared to control cows on d 2, 3, 4, 9 and 10 (Table 3.4C). Cows isolated with Gram-negative bacteria showed no significant deviations prior to or on the day of clinical mastitis diagnosis, however following diagnosis protein concentration was significantly greater on d 1, 2, 3, 4, 5, 9, and 10 compared to control cows (Figure 3.3). All other bacterial groups showed no significant deviations compared to control cows during the 29 d period.

Milk yield

There was a significant bacterial group by day by farm interaction (P<0.0001). Significant differences between the Virginia Tech and University of Florida herds may be due to breed or nutritional differences, or both. There was a significant bacterial group by day interaction (P<.0001). Clinical cows that isolated Gram-positive pathogens had a significantly lower MY concentration compared to control cows on d -14. A covariate was included in the model to account for differences in MY concentrations on d -14 for all bacterial groups. Only those cows which isolated Gram-positive pathogens differed in MY compared to control cows on the day prior to clinical diagnosis. Clinical cows with Gram-positive pathogens were also significantly different as compared to control cows on d 1 and 4 following clinical diagnosis. Cows isolated with Gram-negative bacteria had the most significant drop in milk production on d

0 23.7 kg ± 1.6 kg. Clinical cows which produced samples from which Gram-negative bacteria were isolated never fully recovered in MY from d 1 through d 14 (Figure 3.4). Cows with no growth samples had significantly lower milk yield on d 7 compared to control cows (Table 3.4D). Cows which isolated no pathogen were significantly different in MY compared to control cows on d 3, 12, and 14.

Electrical conductivity

There was a significant bacterial group by day by farm interaction ($P<0.0001$). Significant differences between the Virginia Tech and University of Florida herds may be due to breed or nutritional differences, or both. There was a significant bacterial group by day interaction ($P<0.0001$). Cases in which Gram-positive, Gram-negative and no growth were isolated showed deviations in EC prior to and on the day of clinical mastitis. On d 0 EC (mmho) averaged (11.9 ± 0.2), (11.0 ± 0.2), (11.0 ± 0.1) for Gram-negative, Gram-positive, and no growth groups, compared to control cows (10.2 ± 0.1). Gram-positive cows showed a significant increase in EC 4 d prior to and on the d of clinical diagnosis (Figure 3.5). A significant increase was observed on d 1, 2, 4, 5, 7, 11, and 13 compared to controls (Table 3.4E). Cases with no isolation had significantly increased EC compared to control cows on d -2, -1, 0, 1, 2, 3, 4, 5, 7, 8, and 11 (Figure 3.5). Cases with Gram-negative infections showed significant deviations from control cows on d -1, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 13 (Figure 3.5). Cows in the other group showed a significant increase in EC on d -1, 1, 2, 4, 5, 6, and 8 compared to control cows (Table 3.4E).

Daily steps

There was a significant bacterial group by day by farm interaction (P<0.0001). Significant differences between the Virginia Tech and University of Florida herds may be due to breed or nutritional differences, or both. There was a significant bacteria group by day interaction (P<0.0001). There were no significant differences between bacterial groups and control cows prior to clinical diagnosis (Figure 3.6). After clinical diagnosis (d 0) cows in the Gram-positive and no growth group took significantly more steps per day on d 1(3105 ± 146 steps) and (3184 ± 118 steps), respectively, compared to control cows (2567 ± 77 steps). Cows with clinical mastitis in the no growth group took significantly more daily steps on d 3, d 4, and d 5 following clinical diagnosis as compared to control cows (Table 3.4F).

Daily rest bouts

There was a significant bacteria group by day interaction (P<0.01). There were no significant differences between bacterial groups and control cows prior to clinical diagnosis (Figure 3.7). Cows isolated with Gram-positive bacteria had a greater amount of daily rest bouts (17.9 ± 1.2 bouts) on d 3 as compared to controls cow (14.3 ± 0.6 bouts) (P<0.02).

Daily rest duration

There were no significant interactions between bacteria group and day. No significant deviations were seen in any pathogen category prior to and after clinical onset as compared to control cows (Figure 3.8).

Daily rest time

There were no significant interactions between bacteria group and day. No significant deviations were seen in any pathogen category prior to and after clinical onset as compared to control cows (Figure 3.9).

Cusum Analysis

Lactose

Lactation number had no effect on the model. Case cows had a significant ($P<0.0001$) lactose cusum (0.2% ± 0.0%) compared to control cows (0.0% ± 0.0%) in the 3d prior to clinical mastitis diagnosis. Jersey case cows cusum (0.3% ± 0.1%) compared to Jersey control cows cusum (0.0% ± 0.1%) was numerically different however not significantly different ($P\leq0.05$) (Table 3.5A).

Electrical conductivity

Lactation number had no effect on the model. Case cows had significantly reduced ($P<0.0001$) conductivity scores (mmho). EC cusum values averaged -1.4 ± 0.1 compared to control cows (0.0 ± 0.1). Case Holsteins cows had a significantly reduced cusum (-0.7 ± 0.1) compared to control Holsteins (-0.0 ± 0.1). Case Jersey cows had a significantly lower cusum (-1.8 ± 0.3) compared to control Jerseys (-0.0 ± 0.3). Case Mixed cows had a significantly lower cusum (-1.5 ± 0.2) compared to control Mixed cows (0.2 ± 0.2) (Table 3.5B).

Milk yield

Lactation number was removed from the model due to lack of significance. Case cows had a significantly ($P<0.0001$) higher MY cusum values (kg) (7.8kg ± 1.1) compared to control

cows (0.2 ± 0.1). Jersey case cows had a significantly (P<0.001) higher MY cusum (10.5kg ± 2.8kg) compared to control cows (-1.7kg ± 2.7kg). Mixed case cows had a significantly (P<0.05) higher MY cusum (10.1kg ± 1.9kg) compared to control cows (1.9kg ± 1.8kg) (Table 3.5C).

Daily steps

Case cows had a significantly (P<0.05) higher daily step cusum (4.1 ± 2.7 steps) compared to control cows (-4.2 ± 2.7 steps). Case cows in first lactation had a significantly (P<0.05) higher daily step cusum (6.9 ± 4.0 steps) compared to control cows (-10.1 ± 4.0 steps). There was a significant 3-way interaction between mstatus, breed, and lactation number (P<0.05). Jersey case cows in first lactation had a significantly higher (P<0.05) daily step cusum (17.3 ± 8.0 steps) compared to control Jersey cows in first lactation (-24.1 ± 8.0 steps) (Table 3.5D).

Daily rest duration

Breed had no effect on the model. Case cows had a significantly higher daily rest duration cusum (11.1 ± 5.5 min) compared to control cows (-15.8 ± 5.5 min) (P<0.001). Case cows in first lactation had a significantly higher daily rest duration cusum (18.7 ± 8.8 min) compared to control cows (-15.1 ± 8.8 min) (P<0.05) (Table 3.5E) . No significant differences were seen between cows in second lactation.

Slope Analysis

Lactose

The change in milk protein, milk fat, daily steps, daily rest duration, daily rest time, and daily rest bouts as measured by the slope analysis, in the 3-d prior to clinical onset were not different for case and control cows. The slope of parity did not significantly impact the change in lactose concentrations. The change in milk lactose ($P<0.0001$) was significantly lower for case (-1.22% ± 0.22%) compared to control cows (-0.06% ± 0.18%). There was a significant 2-way interaction between mstatus and breed ($P<0.05$). Holstein cows with mastitis had a greater negative slope of milk lactose (-0.73% ± 0.13%) compared to control Holstein cows (-0.04% ± 0.12%) (Table 3.6A). The change in milk lactose ($P<0.0001$) prior to clinical onset was greater in crossbred cows with mastitis (-2.02% ± 0.35%) compared to control crossbred animals (0.03% ± 0.23%) (Table 3.6A). No significant differences were seen between Jersey case and control cows.

Milk yield

The slope of parity did not significantly impact the change in MY. The change in MY ($P<0.0001$) was significantly lower for case (-8.76% ± 1.06%) compared to control cows (-0.05% ±0.87%). Holstein cows with mastitis had a greater negative slope of MY (-5.81% ± 0.63%) compared to control Holsteins cows (0.01% ± 0.59%) (Table 3.6B). The change in MY prior to clinical onset was greater in Crossbred cows with mastitis (-12.06% ± 1.71%) compared to control Mixed cows (0.02% ± 1.40%) (Table 3.6B). No significant differences were seen between Jersey case and control cows. A positive predictive value (PPV), negative predictive value (NPV), sensitivity, specificity, and threshold analysis were determined for the ability of MY to accurately predict case and control cows. At a threshold cutoff of -2.00%, PPV=68%, NPV=71%, sensitivity=63%, and specificity=75% (Table 3.7A).

Electrical conductivity

The slope of breed did not significantly impact the change in EC. The change in EC ($P<0.0001$) was significantly higher for case (2.29% ±0.24%) compared to control cows (0.13% ± 0.22%). There was a significant 2-way interaction between mstatus and lactation number ($P<0.05$). Primiparous cows with mastitis had a greater positive slope of EC (1.71% ± 0.42%) compared to control cows (0.19% ± 0.39%). Multiparous cows with mastitis had a greater positive slope of EC (2.88% ± 0.24%) compared to control cows (0.06% ± 0.22%) (Table 3.6C). PPV, NPV, sensitivity, specificity, and threshold analysis were determined for the ability of EC to accurately predict case and control cows. At a threshold cutoff of 1.50%, PPV=69%, NPV=60%, sensitivity=48%, and specificity=79% (Table 3.7B).

Pattern Analysis

Fat

Models of daily steps, daily rest time, daily rest bouts, and daily rest duration as measured by the pattern analysis, in the 3-d prior to clinical onset was not different for case and control cows. Twenty-four different patterns for changes in fat concentration in the 3-d prior to clinical diagnosis were observed for clinical and healthy cows. Of the 24 patterns, 4 were found associated with a significantly higher probability of a cow having mastitis (Table 3.8A). Pattern BBC had a mastitic probability of 0.7 ± 0.1 ($P<0.04$), pattern BCC had a mastitic probability of 1.0 ± 0.0 ($P<0.01$), pattern CAB had a mastitic probability of 0.8 ± 0.1 ($P<0.04$), and pattern CCC had a mastitic probability of 0.8 ± 0.1 ($P<0.01$).

Protein

Twenty-six different protein patterns for changes in protein concentration in the 3-d prior to clinical diagnosis were observed for clinical and healthy cows. Of the 26 patterns, 3 were associated with a significantly higher probability of a cow having mastitis (Table 3.8B). Pattern BBB had a mastitic probability of 0.4 ± 0.1 ($P<0.03$), pattern BCC had a mastitic probability of 0.9 ± 0.0 ($P<0.01$), pattern CCC had a mastitic probability of 0.8 ± 0.1 ($P<0.01$).

Lactose

Twenty-seven different lactose patterns for changes in lactose concentrations in the 3-d prior to clinical diagnosis were observed for clinical and healthy cows. Of the 27 patterns, 3 were associated with a significantly higher probability of a cow having mastitis (Table 3.8C). Pattern BBA had a mastitic probability of 0.7 ± 0.1 ($P<0.02$), pattern BCC had a mastitic probability of 0.9 ± 0.1 ($P<0.01$), and pattern CCC had a mastitic probability of 0.8± 0.1 ($P<0.01$) .

Milk Yield

Twenty-seven different fat patterns for changes in MY in the 3-d prior to clinical diagnosis were observed for clinical and healthy cows. Of the 27 patterns, 4 were associated with a significantly higher probability of a cow having mastitis (Table 3.8D). Pattern BBA had a mastitic probability of 0.8 ± 0.1($P<0.01$), pattern BBB had a mastitic probability of 0.4 ± 0.0($P<0.01$), pattern BCC had a mastitic probability of 0.9 ± 0.1 ($P<0.01$), and pattern CCC had a mastitic probability of 0.8 ± 0.1 ($P<0.01$).

Electrical Conductivity

Twenty-six different fat patterns for changes in EC in the 3-d prior to clinical diagnosis were observed for clinical and healthy cows. Of the 26 patterns, 5 were associated with a significantly higher probability of a cow having mastitis (Table 3.8E). Pattern ABB had a mastitic probability of 0.2 ± 0.1 (P<0.02), pattern BBB had a mastitic probability of 0.3 ± 0.0 (P<0.01), pattern BBC had a mastitic probability of 0.8 ± 0.1 (P<0.01), pattern BCC had a mastitic probability of 1.0 ± 0.1 (P<0.01), and pattern CCC had a mean mastitic probability of 0.9 ± 0.1 (P<0.01).

Discriminant Analysis

On d-2 the Virginia Tech model *Wilks' lambda* was 0.82 and this function was significant at P<0.05, the eigenvalue was 0.22, and the canonical correlation was 0.43. Raw canonical coefficients were as follows milk yield, (0.0650); daily rest duration, (0.0083); daily rest time, (0.0001); daily rest bout, (-0.0467); fat, (0.1318); lactation number, (-0.2682); DIM, (0.0000); weight (0.0002); lactose, (1.3017); protein, (0.4412); daily steps, (0.0055); and electrical conductivity, (-0.4894). Class mean canonical values were for case -0.48 and control cows 0.46. On d-1 the Virginia Tech model *Wilks' lambda* was 0.71 and this function was significant at P<0.01, the eigenvalue was 0.41, and the canonical correlation was 0.54. Raw canonical coefficients were as follows milk yield, (0.0262); daily rest duration, (0.0030); daily rest time, -(0.0015); daily rest bout, (0.0532); fat, (-0.2211); lactation number, (0.5734); DIM, (0.0037); weight, (-0.0010); lactose, (1.0361); protein, (0.7190); daily steps, (0.0071); and electrical conductivity, (-0.5313). Class mean canonical variables for case -0.68 and control cows 0.59.

Variables offered to the Virginia Tech and University of Florida model included fat, protein, and lactose percent, lactation number, weight, DIM, electrical conductivity, daily milk

yield, and daily steps. On d-2 Model 2 *Wilks' lambda* was 0.95 and this function was significant at P<0.05. The eigenvalue was 0.05 and the canonical correlation 0.22. Raw canonical coefficients were as follows milk yield, (0.0044); fat, (-0.1618); lactation number, (0.1039); DIM, (0.0009); weight, (0.0025); lactose, (-1.1816); protein, (-1.7057); daily steps, (0.0073); and electrical conductivity, (-0.9286). Class mean canonical variables for case -0.22 and control cows 0.22. On d-1 the Virginia Tech and University of Florida model *Wilks' lambda* was 0.88 and this function was significant at P<0.0001. The eigenvalue was 0.14 and the canonical correlation 0.35. Raw canonical coefficients were as follows milk yield, (-0.0009); fat, (-0.2374); lactation number, (0.5280); DIM, (0.0013); weight, (0.0015); lactose, (1.0419); protein, (0.0637); daily steps, (0.0099); and electrical conductivity (-0.7719). Class mean canonical variables for case -0.39 and control cows 0.37.

On d-1 relative to clinical diagnosis, the candisc discriminant analysis correctly classified Virginia Tech case (n=21) and control (n=20) cows by can1 values at a threshold <1.0 PPV=91%, NPV=63%, sensitivity=48%, and specificity=95% (Table 3.9). On d-2 relative to clinical diagnosis, the candisc discriminant analysis correctly classified Virginia Tech case (n=21) and control (n=20) cows by can1 values at a threshold <2.75 PPV=80%, NPV=65%, sensitivity=57%, and specificity=85% (Table 3.10). On d-2 relative to clinical diagnosis, the candisc discriminant analysis correctly classified Virginia Tech and University of Florida case (n=81) and control (n=67) cows by can1 values at a threshold <0.1 PPV=70%, NPV=60%, sensitivity=63%, and specificity=67% (Table 3.11). On d-2 relative to clinical diagnosis, the candisc discriminant analysis correctly classified Virginia Tech and University of Florida case (n=81) and control (n=67) cows by can1 values at a threshold <0.25 PPV=63%, NPV=53%, sensitivity=56%, and specificity=61% (Table 3.12).

DISCUSSION

Mastitis infections in dairy cows cause several marked changes in milk components and may cause deviations in normal activity and behavior patterns. Daily monitoring of milk components and animal activity may be a useful tool in the early detection of mastitis. The detection of mastitis before clinical signs would allow dairy producers to intervene sooner and may reduce costs associated with this disease, and lessen the severity. To our knowledge, this is the first study to examine changes in both milk component and activity data around the onset of clinical mastitis, as well as the ability to use this combined data to detect mastitis.

Daily Milk Yield

In most studies MY has the greatest loss following the onset of clinical mastitis, but some studies have shown MY to decline prior to clinical signs. Primiparous cows isolated with *Staph aureus* and *Staphylococcus* spp. began to decline in MY 1-2 wk prior to clinical signs, however this data was only numerically different and not significant (Grohn et al., 2004). This same study also found primiparous clinical cows with no pathogen isolated to lose 3.3kg of milk/d 1 wk prior to clinical signs ($P<0.05$) as did multiparous clinical cows with *Streptococcus* spp. isolated (Grohn et al., 2004). While Grohn et al. (2004) examined individual pathogens, the present study examined the effect of groups of bacterial pathogens on MY. Cows isolated with only Gram-positive pathogens showed a decline in MY prior to clinical onset. Those clinical cows with Gram-negative infections began to decline on the day of clinical diagnosis. Prior to the model including a covariate, clinical cows with Gram-positive infections produced significantly less milk 2 wk prior to clinical signs. Clinical cows which isolated Gram-positive pathogens indicate the need for a longer monitoring period as these cows produced significantly

less milk on d -14 compared to control cows. After a covariate was included in the model, cows which isolated Gram-positive pathogens produced significantly less MY as compared to control cows on d -1, 1, and 4. Deluyker et al. (1991) reported similar findings to this study prior to a covariate being included, clinical cows began to decline in MY 1-2 wk prior to clinical onset, although researchers did not control for pathogen (Deluyker et al., 1991). Rajala-Schultz et al. (1999) reported clinical cows in late lactation to decline in milk yield 2-4 wk prior to the onset of clinical mastitis which agrees with results by Hagnestam et al. (2007), however data was only numerically different and neither study controlled for bacterial pathogen. The authors of these papers suggest this decline in MY may be attributed to a subclinical infection.

Cows isolated with Gram-positive pathogens began to decline in MY 2 wk prior to clinical onset in the current study, thus the Gram-positive bacterial group was split into individual pathogens: CNS, *Staph aureus*, and *Streptococcus* spp. Differentiating this bacterial group into individual pathogens allowed the examination of each individual pathogens effect on MY by day. Similar results were seen when the Gram-positive group was split into individual pathogens as compared to when all three pathogens were grouped as Gram-positive. The significant drop in MY prior to clinical signs could not be attributed to any individual Gram-positive bacteria, but rather to Gram-positive infections changing this component farther in advance compared to other bacterial groups. These results indicate the need to monitor MY more than 2 wk prior to clinical onset in control and case cows isolated with Gram-positive infections.

Cows isolated with Gram-negative bacteria upon detection of clinical mastitis produced significantly less milk than controls 1 d prior to diagnosis and lasting throughout the 14 d post-diagnosis period. Furthermore, cows that isolated Gram-negative bacteria had the most severe

milk loss compared to other pathogens which agrees with other studies examining pathogen-specific effects on MY (Grohn et al., 2004, Schukken et al., 2009). Cows with no pathogen isolated from a clinical mastitis case produced significantly less milk on d 0 than controls on d 0, while cows that isolated other pathogen types were not significantly different throughout the 29 d period. Other studies have found differing results in that cows isolated with other microorganisms produced on average 7.1 kg/d less than their healthy herdmates in the week following clinical diagnosis however they had a much larger sample size (n=81) compared to the present study (n=16) which could account for the differences. Differences in MY loss could also be attributed to types of pathogens represented by the other category. Grohn et al. (2004) included *Pasteurella* spp., *Proteus* spp., *Serratia* spp., gram-negative, *Bacillus*, yeast, Gram-positive *Bacillus*, *C. bovis* spp., *Enterobacter* spp., *Strep canis* and *Citrobacter* spp., whereas in the current study pathogens included in the other category were yeast, *Bacillus* spp. in pure culture, Prototheca, and unknown microorganisms.

A cusum analysis, pattern analysis, and slope analysis were conducted to determine the ability of MY to detect case cows prior to clinical signs. While the cusum analysis found case cows to have a higher daily milk yield cusum (7.8kg ± 1.1kg) compared to control cows (0.2kg ± 0.1kg) as well as differences between breeds, it was not able to accurately predict case and control cows. In a study by Lukas et al. (2009), daily MY cumulative sums were calculated for animals experiencing mild mastitis (-2.00kg ± 0.45kg), moderate mastitis (-2.65kg ± 0.31kg), and severe mastitis (-5.39kg ± 0.74kg). In the present study the MY cumulative sum for 3 d was 7.8kg ± 1.1kg, and the average daily MY cumulative sum was 2.65kg which is similar to what Lukas et al. (2009) found in mild and moderate cases of mastitis. The MY pattern analysis produced 27 separate MY patterns for case and control cows; however MY pattern could not be

used as a reliable clinical mastitis detection method. To our knowledge, no studies have conducted a pattern analysis to examine the effect of clinical mastitis on MY.

Although MY cumulative sum and MY pattern were not reliable predictors of clinical mastitis, MY slope predicted case and control cows accurately. At a cutoff of -2.00%, PPV= 68% and NPV=71%. Based on these results a daily milk yield slope threshold of <-2.00% would detect case and healthy cows most accurately.

Electrical conductivity

Electrical conductivity has been shown to be a reliable predictor of mastitis and is commonly used to detect cases of clinical mastitis. In one study, EC changed in 55% of clinical mastitis cases 2 milkings prior to onset of clinical signs, and in 34% of the cases at the same time clots were discovered (Milner et al., 1996). Composite electrical conductivity readings identified 80.1% of case cows and 74.8% of control cows correctly in another study (Norberg et al., 2004).

In the present study several different data analyses were used to examine the change in EC in case and control cows. The degree of change in EC values prior to clinical diagnosis may be pathogen specific. Cows with Gram-positive infections showed an increase in EC 4 d prior to clinical onset, while cows in which no pathogen was isolated from their milk samples EC values increased 2 d prior to diagnosis, and cows with Gram-negative infections and cows isolated with other microorganisms showed increased EC values 1 d before clinical signs. While several studies have examined changes in EC around the onset of clinical mastitis, none have reported EC values during clinical mastitis in individual pathogens or pathogen groups. In the present study EC values (mmho) in control cows on the day of clinical diagnosis (d0) averaged (10.2 ± 0.1), Gram-negative infections (11.9 ± 0.2), Gram-positive infections (11.0 ± 0.2), and cows

which isolated no pathogen (11.0 ± 0.1). A study by Norberg et al. (2004), examined EC values (milliSiemens, mS) in clinical (6.44 ± 1.22), subclinical (5.37 ± 0.02), and healthy (4.87 ± 0.01) cows. Because of the difference in measurement (mmho vs. mS) study results cannot be easily compared.. However, both studies showed that EC values increase during a clinical mastitis infection. Electrical conductivity will increase during mastitis because tight junctions become leaky and allow Na^+ and Cl^- to pass through the junctions and into the lumen of the alveolus, while K^+ moves out of milk (Norberg et al., 2004). Higher changes in clinical cows may also be explained due to the physical changes in milk from clinically sick cows compared to milk from healthy cows. Clinical mastitis infections usually cause clots in the milk which can stick to EC sensors and clots can also cause unstable milk flow which can affect EC measurements (Norberg et al., 2004).

In the slope analysis, case cows showed an increasing slope of EC compared to control cows. Furthermore, primiparous case cows had a significantly greater change in EC compared to pirmiparous control cows, and multiparous case cows had a significantly greater change in EC compared to multiparous control cows (Table 3.7C). EC cumulative sum and EC pattern analyses were not reliable predictors of clinical mastitis, although EC slope predicted case and control cows accurately. At a cutoff of >1.50%, PPV was 69% and NPV was 60%. Based on these results an EC slope threshold of >1.50% would detect case and healthy cows most accurately. Slope predicted 69% of clinical (n=223) cows accurately in the present study, however a different study was able to identify 80.1% of clinical (n=599) cows using EC (Norberg et al., 2004).

Protein

While a small number of studies have examined protein concentrations during mastitis infections, results have varied. In healthy Swedish Red cows, protein concentrations have shown to be 3.47% ± 0.24 throughout lactation (Forsback et al., 2010). In a different study that examined Danish red, Danish Holstein, and Jersey cows, healthy animals showed an average milk protein percent of 3.75% ± 0.50 (Sloth et al., 2003). Whereas in the present study protein concentrations in healthy cows showed an average of 3.1 ± 0.0% and 3.2% ± 0.0 throughout the 29 d monitoring period. Differences between protein concentrations among studies may be due to differences in breeds. When protein concentrations were examined in buffalo experiencing mastitis, overall concentrations decreased (Uallah et al., 2005), whereas in dairy cows, during a subclinical mastitis infection protein concentrations have been shown to increase (Urech et al., 1999). These results agree with another study conducted on dairy cows examining healthy and unhealthy quarters. Unhealthy quarters had significantly greater protein concentrations (3.49% ± 0.14%) compared to healthy quarters (3.18% ± 0.13%) (Nielsen et al., 2005). In the present study 1 d following clinical diagnosis protein concentrations in cows isolated with Gram-negative (3.3% ± 0.0%) pathogens were greater compared to control cows (3.1% ± 0.0%). Protein concentration reported by Nielsen et al. (2005) in healthy quarters (3.18% ± 0.13%) was much lower compared to the concentration reported in healthy quarters (3.47% ± 0.24%) from Forsback and colleagues. However, healthy quarter protein concentrations by Nielsen et al. (2005) (3.18% ± 0.13%) were similar to the present study results (3.1% ± 0.0%). Nielsen et al. (2005) examined 12 different Danish Holstein, whereas this study examined 268 Holstein, Jersey, and Crossbred cows.

In the current study, protein concentrations increased in cows with clinical mastitis. All bacterial groups showed no significant deviations in protein concentrations prior to clinical

diagnosis. When bacterial group was controlled for, cows with Gram-positive infections increased in protein concentration on d 2, 3, 4, 9 and 10 following clinical diagnosis. No studies to our knowledge have reported on pathogen-specific protein concentrations during clinical mastitis. While several studies have reported protein concentrations to increase or decrease during clinical mastitis, we found protein concentrations to increase in cows with Gram-negative and Gram-positive infections. Protein concentrations may increase during mastitis due to leaky tight junctions. Milk proteins can be divided into two primary classes casein and whey, casein being the primary component of protein found in milk. As tight junctions become leaky during an infection, whey proteins such as serum albumin, immunoglobulin's, and transferrin pass through the junctions and into the milk, thus increasing protein concentration (Harmon, 1994).

Protein concentration varied in cows isolated with Gram-positive pathogens prior to clinical onset with no significant differences in other bacterial groups prior to clinical onset. The cusum and slope analyses did not indicate the use of protein concentrations alone as a reliable predictor of clinical mastitis prior to the onset of clinical signs. While 3 different patterns showed significant probability associated with clinical mastitis, classification of cows using solely these 3 patterns was unpredictable. As the general trend in protein concentrations in this study increased, other study results have seen varied data, which further demonstrates the inability of protein alone as a predictor of mastitis.

Fat

Similar to protein, changes in fat concentrations have shown varied results in relation to mastitis infections. Some studies have shown concentrations to decrease after diagnosis including one study where fat concentrations in unhealthy quarters decreased to 4.4% ± 0.33

compared to the concentration in healthy quarters 4.7% ± 0.3 (Nielsen et al., 2005). Another study showed decreased fat concentrations in cows defined different from healthy (n=301) (5.0% ± 1%) compared to concentrations in healthy cows (n=520) (5.18% ± 1.%) (Nielsen et al., 2005, Sloth et al., 2003). In a study examining milk components of cows infected with subclinical mastitis, cows showed increased amounts of fat (54.6g/l ± 4.6g/l) compared to their unaffected contralateral quarter (44.1g/l ± 1.9g/l) (Bruckmaier et al., 2004). These results disagree with many studies that show fat concentration to be reduced during mastitis (Bruckmaier et al., 2004). Researchers from this study attributed a rise in fat yield to a reduction in lactose synthesis and therefore a reduced MY. Fat concentrations decrease during mastitis because increased amounts of lipase are released, causing a breakdown of triglycerides, the predominant type of fat found in milk (Uallah et al., 2005). However, the study by R. M. Bruckmaier, (2004) agrees with the present study which shows fat concentrations to increase during a clinical mastitis infection.

Milk fat content may be affected by milking interval. Cow's experiencing clinical mastitis and milked at shorter intervals may have increased fat concentrations because of the way oxytocin effects active transport of high-fat alveolar milk (Lollivier et al., 2002). Milk fat may also be linked to production drop and not actually to changes in the fat secretion process. Cows which isolated Gram-negative and Gram-positive pathogens showed increased fat concentrations (4.1% ± 0.1%) on the day following clinical diagnosis compared to control cows (3.7% ± 0.0%). To our knowledge no study has reported on fat concentrations during clinical mastitis among different pathogens. Variation in fat percent levels could also be due to factors such as breed and lactation number. Jersey cows have higher amounts of milk fat compared to Holsteins. Cows in one study found higher fat percentages in second lactation cows compared to first lactation cows,

and cows have also been shown to have lower milk fat percent in morning milking compared to night milking (Forsback et al., 2010, Nielsen et al., 2005, Toni et al., 2011).

In the present study, fat concentration was greater in case cows compared to control cows. However, only those cows with Gram-positive infections showed deviations prior to the onset of clinical mastitis, but concentrations were only significantly greater on d-6, -4, and -1 prior to clinical onset. Fat concentration variation in cows isolated with Gram-positive pathogens prior to clinical onset, and no significance in the other analyses indicates fat concentrations alone are not a reliable predictor of clinical mastitis prior to the onset of clinical signs. As the general trend in fat concentrations in this study increased, other study results have seen varied data, which further demonstrates the inability of fat alone as a predictor of mastitis.

Lactose

In healthy dairy cows milk lactose concentration is relatively stable at 4.7% with day-to-day variation of 0.7%, approximately (Forsback et al., 2010, Pyorala, 2003). Therefore deviations could indicate infection. Other studies have reported average lactose concentrations in healthy animals to range from 4.83% ± 0.00% to 4.73% ± 0.22% (Park et al., 2007, Sloth et al., 2003). Lactose concentrations decrease during clinical mastitis. One study reported lactose concentration in clinical quarters to be 4.37% ± 0.06% compared to healthy cows 4.70% ± 0.05%, although this study did not control for bacterial group (Nielsen et al., 2005). When a different study compared major pathogens (all streptococci, *Staphylococcus aureus, Escherichia coli,* and Klebsiella) to cows with minor pathogens (Coagulase-negative staphylococci,micrococci, and other isolates) they reported significantly greater deviations in lactose in animals with a major pathogen (4.75 ± .42) compared to those with a minor pathogen

(4.88 ± .35) (Berning and Shook, 1992). In the present study, on the day of clinical diagnosis cows isolated with Gram-negative pathogens (4.5% ± 0.0%) dropped more compared to Gram-positive pathogens (4.6% ± 0.0%), however both groups were significantly lower compared to control cows (4.8% ± 0.0%). Lactose concentrations from Berning and Shook, (1992) were much higher compared to concentrations found in the present study; however lactose concentrations were higher in healthy cows in their study (4.92% ± 0.25%) compared to the present study (4.8% ± 0.0%) which could account for differences between studies.

Prior to clinical onset, in the present study cows which isolated Gram-positive bacteria showed reduced lactose concentrations on d -8, and d-6, -5, -4, -3, -2, and -1 relative to clinical diagnosis, whereas cows isolated with Gram-negative bacteria showed reduced lactose concentration on the day prior to clinical onset (Table 3.4A). Lactose concentrations may drop during clinical mastitis due to certain types of bacteria, such as coliforms including *E. coli* and *Klebsiella* spp., utilizing this sugar to thrive in milk, thus decreasing overall concentration. Lactose concentrations may also drop during a mastitis infection due to tissue damage. During an infection, enzyme systems of the secretory cells in the mammary gland, will not be fully functioning and the biosynthesis of lactose will be decreased (Pyorala, 2003). While slope, pattern, and cusum analyses showed significant differences between case and control cows, these analyses were not reliable methods to detect clinical mastitis in the study. Perhaps future studies should examine detection of case and control cows on each milking, rather than daily, as this may increase the PPV. Lactose cannot be concluded as a reliable method to detect case and control cows in the present study. However, lactose concentrations and other milk component data, as well as activity measurements may increase the number of detectable cases.

Activity measurements

Activity data has traditionally been used to detect estrus in dairy cows, however more recent appreciation is being given to using these measurements to detect disease and discomfort in animals. While very few studies have been conducted using activity data to detect disease, of the studies that have been conducted, results show activity monitoring as a reliable predictor of disease before clinical onset.

While several studies have shown changes in activity prior to disease onset, in the present study no significant differences were seen in the 14 d prior to clinical diagnosis. Although this study found no significant differences prior to clinical onset, a different study showed that cows with clinical mastitis decreased their daily rest time on d -2 and d -1 (348.5 ± 42.5 min and 390.6 ± 42.5 min, respectively) as compared to non-mastitic cows, although pathogen was not controlled for (Yeiser, 2011). Researchers found on d -5 and d -4 relative to clinical diagnosis (d0) steps/h was significantly decreased for cows later diagnosed with mastitis (3267.3 ± 465.0 steps and 3002.7 ± 459.7 steps, respectively) compared to herdmates who were never diagnosed with mastitis (5553.4 ± 182.6 steps and 4573.3 ± 174.5 steps, respectively) (Yeiser, 2011). In the present study, after clinical diagnosis those cows with Gram-positive mastitis infections took significantly more steps (3105.1 ± 145.8 steps) on d 1 compared to control cows (2567.0 ± 76.5 steps). Those cows with no bacterial growth took more steps following clinical diagnosis on d 1, 2, 3, and 4 compared to control cows (Table 3.4F). No other significant differences were seen between case and control cows following clinical onset.

While no differences were seen in the above analysis prior to clinical onset, those clinically infected cows had a higher daily step cusum (4.1 ± 2.7 steps) compared to control cows (-4.2 ± 2.7 steps). Furthermore, Jersey case cows in first lactation had a significantly ($P<0.05$) higher daily step cusum compared to control Jersey cows in first lactation (Table 3.6). A higher

cusum in the 3 d prior to clinical diagnosis indicates those clinically infected cows were taking less steps/day compared to their previous weekly average, as well as to control cows. These results agree with Yeiser, et al. (2011), on d-5 and d-4 prior to clinical onset cows that were later diagnosed with mastitis had decreased steps/h compared to control cows. In a different study changes in walking activity were identified 7 to 8 d prior to clinical diagnosis of LDA, ketosis, and digestive disorders, indicating the use of walking activity in identifying diseased animals (Edwards and Tozer, 2004).

No other significant differences were seen in activity measurements among clinical and healthy animals in the slope and pattern analyse. Results from this study suggest activity measurements alone are not a sufficient method to detect clinical cows prior to the onset of clinical signs, however other studies have shown animal activity as a reliable predictor of disease (Edwards and Tozer, 2004, Yeiser, 2011). The combination of activity measurements with daily milk component measurements may be a more beneficial method to detect clinical mastitis prior to clinical onset.

Milk component and activity data

Milk component variables (fat, protein, and lactose, electrical conductivity, and milk yield), activity variables (daily rest time, daily rest bouts, daily rest duration, and daily steps), and cows variables (weight, DIM, and lactation number) were used in a linear combination to predict groups of cows with or without clinical mastitis. A canonical threshold was selected for each model based on the amount of correctly classified cows as calculated by PPV, NPV, sensitivity, and specificity.

The candisc discriminant analysis showed the most reliable results for predicting case and control cows prior to the onset of clinical mastitis. The model using animals from the Virginia Tech herd only which included all activity variables was more accurate at predicting case and control cows compared to the Virginia Tech and University of Florida model. The use of activity variables made the model more effective at predicting case and control cows. In both models predictability was more accurate on the day prior to clinical diagnosis. As the threshold level of the canonical values changed, the accuracy of PPV, NPV, sensitivity, and specificity changed. Some producers may want a low number of false-negatives and be willing to accept the false-positives because they would rather bring a cow into the parlor to be examined rather than miss that clinical case. On the other hand, some producers may want a low number of false-positives because they would rather avoid bringing extra animals in to be checked that don't in fact have clinical mastitis. The candisc discriminant analysis is a novel detection method with high PPV, NPV, sensitivity, and specificity. The model worked more effectively on d -1 and when activity variables were included. The use of this model in determining detection milking to milking may be more beneficial and increase detection rates.

In summary, this study worked to distinguish case cows from control cows with a 3 d prior average (cumulative sum), a 3-d prior change in variables (slope), a 3-d prior pattern of daily change (pattern), and a linear combination of variables 2-d prior to separate cows into a clinical group or a control group (canonical discriminant). The pattern and cumulative sum analysis showed the least promising results for classifying cows correctly. The slope and canonical discriminant showed the best results to predict case and control cows prior to clinical onset.

When individual components and activity measurements were examined to solely predict case and control cows accurately milk yield and electrical conductivity were both concluded as reliable predictors. Activity measurements were not able to predict case and control cows accurately when examined individually. Although milk yield and electrical conductivity predicted case and controls effectively, bacterial group may play a role in the effectiveness of using these components to predict accurately. Most changes in components and activity measurements were due to infections caused by Gram-positive and Gram-negative bacteria. Therefore, identification of clinical mastitis prior to clinical onset using the above analyses may be more beneficial when cows are experiencing clinical mastitis due to one of these bacterial groups.

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Bacterial type	Frequency	Percent	Category[1]
No growth	137	51.1	No growth
E. coli	32	11.9	Gram-negative
Env. Strep spp.	27	10.1	Gram-positive
Klebsiella spp.	14	5.2	Gram-negative
CNS	12	4.5	Gram-positive
S. aureus	11	4.1	Gram-positive
Strep dysgalactiae	8	3.0	Gram-positive
Yeast	7	2.6	Other
Citrobacter spp.	5	1.9	Gram-negative
Enterobacter spp.	3	1.1	Gram-negative
Prototheca	3	1.1	Other
Unknown microorganism	3	1.1	Other
Bacillus spp. in pure culture	2	0.8	Other
Gram-negative	2	0.8	Gram-negative
Serratia spp.	1	0.4	Gram-negative
Strep uberis	1	0.4	Gram-positive

Table 3.1 Frequency of bacteria pathogens isolated from naturally occurring cases of clinical mastitis (n=268) at the Virginia Tech dairy Center and the University of Florida research herds.

[1] The Gram-positive bacterial group consisted of *Staphylococcus aureus*, all other *Staphylococcus* spp., and all *Streptococcus* spp. The Gram-negative group contained *Klebsiella* spp., *Escherichia coli*., *Citrobacter* spp., *Enterobacte*r spp., *Serratia* spp., *Pseudomonas* spp., and *Proteus* spp., whereas the "other" group contained *Prototheca*, yeast, and unknown microorganisms. Samples with no bacterial growth remained a separate category.

Bacterial Group	Frequency	Percent
No growth	136	50.8
Gram-positive	59	22.0
Gram-negative	57	21.3
Other	16	6.0

Table 3.2 Frequency of bacterial groups isolated from naturally occurring cases of clinical mastitis (n=268)

Bacterial Group	University of Florida		Virginia Tech	
	Frequency	Percent	Frequency	Percent
No growth	110	62.9	26	28.0
Gram-positive	36	20.6	23	24.7
Gram-negative	19	10.9	38	40.9
Other	10	5.7	6	6.5

Table 3.3 Frequency of bacterial groups isolated from the University of Florida research herd naturally occurring case of clinical mastitis (n=175) and the Virginia Tech research herd naturally occurring cases of clinical mastitis (n=93).

A

Total Milk Lactose (%)									
Clean		**Gram-negative**		**Gram-positive**		**No growth**		**Other**	
Day	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**
-8	4.8 ± 0.0	-8		-8	4.7 ± 0.0	-8		-8	
-6	4.8 ± 0.0	-6		-6	4.7 ± 0.0	-6		-6	
-5	4.8 ± 0.0	-5		-5	4.7 ± 0.0	-5		-5	
-4	4.8 ± 0.0	-4		-4	4.7 ± 0.0	-4		-4	
-3	4.8 ± 0.0	-3		-3	4.7 ± 0.0	-3		-3	
-2	4.8 ± 0.0	-2		-2	4.7 ± 0.0	-2		-2	
-1	4.8 ± 0.0	-1	4.6 ± 0.0	-1	4.6 ± 0.0	-1		-1	
0	4.8 ± 0.0	0	4.5 ± 0.0	0	4.6 ± 0.0	0	4.7 ± 0.0	0	4.6 ± 0.0
1	4.8 ± 0.0	1	4.2 ± 0.0	1	4.5 ± 0.0	1	4.6 ± 0.0	1	
2	4.8 ± 0.0	2	4.3 ± 0.0	2	4.6 ± 0.0	2	4.7 ± 0.0	2	
3	4.8 ± 0.0	3	4.4 ± 0.0	3	4.6 ± 0.0	3	4.7 ± 0.0	3	
4	4.8 ± 0.0	4	4.5 ± 0.0	4	4.6 ±0.0	4	4.7 ±0.0	4	
5	4.8 ± 0.0	5	4.6 ± 0.0	5	4.6 ± 0.0	5	4.7 ± 0.0	5	
6	4.8 ± 0.0	6	4.6 ± 0.0	6	4.7 ± 0.0	6		6	
7	4.8 ± 0.0	7	4.6 ± 0.0	7	4.7 ± 0.0	7		7	
8	4.8 ± 0.0	8	4.7 ± 0.0	8		8		8	
9	4.8 ± 0.0	9	4.7 ± 0.0	9	4.7 ± 0.0	9		9	
10	4.8 ± 0.0	10	4.7 ± 0.0	10		10		10	
11	4.8 ± 0.0	11	4.7 ± 0.0	11		11		11	
12	4.8 ± 0.0	12	4.6 ± 0.0	12		12		12	
13	4.8 ± 0.0	13	4.7 ± 0.0	13	4.7 ± 0.0	13		13	
14	4.8 ± 0.0	14	4.7 ± 0.0	14	4.7 ± 0.0	14		14	

B

Total Milk Fat (%)									
Clean		**Gram-negative**		**Gram-positive**		**No growth**		**Other**	
Day	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**
-6	3.7 ± 0.0	-6		-6	4.0 ± 0.1	-6		-6	
-4	3.7 ± 0.0	-4		-4	4.0 ± 0.1	-4		-4	
-1	3.7 ± 0.0	-1		-1	4.0 ± 0.1	-1		-1	
0	3.7 ± 0.0	0		0	4.1 ± 0.1	0		0	
1	3.7 ± 0.0	1	4.1 ± 0.1	1	4.1 ± 0.1	1		1	
2	3.7 ± 0.0	2	4.3 ± 0.1	2	4.2 ± 0.1	2		2	
3	3.7 ± 0.0	3	4.3 ± 0.1	3	4.1 ± 0.1	3		3	
4	3.7 ± 0.0	4	4.3 ± 0.1			4		4	
5	3.7 ± 0.0	5	4.1 ± 0.1			5		5	
6	3.7 ± 0.0	6	4.2 ± 0.1	6	4.0 ± 0.1	6		6	
7	3.7 ± 0.0	7	4.0 ± 0.1			7		7	
8	3.7 ± 0.0	8	4.0 ± 0.1	8	4.0 ± 0.1	8		8	
9	3.7 ± 0.0	9	4.0 ± 0.1	9	4.0 ± 0.1	9		9	
10	3.7 ± 0.0	10	4.0 ± 0.1	10	4.0 ± 0.1	10		10	

C

Total Milk Protein (%)									
Clean		**Gram-negative**		**Gram-positive**		**No growth**		**Other**	
Day	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**
1	3.1 ± 0.0	1	3.3 ± 0.0	1	3.2 ± 0.0	1		1	
2	3.1 ± 0.0	2	3.4 ± 0.0	2	3.3 ± 0.0	2		2	
3	3.1 ± 0.0	3	3.4 ± 0.0	3	3.3 ± 0.0	3		3	
4	3.1 ± 0.0	4	3.3 ± 0.0	4	3.2 ± 0.0	4		4	
5	3.1 ± 0.0	5	3.3 ± 0.0	5		5		5	
9	3.1 ± 0.0	9	3.3 ± 0.0	8	3.2 ± 0.0	8		8	
10	3.1 ± 0.0	10	3.3 ± 0.0	9	3.2 ± 0.0	9		9	

D

Milk Yield (kg)									
Clean		**Gram-negative**		**Gram-positive**		**No growth**		**Other**	
Day	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**
-1	34.4 ± 0.5	-1		-1	30.4 ± 1.3	-1		-1	
0	34.8 ± 0.5	0	23.7 ± 1.6	0		0		0	
1	34.7 ± 0.5	1	24.2 ± 1.6	1	30.0 ± 1.5	1		1	
2	35.2 ± 0.5	2	25.5 ± 1.2	2		2		2	
3	34.9 ± 0.5	3	25.6 ± 1.4	3		3		3	29.7 ± 2.4
4	34.7 ± 0.5	4	27.1 ± 1.4	4	29.8 ± 1.7	4		4	
5	34.6 ± 0.5	5	27.8 ± 1.2	5		5		5	
6	34.4 ± 0.5	6	28.4 ± 1.4	6		6		6	
7	34.7 ± 0.5	7	28.3 ± 1.4	7		7	31.7 ± 1.0	7	
8	34.3 ± 0.5	8	29.4 ± 1.4	8		8		8	
9	34.4 ± 0.5	9	28.4 ± 1.3	9		9		9	
10	34.0 ± 0.5	10	29.0 ± 1.3	10		10		10	
11	34.1 ± 0.5	11	29.3 ± 1.3	11		11		11	
12	34.2 ± 0.5	12	29.4 ± 1.3	12		12		12	29.7 ± 2.1
13	34.3 ± 0.5	13	30.1± 1.3	13		13		13	
14	34.8 ± 0.5	14	29.7 ± 1.3	14		14		14	30.1 ± 2.1

E

Electrical Conductivity									
Clean		**Gram-negative**		**Gram-positive**		**No growth**		**Other**	
Day	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**
-4	10.3 ± 0.1	-4		-4	10.8 ± 0.2	-4		-4	
-3	10.2 ± 0.1	-3		-3	11.0 ± 0.2	-3		-3	
-2	10.2 ± 0.1	-2		-2	10.8 ± 0.2	-2	10.8 ± 0.1	-2	
-1	10.2 ± 0.1	-1	11.4 ± 0.2	-1	11.2 ± 0.2	-1	10.8 ± 0.1	-1	11.4 ± 0.3
0	10.2 ± 0.1	0	11.9 ± 0.2	0	11.0 ± 0.2	0	11.0 ± 0.1	0	
1	10.2 ± 0.1	1	12.5 ± 0.2	1	11.1 ± 0.2	1	11.0 ± 0.1	1	11.2 ± 0.3
2	10.2 ± 0.1	2	12.1 ± 0.2	2	11.0 ± 0.2	2	11.0 ± 0.1	2	11.5 ± 0.5
3	10.2 ± 0.1	3	12.2 ± 0.2	3		3	10.9 ± 0.2	3	
4	10.2 ± 0.1	4	11.9 ± 0.2	4	11.0 ± 0.2	4	10.8 ± 0.1	4	11.3 ± 0.4
5	10.3 ± 0.1	5	11.7 ± 0.2	5	11.0 ± 0.2	5	10.8 ± 0.2	5	11.5 ± 0.4
6	10.3 ± 0.1	6	11.1 ± 0.2	6		6		6	11.7 ± 0.4
7	10.3 ± 0.1	7	11.1 ± 0.2	7	11.1 ± 0.2	7	10.7 ± 0.1	7	
8	10.3 ± 0.1	8	11.1 ± 0.2	8	10.8 ± 0.2	8	10.7 ± 0.1	8	11.5 ± 0.4
9	10.3 ± 0.1	9	11.0 ± 0.2	9	10.9 ± 0.2	9		9	
10	10.3 ± 0.1	10	11.0 ± 0.2	10	10.9 ± 0.2	10		10	
11	10.3 ± 0.1	11	11.0 ± 0.2	11	11.0 ± 0.2	11	10.7 ± 0.1	11	
12	10.3 ± 0.1	12	11.0 ± 0.2	12		12		12	
13	10.3 ± 0.1	13	11.1 ± 0.2	13	11.0 ± 0.2	13		13	

F

Daily Steps									
Clean		**Gram-negative**		**Gram-positive**		**No growth**		**Other**	
Day	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**	**Day**	**Estimate ± S.E.**
1	2567 ± 77	1		1	3105 ± 146	1	3184 ±118	1	
3	2524 ± 76	3		3		3	2996 ± 120	3	
4	2540 ± 76	4		4		4	2889 ± 119	4	
5	2490 ± 76	5		5		5	2944 ± 119	5	

Table 3.4 Milk component and activity data lsmeans in case (n=268) and control (n=268) cows by category (clean: n=268; Gram-negative: n=57; Gram-positive: n=59; no growth: n=136; and other: n=16) in the 14d prior to clinical diagnosis (d0) and the 14d following clinical diagnosis for lactose (A), protein (B), fat (C), milk yield (D), electrical conductivity (E) and daily steps (F), only significant bacterial by day interactions are shown ($P<0.05$) for each bacterial group or the "other" category. The Gram-positive bacterial group consisted of *Staphylococcus aureus*, all other *Staphylococcus* spp., and all *Streptococcus* spp. The Gram-negative group contained *Klebsiella* spp., *Escherichia coli*., *Citrobacter* spp., *Enterobacte*r spp., *Serratia* spp., *Pseudomonas* spp., and *Proteus* spp., whereas the "other" group contained *Prototheca*, yeast, and unknown microorganisms. Samples with no bacterial growth remained a separate category.

A

Milk Lactose (%) Cusum		
Variable	**Case**	**Control**
Intercept	0.1 ± 0.0	0.0 ± 0.0
Breed (Jersey)	0.3 ± 0.1	0.0 ± 0.1

B

Electrical Conductivity (mmho) Cusum		
Variable	**Case**	**Control**
Intercept	-1.4 ± 0.1	0.0 ± 0.1
Breed (Holstein)	-0.7 ± 0.1	-0.0 ±0.1
Breed (Jersey)	-1.8 ± 0.3	-0.0 ± 0.3
Breed (Crossbred)	-1.5 ± 0.2	0.2 ± 0.2

C

Milk Yield (Kg) Cusum		
Variable	**Case**	**Control**
Intercept	7.8 ± 1.1	0.2 ± 0.1
Breed (Jersey)	10.5 ± 2.8	-1.7 ± 2.7
Breed (Crossbred)	10.1 ± 1.9	1.9 ± 1.8

D

Daily Steps (steps) Cusum		
Variable	**Case**	**Control**
Intercept	4.1 ± 2.7	-4.2 ± 2.7
Lactation Number (1)	6.9 ± 4.0	-10.1 ± 4.0
Breed (Jersey) Lactation (1)	17.3 ± 8.0	-24.1 ± 8.0

E

Daily Rest Duration (min) Cusum		
Variable	**Case**	**Control**
Intercept	11.1 ± 5.5	-15.8 ± 5.5
Lactation Number (1)	18.7 ± 8.8	-15.1 ± 8.8

Table 3.5 Milk component and activity data 3 d cusum intercepts for case (n=268) and control (n=268) cows by lactose (A), electrical conductivity (B), milk yield (C), daily steps (D), and daily rest duration (E). Only significant cusum data is shown as calculated using Proc Glimmix in SAS V. 9.2 ($P<0.05$).

A

Lactose Slope (%)		
Variable	**Case**	**Control**
Intercept	-1.22 ± 0.22	-0.06 ± 0.18
Breed (Holstein)	-0.73 ± 0.13	-0.04 ±0.12
Breed (Crossbred)	-2.02 ± 0.35	0.03 ± 0.23

B

Milk Yield Slope (%)		
Variable	**Case**	**Control**
Intercept	-8.78 ± 1.06	-0.05 ±0.87
Breed (Holstein)	-5.81 ± 0.63	0.01 ± 0.59
Breed (Crossbred)	-12.06 ± 1.71	0.02 ± 1.40

C

Electrical Conductivity Slope (%)		
Variable	**Case**	**Control**
Intercept	2.29 ±0.24	0.13 ± 0.22
Lactation Number (1)	1.71 ± 0.42	0.19 ± 0.39
Lactation Number (2+)	2.88 ± 0.24	0.06 ± 0.22

D

Daily steps Slope (%)		
Variable	**Case**	**Control**
Intercept	-4.84 ± 1.11	0.48 ± 1.08
Breed (Jersey)	-8.64 ± 2.71	2.27 ± 2.62

Table 3.6 Milk component and activity data slope intercepts in the 3 d prior to clinical diagnosis for case (n=203) and control (n=241) cows by lactose (A), milk yield (B), electrical conductivity (C), and daily steps (D). Slope was calculated using the Proc Glimmix procedure in SAS V. 9.2 and only significant slope data is shown ($P<0.05$).

A

Milk Yield		Actual		
		Case	Control	
Threshold >2.00%	Case	197 (43.88%)	169 (37.64%)	PPV 54%
	Control	26 (5.79%)	57 (12.69%)	NPV 69%
		Sensitivity 88%	Specificity 25%	

B

Milk Yield		Actual		
		Case	Control	
Threshold <-4.00%	Case	125 (27.84%)	25 (5.57%)	PPV 83%
	Control	98 (21.83%)	201 (44.77%)	NPV 67%
		Sensitivity 56%	Specificity 89%	

C

Electrical Conductivity		Actual		
		Case	Control	
Threshold >2.5%	Case	85 (18.93%)	22 (4.90%)	PPV 79%
	Control	138 (30.73%)	204 (45.43%)	NPV 60%
		Sensitivity 38%	Specificity 90%	

D

Electrical Conductivity		Actual		
		Case	Control	
Threshold <-1.0%	Case	158 (35.19%)	163 (36.30%)	PPV 49%
	Control	65 (14.48%)	63 (14.03%)	NPV 49%
		Sensitivity 71%	Specificity 28%	

Table 3.7 Slope thresholds were determined for the ability of MY (A, >2.00%), (B, <-4.00%) and EC (C, >2.5%), (D, <-1.0%) to accurately predict case (n=203) and control cows (n=241) as calculated by PPV, NPV, sensitivity, and specificity. ($P<0.05$). Two slope thresholds were chosen, one based off of high sensitivity and one based off of high specificity.

A

Fat			
Pattern	**Mean ± S.E.**	**P-value**	**n**
BBC	0.7 ± 0.1	P<0.04	n=47
BCC	1.0 ± 0.0	P<0.01	n=21
CAB	0.8 ± 0.1	P<0.04	n=13
CCC	0.8 ± 0.1	P<0.02	n=18

B

Protein			
Pattern	**Mean ± S.E.**	**P-value**	**n**
BBB	0.4 ± 0.1	P<0.03	n=250
BCC	0.9 ± 0.0	P<0.01	n=13
CCC	0.8 ± 0.1	P<0.01	n=17

C

Lactose			
Pattern	**Mean ± S.E.**	**P-value**	**n**
BBA	0.7 ± 0.1	P<0.02	n=42
BCC	0.9 ± 0.1	P<0.01	n=15
CCC	0.8 ± 0.1	P<0.01	n=17

D

Milk Yield			
Pattern	**Mean ± S.E.**	**P-value**	**n**
BBA	0.8 ± 0.1	P<0.01	n=56
BBB	0.4 ± 0.0	P<0.01	n=236
BCC	0.9 ± 0.1	P<0.01	n=13
CCC	0.8 ± 0.1	P<0.01	n=15

E

Electrical Conductivity			
Pattern	**Mean ± S.E.**	**P-value**	**n**
ABB	0.2 ± 0.1	P<0.02	n=21
BBB	0.3 ± 0.0	P<0.01	n=222
BBC	0.8 ± 0.1	P<0.01	n=50
BCC	1.0 ± 0.0	P<0.01	n=34
CCC	0.9 ± 0.1	P<0.01	n=22

Table 3.8 Probability estimates and S.E. from pattern combinations in the 3 d prior to clinical diagnosis that showed the best probability of a cow being a clinical case (n=261) compared to control cows (n=258) in the following pattern variables: fat (A), protein (B), lactose (C), milk yield (D), and electrical conductivity (E). Pattern examined 3 changes (d -3 to d -2, d -2 to d -1, d -1 to d 0) to obtain a 3-letter pattern. If the change on any of the given days were greater than 2 standard deviations that change was denoted with the letter A. If the change stayed the same it was denoted with a B and if the change decreased by more than 2 standard deviations it was denoted with a C. All 3 changes were concatenated to give each cow a 3 letter pattern for each variable (fat, protein, lactose, milk yield, and electrical conductivity). P<0.05).

A

Virginia Tech d -1		Actual		
		Case	Control	
Threshold <1.0	Case	10 (24.39%)	1 (2.44%)	PPV 91%
	Control	11 (26.83%)	19 (46.34%)	NPV 63%
		Sensitivity 48%	Specificity 95%	

B

Virginia Tech d -1		Actual		
		Case	Control	
Threshold >2.0	Case	20 (48.78%)	14 (34.15%)	PPV 59%
	Control	1 (2.44%)	6 (14.63%)	NPV 86%
		Sensitivity 95%	Specificity 30%	

Table 3.9 Case (n=21) and control (n=20) cows on d-1 relative to clinical diagnosis correctly classified by can1 values calculated from a training data set (70% original observations) in Proc Candisc in SAS V. 9.2 and applied to a test data set (30% original observations). Two thresholds were chosen, one with high specificity (A) and one with high sensitivity (B). This model contains variables milk lactose, protein, and fat percent, lacation number, DIM, weight, electrical conductivity, milk yield, daily step activity, daily rest bouts, daily rest duration, and daily rest time represented by the Virginia Tech research herd.

A

Virginia Tech d -2		Actual		
		Case	Control	
Threshold <2.0	Case	7 (17.07%)	2 (4.88%)	PPV 78%
	Control	14 (34.15%)	18 (43.90%)	NPV 56%
		Sensitivity 33%	Specificity 90%	

B

Virginia Tech d -2		Actual		
		Case	Control	
Threshold >4.0	Case	15 (36.59%)	16 (39.02%)	PPV 48%
	Control	6 (14.63%)	4 (9.76%)	NPV 40%
		Sensitivity 71%	Specificity 20%	

Table 3.10 Case (n=21) and control (n=20) cows on d-2 relative to clinical diagnosis correctly classified by can1 values calculated from a training data set (70% original observations) in Proc Candisc in SAS V. 9.2 and applied to a test data set (30% original observations). Two thresholds were chosen, one based off of high specificity (A) and one based off of high sensitivity (B). This model contains variables milk lactose, protein, and fat percent, lacation number, DIM, weight, electrical conductivity, milk yield, daily step activity, daily rest bouts, daily rest duration, and daily rest time represented by the Virginia Tech research herd.

A

Virginia Tech & University of Florida d -1		Actual		
		Case	Control	
Threshold <-0.5	Case	38 (26.58%)	7 (4.73%)	PPV 84%
	Control	43 (29.05%)	60 (40.54%)	NPV 58%
		Sensitivity 47%	Specificity 90%	

B

Virginia Tech & University of Florida d -1		Actual		
		Case	Control	
Threshold >0.50	Case	71 (34.46%)	42 (14.86%)	PPV 63%
	Control	10 (20.27%)	25 (30.41%)	NPV 71%
		Sensitivity 88%	Specificity 37%	

Table 3.11 Case (n=81) and control (n=67) cows on d-1 relative to clinical diagnosis correctly classified by can1 values calculated from a training data set (70% original observations) in Proc Candisc in SAS V. 9.2 and applied to a test data set (30% original observations). Two thresholds were chosen, one based off of high specificity (A) and one based off of high sensitivity (B). This model contains variables milk lactose, protein, and fat percent, lacation number, DIM, weight, electrical conductivity, milk yield, and daily step activity represented by the Virginia Tech and University of Florida research herds

A

Virginia Tech & University of Florida d -2		Actual		
		Case	Control	
Threshold >-0.50	Case	24 (16.22%)	8 (5.41%)	PPV 75%
	Control	57 (38.51%)	59 (39.86%)	NPV 51%
		Sensitivity 30%	Specificity 88%	

B

Virginia Tech & University of Florida d -2		Actual		
		Case	Control	
Threshold <1.0	Case	65 (43.92%)	45 (30.41%)	PPV 59%
	Control	16 (10.81%)	22 (14.86%)	NPV 58%
		Sensitivity 80%	Specificity 67%	

Table 3.12 Case (n=81) and control (n=67) cows on d-2 relative to clinical diagnosis correctly classified by can1 values calculated from a training data set (70% original observations) in Proc Candisc in SAS V. 9.2 and applied to a test data set (30% original observations). Two thresholds were chosen, one based off of high specificity (A) and one based off of high sensitivity (B). This model contains variables milk lactose, protein, and fat percent, lacation number, DIM, weight, electrical conductivity, milk yield, and daily step activity represented by the Virginia Tech and University of Florida research herds.

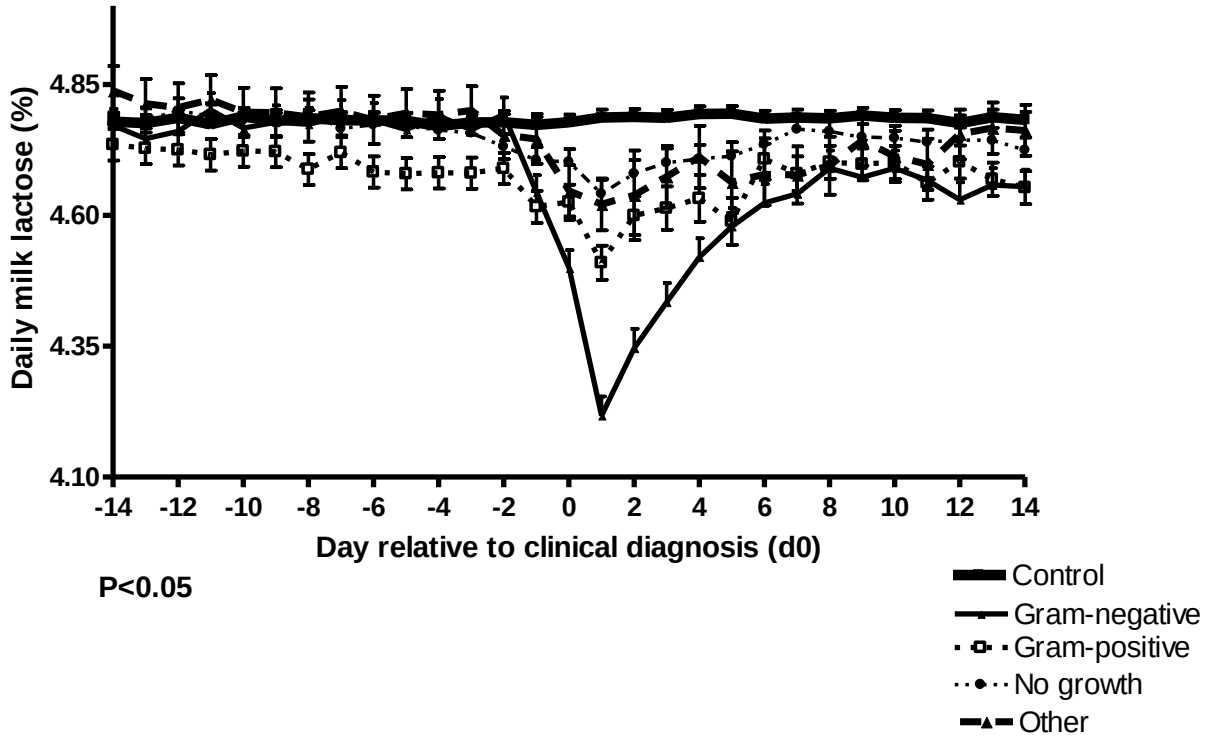

Figure 3.1 Daily milk lactose concentrations in case and control cows relative to clinical diagnosis d(0). Statistical analysis was conducted using Proc Glimmix in SAS V. 9.2 and significance was determined at P<0.05. Bacterial species were classified as control (n=268), Gram-negative (n=57), Gram-positive (n=59), no growth (n=136), and other (n=16). Cows isolated with Gram-negative bacteria significantly differed from control cows on d-1, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14. Cows isolated with Gram-positive bacteria significantly differed from control cows on d-8, -6, -5, -4, -3, -2, -1, 0, 1, 2, 3, 4, 5, 7, 9, 11, 13, and 14. Cows which had no bacterial growth significantly differed from control cows on d0, 1, 2, 3, 4, and 5. Cows which isolated other microorganisms significantly differed from clean cows on d0.

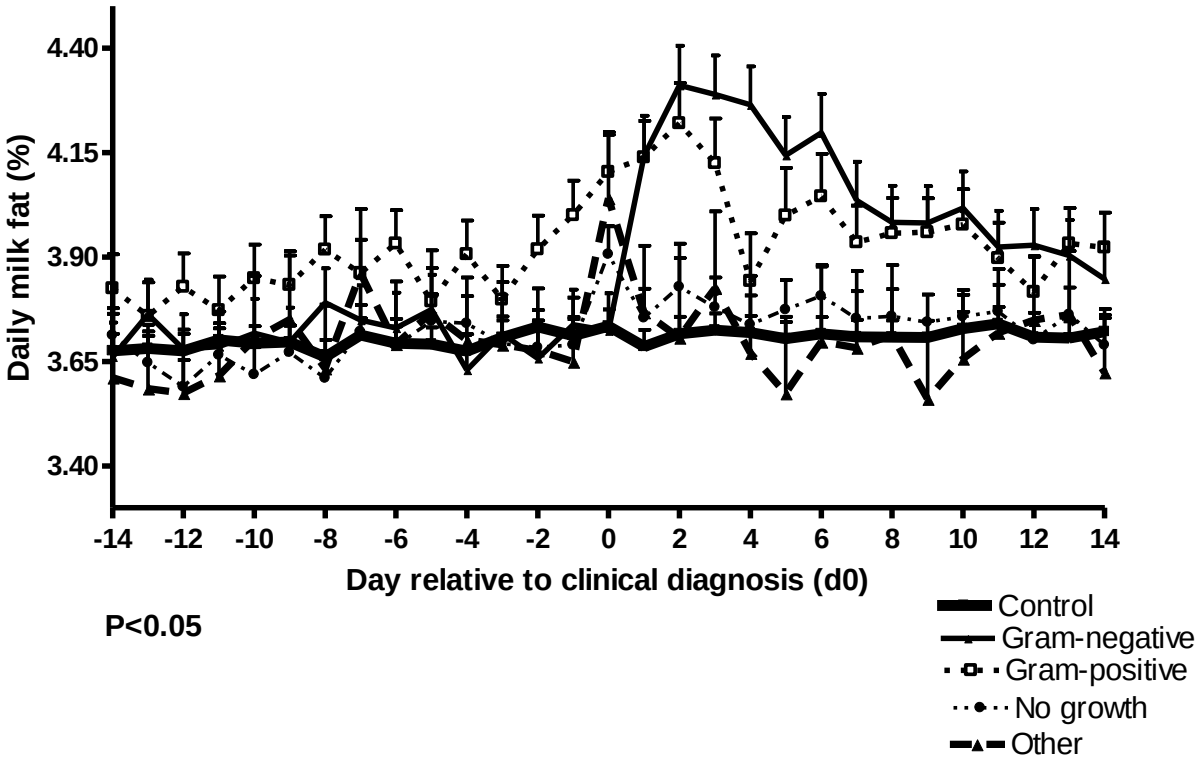

Figure 3.2 Daily milk fat concentrations in case and control cows relative to clinical diagnosis d(0). Statistical analysis was conducted using Proc Glimmix in SAS V. 9.2 and significance was determined at $P<0.05$. Bacterial species were classified as control (n=268), Gram-negative (n=57), Gram-positive (n=59), no growth (n=136), and other (n=16). Cows isolated with Gram-negative bacteria significantly differed from control cows on d1, 2, 3, 4, 5, 6, 7, 8, 9, and 10. Cows isolated with Gram-positive bacteria significantly differed from control cows on d-6, -4, -1, 0, 1, 2, 3, 6, 8, 9, and 10.

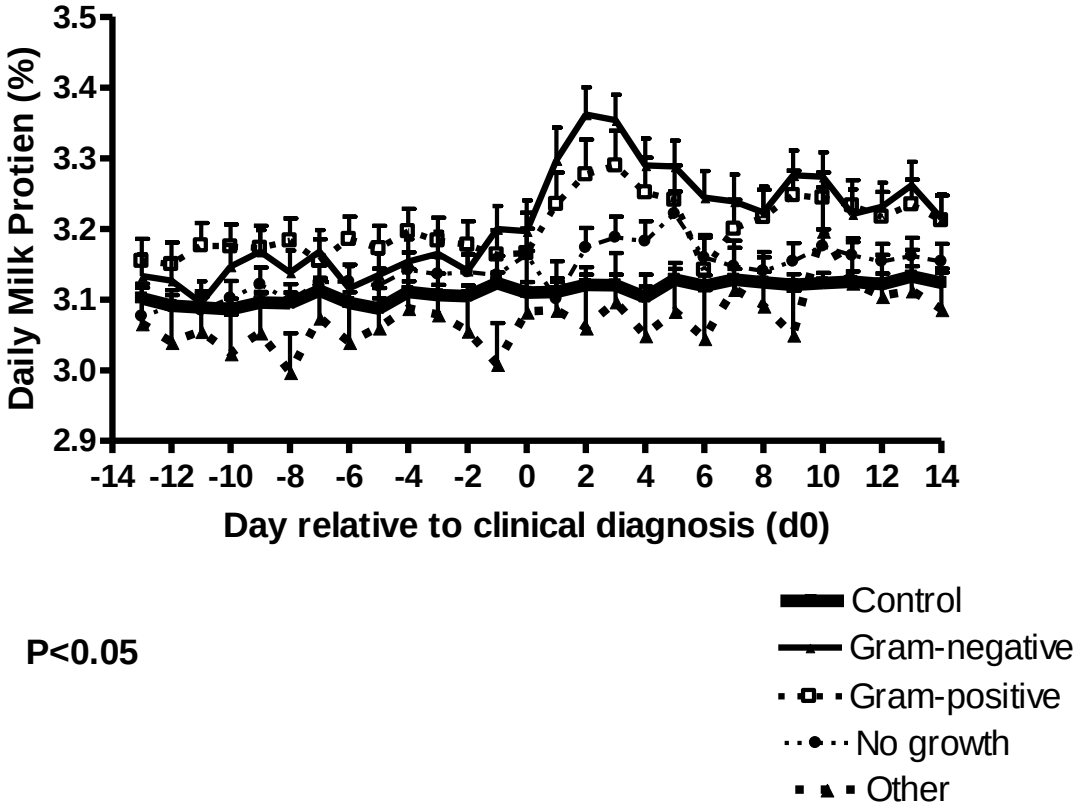

Figure 3.3 Daily milk protein concentrations in case and control cows relative to clinical diagnosis d(0). Statistical analysis was conducted using Proc Glimmix in SAS V. 9.2 and significance was determined at P<0.05. Bacterial species were classified as control (n=268), Gram-negative (n=57), Gram-positive (n=59), no growth (n=136), and other (n=16). Cows isolated with Gram-negative bacteria significantly differed from control cows on d1, 2, 3, 4, 5, 9 and 10. Cows isolated with Gram-positive bacteria significantly differed from control cows on d 1, 2, 3, 4, 9 and 10.

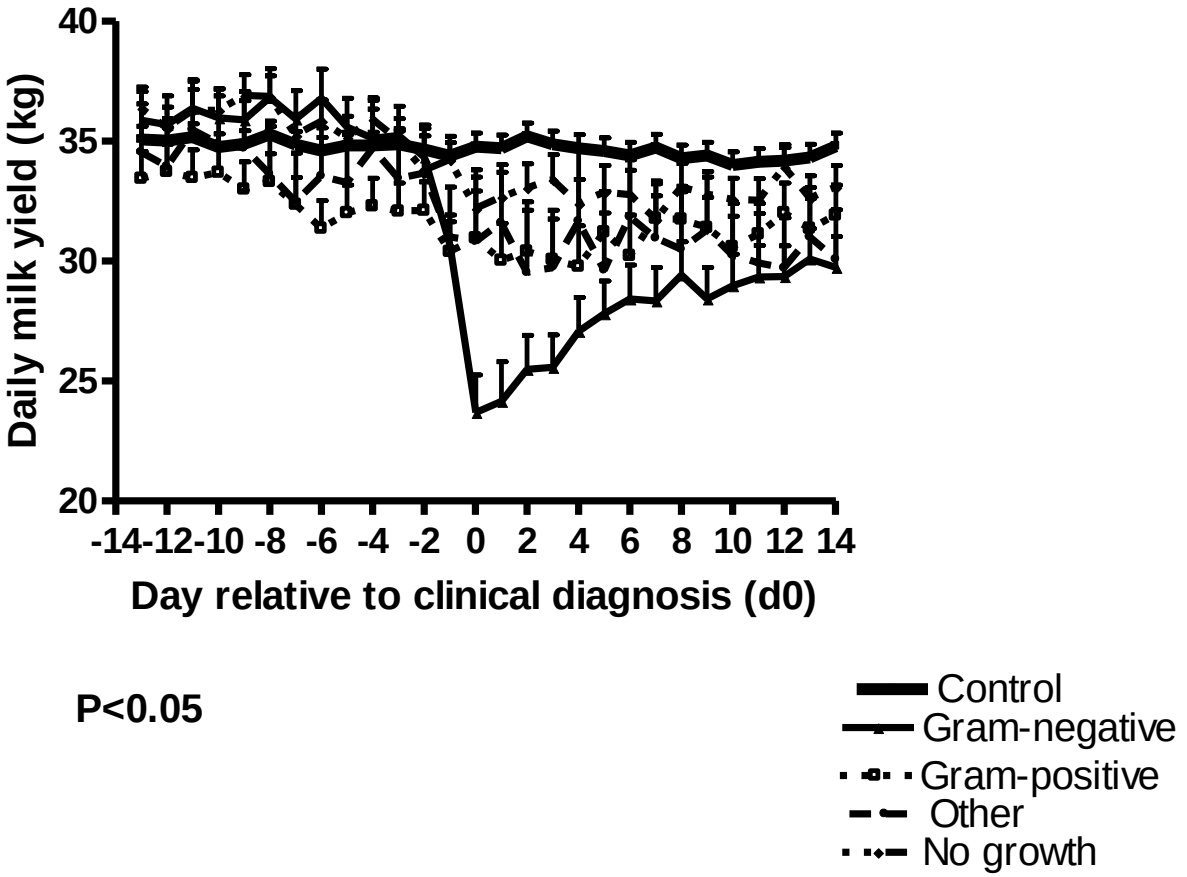

Figure 3.4 Daily milk yield (lb) in case and control cows relative to clinical diagnosis d(0). Statistical analysis was conducted using Proc Glimmix in SAS V. 9.2 and significance was determined at P<0.05. Bacterial species were classified as control (n=268), Gram-negative (n=57), Gram-positive (n=59), no growth (n=136), and other (n=16). Cows isolated with Gram-negative bacteria significantly differed from control cows on d 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14. Cows isolated with Gram-positive bacteria significantly differed from control cows on d -1, 1, and 4. Cows which isolated no pathogen significantly differed from control cows on d 7. Cows which isolated other microorganisms significantly differed from control cows on d 3, 12 and 14.

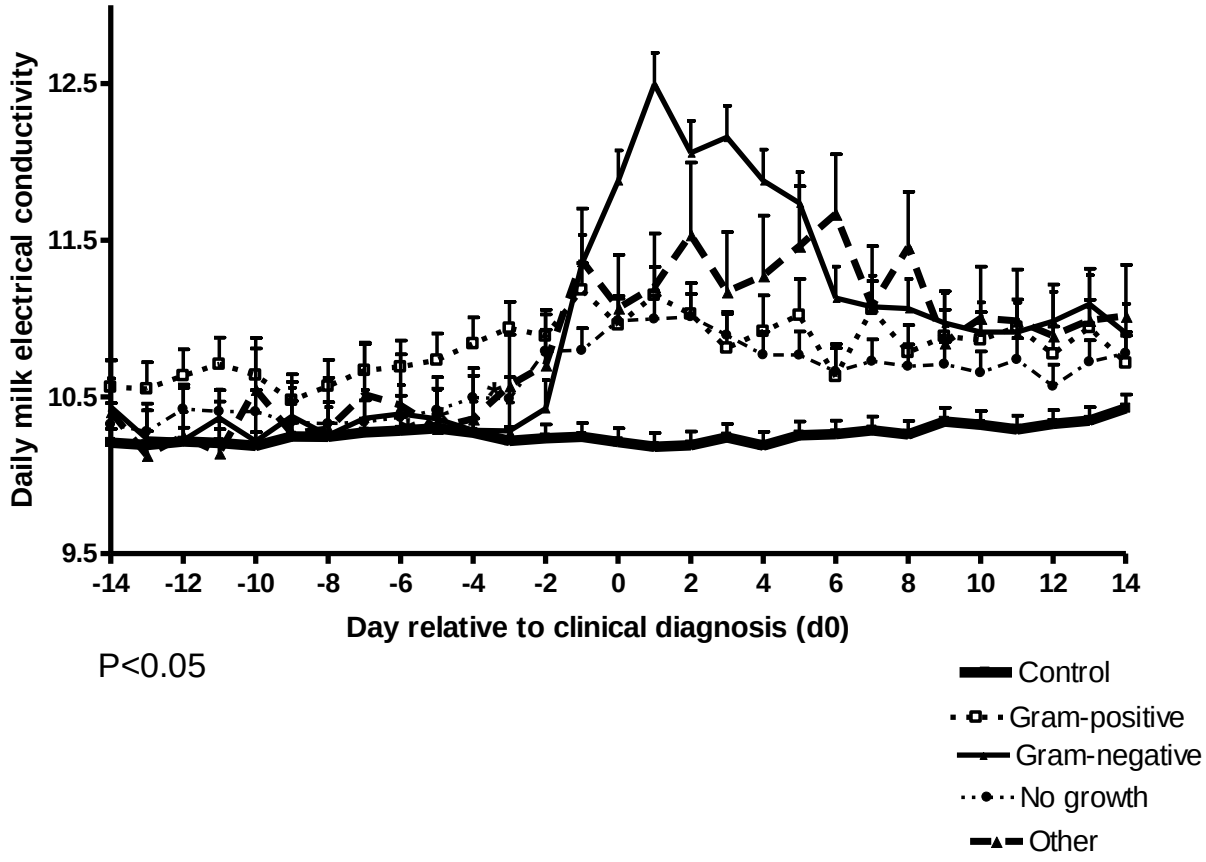

Figure 3.5 Daily electrical conductivity in case and control cows relative to clinical diagnosis d(0). Statistical analysis was conducted using Proc Glimmix in SAS V. 9.2 and significance was determined at P<0.05. Bacterial species were classified as control (n=268), Gram-negative (n=57), Gram-positive (n=59), no growth (n=136), and other (n=16). Cows isolated with Gram-negative bacteria significantly differed from control cows on d-1, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9,10, 11, 12, 13, and 14. Cows isolated with Gram-positive bacteria significantly differed from control cows on d-4, -3, -2, -1, 0, 1, 2, 4, 5, 7, 8, 9, 10, 11, and 13. Cows which showed no bacterial growth differed significantly from control cows on -2, -1, 0, 1, 2, 3, 4, 5, 7, 8, and 11. Cows isolated with other microorganisms significantly differed from control cows on d-1, 1, 2, 4, 5, 6, and 8.

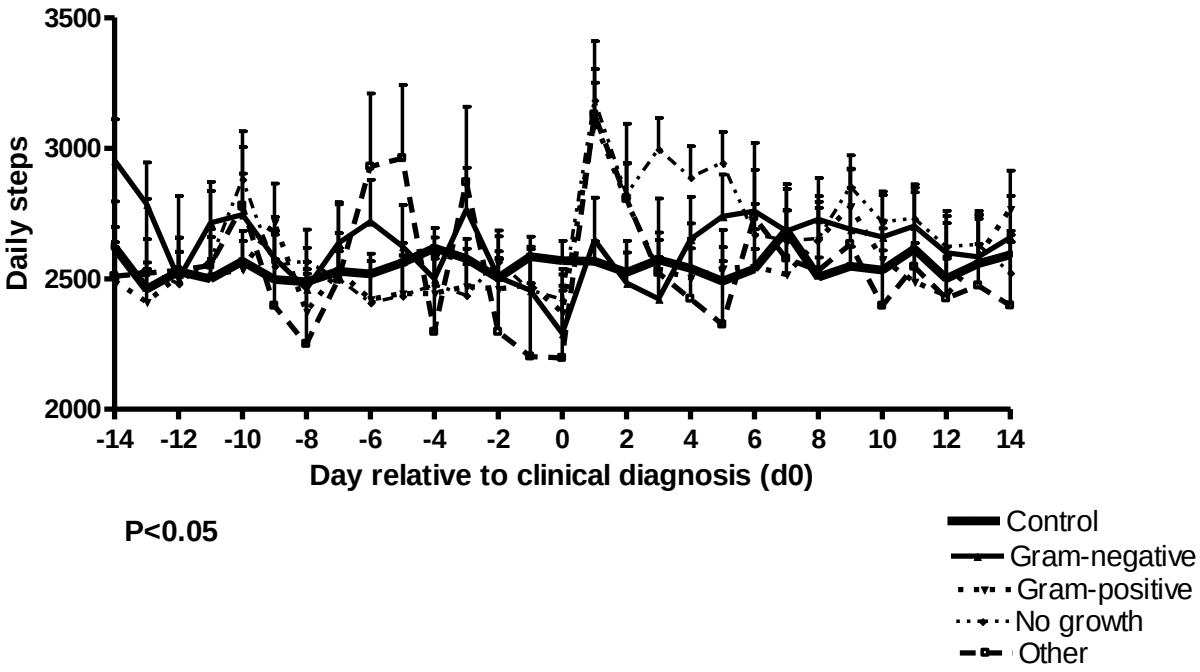

Figure 3.6 Daily step activity in case and control cows relative to clinical diagnosis d(0). Statistical analysis was conducted using Proc Glimmix in SAS V. 9.2 and significance was determined at P<0.05. Bacterial species were classified as control (n=268), Gram-negative (n=57), Gram-positive (n=59), no growth (n=136), and other (n=16). Cows isolated with Gram-positive bacteria significantly differed from control cows on d1. Cows which showed no bacterial growth differed significantly from control cows on d 1, 3, 4, and 5.

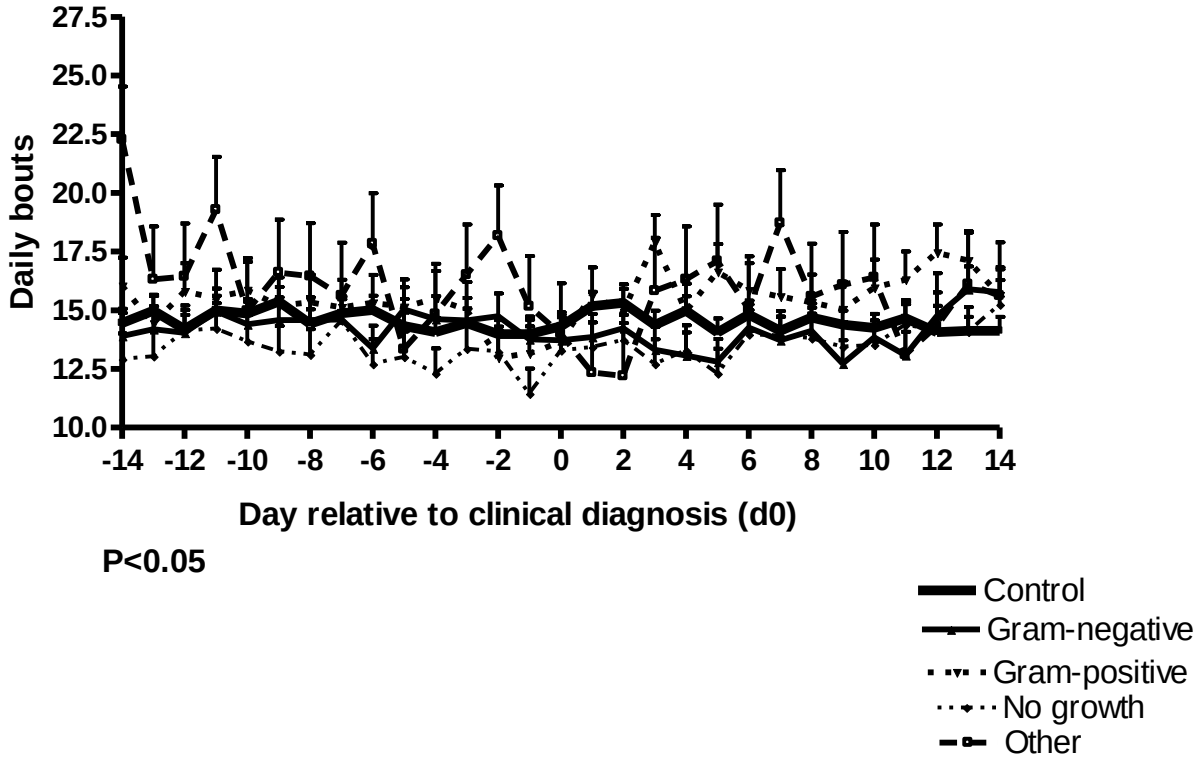

Figure 3.7 Daily rest bouts in case and control cows relative to clinical diagnosis d(0). Statistical analysis was conducted using Proc Glimmix in SAS V. 9.2 and significance was determined at P<0.05. Bacterial species were classified as control (n=268), Gram-negative (n=57), Gram-positive (n=59), no growth (n=136), and other (n=16). Cows isolated with Gram-positive bacteria significantly differed from control cows on d 3.

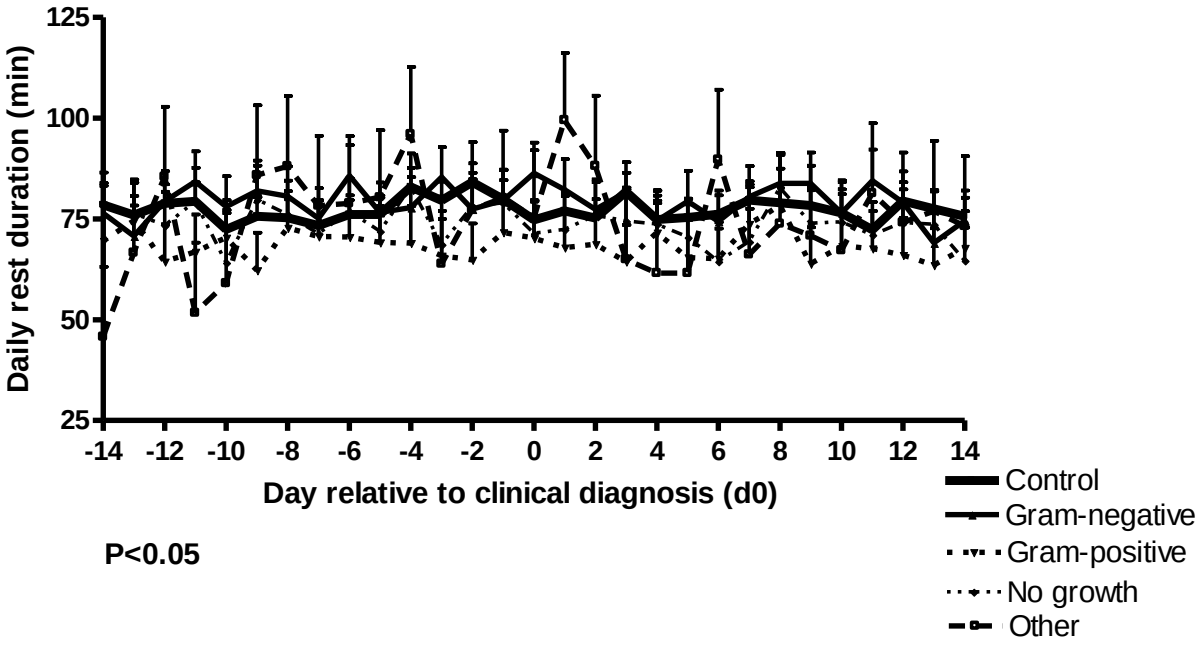

Figure 3.8 Daily rest duration in case and control cows relative to clinical diagnosis d(0). Statistical analysis was conducted using Proc Glimmix in SAS V. 9.2 and significance was determined at P<0.05. Bacterial species were classified as control (n=268), Gram-negative (n=57), Gram-positive (n=59), no growth (n=136), and other (n=16).

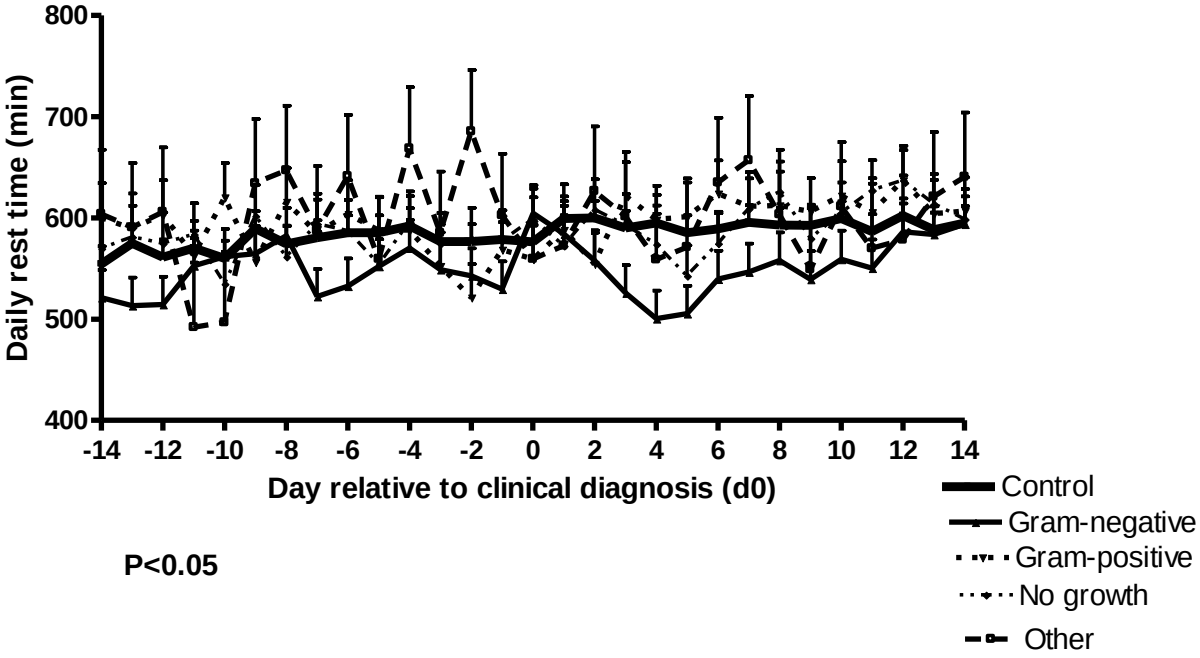

Figure 3.9 Daily rest time in case and control cows relative to clinical diagnosis d(0). Statistical analysis was conducted using Proc Glimmix in SAS V. 9.2 and significance was determined at P<0.05. Bacterial species were classified as control (n=268), Gram-negative (n=57), Gram-positive (n=59), no growth (n=136), and other (n=16).

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Chapter 4: General Conclusions

Every dairy farm is affected by disease with mastitis being the most costly disease affecting this industry today. As a result of mastitis, costs arise due to treatment, reduced milk production, and discarded milk. Producers rely on healthy animals to sustain a profit, while unhealthy animals can substantially reduce profit. The dairy industry is shifting from an era of treatment to an era of proactive management decisions which include better detection and preventions strategies which can aid in keeping producer profit high. Several studies have reported on changes occurring in milk components during clinical mastitis, although some components such as fat and protein have shown varied results to which way these components shift during an infection. Very few have reported on changes in activity measurements, and none have reported on the ability of milk component and activity measurements together to predict mastitis prior to clinical onset.

The need for more research in individual milk component and activity measurements to predict cases and controls, as well as using combined measurements to predict disease prior to clinical onset is evident. Therefore, two studies were conducted that compared compatibility of activity tracking devices and examined milk component and activity data around the onset of clinical mastitis. The first study wanted to determine the compatibility of the Afi PedometerPlus© (S.A.E. Afikim, Israel) to the HOBO© data loggers (HOBO Pendant G Data Logger, Onset; Pocasset, MA) considered the "gold standard" of animal tracking devices. The aim of the second study was to examine changes in individual component and activity measurements in the 14 d prior to clinical diagnosis (d 0) as well as in the 14 d following diagnosis. Trends in components and activity data prior to diagnosis may allow detection of cows more likely to get clinical mastitis in the following days. This study also examined the

feasibility of using individual component and activity measurements to predict clinical disease as well as using combined measurements.

The first study concluded that the high correlation value between daily rest time and lying time confirms the behavior monitoring system to be a valid tracking device in dairy cows for this variable. Furthermore, the behavior monitoring system is a valid method to track activity and behavior data from different breeds of dairy cows for this variable. However, correlation values for rest duration and rest bouts were lower than expected. When individual breeds were examined daily rest duration (R=0.79, R=0.83) and daily rest bouts (R=0.93, R=0.91) from the behavior monitoring system were both validated variables for Jersey and Crossbred cows compared to the data loggers. Daily rest duration and daily rest bouts could not be validated for Holstein cows at this time. Low correlation values could have been due to Holstein animal's involvement in a challenge trial. This challenge trial involved frequent sampling of animals and may have caused animals to move more frequently. Correlation values may have also been lower than expected due to difference in bout recording between tracking devices. Differences in bout recording would also make rest duration correlations low as this is a measurement of average bout time in minutes. Future studies should be conducted that set data loggers to record bouts >3 minutes to assess compatibility with the behavior monitoring system. The novel behavior monitoring system was concluded as a reliable tracking device for daily rest bouts and daily rest duration in Jersey and Crossbred cows, and for daily rest time in Jersey, Crossbred, and Holstein cows.

In the second study when individual components and activity measurements were examined around the onset of clinical mastitis several components showed marked changes while activity measurements showed no significant differences prior to or on the day of

diagnosis. Three separate data analyses (cusum, slope, and pattern) were used to predict case and control cows using individual milk component and activity measurements, and one data analysis (discriminant analysis) was used to predict case and control cows using milk component and activity data together. Of the milk components measured, MY and EC were concluded as reliable predictors of case and control cows when examined individually in the slope analysis. At two separate slope thresholds, MY had a high specificity (89%) and high sensitivity (88%), and similar trends were found for EC, high specificity (90%) and high sensitivity (71%). Activity measurements were not able to predict case and control cows accurately when examined individually. The cusum and pattern analysis were not able to yield high specificities or sensitivities when predicting case and control cows, therefore they were not concluded as reliable analyses for predicting case and control cows.

Although, MY and EC predicted case and controls effectively, bacterial group may play a role in the effectiveness of using these components to detect accurately. Most changes in components and activity measurements were due to infections caused by Gram-positive and Gram-negative bacteria. Therefore, identification of clinical mastitis prior to clinical onset using the above analyses may be more beneficial when cows are experiencing clinical mastitis due to one of these bacterial groups, rather than a miscellaneous organism such as yeast.

The candisc discriminant analysis, which combined milk component and activity measurements together, was able to predict case and control cows most accurately, yielding high specificities and sensitivities. Two different thresholds were examined for each model (VT, VT + UF) in the 2-d prior to clinical diagnosis using can1 values as calculated from this analysis. One threshold produced a high sensitivity whereas a second threshold was chosen that produced a high specificity. On d-1 the Virginia Tech model had a high sensitivity (95%) and high

specificity (95%), and on d-1 the Virginia Tech and University of Florida model had a high sensitivity (88%) and specificity (90%). The discriminant analysis functions better when activity variables such as daily rest time, daily rest bouts, and daily rest duration are included in the model, as higher sensitivities and specificities were achieved. This suggests activity data to be a useful predictor in combination with milk component data to detect clinical mastitis prior to onset.

Producers that implement systems with high sensitivity have more cows correctly classified as a case that actually are a case and their can1 value test results classify them as a case. However, a downfall with a high sensitivity is a high number of false-positives. False-positives are actual control cows, but their can1 values are classifying them as a case cow. A high number of false-positives will create more labor for a producer as they will have to bring in extra cows to be examined for clinical mastitis that are healthy and this could result in reduced costs associated with labor. Whereas producers that rely on systems with high specificity will have a high number of control cows correctly classified. However, a high specificity also produces a high number of false-negatives. A false-negative is a cow that actually has clinical mastitis but her can1 value is indicating she is healthy. Producers who implement a threshold model for high specificity will save in costs associated with labor as they will not have to check as many cows. However, this threshold will have costs associated with producers missing a larger number of cows that actually have clinical mastitis.

The next step in this study would be to implement a candisc discriminant analysis model system into a herd with either a can1 threshold that detected high specificity, or a can1 threshold that detected high sensitivity. If the candisc discriminant analysis was able to detect case and control cows effectively at one of the selected thresholds then an experiment could be setup to

implement therapy treatments. An effective therapy program that intervened prior to clinical signs may alleviate signs of clinical mastitis and reduce costs associated with this disease.

Future studies should take into account the milking in which clinical signs were identified, as this could be useful in increasing detection rates among herds. Clinical diagnosis on a daily basis is useful, however herds that milk 3x a day may not benefit as much from daily data as much as from individual milking data. The candisc discriminant analysis had the highest amount of cows accurately classified among the four different analyses. Perhaps adding SCC to this analysis will even further increase the number of correctly identified cows.

This was the first study to report on the ability to detect clinical mastitis prior to clinical signs using animal activity data and milk components. This study demonstrates the importance of daily milk component monitoring and activity measurements in the early detection of disease, and the need for future research in this area. Dairy producers that implement novel detection methods into herd management decisions will be able to reduce costs associated with disease and intervene before the full onset of clinical disease is present.