Dairy Cow Activity as a Potential Management Tool for Detection of Clinical Mastitis

M.S. Thesis

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Abstract

Mastitis in dairy cattle is considered the most economically devastating disease to the dairy industry. Cows with mastitis have decreased milk yield, decreased fertility, are more likely to be culled, and the disease increases the medical and labor costs of dairy operations. As such, it is imperative that mastitis is diagnosed and treated promptly on modern dairy farms. The objectives of this study were to describe the behavior of healthy dairy cattle during the first week of lactation, and to describe the behavior of cows with mastitis before the clinical diagnosis to evaluate behavior as an indicator of clinical mastitis. The hypothesis was that dairy cows will show altered behavior before diagnosis of naturally occurring mastitis. To study sickness behavior, activity monitors (IceQube™, IceRobotics, Edinburgh, Scotland) were placed on a hind leg of dry dairy cows approximately one week before expected calving and were removed approximately three weeks after calf delivery. The monitors measured number of steps taken, lying and standing time, and number of lying bouts, and the associated IceManager™ software also calculated an overall motion index using the previously listed activity measurements. In healthy cows, the number of steps, lying bouts, and overall motion index were highest on
the day of calving and decreased throughout the first week post-calving. Lying bout length was shortest on the day of calving. Cows spent a longer amount of time standing and a shorter amount of time lying on the day of calving.

Cow activity changed significantly 2 days before a diagnosis of clinical mastitis (CM) was made by the farm personnel. Cows took 398 more steps two days before diagnosis of clinical mastitis ($P=0.03$) as compared to their baseline behavior. The length of an average lying bout two days before CM diagnosis was 16 minutes shorter than at baseline (4-5 days prior to the diagnosis, $P=0.05$). Additionally, cows spent 1.8 more hours standing two days before CM diagnosis than at the baseline ($P=0.04$). Overall, the motion index of CM cows increased by 1810 units two days before CM diagnosis from when compared to their baseline behavior ($P=0.03$). Healthy cows were more active on the day of calving. Cows with mastitis spent significantly more time standing, took more steps, had decreased lying bout lengths, and had an increase in overall motion two days before clinical mastitis diagnosis. The information from this study can aid in early mastitis detection and improving animal welfare.
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Mastitis in dairy cattle is defined as a bacterial intramammary infection in one or more mammary glands resulting in inflammation of the infected quarter (Royster and Wagner, 2015). This disease in dairy cattle is considered the most economically devastating disease to the dairy industry (Huijps, 2008). Cows with mastitis have decreased milk yield, decreased fertility, are more likely to be culled, and the disease increase the medical costs of dairy operations (Rajala-Schultz and Gröhn, 1999a, Rajala-Schultz et al., 1999, Shim et al., 2004, Fuenzalida et al., 2015).

Mastitis is also a painful disease in dairy cattle (Fitzpatrick et al., 2013). The udder pain associated with mastitis leads to significant behavioral changes in dairy cattle. Several studies have shown that cows with mastitis will spend more of their time standing compared to controls (Siivonen et al., 2011, Cyples et al., 2012, Fogsgaard et al., 2015). The results of these studies, however, contradict classic sickness behavior of dairy cows, which include spending less time standing (Hart, 1988). Lying is considered a high priority behavior in dairy cattle, and cows should spend approximately half of their day lying down (Jensen et al., 2005).

Animal welfare is defined as an animal’s ability to feel well, function well, and exhibit natural behaviors (von Keyserlingk et al., 2009). Mastitic cows animals feel pain,
decrease milk production and fertility, and suppress natural behaviors (Rajala-Schultz et al., 1999, Fitzpatrick et al., 2013, Fuenzalida et al., 2015). Therefore, mastitis poses not only an economic, but also a welfare concern.

It is imperative that researchers and producers strive to find ways to better diagnose, treat, and manage mastitis on dairy farms. While there have been many studies concerning the behavior of dairy cows with mastitis, very few studies have used multiple herds in their study, and no studies have used cows as their own control to describe the changes in their behavior. It has been recorded that cows show great variation in behaviors (Müller and Schrader, 2005a). Thus, using cows as their own control may be more useful than pairing cows or using treatment and control groups. Therefore, the objective of this study was to describe the behavior of dairy cattle before diagnosis of clinical mastitis by farm staff, using the cow’s own behavior as the reference to evaluate behavior as a sickness marker in cattle afflicted with mastitis. The hypothesis was that dairy cows will show altered behavior before diagnosis of naturally occurring mastitis.
Chapter 2: Literature Review

**Definition and Pathogenesis of Mastitis**

Mastitis in dairy cattle is caused by an ascending bacterial infection into the mammary gland, and as such, is defined as an intramammary infection in one or more mammary glands resulting in inflammation of the quarter (Royster and Wagner, 2015). The definition of mastitis can be further divided into the categories of clinical and subclinical. Clinical mastitis results in the production of abnormal milk and an elevated somatic cell count (SCC) of the affected quarter, and possibly systemic signs of illness (Ruegg, 2014). Subclinical mastitis results in an elevation of SCC of the infected quarter, but does not result in gross changes in the milk (Ruegg, 2014). Researchers debate over what SCC level should be cut off between a healthy quarter and a quarter inflicted with subclinical mastitis. Royster (2015) states that a composite milk sample of all four quarter above 200,000 cells/dl would indicate that one or more quarters has a subclinical infection. Other researchers take parity into account when defining subclinical mastitis, and consider multiparous cows with a SCC above 250,000 cell/ml and primiparous cows with a SCC above 150,000 cell/ml to be infected (de Haas et al., 2008, Santman-Berends et al., 2012). In the United Stated, an SCC of over 200,000 cells/ml, or in some cases 250,000 cells/ml is often considered a subclinical mastitis infection (Smith, 2009). A precise
definition of an intramammary infection (IMI) based on bacterial culture is ambiguous, with researchers using different CFU/ml thresholds. Sampimon et al. (2009) defined an IMI as 100 CFU/ml for major mastitis pathogens, and 500 CFU/ml for minor mastitis pathogens. Reyher et al. (2012) used a 200 CFU/ml threshold for CNS and 100 CFU/ml for all other pathogens. Green et al. (2002) considered 100 CFU/1 ml to be an infection. Still other studies do not provide a definition of an intramammary infection within their research (Bradley et al., 2013). For this reason, comparison of research in this area can become very difficult.

Mastitis is caused by an ascending bacterial infection into the udder of susceptible dairy cattle. The three weeks before and after calving, known as the transition period, is when dairy cow are most susceptible to mastitis (Pyorala, 2008). During this time, dairy cows undergo great changes in the energy balance, hormonal balance, and daily routines and exposures within the herd (Esposito et al., 2014). Around the time of parturition, the teat canal begins to open again, re-exposing the mammary gland to bacteria (Pyorala, 2008). Cortisol levels will also rise shortly before parturition and remain high during the first three weeks of lactation, reducing the ability of neutrophils to undergo apoptosis and stimulating the bone marrow to release immature neutrophils (Pyorala, 2008, Burnett et al., 2015). The peripartum period is also a time when the cow may go into a state of negative energy balance, where she is using more energy to maintain her pregnancy and produce milk than the energy she can consume in a day. Negative energy balance can depress the immune system and leave the cow vulnerable to new bacterial infections (Esposito et al., 2014)
The bacteria associated with mastitis infections are categorized based on the source and epidemiology of the bacteria. Contagious mastitis infections originate from cows and are transmitted from cow to cow through the milking machine, milkers’ hands, or other shared equipment. These bacteria require a host to live and propagate. Notable examples of contagious mastitis pathogens include *Staphylococcus aureus, Steptococcus agalactiae* and *Mycoplasma spp.* (Barkema et al., 2009). In contrast, environmental mastitis pathogens are capable of living outside of the host. Coliform mastitis pathogens are perhaps the most notorious environmental pathogens. This group of environmental pathogens originates in fecal material and includes *E. coli, Klebsiella ssp, and Enterobacter ssp* (Smith, 2009). This type of mastitis may be most common on farms where the cow’s living conditions are contaminated with a large amount of manure, even if contagious pathogens on the farm are well managed. Other examples of environmental mastitis pathogens include an assortment of *Streptococcus ssp., Salmonella, Pastuerella, and Psuedomonas* (Smith, 2009).

Another increasingly common group of mastitis pathogens include coagulase negative staphylococci (CNS) which are often categorized as environmental pathogens, and they can also pose a threat to udder health. This group contains numerous different species of bacteria, but they are mostly grouped together as CNS, in contrast to coagulase positive *S. aureus.* This group of bacteria does not typically cause a severe mastitis, but can cause long and persistent infections in the mammary gland (Taponen and Pyörälä, 2009). One study on the effects of CNS in dairy cows found the bacteria is capable of increasing the SCC, but not as significantly as other common mastitis pathogens, and that animals infected with CNS do not suffer from a decrease in milk production (Schukken et al.,...
Researchers continue to explore the importance of CNS in bovine mastitis. Research has showed that species of CNS behave differently, so more researchers are beginning to investigate the importance of these differences and stressing the importance of speciating CNS (Ruegg, 2009, Koop et al., 2012).

**Clinical Signs and Diagnosis**

Clinical mastitis is recognizable by the gross changes in milk and clinical signs that accompany the disease. These changes include a change in milk color, abnormally thick or watery milk, and the presence of flakes or even blood (Smith, 2009). As a case of mastitis becomes more severe, the udder will begin to show inflammatory changes, such as redness, swelling, and increased temperature. Clinical mastitis may also become severe enough to result in systemic signs of illness, such as fever, decreased feed intake, decreased milk production, lethargy, and weakness. In the most extreme cases, clinical mastitis will result in a systemic infection, which may render the cow recumbent and dehydrated, and in the worse cases, death may be the outcome of toxic mastitis (Smith, 2009).

Immune cells aid in the detection and diagnosis of mastitis. The number of leukocytes, also known as somatic cells, in milk corresponds to the immune activity and likelihood of a bacterial infection within the mammary gland (Lindmark-Mansson, 2006). Somatic cells in the milk can be searched for and quantified in a number of different ways, most prominent of which would be the California Mastitis Test (CMT) to test for the presence of DNA in milk and the use of various different types of somatic cell counting devices.
Sensitivity and specificity has been reported for the California Mastitis Test. Roy (2009) reports the sensitivity and specificity at the quarter level for the CMT test to be 68.9% and 68.4%, respectively. Others have reported the sensitivity and specificity of the CMT to be closer to 70% and 48%, respectively (Sanford, 2006). When considering composite milk samples, sensitivity and specificity of SCC is 80% and 57%, respectively (Vissio et al., 2014).

Detecting the presence of bacteria in the milk is another common diagnostic technique. Determining the presence of bacteria in the milk is done via bacteriological culture or PCR (Smith, 2009). While bacterial culture can identify specific bacterial agents, which can be useful in making treatment options or identifying mastitis trends within herds, it can take several days to yield results. New Petrifilm technology is being studied and may allow farmers to do rapid bacteriological culture on the farm (Mansion-de Vries et al., 2014). Similarly to bacteriological culture, PCR is also a more expensive option for mastitis diagnosis, although results can be obtained in a matter of hours (Koskinen et al., 2010). While the sensitivity and specificity of PCR deviates depending on the bacteria of interest, PCRs tend to offer moderate specificity but great sensitivity, especially when compared to bacterial culture (Koskinen et al., 2010, Murai et al., 2014, Hiitiö et al., 2015).

One additional test for mastitis is based on increases of sodium and chloride within the milk when cows have mastitis. This method is known as electrical conductivity. The benefit of electrical conductivity monitoring for mastitis in herds is that it can be performed at every milking for all cows in parlors fitted with electrical conductivity equipment (Smith, 2009). Hand held units are also available. The sensitivity and
specificity of electrical conductivity are 75% and 95%, respectively in a survey of 41 studies on the technology (Smith, 2009). Another study found that the sensitivity and specificity of hand held electrical conductivity units were 89.9% and 86.8%, respectively (Fosgate et al., 2013).

**Treatment and Management of Mastitis**

Good management practices are the cornerstone of mastitis prevention in dairy herds.

Guidelines for on-farm management and prevention of mastitis on dairy farms have been proposed over the decades. These guidelines include establishing a dry and clean environment for cows, using proper milking procedure including good teat dipping and milking hygiene, maintaining the milking machine, making wise treatment and culling decisions based on bacteriological culture, using dry cow therapy, and taking biosecurity precautions on the farm (Neave et al., 1969, Eberhart, 1986, Nickerson, 2011).

Antibiotic treatment of cows inflicted with mastitis should be considered on a case by case basis with the causative pathogen in mind (Royster and Wagner, 2015). Cure rates for pathogens such as *Staphylococcus aureus* and *Streptococcus uberis* are considered to be lower than cure rates for many gram-negative pathogens (Royster and Wagner, 2015). Severity, cow history and presence of other systemic symptoms may also prompt addition of systemic treatment (Smith, 2009).

**Economic Impact of Mastitis**

Mastitis is an economically devastating disease to the dairy industry. Costs associated with mastitis include, losses due to discarded milk and decrease in production, increased
cull rates for sick cows, and medical treatment (Huijps, 2008). The cost of mastitis varies depending on farm and the causative agent and the type of mastitis. Not only is clinical mastitis expensive for dairy farms, undetected subclinical infections also account for large amounts of economical losses (Halasa et al., 2009). One study found infections caused by Gram-negative pathogens to be the most costly type of mastitis (Cha et al., 2011). The precise cost of mastitis was also found to be sensitive to changes in prevailing milk prices, replacement prices, treatment costs, pregnancy rates, and herd incidence rates of mastitis (Cha et al., 2011).

Cows with mastitis have been shown to decrease their milk production (Rajala-Schultz et al., 1999, Detilleux et al., 2015). In Finnish herds, it was estimated that cows may lose between 110 and 552 kg of milk per lactation following clinical mastitis (Rajala-Schultz et al., 1999). The effect of subclinical mastitis on milk yield has also been investigated and one study found that cows with subclinical mastitis yield between 0.2 and 0.6 kg less milk per day, depending on parity (Halasa et al., 2009). Not only does milk yield directly impact the profitability of the cow, it also has a significant effect on culling decisions (Rajala-Schultz and Gröhn, 1999b).

The effects of mastitis on culling decisions, and the associated cost thereof, has been thoroughly studied (Reksen et al., 2006, Bar et al., 2008, Heikkilä et al., 2012, Cha et al., 2013). Cows with repeat episodes of mastitis and cows diagnosed with Gram-negative mastitis are at the highest risk of being culled (Bar et al., 2008, Hertl et al., 2011). It is estimated that the cost of culling cows prematurely increases the cost of a case of mastitis by nearly 30% (Heikkilä et al., 2012). In one study comparing cows with chronic clinical mastitis, acute clinical mastitis, and subclinical mastitis to healthy cows, it was found that
those cows that suffered from any one of those three forms of mastitis had lower odds of getting pregnant that healthy cows (Fuenzalida et al., 2015).

Lastly, medical treatment also contributes to the cost of mastitis. While medical treatment is costly, cows not given antibiotics and only provided supportive care end up losing 2 to 3 times as much money as those cow who were treated with antibiotics (Shim et al., 2004).

**Dairy Cow Behavior at Calving**

Dairy cow behavior will vary from their normal behavior during calving. Some of the first behaviors exhibited by cattle as they calve include inspecting the environment and “nesting behavior”, or a rearranging of the bedding material in the calving pen (Wehrend et al., 2006). Further progression of calving leads to an increase in lying bouts, shortening of lying bout lengths, vocalization, rubbing, and alterations in posture (Wehrend et al., 2006, Schuenemann et al., 2011). Healthy cows also increase their standing time on the day of calving, which is a deviation from their higher priority for laying (Jensen et al., 2005, Huzzey et al., 2007, Itle et al., 2015). Additionally, it has been found that heifers appear to be more restless just prior to calving than older cows (Wehrend et al., 2006, Miedema et al., 2011).

**Sickness Behavior of Animals**

It is irrefutable that when animals are sick, they feel painful and will adjust their behavior accordingly (Hart, 1988, Dantzer and Kelley, 2007, Newby et al., 2013a, Offinger et al., 2013). When discussing sickness and pain behavior in dairy cows, it is always important
to remember that cows are prey animals and therefore, are prone to hiding these behaviors (Leslie and Petersson-Wolfe, 2012). Behavior of sick animals is partially mediated at the cellular level. Of particular interest is the relationship between bacteria, cells of the mammary tissue and mastitis in dairy cows. Macrophages are considered to be the sentinel cells of the bovine mammary gland because they are the primary cell present in the healthy udder and are in part responsible for initiating the inflammatory response and neutrophil recruitment (Oviedo-Boys, 2007). Most importantly, they release IL-1, which communicated with the central nervous system and is the primary regulator of the fever response (Hart, 1988). Although fever is considered an important physiological function to fight infections, it also caused animals to become anorexic, depressed, and increase thirst (Hart, 1988). Cows will display these behaviors because it has been noted that sickness behaviors will take precedent over normal behaviors (Dantzer and Kelley, 2007).

Dairy cows, like other sentient animals, are capable of feeling pain, and this pain can be another cause of sickness behaviors. Pain in sick cattle can be measured in both qualitative and quantitative ways. Qualitative measurements include gait, posture, and mental attitude (Leslie and Petersson-Wolfe, 2012). Quantitative measures may include fever, amount of time spent performing a certain activity, cortisol levels, feed intake, and milk production (Leslie and Petersson-Wolfe, 2012). Dairy cows who are lame have been found to increase their overall daily lying time, lying bout length, and to have more variation in lying times (Ito et al., 2010). Lame cows will also alter their gait and walk at slower speeds than healthy cows (Blackie et al., 2011). Cows with metritis will exhibit an
arched back posture and increase their heart rate, especially in response to rectal palpation (Stojkov et al., 2015).

Perhaps one of the most validating ways to evaluate the pain response in cows is to study the effects of non-steroidal anti-inflammatory drugs use. One study found the ketoprofen administration had no effect on lying behavior, BHBA blood levels or heart rate, but was useful in increasing appetite in cows over the first 3 days following surgical displaced abomasum correction, according to farmer’s observations and attitudes (Newby et al., 2013a). A similar study also found that meloxicam administration increased the amount of time cows spend feeding, but did not increase overall DMI or improve other milk production when given in conjunction with an assisted calving (Newby et al., 2013b). In cows who underwent toe amputation, meloxicam was found to increase their daily standing time and number of steps as compared to control cows who did not receive the drug (Offinger et al., 2013). Meloxicam also decreased body temperature, cortisol levels, and lameness scores post operatively after toe amputation (Offinger et al., 2013). These results indicate that while NSAIDs provide some pain relief to animals, sickness behavior is a multifactorial process, controlled by both cell mediated signals to the CNS and an animal’s own aversion to pain.

**Sickness Behavior and Mastitis**

Several studies have previously investigated the effects that clinical mastitis has on the behavior of dairy cows. Cyples et al. (2012) found the cows will lay less on the day of LPS infusion into the udder, but that the number lying bouts and the length of a lying bout will not be altered. Siivonen et. al, (2011) also showed that when cows are
challenged with an acute endotoxin-induced mastitis, they will increase the amount of
time that they spend standing and increase the number of steps that they take on the day
of LPS infusion. Udder tenderness and inflammation was also associated with the day of
experimentally induced mastitis (Siivonen et al., 2011). Fogsgaard et. al. (2015) found
that cows with naturally occurring mastitis in an automatic milking system took more
steps and lying bouts but decreased their total daily lying time around the time of mastitis
when compared to control cows. Cows in that same study also showed decreases in feed
intake and an increase in tripping and kicking at the milking machine (Fogsgaard et al.,
2015). Sepúlveda-Varas et al. (2014a) also found a decrease in feed intake around the
time of naturally occurring mastitis. An additional study found that cows will lay less 2
days after naturally occurring mastitis diagnosis than control cows, although they showed
no preference to which side they rested on in relationship to the mastitic quarter
(Medrano-Galarza et al., 2012). Medrano-Galarza et al. (2012) also showed that cows
with mastitis will increase their kicking in the milking parlor on the first day after
mastitis diagnosis, but will then return to behaviors similar to healthy cows in the
following days. Authors also looked at differences in weight distribution and hock-to-
hock difference in cows with mastitis, but found no difference in these parameters when
compared to healthy cows (Medrano-Galarza et al., 2012).
Lying is considered a high priority behavior in dairy cows, and when made to choose,
cows will prefer to lay down instead of eat or perform social behavior (Jensen et al.,
2005, Munksgaard et al., 2005). It was found than both heifers and mature cows will
spend half of their day, or approximately 12 hours/day, laying down (Jensen et al., 2005).
A cow’s preference to stand during time of mastitis can be an indicator of the pain associated with the illness.

In contrast to the studies that found cows with mastitis to stand more, Fogsgaard et al. (2012) showed an increase in lying time in the acute stages of experimentally induced *E. coli* mastitis. There is also dispute about an animal’s stepping behavior following experimental mastitis, as one study did find that cows decreased the number of steps taken per day after infusion of *Strep. uberis* (Kester et al., 2015). Kester et al. (2015) also found that cows challenged with *Strep. uberis* rested more within the first week after mastitis challenge, which also goes against other research done on this topic. Although Fogsgaard et al. (2012) and Kester et al. (2015) findings are in contrast to other research done on behavior in cows with mastitis, it does agree with the more classic sickness behavior. The study done by Kester et al. (2015) did use a Gram-positive bacteria, as compared to other papers that challenged their cows with LPS, which may account for some of the difference in behavior in cows following challenge. Researchers found animals to increase standing behavior and steps attribute this deviation from classic sickness behavior to be caused by pain in the udder of cows with mastitis, which would be exacerbated by a dairy cow’s natural laying position (Siivonen et al., 2011, Cyples et al., 2012, Medrano-Galarza et al., 2012, Fogsgaard et al., 2015).

Not only is the pain associated with mastitis evident in cow’s reluctance to lay down during the disease, is it also evident when studying the behavior of mastitic cows after the administration of pain relieving drugs. One study evaluated pain as a sequel to LPS induced mastitis by using a pressure algometer on the quarter infused with endotoxin in conjunction with the administration of meloxicam. This study found that both treatment
and control cows exhibited pain in the udder, and that meloxicam reduced the amount of pain in the udder of the treatment cows (Fitzpatrick et al., 2013). Milne et al. (2003) also found that meloxicam will decrease the pain in the udder associated with mastitis. Flunixin meglumine has been shown to be effective in reducing fever in cows with LPS induced mastitis (Chapinal et al., 2013). In a study looking at the effect of meloxicam on naturally occurring mastitis, it was found that meloxicam administration had several beneficial outcomes. Cows that received meloxicam at the time of CM diagnosis had a lower SCC, and were less likely to be culled from the herd although meloxicam had no effect on the milk yield of cows (McDougall et al., 2009).

**Animal Welfare Implications of Mastitis**

A review of animal welfare on dairy cows concluded that the major factors that define animal welfare is the animal’s ability to function well, feel well, and exhibit natural behaviors (von Keyserlingk et al., 2009). As discussed in previous section of this literature review, mastitis directly decreases milk yield and fertility, causes pain and discomfort, and alters the behavior of dairy cows. As such, we can conclude that mastitis is a major welfare concern on dairy farms.

**Activity Pedometer Technology**

An increasing number of farms and researchers are turning to technologies to study and monitor the health and welfare of dairy cows. Many of the studies concerned with behavior in dairy cows use pedometers to measure behavior parameters (Cyples et al., 2012, Fogsgaard et al., 2012, Kester et al., 2015). Pedometers are perhaps most widely
used and studied currently for heat detection in dairy cows, but this technology has also been studied for use in detection of lameness, as well as other peripartum diseases (Mazrier et al., 2006, Chanvallon et al., 2014, Sepúlveda-Varas et al., 2014b). Activity monitors have been validated as an effective way to measure the movement of dairy cows (Ledgerwood et al., 2010, Mattachini, 2013). Pedometers join other automated technologies that aid dairy producers in detecting mastitis, such as electrical conductivity and enzymatic activity measurements (Hogeveen et al., 2010). Overall, technologies in mastitis detection seem to be more valuable as an alert than a true diagnostic method (Steeneveld et al., 2010a, Steeneveld et al., 2010b)
Chapter 3: Dairy Cow Activity as a Potential Management Tool for Detection of Clinical Mastitis

Introduction

It is well established that animals will exhibit sickness behavior when inflicted with disease (Kelley et al., 2003, Dantzer and Kelley, 2007, Weary et al., 2009). This behavior is a reflection of the disease pathology within the animal or an attempt to avoid painful stimuli brought on by disease (Weary et al., 2009). Examples of sickness behavior in animals include decreased feed intake and activity, increased sleep, generalized lethargy, and neglect of personal grooming (Hart, 1988). Sickness behavior may also be a reflection of an animal’s need to maintain social stature within a group (Itle et al., 2015). Prey animals, such as dairy cattle, will attempt to hide their sickness as a natural means of avoiding predation (Weary et al., 2009).

The sickness behavior of dairy cows has been specifically addressed in the literature, particularly for those diseases that occur near parturition. Sepulveda-Varas (2014) showed that dairy cattle that developed one or more periparturient diseases spent more time lying than their healthy counterparts. Similarly, when ill after the peripartum period, cows alter their social behavior and seek solitude (Proudfoot et al., 2014).
Numerous researchers have shown that cows will alter their behavior in relationship to mastitis. Experimentally induced mastitis has been shown to cause decreased feed intake and milk production as well as increased body temperature and time spent demonstrating an idle behavior (Fogsgaard et al., 2012, Kester et al., 2015). Cows with naturally occurring clinical mastitis (CM) have also been documented as showing udder irritation and kicking at the milking personnel and milking machine (Medrano-Galarza et al., 2012, Fogsgaard et al., 2015). Classic sickness behavior in animals include an increase in time spent lying or resting, however, most of the literature on behavior in dairy cows with mastitis contradicts this idea. When challenged with experimental clinical mastitis, cows were found to decrease their time spent lying down (Siivonen et al., 2011, Yeiser et al., 2012). Cows also demonstrated decreased lying time when inflicted with naturally occurring mastitis (Medrano-Galarza et al., 2012, Fogsgaard et al., 2015).

Much of the literature has focused not only on a cow’s behavior during disease, but also on behavior prior to the diagnosis of disease. Cows diagnosed with ketosis in the first three weeks of lactation began to exhibit increased standing time already before calving compared to healthy cows (Itle et al., 2015). Cows with metritis decreased their feed intake relative to healthy cows in the same herd both before and during the disease (Huzzey et al., 2007). The aim of these studies was to use animal behavior as an early detection and diagnostic tool on dairy farms. To the best of our knowledge, similar studies have not been done to evaluate the behavior of dairy cows leading up to diagnosis of naturally occurring clinical mastitis. Mastitis continues to inflict the dairy industry as a common and costly disease and poses a threat to the welfare of those animals that succumb to the disease (Huijps, 2008, von Keyserlingk et al., 2009). For these reasons, it
is important that research continues to address new techniques for identifying mastitic cows as early as possible. Therefore, the objectives of this study were to describe the behavior of cows with mastitis before the clinical diagnosis in an attempt to evaluate behavior as an indicator of clinical mastitis and to describe the behavior of healthy dairy cattle during the first week of lactation. The hypothesis was that dairy cows will show altered behavior before diagnosis of naturally occurring mastitis.

**Materials and Methods**

**Study Population**

One hundred and seventeen cows, including both primi- and multiparous cows, in three central Ohio dairy herds were enrolled into the study between July 2011 and August 2013. Two of the study herds had more than 1000 cows, and one herd consisted of approximately 120 cows. The two larger herds were milked three times a day and the smaller herd was milked twice a day. All herds consisted of Holstein cattle housed in free stall barns. Cows that developed clinical mastitis within the first three weeks of lactation were included in the study. Cows with any concurrent illnesses were excluded from this study.

**Case Definition**

Clinical mastitis was diagnosed on the farms by the farm staff using each farm’s standard detection methods, including visual inspection of cows and udders, and fore-stripping of milk. The farms monitored fresh cows for signs of clinical mastitis, which included
changes in milk, swollen or inflamed udder, fever, and decrease in milk production and feed intake. The farm personnel also diagnosed concurrent diseases on farm.

Activity monitoring
To study sickness behavior, activity monitors (IceQube™, IceRobotics, Edinburgh, Scotland) were placed on a hind leg of pregnant dairy cows approximately one week before expected calving and were removed approximately three weeks after calf delivery. The monitors measured number of steps taken, lying and standing time, and number of lying bouts, and the associated IceManager™ software also calculated an overall motion index using the previously listed activity measurements on a daily basis. Average lying bout length was calculated by dividing the total lying time by the number of lying bouts. Standing and lying time were summarized into hours per day and lying bout length was summarized into minutes per day.

Statistical analysis
Data to evaluate changes in behavior related to CM diagnosis were analyzed using MIXED procedure in SAS, v. 9.3 (SAS Institute Inc, Cary, NC). Using daily summary data from CM cows on different activity parameters as the outcomes, days with respect to diagnosis of clinical mastitis was the main variable to interest. Data from 5 days before the clinical diagnosis of mastitis up to 3 weeks post diagnosis were included. Due to considerable individual variation in activity between cows, activity of each cow on days 4 and 5 before diagnosis was used as the baseline level. Days in milk at CM diagnosis, parity and herd were included in the models as potential confounders. Compound
symmetry covariance structure was used to account for the correlated data structure between the daily observations from cows. Herd was included in the models as a random effect. Additionally, cows with activity monitors that were not diagnosed with mastitis or any other disease were included in statistical analysis to describe normal dairy cow behavior within the first week after calving. Descriptive statistics (mean, standard deviation, range) on all activity parameters by DIM and by herd were generated using STATA 13.0 (Stata Corporation, College Station, TX).

**Results**

A total of 21 cows fitted with the activity monitors were diagnosed with clinical mastitis during the study period. Eight cows were excluded from the analysis due to concurrent disease or because their mastitis occurred outside of the 3-week, post-calving time window. In total, 13 cows were eligible for inclusion into the statistical analysis. All cows included developed clinical mastitis within the first week of lactation. Four cows were diagnosed with mastitis on 1 DIM, three cows developed mastitis at 2 DIM, two cows developed mastitis at 6 DIM, one cow developed mastitis at 4, 5, and 7 DIM each and one cow was diagnosed with mastitis on the day of calving. The study population included four primiparous cows and nine multiparous cows that ranged in parity from two to four. Additionally, 53 cows that were fitted with the activity monitors and were not diagnosed with mastitis nor any other disease and had at least 7 days of their activity after calving recorded by IceQube™ data loggers were used to describe normal dairy cow behavior within the first week after calving.
The behavior of healthy cows in the first 7 days in milk is described in Figure 1. Number of steps, lying bouts, and overall motion index were highest on the day of calving and decreased throughout the first week post-calving. Lying bout length was shortest on the day of calving. Cows spent a longer amount of time standing and a shorter amount of time lying on the day of calving. Figure 1 shows that a majority of the variation in cow activity was at the cow level and the herd means were relatively similar for each activity parameter.

Significant changes in cows’ activity were found two days before herd personnel detected clinical signs of mastitis as compared to cow’s own activity four and five days before the diagnosis. Cows were found to take 398 more steps two days before diagnosis of clinical mastitis ($P=0.03$) as compared to their behavior on days -5 and -4 before CM diagnosis (baseline behavior) and can be seen in Table 1. The length of an average lying bout two days before CM diagnosis was 16 minutes shorter than at baseline (4-5 days prior to the diagnosis, $P=0.05$). Additionally, cows spent 1.8 more hours standing two days before CM diagnosis than at the baseline ($P=0.04$). Overall, the motion index of CM cows increased by 1810 units two days before CM diagnosis from when compared to their baseline behavior ($P=0.03$). Activity on any other days did not significantly differ from the baseline and neither did it differ on the days immediately following the diagnosis. Total daily time spent lying and number of lying bouts did not significantly change before CM diagnosis when compared to the baseline. As expected, days in milk (DIM) was highly significant and a confounder and therefore was kept in all models. Parity, on the other hand, was not significantly associated with the activity parameters and it was not a confounder either in the behavior of cows diagnosed with CM. Interestingly, and
somewhat surprisingly, herd was not significant nor a confounder in the models for any of the activity parameters.

Discussion

Descriptive data from the healthy cows in this study suggest that cows are most active at calving, and then decrease their motion during the first week post partum. Healthy animals in the current study spent more time standing on the day of calving compared to other days in the first week of calving. The results from the current study agree with other studies that found healthy cows to increase their standing time on the day of calving (Huzzey et al., 2005, Ite et al., 2015). We also found that cows would lie down with more frequency, but for shorter amounts of time on the day of calving. This is a classic behavior in calving animals and is well documented in the literature (Schuenemann et al., 2011, Jensen, 2012, Ite et al., 2015). In addition to the increases in standing time and the number of lying bouts, cows also took the most steps on the day of calving. Overall motion index for healthy cows was highest on the day of calving as well. This increase can be attributed to the increase in steps and number of lying bouts on the day of calving, which can be described as a general restless behavior in the calving dairy cow.

The activity of the mastitic cows before the clinical diagnosis changed most two days before the diagnosis of clinical mastitis when compared to cows’ own baseline behavior. Cows spent significantly more time standing and less time lying during a single lying bout, as compared to their baseline activity before diagnosis of CM. Cows also took more steps and showed an increase in their overall motion index when compared to their baseline. These results agree with another study that previously investigated the effects of
mastitis on cow behavior (Cyples et al., 2012). Siivonen et. al (2011) showed that when cows are challenged with an acute endotoxin-induced mastitis, they will increase the amount of time that they spend standing and increase the number of steps that they take. Siivonen et al (2011) found that increased stepping occurred on the day of mastitis challenge. In that same study, increased time spent standing also coincided with day of endotoxin challenge as well as the time period when the udder was inflamed and body temperature of the cows was elevated. Siivonen et al (2011) attributed this increased standing behavior to discomfort in the udder.

With respect to naturally occurring mastitis, several studies also agree with the results of the current study. Fogsgaard et. al. (2015) found that cows with naturally occurring mastitis in an automatic milking system took more steps and decreased their lying time around the time of mastitis when compared to control cows. An additional study also found that cows will lay less 2 days after naturally occurring mastitis diagnosis than control cows, which they explained as a decreased motivation to lay down due to udder pain (Medrano-Galarza et al., 2012).

In contrast to the results of the current study and several other studies of naturally occurring mastitis Fogsgaard et al. (2012) reported an increase in lying time in the acute stages of experimentally induced E. coli mastitis(Medrano-Galarza et al., 2012, Fogsgaard et al., 2015). To the best of our knowledge this is the only study that has shown cows with mastitis to increase their lying time. Reports about animals’ stepping behavior following experimental mastitis are also conflicting, as one study found that cows decreased the number of steps taken (Kester et al., 2015). Although Fogsgaard et. al. (2012) and Kester et al. (2015) findings are in contrast to other research done on
behavior in cows with mastitis, it does agree with the more classic sickness behavior including increased lying time and decreased activity (Hart, 1988).

In the current study, no difference was found in activity parameters between primiparous and multiparous cows which is in agreement with the results of Bewley et al. (2010) who did not find parity to significantly affect lying behavior in dairy cows. Other studies, however, have reported that dairy cow parity alters the behavior of the cow (Wehrend et al., 2006, Azizi et al., 2009).

This is the first study that has used multiple herds to evaluate the effect of clinical mastitis on dairy cow activity, thus, determining the effect of herd on dairy cow behavior was important. Data from the current study showed only small amounts of difference between herds in cows that have been diagnosed with CM. In healthy as well as in mastitic cows, steps and overall standing time showed the most variation between herds, which could easily be attributed to size and design of the individual farms. The data presented in Figure 1 of the study shows wide ranges and occasional outliers in all herds, while many of the herd means are similar. This suggests that most variation of activity is on the cow level instead of the herd level. In contrast to the findings of the current study, another study found the lying behavior in dairy cows is affected by herd factors(Ito et al., 2014).

To the best of our knowledge the current study is the first to use the cow as her own control when analyzing the behavior of cows with naturally occurring mastitis. Several previous studies have matched mastitic cows with their healthy herdmates to study the effects of mastitis on dairy cow behavior in naturally occurring mastitis(Medrano-Galarza et al., 2012, Fogsgaard et al., 2015). Several studies have also looked at cows as their
own control upon studying experimentally induced mastitis (Siivonen et al., 2011, Fogsgaard et al., 2012). Given the large variability in the behavioral parameters among cows and days in the current study, the cow’s own activity level four and five days prior to the diagnosis was used as the reference due to the documented intra-species variation and consistency at which dairy cows will display personalized behavior (Müller and Schrader, 2005b).

Most mastitis cases in the current study occurred in early lactation, within the first week after calving. It has been reported that cows’ behavior significantly changes around calving (Schuenemann et al., 2011, Jensen, 2012, Campler et al., 2015). These behaviors include less lying time and more lying bouts the day before calving and more time spent standing for the first 6 hours post calving (Jensen, 2012). The first three weeks post calving was chosen to study naturally occurring mastitis, because it is the time when cows are most likely to contract the disease (Pyörälä, 2008). Therefore, it was important to account for this altered behavior by including DIM in all the models and as expected, DIM was significantly associated with the activity parameters and it also was a confounder.

A limitation of this current study was a small study population, which was mostly due to the unpredictable nature of naturally occurring mastitis and being able to monitor behavior prior to the diagnosis of clinical cases by the farm staff. Despite that, significant differences were found in cows’ activity and more cases were detected and observed in the current study than has been used in most studies describing dairy cow behavior in conjunction of clinical mastitis.
As use of new technologies, and specifically pedometers, increases on dairy farms, research directed toward the use and applications of this technology becomes increasingly common. Pedometers are currently most widely used and studied for heat detection in dairy cows (Rutten et al., 2013), but this technology has also been studied for use in detection of lameness, as well as other peripartum diseases (Mazrier et al., 2006, Chanvallon et al., 2014, Sepúlveda-Varas et al., 2014b). It is our hope that researchers will continue to investigate ways that this already existing technology can be used to pinpoint illness in dairy cattle and improve animal welfare and producers’ profits.

**Conclusion**

The current study described the behavior of cows shortly before CM diagnosis and in healthy cows within the first week of lactation. Healthy cows were most active on the day of calving. Cows with mastitis spent significantly more time standing, took more steps, had decreased lying bout lengths, and had an increase in overall motion two days before clinical mastitis diagnosis. The information from this study can aid in early mastitis detection, which can be used to improve animal welfare while maximizing profits and minimizing milk and cow losses on dairy farms by initiating treatments as early as possible. Furthermore, this study highlights the important and growing role of technology in modern dairy farming.
Table 1. Mastitic cow data. The least squares mean estimates and standard errors are given for the baseline (days -5 and -4 in respect to day of CM diagnosis), as well as for days -3 to 0 in respect to day of CM diagnosis (Day 0 is the day of diagnosis). The $p$-values in this table represent the values generated from the mixed model whereas the cow is her own control. The $p$-values represent each day’s variation from baseline (day -5 and -4 of CM Diagnosis).
Figure 1. Healthy Cow Data. Box plots describing behavior in healthy dairy cows during the first week post calving, with day 0=day of calving. The x-axis represents the day in respect to calving for herds 1, 2, and 3. In this figure, steps, lying bouts (lbouts), lying bout length (lboutlength, min), and daily time spent standing (standtime, hr) are shown.
Chapter 4: Conclusions and Future Directions

In this study, we have described the behavior of healthy cows in the first week of lactation and the behavior of dairy cows before the diagnosis of naturally occurring clinical mastitis. In healthy cows, number of steps, lying bouts, and overall motion index were highest on the day of calving and decreased throughout the first week post-calving. Lying bout length was shortest on the day of calving. Cows spent a longer amount of time standing and a shorter amount of time lying on the day of calving. Significant changes in cows’ activity were found two days before herd personnel detected clinical signs of mastitis as compared to cow’s own activity four and five days before the diagnosis. Cows were found to take 398 more steps two days before diagnosis of clinical mastitis ($P=0.03$) as compared to their behavior on days -5 and -4 before CM diagnosis (baseline behavior). An average lying bout two days before CM diagnosis was approximately 16 minutes shorter than at baseline (4-5 days prior to the diagnosis, $P=0.05$). Additionally, cows spent 1.8 more hours standing two days before CM diagnosis than at the baseline ($P=0.04$). Overall, the motion index of CM cows increased by 1810 two days before CM diagnosis from when compared to their baseline behavior ($P=0.03$). Total daily time spent lying and number of lying bouts did not
significantly change before CM diagnosis when compared to the baseline. Parity was also not found to be a significant confounder in the behavior of cows diagnosed with CM. In order to properly apply the results in this study, further research is warranted. This study utilized a small number of cases of clinical mastitis. While the current findings were significant, a larger number of naturally occurring clinical mastitis cases would make the results even stronger. Additionally, this study only included 3 herds, 2 of which were very similar. A larger herd variety would also improve the validity of this study. While our finding are in agreement with previous research that little difference exists in the behavior cows display between herds, studying cows in a larger number of herds would strengthen this argument (Müller and Schrader, 2005b). One previous study found experimentally induced *Strep. uberis* to cause different behavioral changes compared to studies looking at mastitis caused by LPS infusion (Kester et al., 2015). Further research could be done to determine the role of mastitis pathogen on the expressed behaviors of dairy cows.

Inter-disciplinary work also needs to continue to allow the current research to be utilized on a large scale on dairy farms. Ideally, software would exist to automatically monitor activity on dairy cows and alert producers to behavior changes and the associated potential disease. Researchers in veterinary, animal science, and technology fields need to work together to efficiently use technology to improve animal welfare.
References


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