AN EVALUATION OF THE IMPACT OF MANAGEMENT PRACTICE ON THE
HEALTH AND WELFARE OF DAIRY HEIFER CALVES

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ABSTRACT:

AN EVALUATION OF THE IMPACT OF MANAGEMENT PRACTICE ON THE HEALTH AND WELFARE OF DAIRY HEIFER CALVES

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The objectives of this thesis were to investigate 1) the use of behavior and activity monitoring for the identification of heifers at risk of disease, 2) the use of group level management practices to reduce the risk of disease, and 3) the identification of long-term impacts of bovine respiratory disease complex (BRD).

For objective 1, lying posture, a decreased willingness to approach an observer and high lethargy scores were associated with diarrhea in calves under 2 weeks of age and a high lethargy score in 4-6 week old calves was associated with decreased average daily gain (ADG) in the first 8 weeks of life (n = 744). In weaned calves (n = 74) increased activity (increase in steps and decrease in lying), standing at the bunk not eating, and lying far from other calves in the first 3 days post-weaning were associated with decreased post-weaning weight gain.

For objective 2, separating social mixing from movement to a novel environment, and administering prophylactic antibiotics to calves at high risk of disease, were investigated. Both mixing and movement to a novel environment increased activity levels in newly weaned dairy calves (n = 64). When calves were mixed prior to movement to a novel environment they had a smaller increase in activity compared to calves that were simultaneously mixed and moved. No
differences in weight gain or calf starter intake were observed. Administration of a prophylactic antibiotic, tulathromycin, to 3 day old calves upon arrival at a heifer raising facility (n = 788) and 8 week old calves at first movement to group housing (n = 1,392) was found to reduce diarrhea and otitis media, and BRD, respectively.

Objective 3 was addressed by monitoring calves that received tulathromycin at 8 weeks of age to determine the long-term impacts of BRD. Bovine Respiratory Disease complex was associated with decreased growth to 9 months of age, decreased survival to first calving, increased risk of dystocia and a greater age at first calving.
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CHAPTER 1: LITERATURE REVIEW

1.1 INTRODUCTION

Bovine respiratory disease complex (BRD) and neonatal calf diarrhea complex are the two most commonly diagnosed diseases in pre-weaned and weaned dairy calves, and account for between 60-80% of dairy heifer mortality in the United States (USDA, 2010b). These diseases are very important due to the impact that they have on animal welfare and productivity.

Animal welfare issues can be categorized into three main ethical concerns (Fraser et al., 1997). The first category is functional, which are concerns focused on deviations from normal physiologic function, such as poor health or growth. The second category stems from a feelings based approach to animal welfare, which are concerns focused on avoiding prolonged or intense pain, discomfort or other negative emotional states, as well as allowing for positive emotional states. The final ethical category is based on the natural view, which focuses on the natural behaviors of animals based on their evolutionary adaptations and capabilities. Although described separately, in practice many of the current animal welfare concerns negatively impact animals under all of the ethical areas. An example of this would be bovine respiratory disease (BRD), which decreases the health and growth of animals. BRD also is likely to be associated with shortness of breath, or ataxia, which is considered to be a negative affective state in many species (Mellor & Stafford, 2004). By considering all three ethical concerns in conjunction with the potential economic impact for the producer, management systems can be improved for the benefit of both producers and animals.

The failure of the dairy industry to address animal welfare concerns can lead to legislative changes, which can force change. An example of this is the European Union directive
which requires that “all calves be group housed by 8 weeks of age and prior to this stalls must not have solid walls, but perforated walls, which allow the calves to have direct visual and tactile contact” (91/629/EEC). In 2006, the state of Arizona passed Proposition 204 (The Humane Treatment of Farm Animal Act), which will require sows and veal calves to be able to “lie down and fully extending its limbs or turn around freely” and will require compliance by December 31st, 2012. California’s Proposition 2 (Treatment of Farm Animals Act) passed in 2008, which requires minimum space allowances for calves based on their ability to “turn around freely, lie down, stand up, and fully extend their limbs” will require compliance by January 1st, 2015.

Voluntary industry changes have been adopted by some parts of the calf rearing agriculture sectors in order to provide guidelines for ethical practices and to avoid legislative changes made by the public, who are less likely to be familiar with the background of many practices. Examples of this include the American Veal Association’s decision to require group housing by December 31, 2017 (American Veal Association, 2007) and the 2009 Canadian Codes of Practice for the Care and Handling of Dairy Cattle, which require that calves be able to “easily stand up, lie down, turn around and adopt normal resting postures, and have visual contact with other calves” (National Farm Animal Council, 2009).

Although no legislation has specifically targeted disease rates in dairy calves, this represents an important area of concern for the dairy industry. The National Animal Health Monitoring System (NAHMS) reported that the average mortality rate are 7.8% and 1.8% in pre-weaned and weaned dairy calves, respectively (USDA, 2009). In comparison, the NAHMS survey of beef cow-calf operations found that pre-weaned calf mortality was 3.2% with 56% of
operations reporting 0% mortality (USDA, 2010a). This high mortality rate represents both a welfare issue and an economic concern for producers.

The objectives of this paper are to review the current status of the literature relating to the use of behavior and automated measures for the identification of animals at risk of disease, the use of group level management to reduce the risk of neonatal calf diseases, and the identification of long-term impacts of respiratory diseases. These factors will be reviewed in the context of the current management systems in North America and the associated animal welfare implications.

1.2 THE USE OF BEHAVIOR FOR EARLY DISEASE DETECTION

Disease detection in animals is frequently based on a coordinated set of responses to infection collectively termed sickness behavior (Hart, 1988). Sickness behavior includes the following behaviours and symptoms: malaise, depression, fever, increased sleep, anorexia, adipsia, decreased exploratory behavior and decreased socializing. Although initially categorized as a maladaptive response to inflammation and infection, Hart (1988) proposed that these behavioral changes are actually an adaptive response that improves the host’s ability to survive colonization by viruses and bacteria. These responses have further been proposed to be based on motivational changes, which have very important implications for the application of behavioral means of disease detection in the field (Aubert, 1999). This motivational organization of sickness behaviour was determined by a series of experiments examining the impact of different physiological and environmental triggers on the responses of rats and mice to infection. Key findings from this research were that maternal behavior, nest building and pup retrieval, were performed in dams challenged with lipopolysaccharide (LPS), which triggers a febrile response, in cooler environmental temperatures. However, at thermoneutral temperatures, the maternal
behaviour of dams challenged with LPS was altered and nest building was not performed. This finding supported the hypothesis that these behaviours were driven by motivation changes rather than reflexes since nest building was not performed in warm environments where the lack of a nest would have minimal impact on pup survival. As such, motivational changes need to be considered when using sickness behavior to identify diseased animals.

Additional factors that have been shown to alter the expression of sickness behavior are reproductive status, social status and gender. The role of reproductive status in the expression of sickness behavior has been shown to interact in free-living song sparrows. Male song sparrows, which are territorial year round, showed decreased territorial aggression during the winter (non-breeding) months in response to a LPS challenge but did not show alterations in aggression during the spring (breeding) months (Owen-Ashley and Wingfield, 2006). The effect of gender on sickness behaviour has also been examined in rats where it was determined that injections with LPS altered the sexual receptiveness, attractiveness, and drive of female rats but did not alter these characteristics in male rats (as reviewed by Avitsur and Yirmiya, 1999). This led to the hypothesis that either interleukin-1 or LPS induced the suppression of ovarian hormone secretion, which may have important implications for dairy cattle given the negative impact on reproduction.

In rodent models, sickness behaviour suppresses social exploration. In laboratory rats and mice, illness reduced the amount of time animals spent socially exploring unknown juveniles (Bluthe et al., 1992; Bluthé et al., 1994). Social rank also appears to play a role in the display of sickness behaviour. When a LPS injection was administered to either a dominant or subordinate animal in a pair of mice, only the dominant mice displayed a reduction in social and agonistic
behavior (Cohn and de Sá-Rocha, 2006). This led to the hypothesis that social status may alter the expression of sickness behavior, especially in confined situations.

Anorexia, adipsia and decreased exploratory behavior associated with classic sickness behavior. Where treatment of illness is not available these behaviours are adaptive since they promote energy conservation which is needed for the febrile response (Hart, 1988). However, in modern animal agriculture, where treatment is occurring, this response is often considered to be detrimental to growth and recovery rather than adaptive. These behavioural changes may be especially problematic for calves post-weaning. When individually housed calves are moved to group housing they need to locate and consume feed and water in a novel environment. If their motivation to locate and consume feed and water is reduced due to sickness associated behavioral changes they will likely be unable to maintain adequate nutrient intake. Additionally, water and grain are necessary for continued rumen development (Kehoe et al., 2007) so animals that are anorexic and consuming less water will be slowing the necessary development of their rumen.

It has also been hypothesized that the evolutionary role of a species will impact the expression of sickness behavior. Cattle, as prey animals, have been identified as ‘stoic’ in response to pain and disease (Weary et al., 2009). The expression of obvious illness in prey animals is likely to draw predatory attention and increase the risk of the sick animal being targeted. This hypothesis has not been formally tested, but it does raise the important issue regarding the role of species on the expression of sickness behavior and indicates the need for research into species specific sickness behaviors for the detection of disease.
In cattle, early disease detection has been identified as a key component of successful disease management. Automated monitoring of health and behavior has been studied for the improvement of disease detection. These automated monitoring systems have been designed in part to overcome the difficulties in identifying sick cattle due to their stoic nature and the decreasing availability of skilled farm labour. Examples of automated disease detection include the use of infrared thermography to detect increased body temperature (Schaefer et al., 2007) and monitoring of eating and drinking (Buhman et al., 2000; Schaefer et al., 2007) in beef calves for the early detection of BRD. In dairy cattle, changes in feeding, drinking, lying and social behavior have been used as early behavioral indicators in cows with a variety of transition diseases including metritis (Huzzey et al., 2007), dystocia (Proudfoot et al., 2009), and subclinical ketosis (Huzzey et al., 2007; Goldhawk et al., 2009; Proudfoot et al., 2009). In dairy calves, automated milk feeder technology has been used to identify sick calves by changes in feeding behavior (Svensson and Jensen, 2007; Borderas et al., 2008; Borderas et al., 2009). These findings have been very useful and have the potential to greatly improve disease detection among group housed, milk-fed dairy calves. However, in the United States 75% of operations house milk-fed dairy calves individually (USDA, 2007); automated feeders are expensive and as such studies that depend on them have limited application for many producers. In addition, these studies do not apply to newly weaned calves, which are also at risk of clinical disease.

1.3 REVIEW OF CURRENT MANAGEMENT PRACTICES TO REDUCE DISEASE

In the United States, 97% of dairy calves are separated from their mother within 24 hours of birth (USDA, 2009). After birth, 75% of operations house calves individually, often with minimal physical contact between animals until calves have been weaned from milk-based diets.
These two practices have been recommended to reduce the spread of disease between adult cattle and neonates.

The two most common diseases in dairy calves are neonatal calf diarrhea complex and bovine respiratory disease complex. Neonatal calf diarrhea complex is a broad category of diseases, which occurs in approximately 24% of dairy calves in the United States (USDA, 2010b). The main diarrheal agents in dairy calves are enterotoxigenic *Escherichia coli* K99, *Salmonella* spp, bovine rotavirus, bovine coronavirus, and *Cryptosporidium parvum*, which may act independently or concurrently (Snodgrass et al., 1986; de la Fuente et al., 1999). These pathogens can cause temporary changes and damage to the intestinal tract through destruction and sloughing of enterocytes, villous atrophy, and inflammation of the submucosa, causing inflammation and sensitization of the visceral afferent nerves. In people, these changes to the intestinal tract are associated with abdominal pain and discomfort (Al-Chaer and Traub, 2002).

The primary treatment for diarrhea is supportive fluid replacement therapy. However, meloxicam (Metacam, Boehringer Ingelheim (NZ) Ltd), a non-steroidal anti-inflammatory drug, has been shown to be effective in reducing recovery time from diarrhea when provided in conjunction with fluid replacement therapy (Todd, 2007). This is a novel approach to treating calf hood disease, since it is aimed at reducing the discomfort and inflammation associated with diarrhea rather than a treatment that targets disease causing organisms. This represents an important change in disease management and animal welfare since it addresses not only the physiologic signs, but also the discomfort likely experienced by the calves during clinical disease. The discomfort felt by calves during a diarrhea outbreak is likely to reduce activity which would promote convalescence. However, if animals are being successfully treated, the
increased rest and decreased appetite associated with intestinal discomfort would be unnecessary since the population of disease causing organisms would already be reduced.

The long term consequences of diarrhea include increased risk of being sold prior to calving, increased age at first calving (Waltner-Toews et al., 1986), and lower first lactation energy corrected 305 d milk production (Svensson and Hultgren, 2008). The combination of production and welfare impacts makes it critical that management practices are instituted to decrease disease spread and the susceptibility of calves to disease, and identify and manage affected animals to reduce the negative consequences of this disease.

To reduce the incidence of disease, three approaches can be used: improve the resistance of the animal to the disease causing agents, reduce or eliminate the disease causing agent, or alter the environment so that the animal cannot come in contact with disease causing agents. Examples of this theory currently practiced in the dairy industry include reducing common stressors, which may inhibit the immune response of calves making them more likely to succumb to disease, reducing the spread of disease causing organisms by vaccination and early treatment, and promoting housing and sanitary practices that limits the exposure of calves to disease.

1.4 STRESS AND THE DAIRY CALF

1.4.1 Background

Stress can be classified into two categories, acute and chronic stress. Acute stress is a short-term stress that is contained within minutes or hours. A chronic stress persists for longer periods of times, anywhere from several hours per day to months at a time (Dhabhar, 2002).
When a stressful stimulus occurs, a stress response is initiated via an increase in the cyclic pulsations of corticotrophin releasing hormone (CRH) and arginine-vasopressin neurons of the paraventricular nuclei of the hypothalamus. This activation of the Hypothalamic–Pituitary–Adrenal (HPA) axis results in increased adrenocorticotropic hormone (ACTH) and cortisol secretory episodes. ACTH is the key regulator of glucocorticoid secretions (Tsigos and Chrousos, 2002). Activation of the sympathetic nervous system occurs simultaneously to HPA activation and redirects blood flow from the interior organs of the body to the exterior where it can be used for rapid response in the flight or fight response (as reviewed by Carroll and Forsberg, 2007). There is also a redistribution of leukocytes from various compartments resulting in increased numbers of white blood cells in peripheral circulation. This results in enhancement of the cell mediated immune response as a result of acute stress (Dhabhar and McEwen, 1997). In the case of chronic stress, there is an increased duration at, which glucocorticoids act. This negates the positive aspects of acute stress on the immune system and results in suppression of interleukin-6 and the cell mediated immune response. This is an adaptive response since repeated stimulation of the HPA axis and the immune system by glucocorticoids could lead to an increased risk of autoimmune disease (Dhabhar and McEwen, 1997). Chronic stress can lead to decreased growth and performance in livestock (Barnett and Hemsworth, 1986; Hemsworth et al., 2000). Two of the main stressors for the milk-fed calf are nutritional stress and social isolation. Two of the main stressors for the post-weaned calf are social mixing and transport stress.
1.4.2 Nutritional Stressors

Dairy calves are primarily fed a milk based diet until weaning is initiated. Energy intake in the pre-weaning period is currently recommended to be 4.8-5.3 Mcal/day for a 50 kg calf under thermoneutral conditions in order to gain 1.0 kg/day (Drackley, 2008). The traditional feeding practice for North American calves is to feed 10% of their body weight; a 45 kg calf is fed 4.5 kg of milk. However, recent data from the National Research Council found that 45 kg calves need to consume approximately 2.5L of whole milk for maintenance and the remaining milk can be used for growth (National Research Council, 2001; Drackley, 2008). These calculations are based on a thermoneutral environment, which does not require animals to exert energy to maintain homeostasis; heat or cold can increase the maintenance demands of calves further reducing the energy available for growth on a restricted feeding regime (as reviewed by Drackley, 2008). For this reason, the recommended caloric intake of calves has begun to change, with the National Farm Animal Council’s Code of Practice for Care and Handling of Dairy Cattle recommending calves be fed 20% of their body weight (National Farm Animal Council, 2009).

To avoid a post-weaning slump in weight gain when calves transition from a primarily milk based diet to a grain based diet it is essential that their rumen is adequately developed to absorb nutrients from a grain based diet. At birth, a calf’s rumen is small and underdeveloped. Highly fermentable dry feed is necessary for it to develop in size, musculature and absorptive capacity (Davis and Drackley, 1998). It takes approximately three weeks of grain and water intake for the rumen to develop to a size and absorptive capacity that is necessary for maintenance of growth and four months for it to reach the size of a mature animal (Davis and
Drackley, 1998). At the time of weaning, it is a general guideline that calves need to consume at least 0.7 to 0.9 kg of calf starter each day for three consecutive days in order to maintain a modest weight gain (Davis and Drackley, 1998). If calves are able to consume this volume their rumen should be adequately developed to sustain growth through the transition from a liquid milk based diet to a solid diet consisting of roughage and calf starter. Rumens can be adequately developed to digest grains and release volatile fatty acids at three weeks of age (Kehoe et al., 2007). Early weaning, defined as 5 weeks compared to 14 weeks of age, has been promoted for many years as a method to reduce the expenses associated with raising calves (Castle and Watson, 1959). This is primarily driven by the high cost of rearing calves prior to weaning (Gabler et al., 2000). However, this practice has been questioned on the grounds of animal welfare since early weaning and low milk volumes are not the natural feeding style of calves and hunger has been shown to occur in restricted fed calves (De Paula Veiera et al., 2008).

Until recently, feeding high volumes of milk to replacement heifers was thought to be associated with decreased mammary development. While early published studies reported that increased growth is associated with decreased mammary development, this finding was restricted to heifers older than 3 months of age (as reviewed by Sejrsen, 1994). However, a lack of association between a higher plane of nutrition and mammary development was first reported by Meyer and colleagues (2006) when age differences at euthanasia were controlled for. Recently, it has also been reported that increased pre-weaning growth is associated with improved mammary development and decreased age at first calving (Raeth-Knight et al., 2009). This contradicts previous research and suggests benefits for high volume milk feeding. An additional concern of limit-feeding dairy calves is the higher energy demand required during a febrile response. In
humans, the metabolic rate increases by 13% for every degree Celsius rise in temperature (Kluger, 1978; Hart, 1988). Calves that have already been through a period of poor nutrition due to restricted feed intake or poor rumen development prior to becoming ill are further disadvantaged since they have lower nutrient availability to fight infection (Carroll and Forsberg, 2007).

A challenge associated with increased nutrient intake pre-weaning, or *ad libitum* milk feeding programs, is the issue of transitioning calves onto solid feed. When calves are offered *ad libitum* access to milk they will consume on average 10 L per day (Appleby et al., 2001). While feeding at this level improves calf welfare by reducing the hunger and frustration experienced during a limited feeding program (De Paula Vieira et al., 2008), it also reduces the motivation for calves to consume calf starter. Some of the methods to encourage starter intake and reduce weaning distress have included reducing the number of milk meals per day (Kehoe et al., 2007), dilution of milk, provision of warm water, extended access to the feeding apparatus (Budzynska and Weary, 2008), extended weaning periods (Sweeney et al., 2010), and rearing calves in pairs (De Paula Vieira et al., 2010). These methods have begun to address the issue of weaning calves from high milk volume feeding regimes, however, research in this area needs to be continued to counteract the negative effects of poor weaning on animal welfare and productivity. Given that there appear to be long-term consequences for the productivity of dairy calves based on rearing procedures, future economic analysis of early milk feeding programs should include these long-term effects and not just the cost of feeding.
1.4.3 Social Stressors

Another stressor occurring around weaning is social stress. In studies of mature cattle, it takes ten days for cattle to establish a social hierarchy (Tennesen et al., 1985). This is a stressful time for adult cattle and is accompanied by decreased milk yield on the day of mixing. It is therefore likely that the initial exposure and mixing of calves reared in isolation to new herd mates is a stressful experience.

Social interactions in established groups are very important to cattle. Operant conditioning can be used to determine how much value an animal places on certain activities. Two-month-old calves are willing to work hard to access full social contact with known conspecifics (Boissy and Le Neindre, 1990; Holm et al., 2002). They still work for nose-to-nose contact, but place a higher value on full contact as shown by an increased willingness to work for the reward of social contact. Yearling heifers were trained to access a feed reward either in isolation, in the presence of conspecifics that they had a strong affinity to, or in the presence of a conspecific they had a weak affinity towards as measured by social contact prior to testing (Boissy and Le Neindre, 1990). In order to eliminate competition heifers only had visual contact with ‘spectator’ heifers during operant testing situations. Results indicate that heifers learn faster when in the presence of conspecifics, even if they are unable to physically interact with them. This result was most noticeable when heifers were in the presence of a spectator heifer they had strong affinity towards. However, heifers trained in the presence of an unfamiliar spectator animal still learned to access the food reward faster than heifers that were trained in isolation. These results indicate that social interactions are beneficial for cattle.
Without human intervention, calves first bond with their dam. Le Neindre (1989) found that beef calves that are left with their dams until they are weaned had preferential attachment to their dams. If calves are allowed to remain with dams through the next calving, the bond between mother-dam is not altered (Veissier et al., 1990). However, due to the nature of the dairy industry, dairy calves do not have this initial bond formation with their dam and must form bonds with their conspecifics without this initial socialization.

Positive Social Interactions

Grooming, pair formation and play behaviour are three examples of positive social interactions that strengthen the bonds between animals within a group. Studies on herds of cattle indicate that grooming behavior is most likely to be directed at animals that are of similar social dominance rank. Frequency of grooming was positively associated with time spent in cohabitation (Sato, 1984; Sato et al., 1991). Environmental factors, such as delayed feeding time and dirty environments, also influence the time spent social licking. However, the preferential partnering and increased growth rates associated with animals that receive social grooming indicates that this likely functions as a bonding and relaxing activity for cattle, as well as for cleaning. The decreased heart rate of cattle that receive allogrooming supports the hypothesis that this is a rewarding behavior, which can improve cattle welfare (Laister et al., 2011). Since individually housed neonates are prevented from contacting conspecifics they are also prevented from performing and receiving this soothing activity, likely affecting their response to stress.

Within groups of cattle preferential partnerships develop. In extensive and feral herds of cattle it is initially the dam-young relationship that is given priority (Lazo, 1994). However, by two weeks of age calves spend the majority of their time with other calves, when nursing time is
removed from the time budget (Wood-Gush et al., 1984). This allows bonds to form between unrelated animals. When 9 male and 9 female prepubertal calves were studied within a large extensive herd where they had minimal human contact, four calf pairs were found. A pair of calves was defined as two animals that mutually preferred to spend time with each other in each of the six months of this study. Interestingly, on average pair members differed in age by 5 days despite the fact that there was an age range of two months within the group (Reinhardt et al., 1978). This indicates that it is likely that age and developmental stage are very important in the development of partners within a herd; Reinhardt and colleagues (1978) concluded that social contact between calves is not random, but also highly unpredictable.

Play behaviour is one aspect of social behavior. Calves move in and out of play behavior quite rapidly and under natural conditions perform this behavior many times throughout the day (Reinhardt et al, 1978). Once play is initiated it appears to elicit this behavior in conspecifics not involved in the original event (Vitale, 1986). Play behaviour occurs between both sexes with males being more active and more likely to be involved in social activity than the females in the group (Reinhardt et al., 1978; Vitale, 1986). There has been interest in using play behavior as an indicator of positive welfare. Play is predicted to be an indicator of positive welfare based on decreases in play behavior during aversive situations, such as hunger and pain. For example, play behavior is decreased in lambs following castration (Thornton and Waterman-Pearson, 2002) and in groups of dairy calves during weaning (Krachun et al., 2010).

**Negative Social Interactions**

In modern dairy systems, cattle are kept in larger groups than they would normally encounter in feral or range conditions. In the mountains of Japan, a feral herd of cattle had an
average group size of three or four cattle and aggressive interactions were minimal except at water, the only limiting resource (Kimura and Ihobe, 1985). However, these animals had a very low-density range of approximately 10 individuals per km$^2$. This is not a stocking density that is viable in the modern dairy industry and as such aggressive interactions are more common in the commercial dairy industry. Cattle have been shown to form a dominance hierarchy, which consists of a range of dominant and submissive animals in each group. This hierarchy is most commonly defined as a linear relationship for post-pubertal beef and dairy cattle (Schein and Fohrman, 1955; Stricklin et al., 1980; Stricklin, 1983).

Benefits of being dominant include better access to resources such as feed, water and stalls. Researchers have found that dominant animals get more rest, can move more freely and eat first and for longer in comparison to submissive animals (Olofsson, 1999). This benefits the dominant cattle by allowing them to better maintain body condition. The health status of cattle and dominance rank is closely linked, and likely feeds back on itself. A healthy cow is more likely to be dominant and subordinate cows are more likely to become ill. Galindo and Broom (2000) found that 28% of high ranking cows became lame during the study period versus 60% of low ranking cows. In a free-stall management system, dominant cows are less likely to be lame, perhaps as a result of an ability to control resources such as stalls and feed bunks. This forces the subordinate animals to walk further and rest less, putting them at increased risk of hoof problems. Agonistic encounters also cause chronic stress in subordinate animals, suppressing the immune system and resulting in increased susceptibility to other diseases (González et al., 2003).

Older animals are more likely to be dominant over younger animals. This predictor is highly influenced by the experience and size of the older animal. Dickson et al (1970) found that
age and body size are correlated with dominance. The ability to win a dominance challenge, however, appears to be highly heritable with genetics being a much larger factor in future dominance than rearing environment. Studies of mononzygotic twins show that when reared separately from the dam the dominance status of twins is highly correlated \( (r=0.93; \text{Purcell and Arave, 1991}) \). Agonistic behaviour develops later in life than affiliative behaviours. Agonistic interactions are rarely observed in pre-pubertal cattle kept in semi-wild conditions with a stable social structure (Reinhardt et al., 1978).

It is not known if young calves are capable of establishing a dominance hierarchy through agonistic interactions. Studies conducted to investigate mixing of calves have produced very different results. When pairs of 11-month old Holstein heifers were mixed weekly over a period of 16 weeks it was found that aggressive interactions at the time of mixing remained at similar levels throughout the study period (Raussi et al., 2005). However, when pairs of 3-14 week old Montbeliard veal calves were repeatedly mixed, they did not display a significant stress response, and although they performed increased activity on the day of mixing, there was a low level of aggressive interactions (Veissier et al., 2001). This would suggest that age plays a role in the development of aggressive interactions. However, it has not been determined when exactly these interactions begin to occur and if they are initiated by physical, environmental or social changes in the calves. For example, milk fed calves will displace each other at the feeder, which indicates that competition for resources can occur in young animals (Jensen and Budde, 2006). This can be alleviated by altering either the ability of competitors to access the resource, such as the milk feeder (Jensen et al., 2008), or by altering the demand for the resource through increasing milk volume fed or the number of teats available (Jensen, 2004; von Keyserlingk et
Group composition and group size also affect the level of aggression with a wider age variation associated with increased levels of aggression and younger animals most likely to be targeted (Færevik et al., 2010). A study comparing pair housed calves to calves housed in groups of 4 found that calves consumed milk faster in groups of four compared to groups of two, indicating increased competition in groups of four (Jensen and Budde, 2006). For groups of four, six and eight calves, feed bunk displacements after mixing were highest in groups of four, but there was no effect of group size on displacements from the lying area (Færevik et al., 2007). When examining established groups, group size was not found to be associated with increased aggression (Kondo et al., 1989). This may indicate that group size only affects agonistic interactions during mixing, when social hierarchy is being determined.

In conclusion, social interactions can either increase or decrease the welfare of calves depending on their status within the group, group composition and group stability. In extensive groups, animals have the potential to join other herds, form different sub-groups and avoid dominant animals. In confined spaces these options are restricted or eliminated. For this reason, management of these social animals needs to focus on refining mixing practices and reducing competition in order to reduce the negative impacts of social life on low-ranking animals.

1.4.4 Transport Stress

It is common practice for calves to be moved to a novel environment post-weaning. This will require calves to either be restrained with a halter or loaded into a trailer in order to relocate them. This will result in acute or chronic stress depending on the duration of travel (Apple et al., 2005). In addition to the stress of travel, calves are taken from an isolated environment that limits disease exposure to being in contact with many heifers of varying age groups, and consequently
exposed to many different disease causing organisms. When this stressor is combined with weaning, disease outbreaks are likely, as shown by the high risk of BRD post-weaning in both dairy calves (McGuirk, 2008) and beef feedlots (Smith, 1998). Efforts to minimize stress associated with transport to a novel environment are important for reducing weaning stress and minimizing the risk of disease.

1.5 CONTROL OF DISEASE SPREAD

At the herd level, management of BRD has included vaccination, removal of stressors, and administration of antibiotics in the water or feed, and prevention of other diseases. Vaccination is a challenge in the face of maternal antibodies received from colostrum; as such, vaccination of young dairy calves for respiratory disease is difficult (Ellis et al., 2001; Fulton et al., 2004). For this reason, disease prevention must continue to address management factors that affect the spread of disease, the stress levels of the calf and reduce the viral and bacterial load in the environment.

Risk factors for BRD include concurrent nutritional, environmental and social changes, as well as increased pathogen exposure from mixing with older animals for the first time. Important control measures include vaccinating cows against the causal organisms, ensuring excellent colostrum management and minimizing acute and chronic stressors such as poor ventilation, mixing of groups, over-crowding and poor nutrition (Kahn, 2005). However, at weaning or during the change of housing after weaning some stressors cannot be avoided, resulting in increased incidence of this disease (McGuirk, 2008).

One approach used by producers to reduce disease is the administration of antibiotics at the group level. There are currently two approaches for group administration of antibiotics. The
first is the addition of antibiotics to the feed and the second is a single injection of antibiotic to all animals in a group at a specific time point. The most recent NAHMS survey reported that 18% of dairy operations used an antibiotic in the feed of post-weaned heifers (USDA, 2009). The addition of antibiotics to the feed is a very convenient method to administer antibiotics to calves; it eliminates the cost of handling animals to administer an injection and when intake is monitored to ensure adequate intake, has been shown to successfully treat clinical disease (Fodor et al., 2000). The main drawback of this approach is the challenge of administering an appropriate dose to all animals, especially those at highest risk. The animals that are most likely to receive the highest dose are those with high feed consumption in the immediate post-weaning period. These animals are already less likely to become ill since high feed consumption reduces or eliminates the negative energy balance that can occur post-weaning. In contrast animals that are not consuming adequate feed, either due to poor adaptation to weaning or the initial stages of disease, will get a lower dose of antibiotics despite a higher risk of disease.

The main drawback of injecting antibiotics is the cost of administering the drugs and the shorter duration of activity. The labour costs to restrain and inject a large group of calves can be very high. Application of proper restraint is costly due to the time required and is likely stressful for the calves. The benefit of this approach is that calves will receive a correct dose based on their size with very little risk of under or over dosing. This approach was first used in the beef feedlot industry for the prevention of respiratory disease in animals expected to be at high risk of morbidity and mortality. Animals considered to be at high risk are animals stressed due to the abrupt severing of the maternal bond, transportation, comingling, and marketing (Duff and Galyean, 2007). In 1999, 27% of small beef feedlots and 81% of large feedlots in the United
States were administering an injectable antimicrobial metaphylactically to all cattle for the prevention of respiratory disease (USDA, 2000). A meta-analysis of field trials indicated that for feedlot calves, administration of oxytetracycline or tilmicosin on the day of arrival at the feedlot consistently reduced morbidity rates attributable to BRD (Van Donkersgoed et al., 1993). The movement of beef cattle into a feedlot is comparable to the movement of dairy calves from individual to group housing. In both cases, calves experience similar stressors, which include nutritional, environmental and social changes, transportation, and increased pathogen exposure.

Regardless of the method used to administer antibiotics, this practice must be carefully evaluated by both researchers and producers to maximize efficacy and avoid unnecessary use given the concern over developing antimicrobial resistance. The ideal circumstances for adopting this prophylactic program are the following: a targeted disease that is highly contagious and is endemic to the region, successful clinical treatment is difficult, and the risk of pathogen exposure is constrained within a short time period. In conjunction with this method, efforts should be made to reduce or minimize manageable risk factors with the goal of reducing or eliminating the need for prophylactic use. For this program to be most effective good record keeping is essential in order to review historical disease trends to identify high risk time periods and environmental triggers. In addition, these records are needed for outcome assessment to measure the success of this program and other management changes made to decrease the risk of disease for efficacy.

1.6 LONG-TERM IMPACTS OF BOVINE RESPIRATORY DISEASE COMPLEX

BRD is a complex of disease processes most commonly resulting from infection with various microorganisms, including Pasteurella multocida, Mannheimia haemolytica and Mycoplasma bovis, Histophilus somni in conjunction with bovine herpesvirus-1 (BHV-1) bovine
viral diarrhea virus (BVDV) bovine respiratory syncytial virus (BRSV) and parainfluenza 3 virus (PI3V) (Kahn, 2005). The development of BRD is typically initiated by an environmental stressor and viral infection, which weakens the resistance mechanisms of the lungs and allows bacterial colonization (Yates, 1984). There are many factors that have been associated with increased risk of BRD, including mixing of animals, transportation, light weight or younger animals within a group and persistent infection with BVDV (as reviewed by Taylor et al., 2010). The National Animal Health Monitoring Survey (NAHMS) dairy heifer study found that 6% of weaned dairy heifers were treated in 2007 for respiratory disease (USDA, 2009). However, the NAHMS study defined post-weaned heifers as including all animals from weaning to first calving. The wide age variation encompassed by this definition would include many older animals that were at low risk of BRD. Other epidemiologic studies in North America have reported BRD rates in milk fed dairy calves ranging from 0.1 cases/100 days in Minnesota (Sivula et al., 1996), 25% of calves in New York State (Virtala et al., 1996) and 39% of calves in Saskatchewan (Van Donkersgoed et al., 1993). This substantial variation in the reported rates of disease probably results from a combination of the differences in the diagnostic criteria used, disease definition, age of the calves, the composition of the viral and bacterial agents, and management conditions.

Costs of BRD were estimated at $14.71 (range: 0 - $119) per pre-weaned calf per year and $1.95 (range: 0-$9.25) per weaned dairy calf stock per year by Michigan producers in 1990 (Kaneene and Hurd, 1990). These costs included labor for detection and treatment of disease, drugs, veterinary fees and cost to replace animals that are died as a result of clinical disease. The higher cost observed in young calves is based on the timing of preventive practices, which
primarily are administered to calves prior to disease outbreaks, and the long follow-up time for calf stock, which included an extended period of low disease risk. However, these estimates likely underestimate the cost of BRD due to the difficulty in associating a disease event with outcomes many months later. The European estimate of 18-57 euros ($25-81 U.S.) per case is likely more accurate since the effect of BRD on growth, long-term survival and productivity were considered (van der Fels-Klerx et al., 2001). However, the effects of BRD on growth, survival and production were based on a small number of studies examining these factors that were conducted 10-20 years ago. Results from these three studies found that BRD increased the risk of mortality prior to calving (Waltner-Toews et al., 1986), decreased growth (Virtala et al., 1996), increased age at calving (Waltner-Toews et al., 1986; Correa et al., 1988) and increased the risk of dystocia at first calving (Warnick et al., 1994). Warnick et al., (1995; 1997) found no association of calfhood BRD with first lactation milk production or survival after first calving.

The welfare costs of BRD can be substantial based on both the clinical state and the long-term implications of this disease. The clinical signs can include inappetance, depression, coughing and shortness of breath (dyspnea), which compromise animal welfare (Mellor and Stafford, 2004; Kahn, 2005). The long-term effects of BRD on body weight gain and survival are of concern since these persistent consequences have the potential to decrease calf welfare. Specifically, decreased growth may be due to a lower dominance status leading to decreased ability to access resources or an ongoing disease challenge, which would be associated with ongoing clinical signs. In both of these scenarios animal welfare would be affected and new management techniques and treatments for affected animals are necessary to improve convalescence in these animals and to reduce the long-term impacts of this disease.
1.7 CONCLUSION AND THESIS OBJECTIVES

In conclusion, the short and long term impacts of dairy calf management on their welfare needs to be evaluated, within the confines of a viable economic model. Currently, there are many gaps in our knowledge and there may be ways to improve management systems, including identifying behaviors that can be used for early identification of disease, and understanding some of the long-term impacts of disease. The goals of this thesis were to investigate the use of behavior and activity monitoring for the identification of animals at risk of disease, the use of group level management practices to reduce the risk of neonatal calf diseases, and the identification of long-term impacts of respiratory disease.

Specifically the research objectives were;

1) To identify behaviors associated with poor weight gain in newly weaned dairy calves.
2) To evaluate the impact of a novel management practice to reduce stress associated with transitioning weaned calves to a novel environment.
3) To identify behaviors associated with disease and poor growth in milk-fed dairy calves
4) To evaluate the impact of a targeted antibiotic approach to reduce disease in milk-fed dairy calves
5) To evaluate the efficacy of tulathromycin on the incidence of BRD and growth in weaned calves following movement to group housing in a novel environment.
6) To evaluate the long-term impacts of BRD on the welfare and productivity of dairy cattle.
1.8 REFERENCES


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CHAPTER 2: PREVALENCE OF SICKNESS BEHAVIOR IN NEONATAL DAIRY CALVES AT A COMMERCIAL HEIFER FACILITY AND ASSOCIATIONS WITH AVERAGE DAILY GAIN AND ILLNESS

2.1 INTRODUCTION

Across species there are sets of predictable physiologic and behavioral responses associated with pathogens, and parasitic infection. These responses include pyrexia, anorexia, depression, lethargy, and decreased grooming and are considered to have evolved because of the increased probability for survival of the host (Hart 1988). In cattle, these changes have been used to identify sick animals. There is interest in using these behaviors to identify and treat animals early in order to avoid the negative effects of disease on calves’ performance and welfare (Millman, 2007; Weary et al. 2009).

Researchers have begun to evaluate technology capable of automatically monitoring and reporting changes in body temperature and behavior that will allow early identification of disease in animals. Some examples include the use of infrared thermography to detect increased body temperature (Schaefer et al., 2007) and monitoring of eating and drinking behavior (Buhman et al. 2000; Schaefer et al., 2007) in beef calves for the early detection of bovine respiratory disease. In dairy cattle changes in feeding, drinking, lying and social behavior have been used as early behavioral indicators in cows with a variety of transition diseases including metritis (Huzzey et al., 2007), dystocia (Proudfoot et al., 2009), and subclinical ketosis (Huzzey et al. 2007; Goldhawk et al. 2009; Proudfoot et al., 2009).

Changes in the feeding behavior of dairy calves have been used to identify sick calves. However, these studies have utilized automated feeders (Svensson & Jensen. 2007) or extended
behavioural observations (Borderas, et al. 2008; Borderas et al. 2009), which are not feasible for many producers. Currently, the primary housing system for milk-fed calves in the United States is individual housing, with 75% of pre-weaned calves housed individually (USDA 2007).

Milk-fed dairy calves, regardless of housing system, are highly susceptible to disease and it is important to have multiple, reliable indicators for disease so early diagnosis can occur. The two most important diseases in pre-weaned dairy calves in the United States are diarrhea and respiratory disease (USDA 2009). Neonatal calf diarrhea complex is a broad category of diseases which occurs in approximately 24% of dairy calves in the United States (USDA 2010). The main diarrheal agents in dairy calves are enterotoxigenic *Escherichia coli* K99, *Salmonella* spp., bovine rotavirus, bovine coronavirus, and *Cryptosporidium parvum*, which may act independently or concurrently (Snodgrass et al. 1986; de la Fuente et al. 1999). The primary sign of this disease complex is diarrhea and the primary treatment for mild cases is supportive fluid replacement therapy. These pathogens can cause temporary changes and damage to the intestinal tract through destruction and sloughing of enterocytes, villous atrophy, and inflammation of the submucosa, causing inflammation and sensitization of the visceral afferent nerves. In people, these changes to the intestinal tract are associated with abdominal pain and discomfort (Al-Chaer & Traub, 2002).

Recognition of key behaviors associated with common diseases and poor growth can allow new and experienced animal caretakers to improve identification of potentially sick animals. The objectives of this research project were to 1) determine the proportion of calves performing five behavioral measures related to sickness behavior at different ages, 2) determine which behavioral measures were associated with reduced growth and 3) determine if these
behaviors can be used to correctly identify calves with clinical disease, specifically diarrhea. The hypotheses for this study were that the five behavioral measures (resting position, standing posture, vigilance, lethargy and human approach test) that were selected based on sickness-driven changes in motivation would be 1) greater in younger calves when the incidence of disease is highest 2) associated with variations in average daily gain (ADG), 3) more beneficial for disease identification at different ages based on motivational changes that occur as calves age and the responses to the behavioral measures will be different in calves with active diarrhea relative to healthy controls.

2.2 MATERIALS & METHODS

2.2.1 Animals and Housing

Between October 2007 and June 2008 744 Holstein heifers housed in individual nursery pens at a commercial heifer raising facility in Western New York were enrolled in the study. Calves were born on 1 of 5 source farms located in New York State. Upon arrival at the heifer raising facility, each calf was processed according to a standard farm protocol, which included collection of a blood sample for measurement of total protein as a surrogate measure of successful passive transfer of immunoglobulins from colostrum. Calves were housed individually in naturally ventilated nursery barns. Calves housed in the same nursery barn simultaneously were considered to be one enrolment group. A nursery barn consisted of 4 rows of pens with 12 pens in each row. Calves were separated by solid partitions between pens to restrict physical contact. Visual contact with calves in the adjacent row of pens was available to all calves. Each pen measured 1.2m by 2.4m. All calves were manually fed 4L of milk replacer (Excel Calf Milk Replacer 26/18, Grober Nutrition, Cambridge, Ontario, Canada) twice daily
and had access to ad libitum calf starter (23% protein; 5% fat) and water. Weaning from milk replacer was initiated at 5 wks and completed at 6 wks of age. Calves were removed from the trial at 8 weeks of age, at which time they moved to group housing.

All calves were weighed on an electronic scale (Tru-test - ID3000, Tru-Test Incorporated, Mineral Wells Texas) upon arrival at the farm (study enrollment) and at exit from the nursery facilities 8 weeks later (study completion). Calves arrived at the heifer farm at 3 ± 2 days of age (mean ± standard deviation) and weighed 41 ± 4 kg. On average, calves gained 0.61 ± 0.13 kg/d over the study period. At the end of the study, calves weighed on average 75 ± 11 kg and were 59 ± 7 days old.

All calves in this study were additionally enrolled in a study examining the impact of an antibiotic administered at upon arrival at the heifer raising facility on the incidence of neonatal calf diseases, specifically otitis media. Calves were injected subcutaneously with either 1.0 mL of tulathromycin (Draxxin®, Pfizer, Animal Health Group, New York, New York, USA) or a placebo at arrival. Draxxin® is a semi-synthetic macrolide antibiotic, containing 100 mg of tulathromycin per mL. The placebo was a visually identical mix of propylene glycol and sterile saline.

2.2.2 Behavioral Measurements

Behavioral observations were performed every other week by the principle investigator, with each calf observed during three time periods (Figure 2.1). Calves were 1- 23 (mean ± SD 10 ± 5), 12- 37 (mean ± SD 24 ± 5), and 26- 50 (mean ± SD 38 ± 5) days of age at observation periods 1, 2, and 3, respectively.
The observer recorded all behaviors for each calf starting with the youngest calf in the group and ending with the oldest. The observer was blind to disease diagnoses. For each calf, the observer stood immediately outside of the individual pen at the front corner, out of sight of the next calf to be observed. Milk feedings occurred at 5am and 5pm each day (Figure 2). Behavioral observations were conducted in the late morning between 10:00 and 12:00 when calves were unlikely to be anticipating their next meal. After behavioral measures were complete for an individual calf, the observer would record if calves were observed with any of the following clinical disease indicators: diarrhea, sunken eyes which indicate dehydration, elevated respiratory effort, coughing, and discharge from the nose or eyes.

Behaviors of interest consisted of standing and lying postures associated with differing levels of comfort, and responses to three tests to measure lethargy relative to competing motivations of vigilance, fear, and exploration (Table 2.1). Behavioral scoring criteria were selected based on behaviors that have been previously associated with pain, discomfort, or tests such as the human approach test that has previously been used as an indicator of fear, which in dairy calves may be an indicator of malaise.

Lying posture was included as a potential indicator of malaise (Hart 1988). The lying postures were evaluated on a visual analogue scale previously validated as a method of assessing acute pain in young lambs (Molony et al. 2002). It was expected that sick animals would conserve energy by restricting their exposed surface area in a short lying position. Alternatively, if there were issues with breathing due to respiratory disease, calves may lie with the neck extended in order to maximize oxygen transfer. Lateral lying was included as a posture which
may indicate abdominal discomfort, due to either diarrhea or umbilical infection, limiting a calf’s willingness to adopt a ventral lying or short lying posture.

To evaluate the value of standing posture as an indicator of illness three potential postures were identified; normal relaxed posture, statue standing, and standing with head below chest. A normal relaxed posture was expected in the majority of calves and would be evidence of health. Statue standing has been observed in lambs following castration and is thought to be an indicator of visceral pain (Kent et al. 2001). Lowered head has anecdotally been observed as an indicator of illness in calves, and may represent malaise and depression during illness.

An initial response test, or “Vigilance Test”, was created to determine each calf’s initial response to the observer. Four types of responses were observed; face, profile, escape and ignore. It was assumed that facing the observer (face) or standing in profile to the observer (profile) were the normal responses to the presence of an observer. Escape was included as a measure of active withdrawal from the observer. The ignore response was expected to be the lowest vigilance response.

As a measure of lethargy, the amount of observer encouragement required for lying calves to rise was measured on a 6 point scale, based on the assumption that calves would respond to observer vocalizations and invasion of their space by rising. Lower response to these signals was hypothesized to indicate that calves are conserving energy and have a strong motivation to remain lying due to exhaustion, discomfort or disinterest, which indicates illness. Due to the low number of calves that required entry into the pen (score ≥ 3) to rise during behavioral scoring at times 2 and 3, these categories were collapsed into a single combined score category of 3+.
The human approach test used in this study is based on the knowledge that these milk-fed calves rely on humans for feed and as such will likely have positive associations with a stationary person. For this reason, it is highly probable that calves will rapidly approach a stationary observer. A previous study showed that at 17 days of age calves that were hand reared without additional positive human contact approached within 1 meter of an observer in an average of 12 seconds (Jago et al. 1999). It was hypothesized that failure to approach an observer by calves in this age group would be an indicator of a decreased interest in the environment, which has previously been associated with sickness behavior (Hart 1988; Dantzer & Kelley, 2007).

2.2.3 Disease Recordings and Weight Gain

All disease treatment events were recorded daily by barn staff. The standard farm practice was to first record all treatments on standardized paper forms and later enter the data into a farm management computer program (DairyComp 305, Valley Agriculture Software Inc., Tulare, CA). Standardized definitions were used for the most common diseases. Neonatal diarrhea complex was defined as “feces soft and does not hold form”. Non-specific fever was defined as “dull and listless with a rectal temperature above 39.5°C”, and was distinct from the definition for respiratory disease, which was “rectal temperature above 39.5°C, together with elevated respiratory rate, nasal discharge or cough”. Neonatal calf diarrhea complex was the most commonly treated disease with 84% of calves treated at a median age of 10 days. Only 4% (27/788) of calves were never treated for disease. Failure of passive transfer was determined based on serum total protein < 5.4 g/dL (Tyler et al. 1998). Failure of passive transfer was
identified in 5% (40/788) of calves. Mortality was 2% (n = 14) during this study. The full description of morbidity and mortality has been published elsewhere (Chapter 5).

Definition of Active Diarrhea

Diarrhea was considered to be active if calves were observed by the principal investigator between 1 day pre- and 3 days post- diagnosis of diarrhea. This was based on the assumption that the farm staff identified clinical disease quickly, and recovery from diarrhea would take a minimum of 3 days.

The behaviours observed in calves with active diarrhea were compared to the behaviours performed by healthy controls. Healthy controls were restricted to calves that were not identified with a clinically apparent disease by barn staff within a specific time period before and after behavioral measures were recorded by the observer (Table 2.2). A longer restriction period was applied to non-specific fever and respiratory disease based on the increased difficulty of detecting these diseases. All calves with non-specific lameness were eliminated as potential controls based on the highly chronic nature of this condition in this population of calves. In addition, on the day of observation, calves observed with any of the following signs of illness were excluded as control calves; loose-watery stool, dehydration (eyes sunken), discharge from the eyes or nose, or elevated respiratory effort.

2.2.4 Statistical Analysis

All statistical analyses were performed using SAS® 9.1 (SAS Institute, Cary, N.C., USA) All frequencies, medians and means were generated using Proc Freq and Proc Means.
Calves that were not observed at all three observation points, or were not weighed upon exit from the study, were excluded from these analyses (n = 52). This was done to allow for comparison of the same populations over time.

**Changes in proportion of calves performing behaviors over time**

The probabilities of a behavior (for example: short lying, ventral lying, human approach test) occurring (n = 692 calves at 3 time points) over time were analyzed using generalized linear mixed models with a logit transformation (Proc Glimmix). Calf id was included as a random effect and observation period as a fixed effect. Significance was based on a $P$-value $\leq 0.05$.

**Associations of behavioral measures with average daily gain at different ages**

The associations between behavior and ADG were evaluated using generalized linear mixed models (Proc Mixed) with ADG as the outcome and random effects for source farm and enrolment group forced into each model. Each behavioral measurement was analyzed separately to determine the utility of specific behavioral measurements for predicting ADG and eliminate any correlation between behavioral measurements. Antimicrobial treatment, age at scoring and weight at study enrolment were included as fixed effects in each model to control for the variation in age at each observation and any effect antimicrobial treatment may have had on disease severity or growth. Behavioral scores at time periods 1, 2, and 3 were included in separate models to evaluate the impacts of behavior on ADG at different ages. Significance was based on a $P$-value $\leq 0.05$.

**Associations of behaviour with neonatal calf diarrhea complex**

Lying position, vigilance test, lethargy, standing posture and human approach test were assessed for their associations with diarrhea. Diarrhea was of interest due to the high prevalence
of diarrhea in calves on this farm and the importance of this disease in calves in North America. The majority of diarrhea events were observed at the first observation time, so analysis was restricted to this observation time point to control for the effect of age on behavior.

Separate chi-square tests were performed to determine if there were differences in the response of calves with diarrhea versus control within each response category within the separate behavioral measures. If there were fewer than 6 observations in a response category a Fisher’s exact test was used. Significance was based on a P-value $\leq 0.05$ and tendencies were discussed if the $P$-value was $\leq 0.10$ and $> 0.05$.

2.3 RESULTS

2.3.1 Changes in response to behavior measures over time and associations with Average Daily Gain

The proportion of calves that were ventral lying and short lying decreased over time ($P < 0.001$, Table 2.3). No calves were observed short lying, lying with neck extended, lateral lying statue standing or standing with a lowered neck at all observation periods.

The ADG of calves that were observed statue standing at observation 1 was 0.11 ± 0.03 kg lower than calves that were observed with a normal posture ($P = 0.002$; Table 2.4). Calves were most likely to ignore the observer at observation 1 ($P < 0.001$). No calves were observed escaping or ignoring the observer at all three observation periods. Less than 1% of calves (3/692) were observed in profile at all three observation periods. There was no difference in the ADG of calves with different responses to the vigilance test at any of the 3 observation times.
For the human approach test, 11% (75/692) of calves never approached the observer, 28% (194/692) approached the observer during only one observation period, 33% (230/692) approached the observer at two of the observation periods, and 28% (193/692) of calves approached the observer during all three observation periods.

Over the three observation periods, 42% (289/692) of calves never had a score of 3+ on the lethargy scale, 45% (309/692) of calves had a score of 3+ at one observation, 11% (78/692) of calves had a score of 3+ at two observations and 2% (16/692) of calves always had a score of 3+. Calves were 4.2 (95% CI: 3.3-5.3) times more likely to have a score of 0 on the lethargy scale at observation 3 relative to observation 1 ($P < 0.001$). The proportion of calves in the different standing postures did not change over time. Calves were 1.6 (CI: 1.3-2.0) times more likely to approach the observer at observation 3 compared to observation 1 ($P < 0.001$).

2.3.2 Behaviors associated with neonatal calf diarrhea complex

The average age of calves with diarrhea and control calves was 10 ± 4 (mean ± standard deviation) days and 9 ± 5 days, respectively. Calves with diarrhea were more likely to be short lying than their healthy cohorts ($P = 0.04$). Ventral lying and short lying were observed in 55% (127/229) and 12% (28/229) of diarrhea calves, respectively. Ventral lying was observed in 45% (21/47) of calves in the control group. There was no difference in the proportion of calves ventral lying in the diarrhea and control groups ($P = 0.17$). Lateral lying and lying with neck extended was observed in 3 calves and 1 calf in the diarrhea group, respectively. One control calf was observed short lying and no control calves were observed lateral lying or lying with neck extended. The control calf that was observed short lying was also observed shivering.
The most common response to the vigilance test was facing the observer regardless of diarrhea status (Table 2.5).

A greater proportion of diarrhea calves had a lethargy score of 3+ compared to the control group \( (P = 0.0004; \text{Figure 2.3}) \). A smaller proportion of calves with diarrhea had a lethargy score of 0 compared to the healthy cohort \( (P = 0.0008) \).

The control calf which exhibited statue standing was 2 days old and was shivering at the time of observation. There were too few calves observed performing either statue standing or lowered neck to perform statistical analysis. Two calves did not have a standing posture recorded due to a failure to maintain a voluntary standing posture, 1 in the control group (2 days old) and 1 in the diarrhea group (7 days old).

The proportion of calves that approached the observer during the human approach test was 46\% \((105/229)\) and 70\% \((33/47)\) in the diarrhea and control groups, respectively \((P = 0.0007)\).

2.4 DISCUSSION

Five measures of behavior were assessed based on the hypothesis that animals alter their behavior during illness. The use of different lying and standing postures have recently been used in the identification of pain associated with illness (Todd 2007) and have previously been validated for use in lambs following castration (Molony et al. 2002). The use of vigilance tests, lethargy scale and human approach tests are novel tests in the field of sickness behavior.

Lying posture was divided into four categories based on the hypothesis that animals during the clinical phase of disease will alter their behavior to conserve their energy or minimize
the discomfort associated with clinical signs. It was hypothesized that short lying would be adopted to conserve energy by reducing the surface area exposed to the environment and reducing the lower critical temperature (Schrama et al. 1993). The hypothesis was supported by the finding that 12% of animals with active diarrhea were observed short lying and only 1 healthy calf (2%) was observed short lying. Due to the lower amount of disease in older calves we cannot confirm if this difference in the proportion of calves short lying was due to the lower prevalence of disease in older animals or if this behavior was restricted to neonatal calves which are the most vulnerable to temperature changes. The lower critical temperature, defined as the environmental temperature at which calves are required to exert energy to maintain their body temperature, for healthy neonates is estimated at 10.8°C and 8.2°C for 10 and 20 day old calves, respectively (Gonzalez-Jimenez & Blaxter, 1962). This lower critical temperature will be higher in animals with either a fever or anorexia. For calves with diarrhea the malabsorption that occurs during this disease complex would mimic fasting, which raises the lower critical temperature of animals (Blaxter, 1962). Short lying was not associated with ADG at any of the three time points. The lower range in critical temperature for older calves may decrease the need of these animals to perform short lying behavior in order to conserve energy. Calves may also be altering their lying behavior during the first observation period in response to discomfort from diarrhea, since short lying was more common in the diarrhea calves.

Calves resting with an extended neck were observed very rarely in the sub-sample of clinically diseased calves and their healthy cohorts. For all calves in the study, the probability of lying with neck extended did not change over time and was not associated with changes in ADG.
at any observation period. For this reason, the hypothesis that lying with neck extended was associated with poor oxygen exchange cannot be confirmed or denied by this study.

Lateral lying was also observed very rarely, and as such no conclusions can be drawn with respect to the usefulness of this behavioral response as a tool for identifying sick animals. However, it is worth noting that no control calves in the diarrhea analysis were observed lateral lying and the calves that were lateral lying at observation 2 and 3 had very low ADG, so future study of this behavioral response may be warranted.

An overall challenge when measuring lying posture in calves was the inability to observe all of the calves while they were lying down. In this study, the proportion of calves that were not observed lying was 4.0 (CI: 3.2-5.0) times greater at time period 3 compared to time period 1. This pattern is consistent with the findings of Panivivat et al (2004), who found that the lying time of calves decreased from 81% to 73% from week 1 to week 6 of life (Panivivat et al., 2004). As expected, a larger number of animals in this study were not lying at the time of observation, given that this was a single measurement taken during the day, when calves were most likely to be active. However, this absence of lying could be beneficial for identifying ill calves because 69% of the calves with diarrhea were observed lying and only 47% of healthy animals were observed lying.

The proportion of animals in the different standing postures did not change over time. Statue standing in period 1 was associated with decreased ADG. If statue standing is a response to clinical disease that has little impact on ADG, for example bloat or hernia which may cause discomfort, the association between statue standing and ADG will be weakened. Statue standing was only performed in calves with diarrhea, and in one calf that was not treated for disease but
was observed shivering at the time of observation. While interpretation of these results is limited due to a small sample size, it does suggest this is an indicator of diarrhea and given the association of statue standing with pain in lambs (Molony et al. 1993) supports the hypothesis that diarrhea is painful in calves.

The vigilance test used in this study had four possible responses based on observations made prior to study initiation. The proportion of calves that faced the observer increased over time such that calves were 2.7 (CI: 1.9-3.7) more likely to face the observer at observation 3 relative to observation 1. It was expected that due to the lethargy and depression that has been observed as part of sickness behavior, sick calves would be more likely to initially ignore the presence of an observer (Hart 1988). Ignoring the observer and holding the head in profile to the observer were less likely at time point 3 relative to time point 1. However, these were not associated with ADG using this schedule of observations. It was hypothesized that calves that tried to escape the observer may be more flighty in temperament. Studies of beef cattle have shown that flight distance is a good measure of fearfulness and is associated with lower ADG (Müller & von Keyserlingk. 2006). In this study, attempting to escape the observer was not associated with lower ADG. This may indicate that flight is not an appropriate measurement of temperament for dairy calves or the temperament of dairy calves is not associated with differences in ADG of this age group. This may be due to decreased fearfulness of dairy calves due to the routine handling and the direct provision of food to the calves by people.

A lethargy score of 3+ was observed in 58% and 30% of calves with diarrhea and the healthy cohort, respectively. This indicates that during active diarrhea calves require a stronger stimulus to rise and this may be useful for identifying sick animals. A score of 3+ at observation
3 was associated with lower ADG relative to a score of 0. Although this finding was not significant at observation 1 or 2, this may indicate that this behavioral measurement is more reliably associated with reduced ADG in older animals. Only 9% of calves had a lethargy score of 3+ at observation 3 versus 49% at observation 1. As animals mature they spend less time resting and this may result in fewer false positives for this behavioral measure as an indicator of illness. There are some limitations for the direct transfer of this measure to other populations because the measure itself is specific to the design of this stall. However, based on the stall dimensions and the difficulty required to enter the pen, similar approaches may be used in other facilities. Where specific cutpoints of this test are designated in other facilities will be determined by the physical barriers to the calves (i.e., gates and fences) which are often noisy to open and may provide the largest inducement for calves to rise. Flight zones and willingness to approach handlers can vary depending on the amount of positive or negative interactions with the animals (de Passille et al. 1996; Breuer et al. 2003). However, a score of 3+ in this study represented a flight zone of less than 1 meter with multiple vocalizations by the observer. The fact that the proportion of animals that had a score of 3+ decreased from 49% to 9% suggests that the flight zone of neonates is smaller than that of older animals. It has previously been shown that calves at two weeks of age show less exploratory behavior and are less fearful in response to a startle test than 6 week old calves (Lauber et al. 2006). The behavioral responses observed are consistent with the finding that calves required less encouragement to rise at older ages. The lethargy test would be related to the startle response as calves are encouraged by vocalization and movement to rise. At younger ages calves are less fearful of sudden movement and as such are less likely to rise and as such unhealthy calves would not stand out in the lethargy test. This
was supported by the lack of association between a lethargy score of 3+ at observation periods 1 and 2 and ADG.

The proportion of calves willing to approach the observer was 70% in the healthy cohort and 44% in the diarrhea cohort. Calves with diarrhea may be in discomfort from damage to the intestinal tract through destruction and sloughing of enterocytes, villous atrophy, and inflammation of the submucosa, which may cause the greatest reluctance to approach. Alternatively, calves with diarrhea may be anorexic and lethargic which would decrease their motivation to approach an observer if they associated people with the provision of feed. The probability of calves approaching the observer was 1.6 (1.3-2.0) times greater at observation 3 compared to observation 1, \( P < 0.001 \). In addition, 10% of calves never approached the observer and had the lowest ADG. This may indicate that these calves are more timid. This would also suggest that this approach may be useful for identifying calves that are more flighty and perhaps is more valuable for determining flightiness in neonatal dairy calves than the escape behavior as a first response to observers. The calves in this study were fed twice daily by farm staff. The lower ADG of calves that did not approach the observer at observations 2 or 3 may represent calves with a higher fear level which may be associated with decreased immunity, and growth (Carroll & Forsberg, 2007). Alternatively, some of these calves may be less likely to approach an observer due to decreased appetite resulting from illness (Aubert, 1999).

There was a higher than normal incidence of disease in this study population. This was due to several factors but primarily due to a focus on identification and treatment of disease, and the mixing of animals from multiple sites. This study was conducted at a commercial contract heifer raising facility, which focuses on high survival rates and maintaining targeted growth rates.
of heifers to allow them to calve at 24 months of age. For this reason, early detection of disease was of extreme importance to this facility manager and this has resulted in a mortality rate of 1.8% in pre-weaned heifers, which is much lower compared to the average mortality rate in the United States of 7.8% (USDA 2010). For this reason, the treatment criteria used on this farm were very sensitive. For example, on this farm diagnosis of diarrhea was based on a single day of diarrhea. Many farmers may not consider a single day of diarrhea to be clinical disease, and as such this would not be recorded. Additionally, calves at this facility were mixed from multiple sites, which is a known risk factor for disease (Maunsell & Donovan, 2008). As well, there was a large number of susceptible animals of the same age, which would allow pathogens to spread quickly. This high rate of disease in this population should not affect the outcomes of this study because individual calves should alter their behavior in a similar manner to disease regardless of the overall incidence of disease in the population. However, these findings should be restricted to this age group of calves due to the changes in responsiveness to novelty and fearful situations with age, as well as the decreasing thermoregulatory demands of older animals. These factors will alter behavioral responses, such as short lying. Lethargy should be studied further in older animals to determine if there is an age at which fearfulness outweighs the motivation of calves during clinical disease to alter their behavior in response to illness or discomfort. If this does not occur, this would be a valuable indicator for the detection of disease in older animals.

The observation schedule during this study was three short observation times over the course of approximately 8 weeks. This schedule allowed for the observation of a large number of sick animals. However, there were limitations in this study design in that the health status and calves response to their internal and external motivators can change rapidly and this observation
schedule may have resulted in these changes not being captured. Average daily gain is an unbiased measure of poor growth and by extension disease, however some illnesses may either not be associated with decreased ADG or calves may experience compensatory growth prior to their final measurement. In these cases, behavioral changes associated with these illnesses would not be detected in this study. However, given these limitations, the behaviors identified would be associated with the most severe illness which was most likely to cause production loss and impair calf welfare.

In conclusion, short lying appears to be a highly specific indicator of diarrhea since it was more likely to be performed during active diarrhea. However, short lying was uncommon. For this reason, behavioral measures such as lethargy score and willingness to approach the observer may be useful to identify animals with decreased ADG and diarrhea. These behaviors could be integrated into future studies of predictors of disease and discomfort as well as used on farm for training of personnel in disease detection.
2.5 REFERENCES


Hart, B. L. 1988. Biological basis of the behavior of sick animals. Neuroscience and biobehavioral reviews, 12, 123.


### Table 2.1: Ethogram of behaviors recognized and recorded during behavioral scoring

#### Lying Posture

<table>
<thead>
<tr>
<th>Posture</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventral Lying</td>
<td>Calf was lying with legs tucked under body or one front leg extended in front of body and head resting in relaxed and upright position</td>
</tr>
<tr>
<td>Short Lying</td>
<td>Calf was lying with head tucked tight to side of body and limbs tucked under body</td>
</tr>
<tr>
<td>Neck Extended</td>
<td>Calf was lying ventrally with neck and nose extended straight in front of body</td>
</tr>
<tr>
<td>Lateral Lying</td>
<td>Calf was recumbent with all four legs extended to the side</td>
</tr>
<tr>
<td>Not lying</td>
<td>Calf was standing at initiation of observation</td>
</tr>
</tbody>
</table>

#### Standing Posture

<table>
<thead>
<tr>
<th>Posture</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Posture</td>
<td>Calf is standing relaxed</td>
</tr>
<tr>
<td>Neck Below Chest</td>
<td>Calf is standing with head and neck below its chest</td>
</tr>
<tr>
<td>Statue Standing</td>
<td>Calf is standing still either trembling, stretching, a hunched back, a tucked in tail and tucked up abdomen (Molony &amp; Kent. 1997)</td>
</tr>
<tr>
<td>Unable to stand</td>
<td>Calf is unable to stand or maintain a standing posture following prompting by observer</td>
</tr>
</tbody>
</table>

#### Vigilance Test

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Face</td>
<td>Calf orients towards observer</td>
</tr>
<tr>
<td>Profile</td>
<td>Calf stands with head and body parallel to observer</td>
</tr>
<tr>
<td>Avoidance</td>
<td>Calf immediately moves to the back of the pen</td>
</tr>
<tr>
<td>Ignore</td>
<td>Calf does not alter behavior in response to observer</td>
</tr>
</tbody>
</table>

#### Human Approach Test

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approach</td>
<td>Calf approached observer within 30 seconds of standing</td>
</tr>
<tr>
<td>No Approach</td>
<td>Calf did not approach observer within 30 seconds of standing</td>
</tr>
</tbody>
</table>

#### Lethargy Test

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Calf is standing or stands prior to any encouragement</td>
</tr>
<tr>
<td>1</td>
<td>Calf stands in response to single vocal encouragement</td>
</tr>
<tr>
<td>2</td>
<td>Calf stands in response after gate opens and 2nd vocal encouragement</td>
</tr>
<tr>
<td>3</td>
<td>Calf stands in response to observer entering pen with 3rd vocal encouragement</td>
</tr>
<tr>
<td>4</td>
<td>Observer must make contact with calf prior to calf standing</td>
</tr>
<tr>
<td>5</td>
<td>Observer must make repeated contact with calf prior to calf standing</td>
</tr>
</tbody>
</table>
Table 2.2: List of exclusions used to select healthy controls for comparison to calves with active diarrhea

<table>
<thead>
<tr>
<th>Disease Identified by barn staff</th>
<th>Calves treated for disease by barn staff within the following time periods were excluded as healthy controls:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal Calf Diarrhea Complex</td>
<td>Between 10 days prior to behavioral observation and 7 days after behavioral observation</td>
</tr>
<tr>
<td>Bloat</td>
<td></td>
</tr>
<tr>
<td>Navel Ill</td>
<td></td>
</tr>
<tr>
<td>Non-specific fever</td>
<td>Between 10 days prior to behavioral observation and 10 days after behavioral observation</td>
</tr>
<tr>
<td>Bovine respiratory disease complex</td>
<td></td>
</tr>
<tr>
<td>Non-specific lameness</td>
<td>Any treatment for lameness</td>
</tr>
</tbody>
</table>
Table 2.3: Prevalence of behavioral responses among 692 milk-fed individually housed heifer calves at a commercial heifer raising facility at three observation periods (average age 10, 24 and 38 days, respectively) with the odds of the response occurring at period 2 and 3, relative to period 1, controlling for calf as a random effect.

<table>
<thead>
<tr>
<th>Behavioural Measures</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
<th>P-value</th>
<th>Odds Ratio</th>
<th>(Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lying Posture</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventral Lying</td>
<td>53% (370)</td>
<td>40% (277)</td>
<td>28% (195)</td>
<td>&lt; 0.001</td>
<td>0.6 (0.5-0.7)</td>
<td>0.3 (0.3-0.4)</td>
</tr>
<tr>
<td>Short Lying</td>
<td>9% (59)</td>
<td>2% (11)</td>
<td>1% (8)</td>
<td>&lt; 0.001</td>
<td>0.2 (0.1-0.3)</td>
<td>0.1 (0.1-0.3)</td>
</tr>
<tr>
<td>Neck Extended</td>
<td>&lt; 1% (2)</td>
<td>1% (6)</td>
<td>1% (4)</td>
<td>0.40</td>
<td>0.3 (0.1-0.3)</td>
<td>0.2 (0.1-0.3)</td>
</tr>
<tr>
<td>Lateral Lying</td>
<td>1% (5)</td>
<td>&lt; 1% (1)</td>
<td>&lt; 1% (1)</td>
<td>0.16</td>
<td>0.5 (0.2-0.8)</td>
<td>0.3 (0.2-0.8)</td>
</tr>
<tr>
<td>Not lying</td>
<td>37% (256)</td>
<td>57% (397)</td>
<td>70% (485)</td>
<td>&lt; 0.001</td>
<td>2.3 (1.9-2.9)</td>
<td>4.0 (3.2-5.0)</td>
</tr>
<tr>
<td><strong>Standing Posture</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Posture</td>
<td>97% (671)</td>
<td>97% (666)</td>
<td>96% (665)</td>
<td>0.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statue Standing</td>
<td>2% (14)</td>
<td>2% (17)</td>
<td>3% (20)</td>
<td>0.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neck Below Chest</td>
<td>1% (4)</td>
<td>1% (6)</td>
<td>1% (7)</td>
<td>0.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vigilance Test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Face</td>
<td>80% (554)</td>
<td>91% (627)</td>
<td>92% (634)</td>
<td>&lt; 0.001</td>
<td>2.4 (1.8-3.3)</td>
<td>2.7 (1.9-3.7)</td>
</tr>
<tr>
<td>Profile</td>
<td>11% (79)</td>
<td>6% (39)</td>
<td>6% (42)</td>
<td>&lt; 0.001</td>
<td>0.5 (0.3-0.7)</td>
<td>0.5 (0.3-0.7)</td>
</tr>
<tr>
<td>Escape</td>
<td>1% (8)</td>
<td>3% (20)</td>
<td>1% (5)</td>
<td>0.006</td>
<td>0.6 (0.1-1.1)</td>
<td>0.6 (0.1-1.1)</td>
</tr>
<tr>
<td>Ignore</td>
<td>7% (51)</td>
<td>1% (6)</td>
<td>1% (9)</td>
<td>&lt; 0.001</td>
<td>0.1 (0.1-1.1)</td>
<td>0.1 (0.1-1.1)</td>
</tr>
<tr>
<td><strong>Human Approach Test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Approach</td>
<td>52% (359)</td>
<td>63% (436)</td>
<td>63% (439)</td>
<td>&lt; 0.001</td>
<td>1.6 (1.3-2.0)</td>
<td>1.6 (1.3-2.0)</td>
</tr>
<tr>
<td>No Approach</td>
<td>48% (333)</td>
<td>37% (256)</td>
<td>37% (254)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lethargy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>41% (284)</td>
<td>61% (422)</td>
<td>74% (514)</td>
<td>&lt; 0.001</td>
<td>2.3 (1.8-2.8)</td>
<td>4.2 (3.3-5.3)</td>
</tr>
<tr>
<td>1</td>
<td>3% (18)</td>
<td>6% (42)</td>
<td>5% (38)</td>
<td>= 0.01</td>
<td>2.4 (1.4-3.9)</td>
<td>2.2 (1.2-3.9)</td>
</tr>
<tr>
<td>2</td>
<td>8% (53)</td>
<td>16% (113)</td>
<td>11% (80)</td>
<td>&lt; 0.001</td>
<td>2.4 (1.7-3.3)</td>
<td>1.6 (1.1-2.3)</td>
</tr>
<tr>
<td>3+</td>
<td>49% (337)</td>
<td>17% (115)</td>
<td>9% (61)</td>
<td>&lt; 0.001</td>
<td>0.2 (0.2-0.3)</td>
<td>0.1 (0.1-0.1)</td>
</tr>
</tbody>
</table>
Table 2.4: The lsmeans ± S.E. of the average daily gain (ADG) (kg/day) of calves (n = 692) in each Behavioral Measure at the three observation periods, controlling for age at observation, antibiotic treatment and weight at arrival as fixed effects, with source farm and enrolment cohort as random effects. P-values are based on the association of the Behavioral Measure with ADG.

<table>
<thead>
<tr>
<th>Behavioral Measure Response</th>
<th>Period 1 ADG (kg/day)</th>
<th>Period 2 ADG (kg/day)</th>
<th>Period 3 ADG (kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lying Posture</td>
<td>$P = 0.60$</td>
<td>$P = 0.27$</td>
<td>$P = 0.05$</td>
</tr>
<tr>
<td>Ventral Lying</td>
<td>0.62 ± 0.02</td>
<td>0.61 ± 0.02</td>
<td>0.60 ± 0.02</td>
</tr>
<tr>
<td>Short Lying</td>
<td>0.60 ± 0.02</td>
<td>0.55 ± 0.04</td>
<td>0.54 ± 0.05</td>
</tr>
<tr>
<td>Neck Extended</td>
<td>0.54 ± 0.09</td>
<td>0.60 ± 0.05</td>
<td>0.54 ± 0.06</td>
</tr>
<tr>
<td>Lateral Lying</td>
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<td>0.30</td>
<td>0.36</td>
</tr>
<tr>
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<td>0.62 ± 0.02</td>
<td>0.62 ± 0.02</td>
</tr>
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<td>$P = 0.29$</td>
<td>$P = 0.14$</td>
</tr>
<tr>
<td>Normal Posture</td>
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<td>0.62 ± 0.02</td>
<td>0.62 ± 0.02</td>
</tr>
<tr>
<td>Statue Standing</td>
<td>0.50 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0.56 ± 0.03</td>
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<tr>
<td>Neck Below Chest</td>
<td>0.55 ± 0.06&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.61 ± 0.05</td>
<td>0.62 ± 0.05</td>
</tr>
<tr>
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<td>$P = 0.22$</td>
<td>$P = 0.73$</td>
</tr>
<tr>
<td>Face</td>
<td>0.61 ± 0.02</td>
<td>0.61 ± 0.02&lt;sup&gt;a,b&lt;/sup&gt;</td>
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<tr>
<td>Profile</td>
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</tr>
<tr>
<td>Escape</td>
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<td>0.61 ± 0.03&lt;sup&gt;a,b&lt;/sup&gt;</td>
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</tr>
<tr>
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<td>0.52 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>$P = 0.11$</td>
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<td>2</td>
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<td>0.60 ± 0.02</td>
<td>0.59 ± 0.02&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3+</td>
<td>0.61 ± 0.02</td>
<td>0.61 ± 0.02</td>
<td>0.57 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup>: If lowercase superscripts are different within a behavioral measure and column then $P < 0.05$ indicating that ADG is significantly different between the different responses of the behavioural measure.
Table 2.5: Proportional responses of diarrhea and control calves to the observer during vigilance testing

<table>
<thead>
<tr>
<th>Response</th>
<th>Control Calves</th>
<th>Diarrhea</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Face</td>
<td>89% (42/47)</td>
<td>77% (176/229)</td>
<td>0.06*</td>
</tr>
<tr>
<td>Profile</td>
<td>2% (1/47)</td>
<td>11% (26/229)</td>
<td>0.06*</td>
</tr>
<tr>
<td>Escape</td>
<td>4% (2/47)</td>
<td>&lt; 1% (1/229)</td>
<td>0.08Ψ</td>
</tr>
<tr>
<td>Ignore</td>
<td>4% (2/47)</td>
<td>11% (26/229)</td>
<td>0.14Ψ</td>
</tr>
</tbody>
</table>

* P-value is based on statistical results from chi-square test

Ψ P-value is based on statistical results from Fisher’s exact test
Figure 2.1: Timeline describing trial milestones and behavioral observations, with average ages (mean ± s.d.) at observation and measurements
Figure 2.2: Method for behavioral scoring of the calves, conducted every other week
**Figure 2.3:** Proportion of calves in each lethargy response category for the active diarrhea (n = 229) and healthy controls (n = 47)
CHAPTER 3: ASSOCIATIONS AMONG ACTIVITY PATTERNS AND WEIGHT GAIN IN NEWLY WEANED DAIRY CALVES

3.1 INTRODUCTION

At the time of weaning dairy calves are usually subjected to a wide variety of stressors. These stressors include not only nutritional changes, through the removal of milk from the diet, but also transportation and changes in both the physical and social environment. These may include moving from individual to group housing with major changes in social interactions as well as the introduction of novel feeding and drinking apparatuses. The first few days following weaning are characterized by changes in activity patterns including a high rate of vocalizations (Jasper et al., 2008), increased walking (Haley et al., 2005) and more time spent standing (Budzynska and Weary, 2008), all of which are thought to be indicators of distress during the post-weaning transition period (Weary et al., 2008).

Different approaches have been used to reduce the stress associated with this transition period. To address the nutritional stress of weaning it is recommended that calves consume at least 0.67 kg of calf starter per day prior to weaning (Davis and Drackley, 1998). However, even when calf starter consumption is above this level, calves still showed increases in vocalizations and in the amount of time standing following weaning (Budzynska and Weary, 2008). Another approach that can be used to reduce the nutritional stress associated with weaning is to provide extended access to the feeding apparatus (pail or bucket) used during the nursery period (Budzynska and Weary, 2008; Jasper et al., 2008). Following abrupt weaning both control animals and animals provided access to a milk feeding apparatus responded with increased vocalizations, and increased time standing. However, access to a milk feeding apparatus resulted
in a lower rate of vocalizations and tended to decrease the number of standing bouts in experimental calves compared to controls, suggesting that this approach reduced the stress response of calves to weaning.

Dairy calves are commonly housed individually prior to weaning and housed in groups after weaning (USDA, 2010). They are often transported from a nursery to another facility, which may be located at the same site or many kilometers away. Transportation of calves has been shown to be stressful and is associated with increased risk of morbidity and mortality (Swanson & Morrow-Tesch, 2001). Following transport, calves are also often exposed to their first social interactions with other calves. First mixing and movement of pair-housed milk-fed calves has been shown to be associated with a decrease in lying, an increase in time spent standing, walking, social aggression, and environmental exploration (Veissier et al., 2001). For newly weaned calves, the new environment may also have different feeding and drinking apparatuses than the calves were previously exposed to. This novelty could also limit their ability to access feed if calves must learn to access the new apparatuses.

Although it is generally accepted that the period of weaning and movement to group housing is a critical time in the management of dairy calves, to date, little research has been conducted to determine the individual variation in the behavioural response to weaning and how this variation is associated with weight gain. The objective of this study was to investigate the association between the individual variation in post-weaning behavioral changes and post-weaning weight gain. To address this objective the following hypotheses were tested: 1) calves that have the greatest change in activity post-weaning will have the lowest weight gain; 2) calves
that are slower to locate and consume feed will have lower weight gains; 3) calves that rest further from their conspecifics will have lower body weight gain.

3.2 MATERIALS AND METHODS

This study used data collected during an unpublished experimental trial conducted between October 2006 and September 2007 in which behavioral and weight gain data were collected for 74 Holstein calves from two research herds. A wide variation in weight gain was observed in this group of calves and is the basis of the current study. Calves were 56 ± 7.4 (mean ± standard deviation) days of age at the time of enrolment.

Enrolment (Day 0) occurred on the day that calves received their last milk meal and were moved from individual to group housing. Enrolment and movement to group housing occurred between 09:00 and 11:00 on Day 0. Calves were transported to group housing at the study site where they were weighed with an electronic scale (Chute Scale, Avery Weigh-Tronix, Fairmont, Minnesota, USA), and height was recorded using a measuring stick. At this time, individual calves were randomly assigned to receive one injection of either the antibiotic tulathromycin (2.5 mg/kg subcutaneous; Draxxin®, Pfizer Animal Health, Montreal, Canada) or a placebo as part of an experimental trial.

Calves were housed at one of two research facilities prior to enrolment in this trial. Calves at Nursery 1 (n = 50) were housed in individual pens in a warm barn, maintained between 7 and 33°C, located at the same site as the trial facility, and were transported less than 0.75 km. Nursery 2 was located 7 km from the study facility. Calves at Nursery 2 (n = 24) were housed outside in individual straw-bedded polyurethane hutches. Both facilities are associated with the University of Guelph and treatment protocols and veterinary care were consistent between the
sites. During the summer months both farms fed calves 2L of whole milk twice per day and provided *ad libitum* water and calf starter (Flordale Standard 20% calf starter with Bovatec; Flordale, Ontario). In the winter months, calves at Nursery 2 were fed 2L of whole milk three times per day but were not provided any water while calves at Nursery 1 were fed as during the summer.

Calf starter intake was measured daily at both facilities for two days prior to weaning to generate an average pre-weaning calf starter intake. Average calf starter intake was based on a single day of calf starter intake for four calves on the trial due to data collection error. Calf starter intake was dichotomized at 0.91 kg/d in the two days prior to weaning based on the nutrient requirements for maintenance and a modest weight gain for a 59 kg calf (Davis and Drackley, 1998).

Following weaning, calves were housed in groups of 4 to 6 calves in a naturally ventilated barn located at the same site as Nursery One. Group size was determined by calving patterns on the farms and the availability of animals to form groups comprised of calves that were within 3 weeks of age of each other with an average variation in age within the group of 12 days. Each pen was 3.6 meters by 7.0m with a shaving bedded pack (3.6m by 4.6m) located at the back of the pen with the remaining pen consisting of a cement section which was scraped daily. The feed bunk was located along the front of the pen and measured 3.6 m long with 9 stanchions. Ambient temperature in the pen was based on a single measurement taken on Day 4 using a handheld digital temperature gage.

Barn staff monitored calves daily. In the event of any health concerns, the herd veterinarian was consulted. Calves were re-weighed on Day 7. Change in calf body weight
ranged from a loss of 4.5 kg to a gain of 11.5 kg with a mean gain of 3.9 ± 3.4 kg (mean ± standard deviation). Three categories of weight gain in the first week after weaning were determined by the first and third quartile of weight gain for all 74 calves. This resulted in a low weight gain category (LOW) with weight change between -4.5 and 2 kg, a moderate weight gain group (MOD) between 2.5 and 6.0 kg and a high weight gain group (HIGH) between 6.5 and 11.5 kg of weight gain.

3.2.1 Behavior Measurements

An IceTag (IceRobotics, UK) was attached to the rear metacarpal of all calves on the study on Day-3 and removed on Day 14. These devices recorded steps, daily lying bouts, daily lying bout duration, and daily lying duration for each day of the trial. Data logger malfunctions occurred for 4 calves and these animals were removed from all analyses of steps, daily lying bouts, daily bout length and daily lying duration.

A subset of calves was recorded using 24-hr time lapse video (Panasonic WV-CP244 Color CCTV Cameras, Heads WV-LZA61/2) between Day 0 and Day 7. One camera was attached above the bunk to record bunk behavior, and 2 cameras were attached to the walls of the pens with overlapping views of the front and the back of the pen. Calves were identified using photographs of unique markings on calves. At the time of enrolment, if calves were determined to be difficult to identify (i.e. all black calves) coloured Vet Wrap (3M, St. Paul, Mn) was wrapped around the girth of the calf. The video tape was analyzed using 5 minute instantaneous scan samples between 12:00-19:00 on Day 0, and 07:00-19:00 on Days 1 and 2. Times were selected based on the completion of all animal handling in the barn on the day of weaning and the hours in which light was sufficient to view calves. Observers were blind to both antibiotic
treatment and weight gain category. Bunk behaviors were observed for the last 65 calves on the trial, and resting behaviors were observed for the last 44 calves on the trial (Table 3.1). Smaller sample sizes were based on the availability of video tapes with earlier groups not recorded. **Bunk engaged** was used to determine if calves stood at the feed bunk but did not consume feed. **Bunk directed** behavior was used as an indicator of reluctance to place the head through the stanchion, which is potential inhibitor of feed intake. **Close** and **Far Lying** were used as measurements of affiliation. One group of 5 calves was excluded from the study of bunk behaviors due to the failure of video recording equipment.

**3.2.2 Experimental Study Design**

The objective of the underlying trial was to identify behavioral and physiological changes associated with bovine respiratory disease complex (BRD) or administration of a long-acting antibiotic. No calves were diagnosed with BRD in the first week post-weaning. On days 0, 4, 7, 11 and 14 of the present study calves were haltered and blood samples were collected via jugular venipuncture for measures associated with the antibiotic trial.

**3.2.3. Statistical Analysis**

All statistical analyses were conducted using Statistical Analysis Software (SAS version 9.1; SAS Institute Inc, Cary, NC, USA).

Means and standard errors for each weight gain category were generated for normally distributed continuous variables (Proc Means). If the variables were not normally distributed upon visual assessment, medians were presented with a range for the 1st and third quartiles (Proc Means).
From the post-hoc visual assessment of the daily activity patterns of calves, the number of steps per day (Figure 3.1), number of daily lying bouts (Figure 3.2) and daily lying duration (Figure 3.3) were divided into 3 time periods: pre-weaning (days -2 and -1), transition (days 0, 1 and 2), and plateau (Days 3-11). Average activity for each of these periods was calculated, resulting in 3 variables per behaviour. All behaviors (number of steps per day, daily lying duration and daily lying bouts) were assessed separately for their association with weight gain, using Day 7 weight with Day 0 weight as a covariate, using generalized linear mixed models (Proc Mixed) with a random effect for group nested within group size. The average activity for each of the 3 time periods were included as fixed effects in the analysis for each of the following activities: number of steps per day, daily lying duration and number of daily lying bouts.

**Eating, bunk engaged, and bunk directed behaviour** between Day 0 and Day 2 were calculated as a percentage of total time at the bunk. The association between these behaviors and Day 7 weight were analyzed in separate models using generalized linear mixed models with Day 0 weight and percentage of total time spent performing all bunk behaviours as covariates, with a random effect of group nested within group size.

The average percentages of time that calves performed **Close** and **Far** Lying between Day 0 and Day 2 were calculated as a percentage of total lying time. The association between these behaviors and Day 7 weight were analyzed in separate models using generalized linear mixed models with Day 0 weight and percentage of total time spent lying as covariates, with a random effect of group nested within group size.

All generalized linear mixed models were built using backwards step-wise regression. All models controlled for fixed effects of Day 0 body weight, nursery location, antibiotic treatment,
pre-weaning calf starter intake, and ambient temperature with a random effect of group nested within group size. Interactions between nursery location and behavioral measures were tested for all behavior models.

For all models statistical significance was defined as a $P$-value $\leq 0.05$. The co-efficients for each variable in the models and their associated p-values were monitored for confounding. Normality of residuals was assessed using boxplots and normal probability plots. No transformations were required. All graphs that presented results of models that included continuous variables were restricted to the interquartile range of the continuous variable.

3.3 RESULTS

The average calf starter intake prior to weaning was $1.21 \pm 0.62$ (mean ± standard deviation) kg, $1.33 \pm 0.79$ and $0.92 \pm 0.74$kg for the High, Average and Low weight gain groups, respectively. Calves from Nursery 1 consumed $0.48 \pm 0.1$ kg more calf starter than calves from Nursery 2 ($P < 0.01$). Consumption of more than $0.91$ kg of calf starter in the pre-wean period was associated with a $1.7 \pm 0.7$ kg increase in Day 7 body weight, controlling for Day 0 weight as a fixed effect and group nested within group size as a random effect ($P=0.03$). Calves that received antibiotic treatment weighed $1.1 \pm 0.6$ kg more than calves that did not receive antibiotic treatment ($P=0.06$).

A total of 23 (31%) calves were treated for clinical disease in the 7 to 30 days following weaning. Of those treated, 91% (21/23) were treated for coccidiosis, and 9% (2/74) were treated for BRD. Day 7 weight was not associated with the probability of being treated in the 30 days following weaning, ($P=0.90$).
### 3.3.1 Activity level in response to weaning and relocation

The average number of steps per day in the pre-weaning, transition and plateau periods were 1,366 ± 43, 3,460 ± 138, and 1,585 ± 43 steps per day, respectively. The average number of steps per day during the transition and plateau periods were associated with Day 7 weight, controlling for Day 0 weight, nursery location and antibiotic treatment as fixed effects and group nested within group size as a random effect (*P* < 0.001 and *P* < 0.001; Table 3.2).

The average lying duration per day during the pre-weaning, transition and plateau periods were 17.4 ± 1.1 (mean ± s.d.), 13.3 ±1.5, and 15.0 ± 1.2 hours, respectively. An interaction between nursery location and daily lying duration during the transition period was significantly associated with Day 7 weight, controlling for daily lying duration during the plateau periods and Day 0 weight as fixed effects and group nested within group size as a fixed effect, (*P*=0.01; Figure 3.4). This result indicates that for Nursery 1 a longer lying duration tended to be negatively associated with body weight compared to Nursery 2 calves which had a positive association between body weight and lying duration. During the plateau period, increased lying duration was associated with increased Day 7 weight (Table 3.2; Figure 3.4).

The average number of lying bouts during the pre-weaning, transition and plateau periods were 19 ± 0.4, 21 ± 5.6, and 13 ± 0.5, respectively. An increase in the number of lying bouts during the plateau period was associated with increased Day 7 weight (*P*=0.03). The number of lying bouts in the transition period was not associated with final weight (*P*=0.15).

### 3.3.2 Bunk behavior

Bunk behavior was examined for 65 calves with 28% (n=18/65), 46% (30/65) and 26% (17/65) of observed calves in the **LOW**, **MOD** and **HIGH** groups, respectively.
Calves spent a total of 13 ± 6 (mean ± standard deviation) percent of their time interacting with the bunk (eating, bunk directed and bunk engaged) during the first three days post-weaning. There was no difference in percentage of time interacting with the bunk by nursery location ($P=0.12$). Calves spent 49 ± 11 and 20 ±11 percent of their time at the bunk eating and bunk engaged, respectively.

An interaction between total time at the bunk and nursery location was significantly associated with Day 7 body weight, ($P=0.03$). More time at the bunk was associated with a lower weight gain in Day 7 in calves from Nursery 1 and a greater Day 7 weight in calves from Nursery 2, controlling for Day 0 weight, antibiotic treatment and pre-weaning calf starter intake as fixed effects and group nested within group size as random effects (Figure 3.5).

There was a non-significant tendency for a 1% increase in the time spent eating to be associated with a 0.07 ± 0.04 kg increase in Day 7 weight ($P=0.07$), controlling for Day 0 weight, antimicrobial treatment, calf starter intake, nursery location and total time at the bunk as fixed effects, and group nested within group size as random effects.

A 1% increase in time in bunk engaged behaviour was associated with a 0.08 ± 0.03 kg decrease in Day 7 body weight ($P = 0.02$), controlling for nursery location, total time at the feed bunk, antibiotic treatment, and calf starter intake as fixed effects and group nested within group size as random effects. Bunk directed behavior was not associated with Day 7 weight ($P = 0.53$).
3.3.3 Resting Behavior

In the subset measured, 30% (13/44), 48% (21/44) and 23% (10/44) of calves were in the LOW, MOD, and HIGH week 1 weight change categories, respectively. The percentage of total lying time as measured by video observation was highly correlated with lying duration as measured by the data loggers during the transition period, \((P < 0.001, R = 0.87)\) which indicated a high degree of agreement between the two measurements.

During the first 3 days after weaning, calves spent 49 ± 10 % (mean ± s.d.) of their time lying down. Calves from Nursery 1 and 2 were observed Close lying 37 ±10% and 25 ± 10 % of their total resting time, respectively \((P = 0.003)\). Close lying was not associated with Day 7 weight, controlling for Day 0 weight and total lying time as fixed covariates and group nested within group size as random effects \((P = 0.38)\). Calves from Nursery 1 and Nursery 2 spent 0.6 (q1-q3: 0-0.9) and 5.3 (q1-q3: 0.8-4.1) percent of total lying time Far lying, respectively \((P = 0.005)\). A 1% increase in Far lying was associated with 1.4 ± 0.6 kg decrease in Day 7 weight, controlling for Day 0 weight and total lying time as fixed effects and group nested within group size as a random effect \((P = 0.02)\). When Nursery Location was included in the above model as a fixed effect, Far lying was not associated with Day 7 weight \((P=0.17)\).

3.4 DISCUSSION

Certain changes in activity are considered to be indicative of distress in newly weaned calves. In the current study, a higher level of activity during the first three days following weaning and movement to a novel environment was significantly associated with decreased short term weight gain. This increased activity in the transition period was also found during two-staged weaning of beef calves. During the first stage of the two-staged weaning process, when
calves were still allowed access to the dam but were restricted from sucking, beef calves walked more than control animals that could still suckle their dams (Haley et al., 2005). In the second stage of the two-staged weaning process, when all calves were removed from the dam, two-staged weaned calves walked less than control animals. While comparison between the results of the two-stage weaning study and the current study must be tempered by different social stressors (isolation versus mixing stress) it does suggest a common response to a weaning and social stress.

In contrast to the transition period, calves that had higher activity between 4 and 11 days post-weaning gained more weight. This may further explain the results of the study of Chua et al (2002), in which pair housed milk fed calves were more active than individually housed calves during the 8 week study period, which included weaning. However, a positive association between growth and activity following an initial adaptation to weaning has not been documented prior to the current study.

A greater lying duration was positively associated with higher body weight gain during the first week post-weaning for calves from Nursery 2 during the transition period and for all calves during the plateau period. In previous studies, time spent standing was not different for calves that were provided warm water post-weaning to alleviate weaning distress in comparison with calves that were abruptly weaned despite other behavioral indicators of decreased weaning distress (Budzynska and Weary, 2008). However, time spent resting was higher after weaning for beef and dairy calves with the two-stage weaning (Haley et al., 2005; Haley, 2006). In addition, the lying time of calves decreases following mixing with unfamiliar animals (Raussi et al., 2005). This indicates that in general, calves respond to these stressors by decreasing resting time.
Although the number of lying bouts decreased in the transition period for all calves, the absolute number of lying bouts was not associated with rate of weight gain. Combined with the results of total lying duration, this finding indicates that the total time resting may be more important than uninterrupted resting time. This is in contrast to the report by Budzynska and Weary (2008), who found that the number of lying bouts decreased in response to changes in weaning management with no change in lying time. However, in that study, calves were individually housed whereas in the present study calves were group housed. It is likely that the group housing influenced their behavior, with the activities of conspecifics disrupting or altering their resting behavior. For this reason, it is important to take into context the environment the calves are housed in when interpreting the relationship between behavior and growth since environment likely alters the response of calves to stimuli.

There was an interaction between total lying duration and nursery location during the transition period. Calves from Nursery 1 that gained less weight also spent more total time lying during the transition period. However, the difference between calves that rested 12 compared to 14 hours was negligible indicating that this behavior was not a strong indicator of poor growth in this group of calves. Greater weight gain was associated with more lying time for calves from Nursery 2. However, Nursery 2 calves had lower rates of gain than calves from Nursery 1. This difference in the association between lying duration and weight gain between nursery locations indicate that facility and management factors should be further investigated. Potential reasons for this difference are the lower calf starter intake of calves pre-weaning, the limited socialization of calves from Nursery 2 pre-weaning and the increased transportation distance of calves from Nursery 2.
It was hypothesized that the more time calves spent at the bunk during the transition period, the more weight they would gain in the week after weaning. This hypothesis was supported by a positive association between increased time at the bunk and post-weaning weight gain for calves from Nursery 2. However, calves from Nursery 1 had lower post-weaning weight gain with more time at the bunk. When the different interactions with the bunk were examined, it was found that, as hypothesized, an increase in time spent eating was positively associated with post-weaning weight gain and the proportion of time spent bunk engaged was negatively associated with post-weaning weight gain. This may indicate that calves are spending time at the bunk due to social facilitation but are not consuming feed. This failure to consume feed may be inappetance due to sub-clinical illness, stress, or a combination of both.

The lack of association between bunk directed behavior and post-weaning weight gain may be due to conflicting motivations for this behavior. Two potential reasons for bunk directed behavior include environmental exploratory behaviors, or unwillingness to place the head through a novel feeding apparatus. These two motivations would be indicators of opposite welfare issues in that environmental exploration is a positive welfare indicator while fear from a novel feeding apparatus hindering feed intake would indicate a negative welfare state. However, using this study design there was no way to identify which motivation was responsible for the bunk directed behavior.

Close lying was not associated with Day 7 weight. However, Far lying was negatively associated with Day 7 weight, when Nursery Location was not included in the model. This negative association is consistent with the original hypothesis that calves which interact less with their conspecifics will have lower weight gain. However, Nursery Location was highly correlated
with Far lying and as such could not be included in the model. These results were interesting in that Close lying was most prevalent in calves from Nursery 1 while Far lying was most prevalent in calves from Nursery 2. Although causation cannot be determined due to the small number of nursery locations used it did suggest an effect of rearing conditions on social interactions. Calves from Nursery 2 were raised in an environment where they had limited visual contact and no physical contact with other calves. At Nursery 1 they had visual contact with a number of calves and physical contact, limited by bars between pens, to other calves. This may have allowed calves from Nursery 1 to become more familiar with each other and have more experience interaction with conspecifics through visual or olfactory cues. This familiarity was less likely to be gained by calves from Nursery 2 given their increased isolation. This difference in prior social experience may explain the difference in the performance of Far lying. Socially isolated cattle appeared to interact less with group reared animals (Broom and Leaver, 1978).

The duration of partial social isolation in the current study was less than 3 months and of lower severity compared to the 8 months of total social isolation in the study by Broom and Leaver (1978). However, it appears that even this short period of isolation might alter the social behavior of dairy calves. The study by Broom and Leaver (1978) found no effect of isolated rearing on weight gain. However, the lower weight gains observed in calves from Nursery 2 and the higher rate of Far lying does suggest that pre-weaning social environment may have an effect on post-weaning behavior and weight gain.

The calves in this study had various levels of calf starter intake with higher pre-weaning calf starter intakes associated with greater post-weaning gain. The consumption of at least 0.91 kg of grain prior to weaning has been recommended (Davis and Drackley, 1998). The failure of
calves to consume sufficient calf starter intake leaves calves vulnerable to hunger (De Passillé et al., 2010). Rumen development takes several weeks, so if calves are not prepared for weaning, there is the potential for calves to experience some degree of hunger for an extended period of time. This would result in calves having poor growth, and suffering from hunger until their rumen develops sufficiently to allow the calf to absorb nutrients.

In conclusion, there are associations between changes in calf behavior and variability in post-weaning weight gain. Far lying was associated with decreased weight gain post-weaning. However, this behavior only occurred in calves from one of the nurseries. The motivation for this behaviour may be a result of pre-weaning housing environment but further research to look into the effect of housing on behaviour and growth is required. Calves with the greatest alterations in behavior in the three days following movement were the most likely to have lower weight gains. This includes steps per day and a rearing environment dependent alteration in lying time. After an initial adjustment period, calves with poor post-weaning welfare took fewer steps, and spent less time lying. In conclusion, the activities of calves in the weeks post-weaning is associated with weight gain during this time period.
3.5 REFERENCES


Table 3.1: Descriptive list of observed behaviours

<table>
<thead>
<tr>
<th>Bunk Behavior (n = 65)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bunk directed</td>
<td>Touching the stanchions of the feed bunk</td>
</tr>
<tr>
<td>Bunk engaged</td>
<td>Head through the bars of the stanchion beyond poll but not lowered</td>
</tr>
<tr>
<td>Eating</td>
<td>Head through stanchions and lowered</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Resting Behavior (n = 44)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Close Lying</td>
<td>Lying while touching another calf</td>
</tr>
<tr>
<td>Far Lying</td>
<td>Lying more than 6 calf lengths from another calf</td>
</tr>
</tbody>
</table>
Table 3.2: Associations between activity and Day 7 weight of calves in the week post-weaning. All models included Day 0 weight and nursery Locations in the model as a fixed effect and group nested within group size as a random effect. All behaviours (steps, lying duration, daily lying bouts) were assessed independently of each other.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Pre-Weaning Period</th>
<th>Transition Period</th>
<th>Plateau Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Steps</td>
<td>$P = 0.68$</td>
<td>$0.11 \pm 0.03 \text{ kg lower /100 step increase (}P &lt; 0.001\text{)}$</td>
<td>$0.62 \pm 0.11 \text{kg higher/ 100 step increase (}P &lt; 0.001\text{)}$</td>
</tr>
<tr>
<td>Total Lying Duration</td>
<td>$P = 0.39$</td>
<td>Interaction with Nursery Location, ($P = 0.01; \text{ Figure 3.4}$)</td>
<td>$0.81 \pm 0.35 \text{ kg higher/1 hour increase (}P = 0.03\text{)}$</td>
</tr>
<tr>
<td>Daily Lying Bouts</td>
<td>$P = 0.21$</td>
<td>$P = 0.15$</td>
<td>$0.30 \pm 0.13 \text{ kg higher/ bout (}P = 0.03\text{)}$</td>
</tr>
</tbody>
</table>
Figure 3.1: The average number of steps per day (mean ± standard deviation) by weight gain category in the first week post-weaning for calves (n = 70) following weaning and relocation to a novel environment.
△ Indicates days calves were weighed

**Figure 3.2**: The total lying duration per day (hours) (mean ± standard deviation) for calves (n = 70) following weaning and relocation to a novel environment, and total lying duration per day by weight gain category in the first week post-enrolment
Figure 3.3: The average number of lying bouts (mean ± standard deviation) per day for calves (n = 70) following weaning and relocation to a novel environment, and number of lying bouts per day by weight gain category in the first week post-enrolment.
Figure 3.4: LSMEANS (±S.E.) of post-weaning weight gain for total lying duration by nursery location interaction ($P = 0.01$)
Figure 3.5: LSMEANS (±S.E.) of post-weaning weight gain for time at the bunk (% of total observations) by nursery location interaction ($P = 0.03$), controlling for Day 0 weight, antibiotic treatment, and pre-weaning calf starter intake, with random effects for group nested within group size.
CHAPTER 4: EVALUATION OF A GRADUATED WEANING PRACTICE ON BEHAVIOR AND PERFORMANCE OF DAIRY CALVES

4.1 INTRODUCTION

Weaning and relocation to group housing is considered to be a time of great stress for dairy calves. The majority of milk-fed calves in the United States are individually housed, and stress associated with weaning and moving to group housing at weaning can result in increased susceptibility of calves to bovine respiratory disease complex (BRD) (McGuirk, 2008). This increased risk of BRD has been attributed to the many changes occurring at this time including nutritional, environmental, and social stress, as well as increased exposure to pathogens (McGuirk, 2008). It has been suggested that separating challenges including vaccination, dehorning, nutritional changes, as well as moving to new social and physical environments could reduce susceptibility to clinical disease (Quigley, 1996). One recommendation is to pre-condition calves by mixing them into small groups after they have been weaned, but before they are moved into group housing in a novel environment. Comparisons of the responses of pair-housed calves with those of individually housed calves indicate that pair-housed calves had lower heart rates during transportation (Lensink et al., 2001) and during the week of weaning did not experience a growth check in contrast to individually housed calves (Chua et al., 2002). However, in these studies, calves were paired for longer than 6 weeks. For managers that house calves individually during the pre-weaning period, preconditioning may require additional space in the nursery to house calves for longer periods of time. Therefore, for economic and practical reasons it is important to determine if housing calves in groups for shorter periods of time (e.g. one week) prior to movement to a new facility will be beneficial for health or growth.
The concept of separating stressors is based on research investigating the effect of multiple concurrent stressors, which has been examined in swine and poultry. Concurrent stressors were found to produce additive effects in pigs on growth rate (Hyun et al., 1998), feed efficiency (Hyun et al., 1998), metabolic stress response (Ritter et al., 2009), and activity (Hyun et al., 1998). In poultry, concurrent stressors were also found to be additive in association with growth and feed conversion efficiency (McFarlane et al., 1989). These researchers investigated a variety of stressors including thermal, social, disease, and confinement stressors. However, this concept has not been examined in dairy calves using dairy specific stressors or management systems.

In a previous study (Chapter 3) higher weight gain in newly weaned and mixed calves was associated with lower activity, measured by steps per day, and more lying during the first 3 days following weaning, and higher activity and more time spent lying from days 4 to 11. This suggests that activity patterns may be used as an indicator of adaptation to weaning, movement to a novel environment, and social mixing. Based on these findings, if mixing calves prior to movement to the environment results in improved adaptation to the environment it was expected that these calves would take more steps and have a greater lying time compared to calves with no previous social experience. The objective of this experiment was to determine the effect of separating post-weaning social mixing and movement into a novel environment on activity patterns and feed intake. The hypothesis was that calves that are preconditioned to a social environment by mixing into a group prior to movement to a novel environment will adapt more easily to the novel environment in comparison to calves that are mixed and moved at the same time.
4.2 MATERIALS AND METHODS

This study was conducted at the Iowa State University dairy research farm in Ames, Iowa. A total of 64 calves were enrolled: 53 Holsteins, 7 Jerseys, 3 Brown Swiss and 1 Milking Shorthorn. All calves were born and raised at the facility under the same management conditions.

4.2.1 Pre-enrolment management

Prior to enrolment, the calves were fed 2L of pasteurized whole milk twice daily by nursery bottle. *Ad libitum* calf starter (custom calf starter: 23% protein and 4% fat: Heartland Co-op, West Des Moines, Iowa) and water were available to all calves. They were housed in a naturally ventilated nursery barn in individual pens from 1 day of age. Weaning was initiated at 4 weeks of age by reducing the number of daily feedings from 2 to 1. Weaning was complete after 1 week, or calves consumed ≥ 0.5 kg of calf starter per day. All calves were monitored daily for clinical disease and any disease events were recorded. All calves were treated in accordance with the standard operating procedures of the herd, and were examined by a veterinarian prior to treatment for clinical disease.

Bovine respiratory disease (BRD) was scored by researchers daily using a standardized scoring system (appendix 4.1; McGuirk, 2005) from 1 to 15, with a score of 0 indicating no signs of BRD. A score ≥ 5 was defined as clinical BRD and triggered treatment by veterinarian. Scores between 1 and 4 were defined as subclinical BRD and calves were monitored but not treated.

4.2.2. Housing

Experimental pens (1.2 x 1.8 meters) were located within the Nursery barn calves were reared in from birth. All pens had 3 solid sides with spindle gates at the front of the pen. All pens
were bedded with straw. While housed individually, calves had no physical access to other calves, but had visual access to calves in pens directly across the alley.

The naturally ventilated barn used as the Novel Environment was located adjacent to the Nursery barn and contained two experimental pens (3.7 m X 6 m), which were bedded with straw. Treatment groups were housed separately and had visual, but no physical access to the other treatment group. No other animals were housed in this barn.

4.2.3. Study Treatment

Calves were randomly assigned to one of two treatments, either traditional management in which they were not mixed prior to movement to a novel environment (Traditional) or mixed prior to movement to a novel environment (Pre-mixed). Eight calves were enrolled at a time with 4 calves in each treatment group. Enrolment occurred when 8 calves were completely weaned from milk, at which time all calves were moved to experimental pens (Day -4) within the nursery barn, which were identical in design to calves’ pre-enrolment housing. Calves were randomly assigned to treatment and experimental pen using a random number generator. Treatment groups were balanced for age and breed (Holstein or non-Holstein).

From day-4 to day 0, all calves were individually housed in experimental pens, within the nursery barn. On day 0, the partitions between 4 experimental pens were removed creating a single pen (4.8 m x 1.8 m) which housed the Pre-mixed treatment group from Day 0 to Day 7. Traditional calves were housed in individual experimental pens until day 7. On day 7, both treatment groups were moved 40 meters to group pens within a naturally ventilated, novel barn. All calves within a treatment group were housed in the same pen. Treatment groups were randomly assigned to 1 of 2 pens for each replicate. Each pen measured 14 meters by 22 meters.
with straw bedding. Calves were housed in the novel barn from day 7 to replicate completion on day 13.

All calves were fed *ad libitum* calf starter (custom calf starter: 23% protein and 4% fat: Heartland Co-op, West Des Moines, Iowa), water and hay during the study period.

**4.2.4. Blood Sampling**

Whole blood samples (3mL) were collected from all calves on the replicate on days 0 (before and after social mixing), 1, 7 (before and after movement to a novel environment) and 8 via jugular venopuncture. The order of blood collection was randomized at the start of each replicate, balanced for treatment and kept consistent throughout the trial. Samples were collected on Days 0, 1, 7 and 8 at 2 pm. After serum-samples were collected on Day 0, pen dividers between the Pre-mixed calves were removed and a second sample was collected from all calves 30 minutes later.

After serum samples were collected on Day 7, both treatment groups were moved to the new barn and second sample was collected 30 minutes after the last calf entered the new barn. Blood samples were centrifuged for 20 minutes. Serum was divided between 2 serum tubes and stored in a -20°C freezer. Duplicate serum samples were analyzed for cortisol using RIA kits (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA.). The co-efficient of variation between the samples was 16.7%, which was determined to be outside the acceptable range as such these results will not be presented.
4.2.5 Data Collection

Calf starter intake was measured daily using an electronic scale. On day -4 calves were offered 3 kg of calf starter per calf. After this initial feeding, fresh calf-starter was offered daily, and increased by 15% based on their previous day’s consumption. The only exception to this was the day of movement to the novel environment in which calves were offered 20% more than their intake in the previous 24 hours to account for potential increases in appetite associated with the move.

Calves were weighed individually on an electronic scale on days -1, 6 and 13. Weight was recorded to the nearest pound. Data were converted to kilograms using SAS 9.1.

On Day -4, IceTags® (IceRobotics, UK) were attached to the rear metacarpal of the last 56 calves on this trial and were removed on day +13. IceTags recorded the number of steps taken, the number of lying bouts and total duration of time spent lying (minutes) on each day. Activity data (steps, lying duration and lying bouts) were collapsed into three time periods: baseline period, pre-mixing period, and novel period. The baseline period was based on the activity of calves on day -2. Day-2 was selected as the baseline period to allow calves 2 days to adjust to the IceTags on their leg. In addition, no study procedures required interaction with calves on this day in comparison to day 1 in which calves were weighed. The Pre-mixing period and Novel Environment Period were based on activity on days 0-5 and 7-11, respectively. Activity on days -1 and 6 were excluded because of the disruptive effect of weighing calves.

4.2.6 Statistical Analysis

All statistical analyses were conducted using Statistical Analysis Software (SAS version 9.1; SAS Institute Inc, Cary, NC, USA). Statistical significance was based on Type III tests for
fixed effects with an associated \( P \)-value of less than 0.10. This cut off was selected since this was a pilot study. All models were built using backwards stepwise elimination. Normality and the distribution of the residuals were visually assessed for all linear models. The effect of BRD at any time during the trial, categorized as no BRD, subclinical BRD, or clinical BRD, was tested in all models.

Due to expected breed differences in weight gain, the analysis of weight gain was restricted to purebred Holsteins. The association between study treatment and weight gain during the Pre-mixing and Novel Environment period was analyzed using linear mixed models (Proc Mixed) with initial body weight as a fixed effect and replicate as a random effect.

Average calf starter intake was determined at the group level (\( n = 16 \)) for three time periods; Baseline (Day -4 to Day -1), Pre-mixing (Day 0 to Day 6), and Novel Environment period (Day 7 to Day 13). The association between study treatment and average calf starter intake for the three time periods was assessed using a linear mixed model with a random effect for replicate. The number of calves identified with a BRD score \( \geq 1 \) within each pen of calves was divided into three categories; no calves with BRD, 1 calf with BRD, and more than 2 calves within a group with BRD.

Associations between treatment and number of steps taken, lying duration and number of lying bouts during the different time periods were analyzed using linear mixed models with repeated measures for individual calf nested within replicate. Normality was assessed using visual assessment of the distribution of the residuals (Proc Univariate). To correct for non-normality, a log transformation was applied to the average number of steps per day. Covariates were selected based on the lowest Akaike information criterion (AIC) for the model.
4.3 RESULTS

On average, calves were 49 ± 9 (mean ± standard deviation) days of age at the time of enrolment.

Treatment had no effect on average daily calf starter intake at the pen level (n = 16) controlling for time period, and number of calves with BRD within a pen as fixed effects and replicate as a random effect ($P = 0.45$). Average daily calf starter intake during the Baseline, Mixing and Novel period was 8.2 ± 0.4 kg, 9.5 ± 0.4 kg, and 10.1 ± 0.4 kg, respectively.

For the Holstein calves on this study (n = 53), the weight gain during the mixing period was 7.4 ± 0.8 kg and 6.7 ± 0.7 kg for the Premixed and Traditional groups, respectively, controlling for BRD, nursery pen location, and initial body weight as fixed effect covariates and replicate as a random effect ($P = 0.28$).

For the Holstein calves on this study, the weight gain in the post-movement period was 6.4 ± 0.4 kg and 7.1 ± 0.4 for the Premixed and Traditional groups, respectively controlling for initial body weight and BRD as fixed effects and replicate as a random effect ($P = 0.15$).

Due to computer errors, activity was only recorded for 18 of the 28 Traditional calves and 21 of the 28 Premixed calves. The average activity of calves during the baseline period was 685 ± 428 (mean ± standard deviation) steps, 19 ± 5 lying bouts and 18.2 ± 0.9 hours lying down per day. During the mixing period, the number of steps per day was higher in the Premixed group relative to the Traditional group ($P = 0.03$, Figure 4.1). In the novel environment, steps per day was higher in the Traditional group compared to the Premixed group ($P = 0.02$). Both Premixed
and Traditional calves increased their activity over time \((P < 0.001)\). BRD had no impact on steps per day \((P = 0.82)\).

The average number of lying bouts was 19 ± 5, 19 ± 5 and 20 ± 5 for the Traditional calves and 20 ± 5, 21 ± 4 and 19 ± 4 for the Premixed calves in the baseline, premix and novel periods, respectively. The number of lying bouts per day did not change by time period \((P = 0.37)\). The average lying number of lying bouts per time period was not significantly different by treatment \((P = 0.39)\). BRD was not significantly associated with the average lying number of lying bouts \((P = 0.87)\).

A time period and study treatment interaction was significantly associated with the daily lying duration per time period \((P = 0.004; \text{Figure 4.2})\). Lying durations for Premixed calves in the mixing and novel environment time periods were 1.6 ± 0.3 and 2.1 ± 0.2 hours less than the baseline lying time, respectively \((P < 0.0001 \text{ and } P < 0.0001, \text{respectively})\). There was no difference in lying duration between the baseline and mixing period for the Traditional calves \((P = 0.85)\). On average, calves with no BRD, subclinical BRD, and Clinical BRD laid down for 16.9 ± 0.3, 17.4 ± 0.3, and 16.5 ± 0.5 hours per day \((P = 0.03)\).

Subclinical BRD (score between 1 and 4) was observed in 8 Traditional calves and 7 Premixed calves. Clinical BRD (score ≥5) was observed in 3 Traditional calves and 2 Premixed calves. Clinical BRD scores varied from 5 to 9. Subclinical disease occurred throughout the trial with the majority occurring in the Premixed time period (Table 4.1). At the group level, 3 pens of calves had no cases of BRD, 5 pens of calves had 1 calf with BRD and 8 pens of calves had 2 or more cases of BRD.
4.3 DISCUSSION

There was no effect of treatment on weight gain, or calf starter intake. The small sample size and the use of multiple breeds within pens may have increased the variability of the results and reduced the ability to detect differences. However, even numerically these results are very similar, indicating that this treatment has minimal impact on the growth and feed intake of calves with the stressors applied in this study.

In the Mixing period, Pre-mixed calves increased the number of steps per day and decreased their daily lying duration compared to Traditional calves. Since the pre-mixing resulted in an increase in pen dimension these alterations in behavior is likely a result of increased play, exploration of a novel environment or increased social interactions. Previous studies have shown that increased space allowance and social contact is positively associated with play (Jensen et al., 1998; Jensen and Kyhn, 2000). However, since the increased activity observed in this study is based solely on pedometry, this increased activity could also be the result of increased displacement and aggression. Anecdotally, both increased play and increased displacement were observed on the day of mixing indicating that both factors may be contributing to the observed increase in activity. A previous study documented decreased lying time, increased steps per day, increased aggression and exploratory behavior in the 3 hours following mixing of pair housed 5 week old bull calves (Veissier et al., 2001). The results of the current study are novel since activity for the 6 days following mixing were evaluated.

Following movement to a novel environment, Pre-mixed calves displayed greater lying duration and fewer steps per day compared to Traditional calves, supporting the hypothesis that Pre-mixed calves would have a lower behavioral response to movement to a novel environment.
The decreased activity of the Pre-mixed calves in the novel environment relative to traditional calves may indicate a less stressful adjustment. However this did not result in greater feed intake or weight gain. An increase in steps per day and lying time in the 4 to 11 days after weaning and movement to a novel environment has previously been associated with increased weight gain in weaned dairy calves (Chapter 3). However, the limitation of this comparison is the differences in the stressors applied to the calves. In the current study calves were moved to a novel environment at least 4 days after weaning, whereas in the previous study calves were both weaned and moved to a novel environment at the same time.

There were no differences in the number of lying bouts in any of the time periods. This could indicate that this factor is not sensitive to management changes and may require larger stressors than those in this study to be altered. Alternatively, an environment that is lacking complexity, such as individual housing, may result in decreased activity due to fewer stimuli. The movement to a novel environment was of very short duration in this study and did not require transport in a vehicle or haltering. If a greater transportation distance was involved the observed differences between treatments in the novel environment may have been greater.

In conclusion, the preliminary data from this study suggests that mixing calves in the nursery prior to moving them to a novel group housed environment does alter their behavior in the novel environment. Based on these findings further study of this management system should be conducted to determine if these behavioural changes indicate increased or decreased calf welfare.
4.4 REFERENCES


Table 4.1: Number of calves identified with bovine respiratory disease complex (BRD) by treatment group and study time period.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>BRD category</th>
<th>Baseline Period</th>
<th>Mixing</th>
<th>Novel Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Day -4 to Day 0)</td>
<td>(Day 0 to 6)</td>
<td>(Day 7 to 14)</td>
<td></td>
</tr>
<tr>
<td>Traditional</td>
<td>No BRD</td>
<td>31</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>n = 32</td>
<td>Subclinical BRD (score 1-4)</td>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Clinical BRD (score 5-15)</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Premixed</td>
<td>No BRD</td>
<td>32</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>n = 32</td>
<td>Subclinical BRD (score 1-4)</td>
<td>0</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Clinical BRD (score 5-15)</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 4.1: Steps per day (Mean and 95% Confidence Interval) for each of the time periods, controlling for breed and age as fixed effects, replicate as a random effect and calf nested within replicate and treatment as a repeated measure.

a,b,c different subscripts within treatments indicates significant differences between time period within treatment groups ($P \leq 0.10$)

1Time Periods

Baseline: Activity of calves on Day -2, while both Traditional and Premixed calves are individually housed

Mixing: Activity of calves during Days 0-6: Traditional calves are individually housed and Premixed calves are housed in groups of 4.

Novel: Activity of calves following movement to a Novel Environment between Day 7-13. Both Traditional and Premixed calves are housed in groups of 4.
Figure 4.2: LSMeans (± S.E.) of total daily lying time (hours), controlling for breed and age with a random effect for replicate and calf nested within replicate and treatment.

a,b: different subscripts indicate a significant difference between time periods with treatments, \(P \leq 0.10\).
Appendix 4.1: Scoring sheet used for health events

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0</strong></td>
<td><strong>1</strong></td>
<td><strong>2</strong></td>
<td><strong>3</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Rectal Temperature</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37.8 to 38.2 °C</td>
<td>38.3 to 38.8 °C</td>
<td>38.9 to 39.4 °C</td>
<td>≤ 39.5 °C</td>
<td></td>
</tr>
<tr>
<td><strong>Cough</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>Induce single cough</td>
<td>Induced repeated coughs or occasional spontaneous cough</td>
<td>Repeated spontaneous coughs</td>
<td></td>
</tr>
<tr>
<td><strong>Nasal Discharge</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal serous discharge</td>
<td>Small amount of unilateral cloudy discharge</td>
<td>Bilateral, cloudy or excessive mucus discharge</td>
<td>Copious bilateral mucopurulent</td>
<td></td>
</tr>
<tr>
<td><strong>Eyes Scores</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>Small amount of ocular discharge</td>
<td>Moderate amount of bilateral discharge</td>
<td>Heavy ocular discharge</td>
<td></td>
</tr>
<tr>
<td><strong>Ear Scores</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>Ear flick or head shake</td>
<td>Slight unilateral droop</td>
<td>Head tilt or bilateral droop</td>
<td></td>
</tr>
<tr>
<td><strong>Fecal Scores</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>Semi-formed, pasty</td>
<td>Loose, but stays on top of bedding</td>
<td>Watery, sifts through bedding</td>
<td></td>
</tr>
</tbody>
</table>

CHAPTER 5: THE EFFECT OF TREATMENT WITH LONG-ACTING ANTIBIOTIC UPON ARRIVAL AT A HEIFER RAISING FACILITY ON DISEASE AND GROWTH IN COMMERCIAL DAIRY HEIFERS

5.1 ABSTRACT

Commercial heifer rearing facilities face unique disease challenges, and offer an opportunity to study the impact and prevention of these diseases. The objectives of this study were to evaluate a single subcutaneous injection of tulathromycin (TUL) in the early postnatal period, administered upon arrival at a commercial heifer raising facility, on the incidence of disease, to describe the risk factors for morbidity and the impact of disease on growth, and to investigate the association of Mycoplasma bovis (M. bovis) with the incidence of otitis media. Calves (n = 788) were randomly assigned to study treatment with TUL or a placebo (CONTROL) upon arrival at the heifer raising facility and were observed for disease daily for 8 weeks by farm staff. Culturing and speciation of M. bovis was performed on nasal swabs collected from a subset of calves (n = 66) at 0, 2 and 4 weeks of age. Analysis of ADG was conducted using linear mixed models with random effects for source farm, housing location, and enrolment group nested within housing location. Analysis of morbidity was conducted using logistic regression with source farm, housing location, and enrolment group nested within housing location as random effects. TUL calves were 60% (OR: 0.41; 95% CI: 0.58-0.82) less likely to be treated for otitis relative to CONTROL calves (P = 0.002). TUL calves were 43% (OR: 0.57; 95% CI 0.39-0.84) less likely to be treated for neonatal calf diarrhea complex (P = 0.005). The ADG of TUL calves was 0.02 ± 0.01 kg greater than CONTROL calves (P < 0.01). Failure of passive transfer, non-specific fever, bovine respiratory disease complex and neonatal calf diarrhea complex decreased ADG. Twenty-six percent (n = 85) of animals sampled tested
positive for *M. bovis* and 4 different strains were identified. *M. bovis* was present in this population but was not associated with otitis media.
5.2 INTRODUCTION

There is a growing trend for dairy heifers to be reared off site from milking herds in commercial heifer raising facilities. In the United States, the proportion of operations that sent calves off-site to commercial heifer raisers increased from 3.6% in 2002 to 4.7% in 2007 (USDA, 2007). In 2007, 11.5% of heifers in the United States were from farms that sent their heifers, primarily pre-weaned calves, off-site to be raised. The commercial rearing industry is an important focus of study since it has unique challenges and opportunities not seen in the traditional dairy farm model. These farms allow personnel to specialize in the recognition, management and treatment of calf diseases, and have reduced the risk of transfer of adult cow diseases to calves after the first week of life. This is in contrast to the traditional dairy model, in which owners or managers must split their time, money and experience between the lactating and non-lactating animals. However, this commercial rearing model also presents challenges since it may involve mixing of many susceptible animals of the same age from multiple source farms. Mixing of animals from multiple sources is a well-documented risk factor for many diseases (Maunsell and Donovan, 2008). However, the incidence of disease in this sector of the industry is not well documented. For instance, participation in the National Animal Health Monitoring System (NAHMS) survey is based on having at least one milking cow, and as such heifer raising facilities are not eligible to participate in the study (USDA, 2009).

The administration of prophylactic antibiotic has been used to reduce infectious disease risk at weaning or movement to group housing in dairy replacement heifers (Stanton et al., 2010) and in veal calves, especially during BRD outbreaks (Catry et al., 2008). However, this practice has been much more thoroughly studied for prevention of bovine respiratory disease in beef...
feedlots (Van Donkersgoed, 1992). In 1999, 83% of feedlots used oral prophylactic or metaphylactic antibiotics and 42% of feedlots administered an injectable antibiotic to high-risk animals to reduce the risk of bovine respiratory disease (USDA, 2000). The experience of beef cattle entering the feedlot has many similarities with that of dairy calves entering a commercial heifer raising facility. Both animal types are vulnerable to infection as they are exposed to disease-causing organisms from multiple sites. Beef cattle are likely to have been recently weaned, and may have been subjected to a lengthy transportation period which could include transit through 1 or more sales barns. These stressors are often associated with immunosuppression (Carroll and Forsberg, 2007). The neonatal dairy calf has a naïve immune system and relies primarily on colostral antibodies received from the dam. This is a high risk period for calves; almost one quarter of all calves develop diarrhea in the pre-weaning period, and 12% of calves experience a respiratory disease event (USDA, 2010). Combining this susceptibility with mixing of animals from multiple sources may put animals at increased risk of morbidity and mortality. To date, no study has investigated the effectiveness of a single injection of a long-acting antibiotic, administered upon arrival at a commercial heifer raising facility, for the control and prevention of clinical disease.

Tulathromycin (Draxxin, Pfizer Animal Health Group, New York, NY) is a tiamilide compound, a subclass of macrolide long-acting antimicrobials. It is distinguished by maintaining therapeutic concentrations against pathogens commonly associated with Bovine Respiratory Disease complex for up to 10 d (Nowakowski et al., 2004). Tulathromycin is approved for treatment and control of BRD in beef cattle and nonlactating dairy cattle. Tulathromycin has
been shown to be effective in the treatment of BRD in 3- to 9-wk-old calves challenged with *Mycoplasma bovis* (Godinho et al., 2005; Godinho et al., 2007).

The primary objective of the study was to evaluate a single subcutaneous injection of tulathromycin (TUL) in the early postnatal period administered upon arrival at a commercial heifer raising facility, on the incidence of disease in young dairy heifers. A second objective was to describe the risk factors for morbidity and the impact of disease on growth of calves, in a commercial heifer raising facility. The third objective was to investigate the association of *Mycoplasma bovis* (*M. bovis*) with otitis media. The primary hypothesis was that a single injection of TUL upon arrival would reduce the prevalence of *M. bovis* and bacterial pathogens and reduce the spread of these pathogens to other animals and so lower the incidence of clinical otitis, diarrhea and non-specific fever in the first 8 weeks of life. The secondary hypothesis was that failure of passive transfer (FPT) and the occurrence of diarrhea, non-specific fever, bovine respiratory disease (BRD), and otitis media would negatively affect average daily gain (ADG).

5.3 MATERIALS AND METHODS

5.3.1 Heifer Management and Housing

All calves were housed at a commercial heifer raising facility in western New York State. Enrollment of 788 heifer calves occurred between October 2007 and June 2008. Calves came from 5 source farms in western New York State. The source farms contributed between 43 and 315 calves to the trial. Calves were between 0 and 10 days of age at arrival at the farm, with a median age of 3 days. Upon arrival at the facility, each calf was processed according to a standard farm protocol, and was then moved to individual housing. Individual housing was available in 6 naturally ventilated nursery barns and 47 individual outdoor hutches. Farm
protocol included the identification of any congenital abnormalities and measurement of serum total protein with a digital refractometer. All calves tested negative for persistent infection with Bovine Viral Diarrhea (BVD) upon arrival at the farm. Calves were vaccinated at 2 and 4 weeks of age with an intramuscular modified live vaccine against Infectious Bovine Rhinotracheitis, BVD Type 1 and Type 2, Bovine Respiratory Syncytial Virus, and Parainfluenza-3 Virus (Vista-5, Intervet Schering-Plough Animal Health, Boxmeer, The Netherlands).

Calves were fed 4L of milk replacer (Excel Calf Milk Replacer 26/18, Grober Nutrition, Cambridge, Ontario, Canada) twice daily (total of 8L/day) and had access to ad libitum calf starter (23% percent protein; 5% fat) and water. Weaning from milk replacer was initiated at 5 wks by decreasing to one milk replacer feeding per day and was complete at 6 weeks of age. Calves were moved from their individual pens or hutchs to group housing 8 weeks after arrival/enrollment, which represented the end of this trial.

### 5.3.2 Study Treatments

Calves were randomly assigned to treatment with tulathromycin (TUL) or a placebo as a negative control (CONTROL) using a random number table and balancing allocation in blocks of 20 based on the order they were processed by farm staff upon arrival at the heifer raising facility. Researchers and barn staff were blind to treatment. All TUL calves were injected with 1.0 mL containing 100 mg of tulathromycin (Draxxin®, Pfizer, Animal Health Group, New York, New York, USA) by subcutaneous injection on the day of arrival at the heifer raising facility. The placebo was a 1 mL visually identical mix of propylene glycol and sterile saline.
5.3.3 Outcome Measures

All calves were monitored twice daily for signs of clinical disease by trained barn staff using standardized disease definitions (Table 5.1). The incidences of disease in this study were calculated based on the number of new cases of a defined disease over the study period (0-8 weeks) (Dohoo et al., 2003). Failure of passive transfer (FPT) was determined based on serum total protein < 5.4 g/dL (Tyler et al., 1998). The established farm practice was to record all treatments on standardized forms at the time of treatment and these data were later entered into a farm management computer program (DairyComp 305, Valley Agriculture Software Inc., Tulare, CA). In addition to the routine diseases recorded, farm staff were additionally asked to indicate if calves affected with ear droop showed signs of unilateral ear droop (UNI), bilateral ear droop (BIL), or if the head was held in an unusually tilted position (Figure 5.1).

All calves were weighed on an electronic scale (Tru-test - ID3000, Tru-Test Incorporated, Mineral Wells Texas) and measured for height at the withers (to the nearest inch) using a yardstick upon arrival at the farm (study enrollment) and at exit from the nursery facilities 8 weeks later (study completion).

5.3.4 Sampling for Mycoplasma testing

Nasal swabs were collected from a subset of study calves. Every other week, the youngest five calves with no history of ear droop were enrolled by study personnel for mycoplasma sampling and were re-sampled two and four weeks later. Additionally, nasal samples were collected twice a month from clinically affected and healthy calves. At each visit, two calves with that were identified and treated for ear droop by farm staff within 24 hours.
(cases) and two calves closest in age to the affected calves, but with no history of ear droop, were sampled. Case and control calves were not re-sampled.

Study personnel wearing sterile disposable gloves used swabs moistened with mycoplasma enrichment medium to swab the nares of selected calves. Swabs were placed in enrichment medium and shipped to the Mastitis Research Laboratory at Washington State University in Pullman, Washington. Specimens were incubated at 37°C in 10% CO₂ for 4 days. On the fourth day, 100 µL of enrichment medium was streaked on a Mycoplasma agar plate (Hogan et al., 1999). Plates were incubated at 37°C in 10% CO₂ for 7 to 14 days, then examined with a 15X dissecting microscope for colonies with the distinctive “fried-egg” appearance (Hogan et al., 1999). Results were considered positive if any Mycoplasma colonies were seen and negative in the absence of Mycoplasma growth. Speciation was done using PCR analysis, and fingerprinting for type was performed using pulsed-field electrophoresis patterns of the positive isolates as described by Biddle et al, ((Biddle et al., 2005).

5.3.5 Statistical Analysis

All analyses were performed using SAS® statistical software (SAS Institute Inc., Cary, NC) v 9.1, with statistical significance based on Type III test for fixed effects having a $P$-value ≤ 0.05. All models were built using backward step-wise elimination. Normality was assessed for all linear models.

The associations between disease events and ADG and height at study completion were analyzed using a linear mixed model (PROC MIXED) with random effects for source farm, location of barn or hutch system, and cohort group nested within housing location. A cohort group was defined as animals in the same housing system at the same time. Disease outcomes
were neonatal calf diarrhea complex, otitis (bilateral and unilateral), non-specific fever, non-specific lameness, navel ill, and bovine respiratory disease (BRD). The associations of study treatment, difficulty with the calf’s birth, housing type (hutch versus barn) and FPT with growth were tested in separate models from those that examined the association of growth with disease events because disease events are likely to be intervening variables in the path from risk factors to decreased growth and as such cannot be included in the same model. Models of ADG and height included weight and height at arrival, respectively and age at final measurement as fixed effects.

Potential predictors of first treatments for neonatal calf diarrhea complex, unilateral ear droop, bilateral ear droop and fever were evaluated using generalized linear mixed models with a logit transformation (PROC GLIMMIX). Source farm, location of barn or hutch system, and cohort group nested within housing system location were included as random effects. A cohort group was defined as all animals in the same housing system at the same time. Models of unilateral and bilateral ear droop were built separately since it is not known if the unilateral and bilateral ear droop have the same risk factors. The model for unilateral ear droop included calves with unilateral ear droop versus calves with no recorded history of any ear droop, while the model for bilateral ear droop included calves with bilateral ear droop versus calves with no recorded history of any ear droop. Study treatment, birth difficulty score, housing system type (hutch versus barn) and FPT were tested as predictors in all disease models.

The relationship between study treatment and mortality was evaluated using a chi-square test (PROC FREQ).
No statistical analysis was performed to evaluate the association between source farm and Mycoplasma due to the low number of positive samples.

5.4 RESULTS

At enrolment, calves were 3 ± 2 days old (mean ± standard deviation), weighed 41.2 ± 4.4 kg and were 80±3 cm tall at the withers.

5.4.1 Health Events in the Study Population

The most frequent health conditions were neonatal calf diarrhea complex (84%) and otitis media (78%) (Table 5.2).

Unilateral and bilateral ear droop were observed in 4% (32/788) and 52 % (414/788) of calves, respectively. Farm staff failed to record whether ear droop was unilateral or bilateral at first treatment for 170 of the 616 calves treated for ear droop. Analyses of ear droop excluded calves that did not have a record of the numbers of ears affected. None of the calves showed signs of ataxia during this trial. Serum total protein was not measured for 10 calves on this trial but the prevalence of FPT (total protein ≤ 5.4) was only 5% overall.

5.4.2 Morbidity

Age, weight and height at enrolment were not significantly different between TUL and CONTROL calves. The incidence of diarrhea was 87% (345/395) and 80% (314/393) in the CONTROL and TUL calves, respectively. CONTROL calves were 1.8 (95% Confidence Interval: 1.2-2.6) times more likely to have neonatal calf diarrhea complex than calves that received TUL, (P = 0.005), controlling for source farm, housing location and group nested within
housing location as random effects. There was a tendency for calves from the nursery barn to be at greater risk for diarrhea compared to calves from the hutches (OR: 2.4; 0.9-6.4; \( P = 0.08 \)).

The incidence of unilateral, bilateral and unrecorded ear droop were 3% (11/393), 49% (193/393) and 22% (86/393) in TUL calves, and 5% (21/395), 56% (221/395) and 21% (84/395) in CONTROL calves, respectively. The probability of TUL calves being treated for otitis (either unilateral or bilateral ear droop) was 60% lower (OR: 0.41; CI: 0.60-0.82) than CONTROL calves (\( P = 0.002 \)) controlling for source farm, housing location and group nested within housing location as random effects. Otitis was observed in 5% (1/19) of calves in the hutch and 17% (31/185) of calves in the nursery barn (\( P = 0.006 \)). Study treatment was the only variable that was significant in the final models for unilateral and bilateral ear droop that controlled for group and source farm as random effects. TUL calves were 0.26 (CI: 0.11-0.65) times as likely to display unilateral ear droop relative to CONTROL calves (\( P = 0.03 \)). The probability of TUL calves displaying bilateral ear droop was 40% lower (OR: 0.58; CI: 0.40-0.83) than CONTROL calves (\( P = 0.003 \)).

The incidence of Non-Specific Fever (NSF) in the TUL group was 13% (52/393) and 18% (71/395) in the CONTROL group. Calves in the CONTROL group tended to have greater odds of having NSF compared to calves in the TUL group (OR: 1.5; CI: 1.2-2.2; \( P = 0.07 \)), controlling for source farm, housing location and group nested within housing location as random effects. There was no difference in the incidence of NSF between hutch and nursery barn housed calves (\( P = 0.12 \)). The incidence of BRD was 2% (9/393) and 3% (12/395) in TUL and CONTROL groups, respectively (\( P = 0.52 \)). The incidence of lameness was low with 0.8% (6/393) and 1.3% (10/395) in the TUL and CONTROL group, respectively (\( P = 0.32 \)).
5.4.3 Growth

Three calves returned to their source farm at the end of the study without being weighed and were excluded from analyses of ADG and height. Prior to the end of the trial, 14 calves died and were excluded from analyses of ADG and height.

At the end of the study, calves were 58 ± 7 (mean ± standard deviation) days old and weighed 75 ± 11 kg. Average daily gain over the study period was 0.61 ± 0.13 kg/d.

Relationship between morbidity and average daily gain

Diarrhea, lameness, BRD and NSF were significantly associated with ADG, controlling for initial body weight and age at final measurement as fixed effects and source farm, group and group within barn as random effects. Diarrhea was associated with a decrease in ADG of 0.03 ± 0.01 kg relative to non-diarrheic calves ($P = 0.01$). NSF was associated with a decrease in ADG of 0.07 ± 0.01 kg relative to calves that were not diagnosed with NSF ($P < 0.001$). Calves with lameness had a decrease in ADG of 0.16 ± 0.03 relative to non-lame calves ($P < 0.001$). Calves with BRD had a decrease in ADG of 0.10 ± 0.03 kg relative to non-BRD calves ($P < 0.001$). Calves with unilateral ear droop had a decrease in ADG of 0.05 ± 0.02 kg compared to unaffected calves, ($P = 0.04$). The ADG of calves with bilateral and unknown ear droops were not significantly different from unaffected calves, ($P = 0.99$ and $P = 0.98$, respectively).

Relationship between disease predictors and average daily gain

Failure of passive transfer and study treatment were associated with ADG, controlling for arrival weight and with the random effects of group and source farm. Calves with FPT had lower ADG by 0.04 ± 0.02 kg/day ($P = 0.03$). Calves from the TUL group had greater ADG of 0.02 ± 0.01 kg/day compared to CONTROL calves ($P = 0.01$). TUL and FPT did not interact in this
model \( (P = 0.54) \). The ADG of calves in hutch housing were not different than calves housed in barns \( (P = 0.79) \).

### 5.4.4 Height at movement to group housing

On average, calves were \( 92 \pm 3 \) (mean \( \pm \) standard deviation) cm tall at the end of the study, an average growth of \( 11 \pm 3 \) cm over the course of the trial.

**Relationship between disease and height**

Diarrhea, otitis and NSF were associated with decreased height upon exit from the nursery, controlling for arrival height as a fixed effect, and source farm, housing location and group nested within housing location as random effects. Calves with diarrhea were shorter by \( 1 \pm 0.2 \) cm relative to calves without diagnosed diarrhea, \( (P = 0.01) \). Calves with unilateral ear droop were shorter by \( 2 \pm 0.5 \) cm relative to calves without diagnosed ear droop, \( (P = 0.001) \). Calves with bilateral and unrecorded ear droop did not differ in height from unaffected calves, \( (P = 0.32 \) and 0.12, respectively). Calves with NSF were \( 1 \pm 0.2 \) cm shorter relative to calves without diagnosed NSF, \( (P < 0.001) \).

**Relationship between disease predictors and height**

Failure of passive transfer was a significant predictor of height at movement from the nursery barn. Controlling for height at arrival and age at measurement, with random effects to control for source farm, housing location and group nested within housing location, calves with FPT were \( 1.2 \pm 0.4 \) cm smaller than calves with successful passive transfer \( (P < 0.001) \). There was a tendency for TUL calves to be \( 0.3 \pm 0.2 \) cm taller at exit from the study \( (P = 0.08) \). There was no difference in height between calves housed in hutches and calves housed in barns \( (P = 0.20) \).
5.4.4 Testing for Mycoplasma

Of the 66 calves that were enrolled upon arrival at the heifer raising facility and sampled at 0, 2, and 4 weeks, 17 (26%) calves had one positive test for Mycoplasma. Of the sampled calves, 89% (59/66) were treated for neonatal calf diarrhea and 74% (49/66) had ear droops during the study (Table 5.3). No samples collected in week 0 were positive for Mycoplasma. Positive samples were collected from two calves in week 2 and from 15 calves in week 4. Of the positive calves, 9 out of 17 were from the TUL group. Ear droop was recorded in 10 out of 17 of the calves that had positive samples collected. Of the 10 calves with ear droop, 90% were diagnosed with ear droop between week 2 and week 4 samples. Positive cultures were found in calves from 4 out of the 5 source farms (Table 5.4). The fifth farm was a small facility and only 2 calves were cultured from this farm during the study.

*M. bovis* samples were strain typed and 5 distinct strains identified with 4 out of 5 source farms having at least 2 strains. Due to the fragility of *M. bovis*, there was not enough DNA to complete fingerprinting on 5 samples, and as such, these samples could only be confirmed as *M. bovis*. In addition, 4 samples did not survive frozen storage prior to confirming strain of fingerprinting and can only be confirmed as *Mycoplasma* species.

Nasal samples were taken from 42 cases and 43 control animals. One of the active cases from source farm 1 and 1 of the control animals from source farm 4 tested positive for *M. bovis*. The case from farm 1 was identified as strain A. The control animal showed signs of ear droop within 24 hours of the positive sample. The strain *M. bovis* from farm 4’s case did not have enough DNA to complete fingerprinting, and as such, this sample could only be confirmed as *M. bovis*. 

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5.4.4 Mortality

Eight calves from CONTROL and 6 calves from TUL died during the trial for a mortality risk to 8 weeks of age of 2% (14/788). The main causes of mortality as determined by farm staff were diarrhea (n = 3), bloat (n = 2), high fever (n = 2) and chronic poor health (n = 2). Other causes included paralysis, stomach ulcer, and swelling of neck. No deaths were directly associated with ear droop.

5.5. DISCUSSION

Disease pressures and management decisions that affect calf disease are likely to be different on a commercial heifer raising facility than on a traditional farm which raises its own replacement animals. Heifer raising facilities are likely to be more focused on monitoring of growth and disease than traditional farms based on their need to report and justify the cost of rearing calves to the contracted source farms; in addition their risk factors are different as a result of completely isolating calves from older animals but exposing them to animals from multiple sources. In comparison to published reports, this study herd had a much higher incidence of diarrhea, a similar incidence of respiratory disease and navel ill and a much lower incidence of hernia and FPT, (Table 5.5). These differences are likely due to a combination of factors that make commercial heifer raising facilities unique. For diarrhea, the high level of vigilance by the farm staff and the unrestricted definition of diarrhea to any observation of soft feces lacking in form likely increased the apparent incidence. The transport and mixing of multiple animals shortly after birth would also increase disease pressure. This study farm defined non-specific fever as dull and listless with a rectal temperature above 39.5C. This definition lacks symptoms specific to BRD, and as such may either represent a response to another unrelated infection or
may be an early sign of respiratory disease. As such, the decision was made not to lump BRD and fever together in analysis. However, when the incidences of BRD and fever are combined they are similar to the incidences reported in the literature. In this herd, navel ill and hernias were on the low end of reported incidences. Failure of passive transfer was very low for these calves in comparison to the literature. This is likely due to the incentive and education program offered by the manager of the facility which educates producers on colostrum management and penalizes FPT by increasing the cost of rearing calves with FPT. The risk of mortality of pre-weaned heifers in the present study is substantially lower in comparison with the NAHMS Survey, which recently documented mortality of 7.8% in pre-weaned calves (USDA, 2010).

In the 2007 survey, NAHMS reported that the median weight of Holstein heifer calves between 56 and 62 days of age in the United States was 80 kg (USDA, 2010). The calves in this study were slightly below the median calf weight, with an average weight at 8 weeks of 75.4 kg. BRD, fever, diarrhea and lameness affected ADG in this study. BRD and fever were found to reduce ADG by 0.10 and 0.07 kg, respectively. The reduced impact of fever relative to BRD and the similarity between the two measurements does support our theory that fever is an earlier stage of BRD. The impact of these health conditions on ADG has been documented in two other studies. Virtala et al (1996) found that ADG decreased by 0.07 kg for calves with BRD in the first month of life and Donovan et al (1998a) found that BRD decreased ADG by 0.01 kg in the first 6 months. The 6-month follow up period of the Donovan et al (1998a) study allowed for factors such as recovery from BRD, other diseases, diet, social interactions, and movement to group housing to affect the growth of calves. Therefore the effect of BRD was likely diluted.
The present study found that diarrhea decreased ADG by 0.03kg (range of 0.01 to 0.05 kg). This is consistent with the findings of Donovan et al (1998a) who found that diarrhea decreased ADG by 0.013 kg. However, these two studies were in conflict with the finding of Virtala et al (1996) of no effect of diarrhea. This may be due to the methods used to measure weight gain in the latter study. The studies which found an effect of ADG measured weight gain using electronic scale while the one which did not estimated weight gain using weight tape measurement. The measurement error introduced from using this measurement may have masked the effect of diarrhea.

Otitis did not have an effect on ADG. This is inconsistent with Sockett, et al (Sockett et al., 2008) who found a 4.68kg decrease in body weight gain in calves with clinical otitis media. However, the current findings are consistent with Stipkovits et al (Stipkovits et al., 2005), in which an experimentally induced Mycoplasma bovis infection did not have a significant effect on ADG during the 18 days following infection when treated with antibiotics. These differences in the reports on the effect of otitis media on ADG may be due to several factors. No statistical methods were reported in Sockett et al (2008), so comparisons between studies are limited. Early detection of disease is associated with a decrease in the severity of signs of other diseases (McGuirk, 2008), and the high vigilance of farm staff and early treatment in the present study may have minimized the effect of otitis on average daily gain. Alternatively, our definition of otitis needed to be very simple since this was a field study on a commercial farm and did not allow for advanced diagnostics. Given that our definition of otitis was based on ear droop, it is possible that some of the observed bilateral ear droops may have been associated signs of depression from other diseases such as diarrhea and may have been an indicator of malaise. The
small number of unilateral ear droops limited our ability to determine the impact of this condition on average daily gain. However, given that there was a tendency for unilateral ear droop to reduce ADG, further research into the effect of this condition on ADG is warranted.

Failure of passive transfer decreased ADG by 0.04 kg. This is consistent with Virtala et al (1996), who found that FPT decreased ADG by 0.05 kg during the first month of life. TUL increased ADG in this study by 0.02 kg. Both passive transfer of antibodies and TUL decreased the risk of disease. Calves with FPT are more susceptible to diseases that negatively affect ADG (Donovan et al., 1998). Tulathromycin is an antibiotic that is primarily targeted against respiratory disease. However, this study demonstrated a beneficial impact of TUL treatment on the incidence of neonatal calf diarrhea complex, perhaps by reducing the incidence of BRD and otitis media, thereby enabling calves to mount a stronger immune response against diarrhea-causing organisms without the additional burden of other diseases. There was no interaction between TUL and successful passive transfer, with both conferring protection against disease. This may be due to the low number of calves with FPT in this population limiting the power to investigate interactions. In this study herd, TUL reduced the incidence of both unilateral and bilateral ear droop, diarrhea and tended to reduce the incidence of non-specific fever. In a similar study, Sockett et al (2008) also found that tulathryomycin, administered prophalactically, reduced the incidence of otitis media in milk-fed calves. However, tulathromycin was administered at 1 and 7 days post arrival, whereas we used a single treatment. If an odds ratio is calculated from the results reported by Sockett et al (2008), the incidence of otitis media was 3.8 (CI: 1.8-9.8) times greater in placebo calves compared to calves that received tulathromycin on day 1 and 7. This finding is similar to our finding that CONTROL calves were 3.7 (CI: 1.6 - 9.1)
and 1.7 (CI: 1.2-2.5) times more likely to display unilateral and bilateral ear droop compared to calves that received TUL, respectively. The results from these two studies suggest that a single injection of tulathromycin reduces the incidence of otitis in neonatal calves; further study is warranted to confirm these findings.

The results from the cultures indicated that *M. bovis* was present in this herd and that calves were more likely to test positive for *M. bovis* when they were greater than 2 weeks of age. This may indicate that calves were not contracting Mycoplasma until after they arrived at the heifer raising facility, or they were not shedding this organism until they were older. However, clinical otitis media was primarily identified in calves less than 2 weeks of age.

There are two potential explanations for the lack of association between *M. bovis* and otitis. The first is that *M. bovis* is not an important pathogen in clinical otitis media in young dairy calves and the second is that due to the sampling schedule and antibiotic use on this farm false negative were generated. Prior research has found that *M. bovis* is associated with respiratory disease, arthritis and otitis (as reviewed by Maunsell and Donovan, 2009).

However, while *M. bovis* can result in clinical disease in a challenge study, in the field this relationship is much more complex. Since the farm treatment protocol that required the staff to administer antibiotics at the time of diagnosis, which likely inhibited culture growth since samples were collected 2-12 hours after treatment, a higher rate of false negatives is probable. Based on the findings of this study, we cannot determine which of these explanations is accurate.

The use of mass antibiotic treatments can be controversial and spark worries about antimicrobial resistance. This is a valid concern and as such the use of antibiotics must be fully
evaluated for efficacy and carefully targeted for rational use. Research into the efficacy of strategic antibiotic treatments is important to prevent producers from using antibiotics ineffectively. In order to minimize the risk of antimicrobial resistance the antibiotics should be used strategically on high risk animals within a confined time period of high risk. This method of antibiotic treatment should not be a blanket treatment undertaken for the purpose of growth promotion but rather a strategic intervention to prevent the outbreak and spread of disease in a highly vulnerable population. In conjunction with this method efforts should be made to reduce or minimize the risk factors that are manageable such as cleanliness, ventilation, and colostrum management which can lead to increased disease susceptibility with the aim of reducing or eliminating the need for this program. For this to occur, good record keeping is needed in order to identify the risk factors and appropriate conditions to use this method and to monitor the success of this program. The ideal circumstance for metaphylactic use of anti-infectives is one in which the risk of developing the targeted disease is high in a constrained period of time and treatment success of clinical disease is limited, representing an animal welfare and production concern. *Mycoplasma bovis* contracted at a source farm would fit these criteria based on the high incidence of disease following movement to the heifer raising facility and the difficulty of treating *Mycoplasma bovis* associated disease (as reviewed by Caswell and Archambault, 2007). However, based on the previously listed criteria, the lack of association between study treatment, or clinical otitis and *M. bovis* does suggest that on this farm otitis media does not fit the criteria for the use of metaphylaxis.

In conclusion, TUL decreased the incidence of diarrhea, unilateral and bilateral ear droop in the early post-natal period. In addition, TUL tended to decrease the incidence of non-specific
fever. Non specific fever, respiratory disease, FPT, lameness and neonatal calf diarrhea complex had negative effects on average daily gain. Finally, despite 26% of sampled animals testing positive for *M. bovis* confirming the presence of this organism at the facility, this organism was not associated with otitis. With improved culturing methodology and decreased antibiotic treatments the ability to detect an association between clinical disease and positive *M. bovis* cultures may be altered.
5.6 REFERENCES


USDA:APHIS:VS, CEAH. Fort Collins, CO #N517.0208

USDA:APHIS:VS, CEAH. Fort Collins, CO. #N336.1200


**Table 5.1:** Disease definitions used by farm staff

<table>
<thead>
<tr>
<th>Disease</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ear Droop</td>
<td>One or both ears are visibly drooping or head is maintained in a tilted position, Figure 5.1</td>
</tr>
<tr>
<td>Neonatal Calf Diarrhea Complex (Diarrhea)</td>
<td>Feces are soft and do not hold form</td>
</tr>
<tr>
<td>Bovine Respiratory Disease (BRD)</td>
<td>Rectal temperature above 39.5 C plus one or more of the following: elevated respiratory rate, nasal discharge, cough</td>
</tr>
<tr>
<td>Non-Specific Fever (fever)</td>
<td>Dull and listless with a rectal temperature above 39.5C</td>
</tr>
<tr>
<td>Non-Specific Lameness (lameness)</td>
<td>Abnormal gait with unknown cause</td>
</tr>
<tr>
<td>Navel infection</td>
<td>Navel is swollen or tender</td>
</tr>
</tbody>
</table>
Table 5.2: Incidence and median age at first treatment for all health events for calves at a commercial heifer raising facility during the first 8 weeks of life.

<table>
<thead>
<tr>
<th>Health Events</th>
<th>Incidence (%)</th>
<th>Median Age at first treatment (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal calf diarrhea complex</td>
<td>659/788 (84%)</td>
<td>10</td>
</tr>
<tr>
<td>Otitis Media</td>
<td>616/788 (78%)</td>
<td>8</td>
</tr>
<tr>
<td>Unilateral Ear Droop</td>
<td>32/788 (4%)</td>
<td>8</td>
</tr>
<tr>
<td>Bilateral Ear Droop</td>
<td>414/788 (52%)</td>
<td>7</td>
</tr>
<tr>
<td>Unspecified Ear Droop</td>
<td>170/788 (22%)</td>
<td>11</td>
</tr>
<tr>
<td>Non-specific fever</td>
<td>115/788 (15%)</td>
<td>28</td>
</tr>
<tr>
<td>Bovine Respiratory Disease</td>
<td>21/788 (3%)</td>
<td>35</td>
</tr>
<tr>
<td>Lameness</td>
<td>16/788 (2%)</td>
<td>17</td>
</tr>
<tr>
<td>Navel ill</td>
<td>16/788 (2%)</td>
<td>7</td>
</tr>
<tr>
<td>Hernia</td>
<td>5/788 (1%)</td>
<td>19</td>
</tr>
<tr>
<td>Bloat</td>
<td>2/788 (&lt; 1%)</td>
<td>17</td>
</tr>
<tr>
<td>Failure of Passive Transfer</td>
<td>40/778 (5%)</td>
<td>10</td>
</tr>
<tr>
<td>No Disease Treatments</td>
<td>27/788 (3%)</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 5.3: Incidence of health events for calves sampled for Mycoplasma at 0, 2 and 4 weeks of age.

<table>
<thead>
<tr>
<th>Health Condition</th>
<th>Health conditions with 1 positive culture of Mycoplasma at 2 or 4 weeks old (n = 17)</th>
<th>Health conditions in Mycoplasma negative calves (n = 49)</th>
<th>P-Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea¹</td>
<td>14/17 (82%)</td>
<td>45/49 (92%)</td>
<td>0.36</td>
</tr>
<tr>
<td>Ear Droop</td>
<td>10/17 (59%)</td>
<td>39/49 (78%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Fever²</td>
<td>1/17 (6%)</td>
<td>7/49 (14%)</td>
<td>0.67</td>
</tr>
<tr>
<td>BRD³</td>
<td>1/17 (6%)</td>
<td>1/49 (2%)</td>
<td>0.45</td>
</tr>
<tr>
<td>Lameness⁴</td>
<td>0/17 (0%)</td>
<td>2/49 (4%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Navel ill</td>
<td>1/17 (6%)</td>
<td>2/49 (4%)</td>
<td>1.0</td>
</tr>
<tr>
<td>FPT⁵</td>
<td>0/17 (0%)</td>
<td>5/49 (10%)</td>
<td>0.32</td>
</tr>
<tr>
<td>No health conditions</td>
<td>2/17 (12%)</td>
<td>3/49 (6%)</td>
<td>0.60</td>
</tr>
</tbody>
</table>

¹ Diarrhea: Neonatal Calf Diarrhea Complex
² Fever: Non-specific Fever
³ BRD: Bovine Respiratory Disease Complex
⁴ Lameness: Non-specific Lameness
⁵ FPT: Failure of Passive Transfer
Table 5.4: Breakdown of the number of positive *M. Bovis* samples collected from calves at repeated intervals (0, 2, and 4) by source farm and strain type

<table>
<thead>
<tr>
<th>Farm</th>
<th>Sample Types</th>
<th>Proportion of calves with positive Mycoplasma samples (pos/n sampled)</th>
<th>Strain</th>
<th>M. Bovis (strain unknown)</th>
<th>Freezer Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Repeated</td>
<td>22% (8/36)</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Repeated</td>
<td>20% (3/15)</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Repeated</td>
<td>38% (3/8)</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Repeated</td>
<td>60% (3/5)</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Repeated</td>
<td>0% (0/2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>66</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>
### Table 5.5: Incidence of health conditions as reported in the literature for calves under 6 months of age

<table>
<thead>
<tr>
<th>Health Condition</th>
<th>Study Incidence</th>
<th>Incidence in Literature</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal Calf Diarrhea Complex</td>
<td>84%</td>
<td>9.8-35%</td>
<td>Waltner-Toews et al., 1986; Curtis et al., 1988; Van Donkersgoed et al., 1993; Sivula et al., 1996; Virtala et al., 1996a; Donovan et al., 1998; Svensson et al., 2003; USDA, 2010</td>
</tr>
<tr>
<td>Bovine Respiratory Disease</td>
<td>3%</td>
<td>7-39%</td>
<td>Waltner-Toews et al., 1986; Van Donkersgoed et al., 1993; Sivula et al., 1996; Virtala et al., 1996b; Donovan et al., 1998; Svensson et al., 2003; USDA, 2010</td>
</tr>
<tr>
<td>Non-specific fever</td>
<td>15%</td>
<td></td>
<td>Virtala et al., 1996b; Donovan et al., 1998; Svensson et al., 2003; USDA, 2010; Svensson et al. 2007,</td>
</tr>
<tr>
<td>Navel ill</td>
<td>2%</td>
<td>1.3-14%</td>
<td>Virtala et al., 1996b; Donovan et al., 1998; Svensson et al., 2003; USDA, 2010; Svensson et al. 2007,</td>
</tr>
<tr>
<td>Hernia</td>
<td>1%</td>
<td>15%</td>
<td>Virtala et al., 1996a</td>
</tr>
<tr>
<td>Failure of passive transfer</td>
<td>5%</td>
<td>19-37%</td>
<td>Van Donkersgoed et al., 1993; Virtala et al., 1996a; Trotz-Williams et al., 2008; USDA, 2010</td>
</tr>
<tr>
<td>Mortality</td>
<td>1.9%</td>
<td>3.8-12%</td>
<td>Waltner-Toews et al., 1986; Curtis et al., 1988; Sivula et al., 1996; USDA, 2010</td>
</tr>
</tbody>
</table>
Figure 5.1: Images taken from farm prior to the start of the trial and used as guidelines to identify ear droop. Shown: from left to right: Unilateral ear droop and ear droop with abnormal head position
CHAPTER 6: THE EFFECT OF TREATMENT WITH LONG-ACTING ANTIBIOTIC AT POSTWEANING MOVEMENT ON RESPIRATORY DISEASE AND ON GROWTH IN COMMERCIAL DAIRY CALVES

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Key words: (antibiotic; weaning; respiratory disease; heifer)

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6.1 ABSTRACT

Bovine respiratory disease (BRD) is a major concern when raising replacement heifers because of the high incidence and long-term effects of this disease, such as decreased growth and increased time to first calving. The objective of this study was to determine the effect of tulathromycin (TUL) treatment at postweaning movement on the incidence of BRD in dairy replacement heifers. A total of 1,395 heifers were enrolled between November 2006 and June 2007 at a commercial heifer-raising facility. Calves were randomly assigned either to treatment with TUL or to a positive control group treated with oxytetracycline (TET). Calves treated with TUL were 0.5 times (95% CI: 0.4 to 0.7) less likely to be treated for BRD in the 60 d following enrollment than calves treated with TET. For calves that had no history of BRD in the pre-enrollment period, TET calves weighed 4.9 ± 0.5 kg less than TUL calves after 6 wk in group housing. If calves were treated for BRD in the pre-enrollment period, there was no treatment effect on growth. Calves with clinical BRD in the 60 d following movement weighed 7.9 ± 0.6 kg less than calves without BRD after 6 wk in group housing. Treatment with TUL at the time of movement to group housing had a beneficial effect on the health and performance through the prevention of BRD in dairy calves with no prior history of the disease. Moreover, BRD after movement to group housing after weaning had a significant effect on the growth of dairy calves.
6.2 INTRODUCTION

Dairy replacement heifers undergo numerous challenges from birth until they enter the lactating herd, with many of these challenges coinciding with management changes. In the North American dairy industry, preweaned dairy heifers are raised primarily in individual housing and after weaning are primarily housed in groups (USDA, 2007a). Potential stressors involved in this move from individual to group housing include social, environmental, transportation, and nutritional changes. The environmental stressors include increased pathogen exposure and temperature changes. If calves are required to mount a prolonged response to these multiple stressors, suppression of their immune response and disease outbreaks can occur (Carroll and Forsberg, 2007). Bovine respiratory disease (BRD) is the primary disease to which calves are susceptible at this time.

In cattle, BRD is a complex of disease processes most commonly resulting from infection with various microorganisms, including Pasteurella multocida, Mannheimia hemolytica, and Mycoplasma spp. The development of BRD is typically initiated by an environmental stressor and viral infection, which weakens the resistance mechanisms of the lungs and allows bacterial colonization of the lung (Yates, 1982). In an Ontario study, 15% of calves were treated for pneumonia before weaning (Waltner-Toews et al., 1986a). In a Minnesota study, the case fatality rate for BRD was about 9% (Sivula et al., 1996). The direct costs of treatment and prevention of this disease have been estimated at US$14.71 per calf per year in milk-fed calves, as well as $1.95 per calf per year in replacement heifers and $9.08 per cow per year over the entire herd (Kaneene and Scott Hurd, 1990; Miller and Dorn, 1990). These studies were not able to take into account the indirect costs of this disease, which include an increased risk of culling or death.
before entry into the milking herd or the losses associated with delayed entry into the milking herd (Waltner-Toews et al., 1986b). Important control measures include vaccinating cows against the causal organisms, ensuring excellent colostrum management, and minimizing acute and chronic stressors such as poor ventilation, mixing of groups, overcrowding, and poor nutrition (Kahn, 2005). However, at weaning or first movement, some of these stressors cannot be avoided. As such, calves are placed at increased risk of developing BRD and additional steps may be necessary to prevent BRD.

In 1999, 27% of small beef feedlots and 81% of large feedlots in the United States were administering an injectable antimicrobial metaphylactically to feedlot cattle for the prevention of respiratory disease (USDA, 2000). A meta-analysis of field trials indicated that for feedlot calves, the administration of oxytetracycline or tilmicosin on the day of arrival at the feedlot consistently reduced morbidity rates attributable to BRD (Van Donkersgoed et al., 1993). The movement of beef cattle into a feedlot is comparable to the movement of dairy calves from individual to group housing in that they experience similar potential stressors, including nutritional and social changes, transportation, and increased disease exposure. However, the management systems and the age and size of the animals are different between beef and dairy calves. As such, research into the use of injectable antimicrobials at the time of calf movement to prevent BRD in weaned dairy calves is necessary to determine the efficacy of this approach in the context of a dairy replacement heifer management system.

The objective of this randomized blinded clinical trial was to compare the effects of tulathromycin under commercial conditions on calf health and performance to an existing oxytetracycline treatment. Tulathromycin (Draxxin, Pfizer Animal Health Group, New York,
NY) is a tiamidine compound, a subclass of macrolide long-acting antimicrobials. It is distinguished by maintaining therapeutic concentrations against pathogens commonly associated with BRD for up to 10 d (Nowakowski et al., 2004). Tulathromycin is approved for treatment and control of BRD in beef cattle and nonlactating dairy cattle. Tulathromycin has been shown to be effective in the treatment of BRD in 3- to 9-wk-old calves challenged with *Mycoplasma bovis* (Godinho et al., 2005, 2007). Oxytetracycline is a broad-spectrum tetracycline antibiotic that maintains therapeutic concentrations against pathogens associated with BRD for 3 d (Biomycin 200, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO). Oxytetracycline is approved for use in beef and dairy cattle. The hypothesis for this study was that administration of tulathromycin at a time of high risk of BRD would reduce the pathogen challenge and the risk of clinical respiratory disease in the weeks following a postweaning movement and housing change in dairy calves.

**6.3 MATERIALS AND METHODS**

**6.3.1. Heifer Management and Housing**

This study was conducted at a commercial contract heifer raising facility in New York State. Enrollment in the study occurred between November 2006 and June 2007, with a total of 1,395 heifers being enrolled.

Heifer calves arrived at the farm from 7 commercial dairy farms at 1 to 5 d of age. Upon arrival at the facility, each calf was processed according to a standard farm protocol and was then moved to individual housing in a naturally ventilated nursery barn or an outdoor hutch. The farm protocol included the identification of any congenital abnormalities, measurement of serum total protein, tail docking, and the collection of a tissue sample for the detection of persistent infection
with bovine viral diarrhea (BVD). Over the course of this study, no animal tested positive for BVD. Calves were vaccinated at 2 and 4 wk of age with an intramuscular modified live vaccine against infectious bovine rhinotracheitis, BVD type 1 and type 2, bovine respiratory syncytial virus, and parainfluenza-3 virus (Vista-5, Intervet Schering-Plough Animal Health, Boxmeer, the Netherlands).

Preweaned calves were fed 4 L of milk replacer (Excel Calf Milk Replacer 26/18, Grober Nutrition, Cambridge, Ontario, Canada) twice daily and had access to ad libitum calf starter and water. Weaning from milk replacer was initiated at 5 wk and completed at 6 wk of age. Calves remained in their original individual housing for approximately 2 wk after weaning, at which time they were moved to group housing in the weaning barn and enrolled in the trial. Calves that were moved to group housing after 80 d of age were excluded from this trial. A schematic representation of the trial methods and events is shown in Figure 6.1.

For the first 6 wk of the study, calves were housed in the weaning barn in groups of 8 to 10 calves. For the initial 3 to 4 wk in the weaning barn, calves received dry hay and pelleted heifer grain ad libitum. Calves were then transitioned onto a TMR. After approximately 6 wk in the weaning barn, calves were moved to the transition barn and housed in groups of approximately 50 calves.

6.3.2 Study Treatments

Before the start of the study, the farm had a pre-existing treatment protocol at movement. As such, the current study was designed as a positive control trial, with calves being assigned to treatment with tulathromycin (TUL) or the previously established oxytetracycline protocol (TET). Each calf was assigned to treatment group using a systematic random assignment method.
based on the unique identification number assigned to each calf on arrival, determined by their order of arrival at the farm. All TUL calves were injected with 2.0 mL of tulathromycin (Draxxin, Pfizer Animal Health Group) by subcutaneous injection on the day of movement to group housing. Draxxin is a semi-synthetic macrolide antibiotic, containing 100 mg of tulathromycin per milliliter. All TET calves received 5.0 mL of oxytetracycline (Biomycin 200, Boehringer Ingelheim, St. Joseph, MO) intramuscularly on the day of movement to group housing. Biomycin 200 is a tetracycline antibiotic containing 200 mg of oxytetracycline per milliliter. The standard dose for TUL was determined based on the weight of the upper quartile of calves (78 kg) at enrollment. The dose of TET was part of the established weaning management protocol on the farm and was two-thirds of a full label dose. During the study, no antibiotics were administered in the feed or water. The 2 trial treatments were very different in appearance and volume used; therefore, the farm staff members responsible for administration of treatment at the time of enrollment were not blinded to treatment assignment. However, the decision as to which drug to administer was predetermined. As such, randomization to treatment group was not influenced by preconceived bias of the farm staff. Outcomes, such as detection of BRD and follow-up weight measurements, were assessed by a different set of staff members than those administering the treatment at enrollment, who were blind to treatment group allocation.

The distribution of calves in the TET and TUL groups within a pen of 10 calves in the weaning barn was random, based upon the order in which calves were processed. Pens were open-sided. Thus, nose-to-nose and oral contact was possible between pens.
6.3.3 Outcome Measures

All calves were monitored daily in the nursery, weaning, and transition barns by trained barn staff for the occurrence of disease, using consistent case definitions. All disease events and treatments were recorded in the farm management database (DairyComp 305, Valley Agricultural Software Inc., Tulare, CA). The farm staff recorded fever and respiratory disease as 2 separate events. Fever was defined as an animal that was dull, off-feed, listless, with a rectal temperature > 39.5°C and normal respiratory rate with no nasal discharge; respiratory disease was defined as the animal being dull, listless, with elevated respiratory rate, nasal discharge, or both. However, for the purpose of this trial, we combined these 2 diseases into one category called BRD. This approach was based on the likelihood that these 2 events are the same disease at different stages or represent animals with a slightly different clinical presentation of the same underlying condition. All calves in individual housing were treated for disease in accordance with the previously established treatment protocols of the farm. Calves were not eligible for BRD treatment for 5 d after the experimental treatment. Group-housed animals identified with BRD were treated with ceftiofur crystalline suspension (Excede Sterile Suspension, Pfizer Animal Health Group) administered at the base of the ear, according to label instructions.

All calves were weighed upon arrival at the farm, upon enrollment in the study (approximately 8 wk of age), and at 6 wk after enrollment. Height at the withers (to the nearest inch) was measured at the same times as weight.

Farm staff performed a gross necropsy for those calves with signs of BRD that died during the first 6 wk post-enrollment. To complete this gross postmortem examination, specific digital images of the heart, lung, intestines, and trachea were taken. These images, along with a
case history, were sent electronically to a pathologist at the University of Guelph (Ontario, Canada) for a morphological diagnosis. Calves that died as a result of a known problem such as physical injury or were culled from the herd did not have a necropsy performed. If necropsy results were not available, staff-designated clinical findings and diagnosis were used as the most likely cause of death.

6.3.4 Statistical Analysis

All analyses were performed using SAS statistical software (SAS Institute Inc., Cary, NC). Descriptive statistics and residual diagnostics were performed using Proc Freq and Proc Univariate procedures. Regression analyses were conducted using a mixed linear model (Proc Mixed) for height and weight at exit from the weaning barn, and a generalized linear mixed model with a logit-transform (Glimmix) was used to analyze the probability of BRD in the first 60 d post-enrollment. The effects of treatment and BRD on weight and height were modeled separately. The models for weight controlled for enrollment weight and age at exit measurement. The models for height controlled for enrollment height and age at exit measurement. Clustering by weekly enrollment cohort and farm of origin were included in all models as random effects. Disease variables considered for analysis are described in Table 6.1. In preliminary analysis, pre-enrollment disease events were divided into 2 time periods, with the disease occurring either 2 wk before enrollment (between weaning and enrollment) or from birth to 2 wk before enrollment (weaning). Variables that were significant were found to be of similar magnitude and direction in the model; as such, these time periods were combined into one pre-enrollment category. All variables that were potentially associated \( (P < 0.2) \) with the outcomes in univariate analyses were included in the final model and removed by backward step-wise elimination based on partial \( F- \)
tests, or multiple partial $F$-tests, with a $P$-value < 0.05. Confounding was assessed if the exclusion of a variable changed the remaining parameter estimates by 30%. Disease variables were tested for potential interactions with treatment. All data were assessed for the assumption of normality. Weight and height models did not include calves that died before their final measurement.

6.4 RESULTS

Three calves were removed from all analysis. One TUL calf was diagnosed and treated for BRD at 1 d post-enrollment, which violated the protocol. One TET calf and one TUL calf were removed from analysis because they were more than 80 d of age at the time of enrollment on the trial. In total, 1,392 animals were included in the analysis of final outcomes.

Calves arrived at the trial facility at $3 \pm 2.1$ (mean $\pm$ SD) d of age and were $56 \pm 7$ d of age at enrollment. At enrollment, calves weighed $71 \pm 11$ kg and were $90 \pm 4$ cm at the withers. Each week, $40 \pm 11$ calves were enrolled in the trial. Pre-enrollment, 63.7% (887/1,392) of calves were treated for scours, 59.1% (822/1,392) were treated for otitis media, 14.3% (199/1,392) were treated for BRD, and 1.7% (24/1,392) were treated for lameness. Before enrollment, 73.3% (147/199) of calves treated for pre-enrollment BRD were also treated for otitis media. Of the calves treated for both otitis media and BRD pre-enrollment, 74.0% (108/147) were treated for otitis media before BRD. Age, weight, and height at enrollment, and pre-enrollment diseases were not significantly different between TET and TUL calves.

6.4.1 Incidence of BRD in the 60 d Post-Movement

Risk of treatment for BRD for the first 60 d following enrollment was evaluated for 1,392 calves. In the 6 wk after enrollment, the frequency of treatment for BRD was 22.4% (156/695) in
the TET calves and 13.2% (92/697) in the TUL calves. The mean time of treatment of BRD was 24.7 ± 13.4 d after enrollment and was similar ($P = 0.58$) among calves in the TET and TUL groups. The model of the outcome BRD included 3 significant fixed effects: treatment, pre-enrollment lameness, and pre-enrollment average daily gain (Table 6.2). There were no significant interaction terms. Controlling for the other variables in the model, TET calves were 2.0 times (95% CI: 1.5 to 2.6) more likely to be treated for BRD in the 60 d following enrollment than were TUL calves ($P < 0.0001$). There was a tendency for calves treated for preweaning lameness to be 2.3 times (95% CI: 0.9 to 6.0) more likely to be treated for BRD ($P = 0.07$). As pre-enrollment average daily gain increased, risk of BRD decreased ($P < 0.005$). For example, a calf with a pre-enrollment average daily gain of 0.5 kg/d was 1.5 times more likely to be treated for BRD than a calf with a pre-enrollment average daily gain of 0.75 kg/d.

### 6.4.2 Growth in Postweaned Dairy Calves

Twenty-one calves died before being weighed 6 wk after enrollment. Therefore 1,371 calves had exit weights and heights and were included in this analysis. The data were analyzed to determine the effect of treatment and the effect of BRD on the weight and height of calves upon exiting the weaning barn, 6 wk after enrollment. Overall, calves gained 37.3 ± 11.8 kg during the 6 wk post-enrollment and grew on average 8 ± 0.1 cm.

The effect of treatment on weight after exiting the weaning barn had a significant interaction with pre-enrollment BRD. If calves were not treated for BRD pre-enrollment, the TUL treatment increased the exit weight of calves by 4.9 ± 0.5 kg relative to TET calves ($P < 0.001$; Table 6.3). If calves were treated for BRD in the pre-enrollment period, treatment had no effect on BW ($P = 0.38$).
The effect of treatment on height after exiting the weaning barn had significant interactions with pre-enrollment scours and pre-enrollment BRD. Among calves with no history of BRD, TUL increased the exit height of calves by 0.8 ± 0.1 cm ($P < 0.001$) and, among calves with no history of preweaning scours, TUL increased the exit height of by 0.7 ± 0.3 cm ($P < 0.05$) compared with TET calves (Table 6.4). Treatment had no significant effect on calves with a history of pre-enrollment scours or pre-enrollment BRD.

Bovine respiratory disease in the pre-enrollment or post-enrollment period affected the weight of calves when they exited the weaning barn. Accounting for weight at enrollment, age at measurement, and random effects of source farm and enrollment cohort, pre-enrollment BRD decreased the exit weight by 2.9 ± 0.7 kg ($P < 0.001$) and post-enrollment BRD decreased exit weight by 7.9 ± 0.6 kg ($P < 0.001$).

Bovine respiratory disease in the pre-enrollment or post-enrollment period decreased the height of calves when they exited the weaning barn. Accounting for height at enrollment, age at measurement, and random effects of source farm and enrollment cohort, pre-enrollment BRD decreased the exit height by 0.6 ± 0.2 ($P < 0.005$) and post-enrollment BRD decreased the exit height by 1.2 ± 0.2 ($P < 0.001$).

### 6.4.3 Death Loss

Calves were followed for 60 d following enrollment. During this time, 27 calves (1.9%) were removed from the herd because of mortality or culling. Assignment to TUL or TET groups was not associated with mortality, in that 11 and 16 calves from the TUL and TET groups were removed, respectively ($P = 0.33$). The cause of death for these calves was most frequently attributed to BRD (Table 6.5). Ten calves were examined with a digital necropsy because of
suspected respiratory disease or unknown cause of death. Of these cases, BRD was determined to be the most likely cause of death in 9 of the 10 cases, with one case of probable enteritis. Morphologically, bronchopneumonia was present in 8 out of 9 cases, and there was one case of probable septicemia due to fibrinous pleuritis. Of the 8 cases of bronchopneumonia, *Mycoplasma bovis* was the most likely etiologic agent in 3 of the cases; the remaining 5 cases were diagnosed as having a nonspecific bacterial cause based on the gross appearance of the lesions. Other than BRD, deaths were attributed to injury, joint infection, or bloat. Other calves were sold because of poor growth and conformation. Death or removal from the farm occurred at 38.7 ± 2.7 d after enrollment and did not differ by treatment (*P* = 0.51).

### 6.5 DISCUSSION

This study evaluated a novel approach to reducing the incidence and effect of BRD in dairy calves, examining the effect of a long-acting prophylactic treatment relative to a shorter acting prophylactic treatment, which is a practice that has been examined primarily in beef cattle on arrival in feedlots. The time period immediately following movement from individual to group housing is particularly critical for dairy calves. At this time, there is an increased exposure to pathogens, an immature immune system, and many social, environmental, and dietary changes. All of these stressors put dairy calves at increased risk for the development of BRD during this time period, similar to beef calves arriving in feedlots.

The objective of this study was to compare the effect of administering a long-acting antibiotic, tulathromycin, at the time of movement to group housing on the incidence of BRD and effects on growth in dairy calves relative to a shorter acting antibiotic, oxytetracycline. The study was designed as a positive control study. It should be noted that the positive control used in
this study involved continuing with an existing practice at the study farm of administering oxytetracycline around the time of weaning to all calves. The dose of oxytetracycline used was derived from the previously established program and was continued into the current study. Calculation of the dosage of oxytetracycline that would be appropriate based on the weights of calves in this study indicates that the dose of TET was lower than is considered to be optimal. As such, the underdosing of oxytetracycline may not represent the effects that would be expected with a full dose.

The National Animal Health Monitoring and Surveillance (NAHMS) reports on dairy heifers found that 5% of postweaned heifers in the United States were treated for BRD in 2002, and 6% were treated in 2007 (USDA, 2005, 2007b). However, the NAHMS study defined postweaned heifers as including all animals from weaning to first calving. The wide age variation encompassed by this definition would include many older animals that were at low risk of BRD. Preweaned calves have reported rates of disease ranging from 0.1 cases/100 d in Minnesota (Sivula et al., 1996) to 39% of calves in Saskatchewan (Van Donkersgoed et al., 1993). A study of herds in New York found that 25% of calves in the first 3 mo of life were treated for BRD (Virtala et al., 1996). This substantial variation in the reported rates of disease probably results from a combination of the differences in the diagnostic criteria used, age of the calves, and management conditions. In large beef feedlots, 15.5% of cattle are treated for BRD, and on small beef feedlots, 8.7% of cattle are treated for BRD (USDA, 2000). During the course of this study, 22% of calves in the TET group developed BRD, which is consistent with the above observational studies of dairy calves but is higher than what is found in beef feedlots. The
elevated incidence of respiratory disease in postweaned dairy calves relative to feedlot cattle stresses the importance of finding effective techniques to manage this disease.

The results of this study indicate that calves in the TUL group were half as likely to be treated for BRD as calves in the TET group during the 60 d following movement. However, treatment did not affect mortality in the 60 d following movement. This finding may be a result of several factors. First, this was a positive control study so both groups of calves were receiving antibiotic therapy. Second, the overall mortality rate was low and if a difference between the 2 treatment groups did exist, the sample size would not be sufficient to detect it. Finally, given the long-term increased risk of death associated with clinical BRD documented by Waltner-Toews et al., (1986b), it is also possible that any difference in mortality risk between groups would not be seen until animals are older.

The failure of calves treated for pre-enrollment BRD to respond to TUL with improved growth indicates that this treatment does not compensate for an earlier occurrence of the disease. This may be because of the effect that BRD has already had on the calf’s future health and performance. Despite its high incidence, otitis media was not a significant risk factor for post-enrollment BRD or decreased growth in this population of calves. There may be several reasons for this, including a program for prompt detection and treatment of otitis media on this farm that may have limited the effect of this disease. In addition, given that most calves treated for pre-enrollment BRD were also treated for otitis media, the effect of otitis media may have been accounted for when pre-enrollment BRD was included in the model. In this study, the implementation of the prophylactic therapy program at the time of movement and grouping is of the greatest benefit for calves that do not have a history of BRD before enrollment. Therefore, it
is important to emphasize that the health of pre-enrollment calves should be closely monitored to minimize this disease in the pre-enrollment period as well.

Among calves that were not treated for BRD in the pre-enrollment period, at 6 wk post-enrollment, calves in the TUL group weighed more and were taller compared with calves in the TET group. This further supports the hypothesis that TUL calves had a decreased incidence of BRD. A previous study documented a decrease in average daily gain of 0.14 g/d in calves treated for BRD during their third month of life and a decreased height of 0.2 cm for each week of BRD (Virtala et al., 1996). When this value is converted to a 6-wk time period, it becomes a loss in potential weight of 5.9 kg and potential height of 1.2 cm. In this study, calves with BRD had lower weight (7.8 ± 0.5 kg) and height (1.3 ± 0.2 cm). Calves in the TUL group showed improved weight gain of 4.8 ± 1.2 kg and height of 1.5 ± 0.3 cm compared with the TET group. The results of the current study indicate that BRD has a highly detrimental effect on the growth of dairy calves and that the administration of TUL at the start of this high-risk period can decrease the incidence of the disease and mitigate the negative outcomes associated with it. Knowledge of the benefits and limitations of a strategic antimicrobial intervention would be beneficial when management efforts are unable to overcome the risk of BRD. In addition, this approach would be beneficial to protect calves that are at high risk of BRD while contributing factors such as housing and pre-weaning disease are being addressed by farm management.

In conclusion, the use of TUL at the time of movement to group housing had a beneficial effect on the health and performance of dairy calves compared with treatment with TET, primarily through the prevention of respiratory disease in the period immediately following movement from individual to group housing.
6.6. ACKNOWLEDGMENTS

The authors acknowledge Jeff Caswell (Department of Pathobiology, University of Guelph, Ontario, Canada) and the management and staff at CY Heifer Farm (Elba, NY) for their invaluable contribution to this research. Funding was provided by Pfizer Animal Health (New York, NY) and the National Science and Engineering Research Council of Canada (Ottawa, Ontario).
6.7 REFERENCES


Table 6.1: Disease variables considered in models for weight, height, and risk of bovine respiratory disease (BRD)

<table>
<thead>
<tr>
<th>Disease event</th>
<th>Clinical Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRD</td>
<td>Dull and listless with a rectal temperature &gt; 39.5°C, and/or elevated respiratory rate and/or nasal discharge</td>
</tr>
<tr>
<td>Scours</td>
<td>Feces are soft and do not hold form</td>
</tr>
<tr>
<td>Otitis media</td>
<td>One or both ears are drooping or head is tilted and calf is unable to correct position</td>
</tr>
<tr>
<td>Lameness</td>
<td>Abnormal gait with unknown cause</td>
</tr>
<tr>
<td>Navel ill</td>
<td>Navel is swollen or tender in newborn calf</td>
</tr>
</tbody>
</table>
**Table 6.2:** The probability of respiratory disease in the 6 wk after movement in 1,392 weaned dairy calves, randomly assigned to treatment with tulathromycin (TUL) or oxytetracycline (TET) at the time of movement to group housing

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>SE</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>−0.44</td>
<td>0.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment with TUL (relative to TET)</td>
<td>−0.68</td>
<td>0.15</td>
<td>0.51</td>
<td>0.38–0.68</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pre-enrollment lameness (relative to calves without lameness)</td>
<td>−0.85</td>
<td>0.47</td>
<td>2.35</td>
<td>0.93–5.94</td>
<td>0.07</td>
</tr>
<tr>
<td>Weight gain pre-enrollment (kg/d)</td>
<td>−1.53</td>
<td>0.57</td>
<td></td>
<td></td>
<td>0.007</td>
</tr>
</tbody>
</table>

1 Logistic regression model, accounting for source farms and weekly enrollment cohort with random effects.
Table 6.3: Least squares means (± SEM) of the weight (kg) of commercial heifers recorded upon exit from the weaning barn controlling for age at final measurement and weight at enrollment with random effects for source farm and weekly enrollment cohort

<table>
<thead>
<tr>
<th>Preweaning BRD</th>
<th>n</th>
<th>Treatment</th>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TUL</td>
<td>TET</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>190</td>
<td>105.5 ± 1.4</td>
<td>104.4 ± 1.7</td>
<td>0.38</td>
</tr>
<tr>
<td>No</td>
<td>1,181</td>
<td>110.6 ± 1.1</td>
<td>105.7 ± 1.1</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

1 Bovine respiratory disease.
2 Treatment with tulathromycin (TUL) or oxytetracycline (TET) at the time of movement to group housing.
Table 6.4: Least squares means (± SEM) of the height (cm) of commercial heifers upon exit from the weaning barn controlling for age at final measurement and height at enrollment with random effects for source farm and weekly enrollment cohort

<table>
<thead>
<tr>
<th>Health Event</th>
<th>N</th>
<th>Treatment</th>
<th></th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TUL</td>
<td>TET</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preweaning scours</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>872</td>
<td>97.8 ± 0.3</td>
<td>97.7 ± 0.3</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>499</td>
<td>98.3 ± 0.4</td>
<td>97.6 ± 0.4</td>
<td>&lt; 0.005</td>
<td></td>
</tr>
<tr>
<td>Preweaning BRD²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>190</td>
<td>97.5 ± 0.4</td>
<td>97.5 ± 0.4</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1,181</td>
<td>98.6 ± 0.3</td>
<td>97.8 ± 0.3</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

¹ Treatment with tulathromycin (TUL) or oxytetracycline (TET) at the time of movement to group housing.
² Bovine respiratory disease.
Table 6.5: Reasons for death loss and culling in the 60 d following movement to group housing

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>BRD confirmed by digital necropsy</th>
<th>BRD diagnosed by farm staff (unconfirmed)</th>
<th>Other</th>
<th>Total deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>TET</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>TUL</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>11</td>
</tr>
</tbody>
</table>

1 Treatment with tulathromycin (TUL) or oxytetracycline (TET) at the time of movement to group housing.
2 Bovine respiratory disease.
Figure 6.1: Timeline of trial events
CHAPTER 7: THE IMPACT OF BOVINE RESPIRATORY DISEASE AND A PREVENTATIVE ANTIBIOTIC TREATMENT ON GROWTH, SURVIVAL, AGE AT FIRST CALVING, AND PRODUCTIVITY HEIFERS

7.1 ABSTRACT

Bovine respiratory disease complex (BRD) is a common disease in weaned dairy calves that incurs economic and welfare costs. This study was an extension of a randomized clinical trial in which a single injection of tulathromycin (TUL) or oxytetracycline (TET) was administered at first movement to group housing for the prevention of BRD in the 60 days following antimicrobial treatment (BRD60). Calves treated with TUL were 0.5 times (95% CI: 0.4 to 0.7) as likely to be treated for BRD60 as calves treated with TET. The objectives of the current study were to evaluate the long-term effects of BRD and antibiotic treatment on growth of heifers until breeding age, age at first calving, incidence of dystocia, milk production and mortality prior to first calving and mortality prior to 120 days in milk. Body weight and average daily gain were analyzed using generalized linear mixed models (Proc Mixed, SAS) controlling for source farm and enrolment cohort as random effects. Survival to calving and calving prior to 25 months were analyzed using mixed models with binary distribution and logit link function (Proc GLIMMIX) in SAS (Version 9.1). A p-value of less than 0.05 was considered to be statistically significant. At entry to the breeding barn (382 days of age) calves that experience BRD60 weighed 16.0 ± 2.3 kg less than calves that did not. For TET and TUL calves, calves without BRD60 were 3.4 {Confidence Interval: 2.2-5.2} and 1.7 {CI:1.0-2.9} times more likely to survive to first calving compared to BRD60 calves, respectively. BRD60 calves were 0.6 (95% CI: 0.4-0.8) times as likely to calve before 25 months of age and 1.5 (95% CI: 1.1-2.2) times more likely to have a calving ease score ≥ 2 at their first calving. BRD may affect first test
milk production depending on farm management factors, but had no effect on projected 305 day milk production. BRD has many long-term effects on the productivity of heifer calves. The administration of TUL at movement to group housing may have a role in the prevention of BRD and mediating some of the long-term effects of this disease.
7.2 INTRODUCTION

Bovine respiratory disease complex (BRD) is of great concern due to the high economic and welfare costs associated with this condition. According to the 2007 U.S. National Animal Health Monitoring Survey (NAHMS) the incidence of post-weaning respiratory disease in dairy heifers was 5.9%, and was the predominant cause of reported deaths of weaned heifers (46.5%) (USDA, 2010). This disease is often observed following first movement to group housing after weaning (McGuirk, 2008). Risk factors for BRD include concurrent nutritional, environmental and social changes, as well as increased disease exposure from mixing with older animals for the first time.

Severe, undetected or untreated BRD may result in death. Prompt therapy should result in a low case fatality risk, but this disease can represent an economic drain for producers. In the short-term, costs of the disease include labor for detection and treatment of disease, drugs, veterinary fees and reduced performance and cost to replace animals that are died as a result of clinical disease. Costs of this disease were estimated at $14.71 (range: 0 - $119) per pre-weaned calf per year and $1.95 (range: 0-$9.25) per weaned dairy calf stock per year by Michigan producers in 1990 (Kaneene and Hurd, 1990). The higher cost observed in young calves is based on the timing of preventive practices, which primarily are administered to calves prior to disease outbreaks, and the long follow-up time for calf stock which included an extended period of low disease risk. However, the long-term costs of BRD are likely underestimated, since generally poor disease records available for heifers make it difficult for producers to associate BRD with outcomes many months later. Costs of long-term effects were estimated between 18-57 euros ($15-49 U.S.) in a partial budget of the cost of BRD on a typical Dutch dairy farms, in which 60%
of dairy heifers were affected by mild to chronic BRD (van der Fels-Klerx et al., 2001). However, the effects of BRD on growth, survival and production were based on a small number of studies examining these factors. These studies have documented different long-term costs include increased risk of mortality prior to calving (Waltner-Toews, et al, 1986), decreased growth (Virtala et al, 1996), later age at calving (Waltner-Toews, et al, 1986; Correa et al, 1988) and increased risk of dystocia (Warnick et al, 1994). Warnick et al, (1995; 1997) found no association of calfhood BRD with first lactation milk production or survival after first calving. However, calves with more than 4 treatments for BRD prior to calving were 2 times more likely to survive first lactation compared to calves with no BRD(Bach, 2011). BRD in beef feedlots is associated with decreased ADG, marbling, meat quality, and subcutaneous fat cover and increased mortality (Smith, 1998; Reinhardt et al., 2009; Schneider et al., 2009). There is a need re-examine the effects of BRD on dairy heifer growth, age at first calving, dystocia, mortality and milk production in first lactation due to the likely changes in management practices, BRD treatments and animal genetics.

This study is an extension of a randomized clinical trial, in which a single injection of tulathromycin (TUL) or oxytetracycline (TET) was administered to dairy heifers at first movement to group housing for the prevention of BRD in the 60 days following antimicrobial treatment (BRD60) (Stanton et al., 2010). Briefly, 1,392 calves, raised in a commercial heifer raising facility were enrolled at first movement to group housing when they were 8 weeks of age. A total of 248 calves were treated for BRD in the 60 days following enrollment. Calves assigned to the TUL group were 0.5 times (95% CI: 0.4 to 0.7) as likely to be treated for BRD in the 60
days following study enrolment than calves assigned to the TET group. That study was restricted to the first 60 days following movement to group housing.

The objectives of the present study were to evaluate the long-term effects of BRD and antimicrobial treatment performance. Specifically, growth of heifers until breeding age, age at first calving, incidence of dystocia, milk production and mortality prior to first calving and mortality prior to 120 days in milk were examined.

7.3 MATERIAL AND METHODS

7.3.1 Heifer Management and Housing

Heifers enrolled in this study (1,392) were reared at a commercial heifer raising facility in Western New York. They arrived at the facility from 7 separate source farms at an average age of 3 ± 2.1 (mean ± SD) days of age. Calves were enrolled following movement from individual housing in hutches or nursery barns during the milk-fed period to group housing post-weaning. The average age at enrolment was 56 days of age. Calves were randomly assigned to treatment with either 2cc of tulathromycin (TUL) or 5cc of oxytetracycline (TET). Calves were monitored for disease for 60 days following study enrollment. The definition of BRD was dullness, decreased appetite or listlessness, with at least one of the following; rectal temperature > 39.5 °C, nasal discharge, or elevated respiratory effort. Bovine respiratory disease was diagnosed in 13.2% (92/697) of TUL calves and 22.4% (156/695) of TET calves during the 60 days following first movement to group housing.

All heifers remained at the heifer raising facility until they were confirmed pregnant. After confirmation of pregnancy, heifers were eligible to return to their source farm and did so.
based on the preset preferences of the original source farm. All heifers were returned to their
original source farms unless they died or were culled prior to calving, (Figure 7.1).

7.3.2 Outcome Measures

Body weight, height and average daily gain

All calves were monitored daily in the nursery, weaning and transition barns by trained
barn staff for the occurrence of disease, using consistent case definitions. All disease events and
treatments were recorded in the heifer farm management database (DairyComp 305, Valley
Agricultural Software Inc., Tulane, CA.) prior to returning to the source farms. When heifers
were returned to their source farms to calve and to enter the milking herd, all disease, production,
calving and mortality information were recorded by the farms’ staff using DairyComp 305.

Weights and heights of calves were measured at standard times using an electronic scale
(Tru-test - ID3000, Tru-Test Incorporated, Mineral Wells Texas) as part of the standard
operating procedure of the commercial heifer rearing facility. Height at the withers (to the
nearest inch) was measured at the same time as weight.

Measurements were taken at entry into the following barns; Nursery Barn (mean ± s.d.
age in days; 3 ± 2), Weaning Barn (56 ± 7), Transition Barn (98 ± 8), Grower Barn 1 (181 ± 13),
Grower Barn 2 (269 ± 31) and Breeding Barn (382 ± 33).

Survival to first calving

Survival was deemed to occur if heifers calved in their original source herd.
**Age at First Calving**

Since age at first calving is not normally distributed, median and quartiles are presented to describe the age at first calving for the population. The outcome age at first calving was ultimately divided into two categories, heifers that calved up to and including 25 months of age and those that calved after 25 months of age. This is based on the widely used economic target of a calving age of 24 months with an additional month added to allow for minor deviations from this goal (Le Cozler et al., 2008).

**Dystocia**

Calving ease was scored in accordance with the standard 5 point system of the National Dairy Herd Information Association (DHIA) as part of the source farms’ standard practices (Table 7.1). Cutpoints of calving ease scores \( \geq 2, \geq 3, \geq 4, \) and \( \geq 5 \) were independently assessed as potential indicators of dystocia since there is no validated cutpoint for dystocia using on this calving ease scale.

**Survival to 120 days in milk**

Survival to 120 days in milk was deemed to occur if heifers were present in their source herd for \( \geq 120 \) days after their first calving.

**Milk Production**

Regular DHIA milk testing was performed at monthly intervals on 4 of the 6 farms and every other month on the remaining farms. First test milk production and third test projected 305- day milk production were collected for the 4 farms using monthly testing. First test milk production and second test projected 305 –day milk production were collected for the 2 farms
that were testing every other month in an attempt to standardize days in milk at test days among the 6 farms.

**Culling and Mortality**

The causes of culling and mortality were entered into DairyComp305 by the staff of the heifer raising facility or of the individual source farms, depending on the location of the animal at the time the determination was made. Producer-attributed cause of death or culling was recorded as respiratory disease, injury, other, unknown. Culling was divided into the following categories based on the primary reason given by producers: respiratory, other diseases, sold for dairy, reproduction, injury, poor growth, other.

**7.3.3 Statistical Analysis**

All analyses were conducted using SAS statistical software Version 9.1 (SAS Institute Inc., Cary, NC). For all models, statistical significance was based on a p-value ≤ 0.05.

Season was offered to all models as a fixed effect. Season was divided into three periods based on a visual assessment of daily temperature recordings obtained from the Rochester, New York Weather Station (Weather Underground, 2010) (Appendix 1): *Moderate* (5 ± 4 °C (mean ± standard deviation); November 1\textsuperscript{st}, 2006 to January 15\textsuperscript{th}, 2007); *Cold* (-2 ± 7 °C: January 16\textsuperscript{th} to April 15\textsuperscript{th}, 2007); *Warm* (18 ± 6 °C; April 16\textsuperscript{th}, 2007 to the end of the trial in July 2007). The Rochester Weather Station is located approximately 65 km from the heifer raising facility.

*Body weight, height and average daily gain*

The impacts of antimicrobial treatment and BRD60 on body weight and height were evaluated separately at each management time point, corresponding to entry into Rearing Barns.
(Weaning, Transition, Grower 1, Grower 2, and Breeding). The impact of antimicrobial treatment and BRD60 on ADG were evaluated separately for each time period corresponding to time in the Rearing Barns (Weaning, Transition, Grower 1, and Grower 2). In addition, the impact of antimicrobial treatment and BRD60 on ADG between the Weaning Barn and the Breeding was evaluated. The impact of antimicrobial treatment on ADG, weight and height was evaluated separately from BRD60 since BRD60 is an intervening variable for antimicrobial treatment and growth.

Due to inconsistent recording of breeding barn exit weights, the ADG of heifers in the breeding barn were not analyzed. Body weight, height and ADG were evaluated using linear regression mixed models (PROC MIXED) with random effects for source farm and weekly enrolment cohort. The models for body weight controlled for birth weight, pre-enrollment average daily gain (pADG), pre-enrollment respiratory disease (pBRD), season and age at entry into the rearing barn. The models of height controlled for height at birth, pBRD, and age at entry into the barn. The models of ADG controlled for birth weight, pBRD, pADG, season and age at exit from barn.

**Dystocia, survival to first calving, and first lactation survival (120 days)**

First lactation survival to 120 days following calving, as well as dichotomous outcomes for dystocia at various cutpoints, age category at first calving and survival to first calving, were analyzed using mixed models with binary distribution and logit link function (Proc GLIMMIX). Source farm and weekly enrollment cohort were included as random effects. Antimicrobial treatment, BRD, pBRD and season were included as fixed effects. In addition, the association between age at first calving and calving ease with first lactation survival was modeled, without
BRD or antimicrobial treatment, since these are intervening variables between BRD and survival.

*Age at First Calving*

Time to first calving was analyzed using a Cox Proportional Hazard survival analysis model (PROC tphreg), controlling for source farm and group as random effects, and using a non-parametric estimate of the survival distribution function (PROC Lifetest).

*Milk production*

Milk production, first test DHIA milk weight and 305d milk, were analyzed using linear regression mixed models (PROC MIXED) with random effects for source farm and weekly enrolment cohort. Two models were built for milk production. The first model evaluated the impact of BRD60 and antimicrobial treatment on milk production, and the second model evaluated age at first calving and calving ease score. These two sets of predictors were modeled separately since age at first calving and calving ease score are intervening variables for BRD60. The models for milk production controlled for days in milk at DHIA testing and source farm as fixed effects. Source farm was included as a fixed effect in this model to determine if management factors were interacting with milk production. Residual diagnostics and normality was assessed using Proc Univariate.

**7.3.4 Exclusion Criteria**

One source farm did not provide records after return to the farm for calving. Animals from this farm were excluded from analysis of survival to first calving and from analyses of all events that occurred after calving. These calves were included in analysis of average daily gain.
The excluded source farm resulted in a loss of 3% of calves from the original database (48/1393).

**7.4 RESULTS**

The weights and heights of calves upon entry to the six rearing barns are presented in Table 7.2. Four heifers were not weighed or measured at one of the measurement time points (transition barn (n = 1), grower barn 1 (n = 1) and grower barn 2 (n = 2)) and were excluded from all analyses of ADG, weight and height that require the use of this data.

**7.4.1 Average Daily Gain**

Effects of BRD60 on ADG are presented in Table 7.3. BRD60 was associated with a 0.16 ± 0.01 kg decrease in ADG in the weaning barn (P < 0.001). Similarly, BRD60 was associated with reduced ADG in the Transition Barn and Grower Barn 1. Overall, BRD60 was associated with a decrease in ADG between the weaning barn and the breeding of 0.03 ± 0.01 kg (P < 0.001).

An antimicrobial treatment and pBRD interaction was significant in the weaning and transition barns, (P < 0.005 and P=0.05, respectively). The interaction between antimicrobial treatment and pBRD did not have a significant effect on ADG in Grower Barn 1 or Grower Barn 2, (P = 0.51 and P = 0.25, respectively). In the weaning barn, calves without pBRD gained 0.97 ± 0.01 kg/day and 0.86 ± 0.01 kg/day in the TUL and TET group, respectively (P < 0.001). In the transition barn, calves without pBRD gained 0.98 ± 0.01 kg/day and 0.96 ± 0.01 kg/day in the TUL and TET group, respectively, (P = 0.03). In calves with pBRD, antimicrobial treatment had no effect on ADG, (P = 0.57).
7.4.2 Body weight

Calves treated for BRD60 had lower body weights starting at entry into the transition barn (3.5 months) and continuing until the last measurement at entry into the breeding barn, \( P < 0.001 \); Figure 7.2). The maximum difference in body weight observed between BRD60 and non-BRD60 calves was 15.4 ± 2.3 kg upon entry to the grower barn 2 at 269 days of age \( P < 0.001 \).

Antimicrobial treatment and pBRD interactions were significant for all weights taken after entry into the transition barn, \( P < 0.05 \). For calves with no pBRD, the greatest difference in body weight observed between TUL and TET calves was 7.3 ± 1.4 kg, which occurred at entry into Grower Barn 2 at 269 days of age \( P < 0.001 \); Figure 7.3).

7.4.3 Height

Calves treated for BRD60 were 1.3 ± 0.2, 2.0 ± 0.2, 1.5 ± 0.2, and 1.6 ± 0.4 cm smaller than calves without BRD60 when measured at entry into the Transition Barn, Grower Barn 1, Grower Barn 2 and Breeding Barn, respectively \( P < 0.001 \).

In grower barn 1 and breeding barn, antimicrobial treatment and pBRD interacted \( P = 0.01 \) and \( P = 0.0003 \), respectively). For calves without pBRD, TUL calves were 1.0 ± 0.2 cm taller than TET calves in grower barn 1, \( P < 0.001 \); Table 7.4). In grower barn 2, pBRD and antimicrobial treatment were not significant \( P = 0.61 \) and TUL calves were 1.1 ± 0.3 cm taller than TET calves \( P < 0.001 \).

7.4.4 Survival to First Calving

One heifer returned to her source farm but was lost to follow-up prior to calving. A total of 1,088/1343 (81%) heifers survived to first calving at their source farms. Only 66% (158/238)
of calves with BRD60 survived to first calving and 84% (931/1105) of calves without BRD60 survived to first calving. Survival to first calving was 83% (559/676) and 79% (530/667) for TUL and TET calves, respectively. Of the 237 calves with BRD60, 73% (64/88) of the TUL calves survived to first calving and 62% (93/149) of the TET calves survived to first calving.

Survival to first calving was affected by BRD60 and antimicrobial treatment with a significant interaction between antimicrobial treatment and BRD60, $P < 0.03$. For TET and TUL calves, calves without BRD60 were 3.4 (2.2-5.2) and 1.7 (1.0-2.9) times more likely to survive to first calving compared to BRD60 calves, respectively. Calves treated for BRD60 were 2.0 (95% CI: 1.1-3.6) times more likely to survive to first calving if they had received TUL at movement to group housing compared to TET. Antimicrobial treatment did not have an effect on the probability of non-BRD60 calves surviving to first calving, (OR: 1.0; CI: 0.7-1.5, $P = 0.80$). Survival to first calving by source farm is reported in Table 7.5. There was no interaction of farm with the effect of BRD60 on survival.

7.4.5 Culling and mortality prior to first calving

Of the 255 heifers that did not survive to first calving, 36% (93/255) died and 64% (162/255) were culled. The primary reason for mortality was BRD, with 47% (44/93) of all deaths attributed this disease (Table 7.6). For the deaths attributed to BRD, 61% (27/44) were treated for BRD in the 60 days following movement to group housing with 34% (15/44) and 66% (29/44) of deaths due to BRD in the TUL and TET groups, respectively ($P = 0.04$). Poor reproduction was the primary reason for culling of heifers (Table 7.7).
7.4.6 Age at First Calving

Of the 1,088 heifers that returned to calve at their source farms, the median age at first calving was 703 (First and third quartile; 683-737) days. The median age at first calving was 714 (q1 - q3; 705-723) and 702 (699 - 706) days for BRD60 and non-BRD60 calves, respectively. Only 19% (205/1088) of heifers failed to calve prior to 25 months of age. The proportion of BRD60 and non-BRD60 heifers that failed to calve prior to 25 months of age was 27% (43/158) and 17% (162/931), respectively. Controlling for source farm, enrollment cohort and antimicrobial treatment, calves with BRD60 were 0.6 (0.4-0.8) times as likely to calve by 25 months of age ($P = 0.01$). There was no association ($P = 0.98$) of antimicrobial treatment with probability of calving by 25 months of age. Based on the results of the survival analysis, heifers with BRD60 were 0.8 (95% CI: 0.7-1.0) times as likely to calve as heifers without BRD60 ($P = 0.02$; Figure 7.4).

7.4.7 Dystocia

Calving ease scores were recorded for 98% (1066/1089) of heifers which calved at their source farms (Table 7.8). A calving ease score $\geq 2$ was recorded for 48% (509/1066) of heifers. Fifty-five percent of the heifers with BRD60 had a calving ease score $\geq 2$ (85/155) compared to 46% (424/911) of calves without BRD60. Controlling for source farm, enrollment cohort and antimicrobial treatment, calves with BRD60 were 1.5 (95% CI: 1.1-2.2) times more likely to have a calving ease score $\geq 2$ at their first calving than calves without BRD60 ($P = 0.03$). However, BRD60 was not associated with the probability of calving scores $\geq 3$ (Table 7.9). There was no association of antimicrobial treatment with the probability of a calving score $\geq 2$, $\geq 3$, or $\geq 4$ ($P = 0.71$, $P = 0.98$, and $P = 0.18$, respectively).
7.4.8 Milk Production

First Test Day Milk production

A total of 1,041 heifers had first DHIA test milk production. Eleven heifers were removed from this analysis due to abnormalities identified by the producer at the time of milk testing. There was a significant interaction of source farm with the effect of BRD60 on first test milk production ($P = 0.04$). For two of the farms, heifers with BRD60 had significantly lower milk production than heifers without BRD60 (Table 7.10). For one of the source farms, heifers with BRD60 had significantly higher milk production than heifers without BRD60 ($P = 0.05$). For the remaining three source farms there was no statistically significant difference in milk production.

Calving ease and age at first calving were both associated with milk production at first milk test, ($P = 0.1$ and $0.7$, respectively). Average first test milk production for calving ease scores 1,2,3,4 and 5 were $28.8 \pm 0.3$, $28.8 \pm 0.4$, $27.8 \pm 0.6$, $27.6 \pm 0.9$ and $26.0 \pm 1.2$ kg, respectively. Heifers with a calving ease score of 1 and 2 had significantly higher milk production at first test compared to heifers with a calving ease score of 5 ($P < 0.05$).

Projected 305 D milk production

A total of 1,019 cows had projected 305d milk. For every day age at first calving increased, 305d milk production decreased by $5.2 \pm 1.3$ (lsmeans ± S.E.M.) kg. BRD60 and antimicrobial treatment were not significantly associated with 305d milk production ($P = 0.75$ and $P = 0.46$, respectively). Calving ease score was not significantly associated with 305d milk production ($P = 0.92$).
7.4.9 Survival to 120 days in milk

Twenty-four animals (2% of total) were lost to follow-up or did not complete 120 days in milk. Of the animals that survived to first calving, 92% (1001/1064) survived to 120 days in milk. BRD60 and antimicrobial treatment did not significantly affect the survival to 120 days in milk, ($P = 0.94$ and $P = 0.36$, respectively). Age at first calving was not associated with post-calving survival ($P = 0.70$). Calving ease scores were recorded for 1,042 of the heifers that survived to their first calving. Calving ease score was associated with post-calving survival ($P = 0.04$). Heifers with a calving ease score of 5 were 0.2 (95% CI: 0.1-0.9) times as likely to survive to 120 days post-calving compared to heifers with a calving ease score of 1 ($P = 0.04$; Table 7.11).

7.5 DISCUSSION

This study evaluated the long-term effects of BRD under modern management conditions and the long-term effects of a novel approach to reducing the incidence and effect of BRD in dairy calves. Specifically, the effect of a long-acting prophylactic treatment was compared to a shorter acting prophylactic treatment. Respiratory disease has previously been documented to have many long-term effects on the health and productivity of dairy heifers. However, these studies have become dated and may not reflect current management practices.

The negative effects of BRD60 following movement to group housing on ADG were seen until approximately 9 months of age, or 7 months after movement to group housing. This difference in average daily gain resulted in a 15 ± 2 kg decrease in body weight for calves with BRD60 at entry into the breeding barn at this facility (approximately 382 days of age). This finding is consistent with previous studies which found that the number of days treated for
BRD before 6 months of age decreased ADG between 6 and 14 months by 0.02 kg/day (Donovan et al., 1998). However, direct comparison is not possible given the variation in follow-up time and the differences in outcomes. Donovan et al (1998) looked at number of days treated while this study assessed the presence or absence of a BRD event within the first 60 days of movement to group housing. Both of these studies indicate that BRD has a negative impact on the growth of dairy heifers for many months after initial treatment. This is inconsistent with findings from the beef feedlot industry that BRD does not have a significant long-term effect on ADG following an initial clinical phase (Thompson et al., 2006; Holland et al., 2010). This discrepancy may be due to differences in the stage of the animal’s physical development at onset of disease, or a difference in the type or incidence of pathogens. In the study by Holland et al., (2010), beef animals entered the study at 241 kg and they contracted respiratory disease in the pre-conditioning phase which occurred over the next 63 days. Following the pre-conditioning phase, there was no effect of BRD treatment on ADG. The disparity in size between the calves on the present study (enrolled at 71 kg) may indicate that that the age and weight of the animal at disease onset plays a role on the impact of BRD. If the majority of structural growth has already occurred at the onset of disease then the long-term impact of BRD may be reduced.

BRD60 decreased the height of heifers up to entry into the breeding barn by 1.6 ± 0.4 cm at approximately 382 days of age. The maximum observed difference in height between heifers with and without a recorded case of BRD60 was -2.0 ± 0.2 cm at approximately 180 days of age, upon entry into Grower Barn 1. This is consistent with the findings of Donovan et al (1998) that the impact of BRD on height gain is primarily seen in the first 6 months of life. However, as shown in this study, the effect on delayed growth in stature can remain beyond 1 year of life.
For calves without BRD prior to enrolment, calves treated with TUL at first movement to group housing had a $0.11 \pm 0.01$ and $0.02 \pm 0.01$ kg increase in ADG in the weaning and transition barns relative to calves treated with TET. This difference in ADG was not observed after calves left the transition barn. This suggests that TUL had a short-term effect on the ADG of dairy calves in calves with no history of BRD prior to antimicrobial treatment. This improvement in body mass was maintained until all surviving heifers entered the Breeding Barn at 382 days of age. For heifers with a history of BRD prior to enrolment, antimicrobial treatment had no effect on growth. This absence of antimicrobial treatment effect in calves with a prior history of BRD indicates that antimicrobial treatment does not compensate for an earlier disease occurrence, likely due to the effect BRD has already had on heifer health and performance. This indicates that treatment with TUL is most beneficial for animals that are at low risk of BRD pre-movement to group housing and high risk of BRD in the period following movement to group housing.

It should be noted that the comparison between antimicrobial treatments is limited by the discrepancies in dosages. The dosage of oxytetracycline was based on the previously established farm protocol of the commercial heifer raising facility. Calculation of the dosage of oxytetracycline that would be appropriate based on the weights of calves in this study indicates that the dose of TET was lower than is considered to be optimal. As such, the under dosing of oxytetracycline may not represent the effects that would be expected with a full dose (Stanton et al., 2010). However, the value of the reduced incidence of BRD and improved growth show a significant benefit of tulathromycin for improved prevention of clinical BRD and some of the long-term consequences of BRD.
BRD following movement to group housing had a negative effect on the survival of heifers to first calving. A reduction in survival to first calving has two main impacts on the productivity of a farm. It reduces the ability of producers to make genetic gains by reducing the pool of animals from which to select and retain replacements. Furthermore, for heifers that die there is a loss of the future value of the cow, as well as the wasted costs associated with rearing the animal up to its death without any financial return. As shown in this study, heifers with BRD60 were 0.3 to 0.6 times as likely to survive to first calving. This is consistent with the findings of Waltner-Toews, et al (1986) which found that heifers treated for BRD in the first 90 days of life were 2.5 times more likely to die between 90 days of age and calving. These two studies differ from Curtis et al, (1988) who found that respiratory disease was not associated with survival to calving. These differences may be due to the severity of the disease events considered. Curtis et al, (1988) included any BRD event regardless of treatment, whereas both this study and Waltner-Toews et al, (1986) only included calves that were ill enough to be treated.

Calves treated for BRD60 were 2.0 (95% CI: 1.1-3.6) times more likely to survive to first calving if they had received TUL at movement to group housing compared to TET (i.e. prior to disease occurrence). A possible explanation for this interaction is that the longer duration of activity of tulathromycin relative to oxytetracycline may have reduced the severity of the infection of BRD60 in some calves. However, the comparison between these two groups must be taken cautiously due to the lack of a true positive control in this trial. However, these results do suggest that further study is warranted on the effect of TUL administered around weaning on the survival of calves that developed BRD.
Age at first calving is an important factor for producers since it has a significant impact on the lifetime productivity of heifers (Le Cozler et al., 2008). In 1985, BRD was shown to increase the average age at first calving by Warnick et al (1994), who found that heifers with BRD in the first 3 months of life calved three months later than heifers without BRD. The present study found that heifers treated for BRD in the 60 days following movement to group housing were almost half as likely to calve prior to 25 months (OR: 0.6; CI:0.4- 0.8) than heifers without BRD. Waltner-Toews et al (1986) found no effect of BRD on age at first calving which may be due to in part from dichotomizing age at first calving using a cutpoint of 30 months, and a sample size which is approximately half that of the present study. In addition, it is likely that genetic potential of dairy animals and management practices in the dairy industry have changed since the 1980’s.

Dystocia is an important issue in the dairy industry. According to the 2007 NAHMS report, difficulty in calving occurred in 18% of heifers (USDA, 2009). Dystocia is most common in heifers, and is commonly associated with body weight at calving, age at calving and sex of the calf (Mee, 2008). In addition, calves born from dystocia events are more likely to be stillborn. Difficulty in calving can cause damage to the reproductive tract of the cow, prolong calving and can result in surgical intervention. Dystocia can increase the risk of retained placenta and displaced abomasum (Correa et al., 1993; Laven and Peters, 1996). Defining dystocia is challenging and subject to interpretation. In this study, various cutpoints were used on a standardized scale of calving ease scores to assess the impact of BRD on dystocia. BRD did not significantly affect dystocia rates if the calving ease cutpoints were ≥3. However, the small number of animals with calving ease scores ≥ 3 does present an issue with power. In this study
calves with BRD60 were 1.5 (95% CI: 1.1-2.2) times more likely to have at least mild dystocia (calving ease score ≥ 2). This is lower than in Warnick et al, (1994) who found that calves with BRD were 2.4 times more likely to have dystocia, but does add support to the association of calfhood respiratory disease with dystocia.

BRD60 decreased first test milk weight by 2.3 to 2.9 kg for 2 of the 6 farms. For 3 of the farms, the effect of BRD60 was not significant and on the remaining farm heifers with BRD60 had higher milk production (P = 0.05). This indicates that the effect of BRD60 in calves on early lactation milk production nearly 2 years later depends on management factors at the herd level. One potential reason for differences by farm is the rate at which BRD positive heifers were culled prior to entry into the milking herd. It is worth noting that the herd with a tendency for BRD60 to have a positive effect on milk production had only 54% of BRD60 calves surviving to first calving and 85% of calves without BRD60 surviving to first calving. This different rate in survival between BRD60 and non-BRD60 heifers on this farm increases the risk that only the superior and well performing animals are retained in this herd, impacting the ability to determine the association between BRD60 and milk production. This interaction of farm with BRD60 was not significant for the projected 305 day milk production. These results from 305 day milk production are consistent with a previous study which found no long term effects of BRD following entry into milk production (Warnick et al., 1995). This may be due to many factors including culling of low producers, a high level of variation in the estimate of 305d milk production and the large number of factors which influence longer term milk production.

Among heifers that calved, BRD60 did not affect survival to 120 days in milk. This could be due to many factors including selection for healthy animals, such that by the time animals
have calved the most severely affected animals had been removed from the herd. Further culling may occur after 120 days and bears further examination. Alternatively, dystocia significantly decreased survival to 120 days of milk. Therefore, BRD may indirectly increase the risk of a heifer being removed from the herd prior to 120 days in milk through its association with dystocia.

In conclusion, TUL results in improved growth post-enrolment for calves with no history of BRD, likely through the mechanism of prevention of subclinical disease. In addition, TUL has a modifying effect on the impact of BRD60 on survival to first calving which may indicate a reduction in the severity of clinical disease. BRD60 had several negative effects on the long-term productivity and welfare of dairy heifers including decreased growth, decreased survival to first calving, increased age at first calving, increased risk of dystocia, and depending on farm management perhaps a short-term impact on milk production.

7.6 ACKNOWLEDGEMENTS

The authors acknowledge Dr. Suzanne T. Millman and Tina M. Widowski, the staff and management of CY Heifer Farms (Elba, NY) and participating source farms for their invaluable contribution to his research. Funding was provided by Pfizer Animal Health (New York, New York) and the National Science and Engineering Research Council of Canada (Ottawa, Ontario)


Table 7.1: Calving Ease Scoring System recorded in farm DairyComp records and reported to DHIA

<table>
<thead>
<tr>
<th>Score</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No problem</td>
</tr>
<tr>
<td>2</td>
<td>Slight Problem</td>
</tr>
<tr>
<td>3</td>
<td>Needed Assistance</td>
</tr>
<tr>
<td>4</td>
<td>Considerable Force</td>
</tr>
<tr>
<td>5</td>
<td>Extreme Difficulty</td>
</tr>
</tbody>
</table>
Table 7.2: Weight and height of 1,392 calves upon entry in the 6 rearing barns at the commercial heifer raising facility (mean ± S.D.)

<table>
<thead>
<tr>
<th>Rearing Barn</th>
<th>N</th>
<th>Age (days)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nursery Barn (arrival)</td>
<td>1,392</td>
<td>3 ± 2</td>
<td>40 ± 5</td>
<td>80 ± 3</td>
</tr>
<tr>
<td>Weaning Barn</td>
<td>1,392</td>
<td>56 ± 7</td>
<td>71 ± 11</td>
<td>90 ± 4</td>
</tr>
<tr>
<td>Transition Barn</td>
<td>1,373</td>
<td>98 ± 9</td>
<td>109 ± 16</td>
<td>98 ± 4</td>
</tr>
<tr>
<td>Grower Barn 1</td>
<td>1,333</td>
<td>181 ± 13</td>
<td>190 ± 28</td>
<td>109 ± 4</td>
</tr>
<tr>
<td>Grower Barn 2</td>
<td>1,301</td>
<td>270 ± 34</td>
<td>273 ± 41</td>
<td>117 ± 4</td>
</tr>
<tr>
<td>Breeding Barn</td>
<td>1,271</td>
<td>382 ± 33</td>
<td>370 ± 39</td>
<td>126 ± 6</td>
</tr>
</tbody>
</table>
Table 7.3: Least-square means (± SE) of the average daily gain (kg/day) of 1,392 heifers recorded during housing in 4 barns and between enrolment and exit from Grower Barn 2, controlling for age at exit from each barn, birth weight, pre-enrolment average daily gain, pre-enrolment bovine respiratory disease and season with random effects for source farm and weekly enrolment cohort.

<table>
<thead>
<tr>
<th>Growing Period</th>
<th>BRD60-(^a)</th>
<th>BRD60+(^b)</th>
<th>Difference of least-square mean (BRD60-(^a) - BRD60+(^b))</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>(n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning Barn</td>
<td>0.91 ± 0.01</td>
<td>0.73 ± 0.02</td>
<td>0.16 ± 0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(1,133)</td>
<td>(240)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transition Barn</td>
<td>0.97 ± 0.01</td>
<td>0.90 ± 0.02</td>
<td>0.06 ± 0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(1,119)</td>
<td>(213)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grower Barn 1</td>
<td>0.93 ± 0.01</td>
<td>0.89 ± 0.02</td>
<td>0.04 ± 0.01</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td></td>
<td>(1,093)</td>
<td>(207)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grower Barn 2</td>
<td>0.87 ± 0.01</td>
<td>0.87 ± 0.02</td>
<td>&gt; 0.01 ± 0.01</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>(1,071)</td>
<td>(198)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between enrolment and</td>
<td>0.88 ± 0.01</td>
<td>0.84 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>exit from Grower Barn 2</td>
<td>(1,072)</td>
<td>(199)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)BRD60-: No clinical bovine respiratory disease complex in the 60 days following enrolment

\(^b\)BRD60+: treated for clinical bovine respiratory disease complex in the 60 days following enrolment
Table 7.4: Differences of least squares means (± SE) in the wither height (cm) between calves administered tulathromycin or oxytetracycline at enrolment at a commercial heifer raising facility. The linear mixed model controlled for age at final measurement, bovine respiratory disease prior to trial enrolment (pBRD), height at birth, season, and random effects for source farm and weekly enrollment cohort.

<table>
<thead>
<tr>
<th>Rearing Barn</th>
<th>P-value for interaction term</th>
<th>Difference between tulathromycin and oxytetracycline groups</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transition Barn</td>
<td>0.08</td>
<td>No pBRD 0.7 ± 0.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pBRD -0.1 ± 0.4</td>
<td>0.80</td>
</tr>
<tr>
<td>Grower Barn 1</td>
<td>0.01</td>
<td>No pBRD 1.0 ± 0.2</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pBRD 0.3 ± 0.5</td>
<td>0.60</td>
</tr>
<tr>
<td>Grower Barn 2</td>
<td>0.61</td>
<td>1.1 ± 0.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Breeding Barn</td>
<td>&lt; 0.005</td>
<td>No pBRD 0.5 ± 0.3</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pBRD -3.0 ± 0.9</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
Table 7.5: Proportion of calves surviving to first calving by source farm and bovine respiratory disease status

<table>
<thead>
<tr>
<th>Source Farm</th>
<th>BRD60 +(^1)</th>
<th>BRD60 -(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64% (37/58)</td>
<td>80% (238/297)</td>
</tr>
<tr>
<td>2</td>
<td>87% (20/23)</td>
<td>91% (77/85)</td>
</tr>
<tr>
<td>3</td>
<td>61% (19/31)</td>
<td>92% (132/143)</td>
</tr>
<tr>
<td>4</td>
<td>57% (13/23)</td>
<td>67% (87/129)</td>
</tr>
<tr>
<td>5</td>
<td>71% (55/77)</td>
<td>89% (307/346)</td>
</tr>
<tr>
<td>6</td>
<td>54% (14/26)</td>
<td>85% (89/105)</td>
</tr>
</tbody>
</table>

\(^1\) Calves treated for bovine respiratory disease in the 60 days following enrolment

\(^2\) Calves without bovine respiratory disease in the 60 days following enrolment
**Table 7.6**: Producers’ primary reason for the 7% (93/1293) of heifers that died between enrolment (8 weeks of age) and calving.

<table>
<thead>
<tr>
<th>Producer Attributed Cause of Death</th>
<th>Proportion of Total Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory Disease</td>
<td>47% (44/93)</td>
</tr>
<tr>
<td>Injury</td>
<td>27% (25/93)</td>
</tr>
<tr>
<td>Non-respiratory Diseases</td>
<td>14% (13/93)</td>
</tr>
<tr>
<td>Other</td>
<td>1% (1/93)</td>
</tr>
<tr>
<td>Unknown</td>
<td>11% (10/93)</td>
</tr>
</tbody>
</table>
Table 7.7: Producers primary reason for the 12% (162/1,392) of heifers culled from herd between enrolment (8 weeks of age) and calving.

<table>
<thead>
<tr>
<th>Primary Reason for Culling</th>
<th>Proportion of Cullings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor reproduction</td>
<td>32% (51/162)</td>
</tr>
<tr>
<td>Poor growth</td>
<td>22% (36/162)</td>
</tr>
<tr>
<td>Dairy</td>
<td>22% (35/162)</td>
</tr>
<tr>
<td>Non-respiratory diseases</td>
<td>16% (26/162)</td>
</tr>
<tr>
<td>Injury</td>
<td>4% (6/162)</td>
</tr>
<tr>
<td>Bovine Respiratory Disease Complex</td>
<td>2% (3/162)</td>
</tr>
<tr>
<td>Other or unknown</td>
<td>3% (5/162)</td>
</tr>
</tbody>
</table>
Table 7.8: Distribution of calving ease score recorded by source farms in 1066 heifers

<table>
<thead>
<tr>
<th>Score</th>
<th>Definition</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No problem</td>
<td>52% (557)</td>
</tr>
<tr>
<td>2</td>
<td>Slight Problem</td>
<td>24% (259)</td>
</tr>
<tr>
<td>3</td>
<td>Needed Assistance</td>
<td>14% (151)</td>
</tr>
<tr>
<td>4</td>
<td>Considerable Force</td>
<td>6% (60)</td>
</tr>
<tr>
<td>5</td>
<td>Extreme Difficulty</td>
<td>4% (39)</td>
</tr>
</tbody>
</table>
**Table 7.9**: Effect of BRD60 on calving ease score using multiple cut-points as evaluated in a logistic model with random effects for source farm and enrolment cohort.

<table>
<thead>
<tr>
<th>Dystocia cut-points</th>
<th>Incidence of Dystocia Events</th>
<th>Odds Ratio : 95% Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ease $\geq 2$</td>
<td>55% (85/155)</td>
<td>1.5 : 1.1-2.2</td>
<td>$P &lt; 0.05$</td>
</tr>
<tr>
<td>Ease $\geq 3$</td>
<td>27% (42/155)</td>
<td>1.3 : 0.9-2.0</td>
<td>$P = 0.17$</td>
</tr>
<tr>
<td>Ease $\geq 4$</td>
<td>11% (17/155)</td>
<td>1.4 : 0.8–2.5</td>
<td>$P = 0.32$</td>
</tr>
<tr>
<td>Ease $\geq 5$</td>
<td>5% (7/155)</td>
<td>1.4 : 0.6–3.4</td>
<td>$P = 0.44$</td>
</tr>
</tbody>
</table>
Table 7.10: The number of cows with and without bovine respiratory disease in the 60 days following enrollment (BRD60) from each source farm in the analysis of first milk test, the least-squares mean (± standard error of the mean) age at first milk test and the effect of BRD60 on first milk test date.

<table>
<thead>
<tr>
<th>Source Farm</th>
<th>BRD60 + (n=)</th>
<th>BRD60 – (n=)</th>
<th>Average days in milk at first DHIA test</th>
<th>Effect of BRD60 on Milk Test Weight (LSMean ± S.E.M)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>232</td>
<td>33 ± 17</td>
<td>28.9 ± 0.5</td>
<td>26.0 ± 1.1</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>74</td>
<td>17 ± 12</td>
<td>31.6 ± 1.1</td>
<td>29.1 ± 1.5</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>129</td>
<td>21 ± 10</td>
<td>30.3 ± 0.6</td>
<td>28.5 ± 1.6</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>85</td>
<td>25 ± 13</td>
<td>26.5 ± 0.7</td>
<td>28.1 ± 1.9</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>290</td>
<td>18 ± 11</td>
<td>26.9 ± 0.4</td>
<td>26.3 ± 0.9</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>86</td>
<td>19 ± 12</td>
<td>27.4 ± 0.7</td>
<td>31.1 ± 1.8</td>
</tr>
</tbody>
</table>
**Table 7.11:** Distribution of heifers that survived to 120 days post-calving recorded by calving ease score and the probability of survival relative to a calving ease score of 1

<table>
<thead>
<tr>
<th>Calving Ease Score</th>
<th>Proportion of heifers that survived to 120 days in milk</th>
<th>Odds Ratio: 95% Confidence Interval relative to score = 1</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95.4% (519/544)</td>
<td>0.5: 0.3-1.0</td>
<td>0.05</td>
</tr>
<tr>
<td>2</td>
<td>93.2% (234/251)</td>
<td>0.5: 0.2-1.0</td>
<td>0.05</td>
</tr>
<tr>
<td>3</td>
<td>92.5% (137/148)</td>
<td>0.5: 0.2-1.0</td>
<td>0.03</td>
</tr>
<tr>
<td>4</td>
<td>91.7% (55/60)</td>
<td>0.3: 0.1-0.9</td>
<td>0.04</td>
</tr>
<tr>
<td>5</td>
<td>92.3% (36/39)</td>
<td>0.2: 0.1-0.9</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Figure 7.1: Timeline of trial events
**Figure 7.2:** Difference in least squares means (± SE) of the weight (kg) of heifers with BRD60 relative to calves without BRD60 at 5 time points at a commercial heifer raising facility, controlling for birth weight, age at measurement and season with random effects for source farm and weekly enrolment cohort.

**Rearing Barn**

<table>
<thead>
<tr>
<th>Weaning Barn</th>
<th>Transition Barn</th>
<th>Grower Barn 1</th>
<th>Grower Barn 2</th>
<th>Breeding Barn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difference in Body weight (KG)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>98</td>
<td>181</td>
<td>269</td>
<td>382</td>
</tr>
</tbody>
</table>

Average age (days) at measurement

** P < 0.001

1 BRD60: Bovine respiratory disease occurring within 60 days of study enrolment
**Figure 7.3:** Differences of least squares means (± SE) of the weight (kg) of heifers with no prior history of bovine respiratory disease that received tulathromycin at study enrolment relative to heifers that received oxytetracycline at study enrolment at entry at 5 time points at a commercial heifer raising facility. The linear mixed models controlled for birth weight, age at measurement and season as fixed effects and for source farm and weekly enrolment cohort as random effects.

**P < 0.001**
**Figure 7.4:** Time to event analysis for age at first calving stratified by BRD60 status with culled heifers censored ($P = 0.02$).
Appendix 7.1: Daily minimum and maximum temperature at the Rochester, New York weather station, located approximately 65 km from study site, by season.

Reference

CHAPTER 8: CONCLUSIONS AND FUTURE RESEARCH

8.1 GENERAL DISCUSSION

There are many challenges associated with rearing healthy dairy heifer calves that can result in high mortality rates and welfare problems. Multidisciplinary approaches of evaluating management practices, identifying animals at risk of disease, and quantifying the impact of diseases are valuable tools for improving calf welfare and productivity. High mortality rates in dairy calves are important concerns for producers, due to the monetary costs of calf loss, the welfare costs to animals during illness and convalescence, and the impact of mortality and calf suffering on public perception of the dairy industry. Two diseases are primarily responsible for mortality in neonatal dairy calves: neonatal calf diarrhea complex (diarrhea) and bovine respiratory disease complex (BRD). These diseases are responsible for 79% of the deaths in milk fed calves and 59% of the deaths in weaned dairy calves (USDA, 2007). To reduce the impact of these two diseases, new and improved methods of disease identification and animal management are needed. The three main areas of focus of this doctoral thesis are the use of behavior to identify animals at risk of disease, group level management of disease risk, and identification of long-term impacts of BRD.

Animal diseases have many negative impacts on animal welfare and production (Chapter 1). The early identification of animals at risk of disease allows for interventions either through reducing or eliminating risk factors. Examples include altering weaning management practices (Chapter 4), or in the absence of controllable risks, such as poor weather, administering a prophylactic antibiotic (Chapter 5 and 6). Even if risk factors are reduced, some disease still occurs. Therefore, to improve recovery and reduce welfare impacts, early identification of
clinical diseases is important. For this reason, behavior alterations associated with sickness are of value to identify at risk animals. This thesis identified two predictors of poor growth in individually reared calves; 1. altered postures in calves under 2 weeks of age, 2. high lethargy scores during clinical diarrhea in calves under 2 weeks and calves between 4 and 6 weeks of age (Chapter 2). These measures of behavior were originally selected to identify calves with low motivation to explore their environment and decreased appetite based on known motivational changes associated with sickness (Aubert, 1999) as well as indicators of visceral pain, previously identified in lambs (Molony et al., 2002). Poor growth was evaluated based on its association with disease in neonatal dairy calves (Chapter 5). The association of posture and lethargy scores with poor growth supports the hypothesis that these are indicators of sickness in individually housed dairy calves. The main challenge for this study was that behavioral measures were taken every other week, which may have allowed for illness and recovery between measures, decreasing the sensitivity of these tests. In the future, this could be addressed by measuring the selected behaviors in a smaller, more intensive study in which daily behavioral scores and weekly body weights are measured. However, the behavior measures examined here do provide a valuable starting point for the identification of sickness behaviors that are most beneficial for identifying disease. These behavior measures should be further refined for use by calf caretakers to improve disease recognition.

Milk fed calves are at greatest risk of diarrhea (USDA, 2010). However, a second disease risk period, frequently associated with BRD, is weaning and the change to group housing. For this thesis, poor post-weaning growth was chosen as an indicator of poor adaptation to weaning and movement based on the hypothesis that these animals are the most likely to contract BRD.
Heifers with poor post-weaning growth were identified as those that were most active in the three days following weaning (Chapter 3) and that spent greater amounts of time at the bunk without eating (bunk engaged behavior). The effect of weight gain and lying distance was confounded by nursery housing system and location; Calves from Nursery 2, who had been housed in individual outdoor calf hutches, gained less weight and spent more time lying far from other calves compared to calves from Nursery 1, who had been housed in adjacent individual pens in a barn. This resulted in limitations to our ability to interpret the results, but does suggest that social lying distance is associated with weight gain and nursery housing system may impact weight gain and behaviour. From these findings several areas of study were identified. The variation in behavioral response observed in this study suggests that there is potential to intervene prior to weaning to improve adaptation and reduce stress associated with these experiences. For this reason, focusing future research on preparing calves for weaning by decreasing stress and encouraging calf starter intake are important areas of focus for disease risk management and animal welfare.

Disease management is an important area of study for the welfare and productivity of dairy calves. There are many approaches to reducing disease risk; in this thesis management systems currently in use in modern dairy farms were evaluated. Two approaches were used to reduce the risk of calves succumbing to clinical disease, the first was reduction of disease susceptibility through the use of graduated weaning (Chapter 4) and the second was the use of prophylactic antibiotics at a group level during periods of high risk (Chapter 5 and 6). Graduated weaning programs have been promoted to reduce the stress of dairy calves during the post-weaning and movement time period. The findings from this experiment were that mixing calves,
and movement to a novel environment was associated with increased steps and decreased lying time. If mixing and movement to a novel environment occurred a week apart the increase in the number of steps and the decrease in lying time was reduced compared to mixing and movement occurring simultaneously. Further research in needed to determine if the decreased activity in the novel environment performed by pre-mixed calves was a result of decreases agonistic behaviours or if the agonistic behaviours occurred whenever calves were mixed. This information would determine if premixing calves reduces the stress associated with movement to a novel environment. Further study of this management procedure would be valuable to identify the types of behaviors occurring upon movement to a novel environment and determine the effect of increased transportation stress and grouping on adaptation to a novel environment and the risk of disease.

A second approach, which can be used in conjunction with minimizing weaning stress, is the strategic use of antibiotics at the group level. The primary criteria for consideration of this approach were the use of antibiotics during a short time period of high risk of disease that cannot be eliminated through other methods, and the welfare impacts of disease, particularly when recovery is poor or prolonged. Prophylactic antimicrobial use was reviewed based on its established efficacy in beef feedlots and its previously unstudied use in dairy calves. Two time periods were evaluated; the first was shortly after birth when calves arrived at a heifer raising facility and the second was upon first movement to group housing following weaning. Prophylactic antimicrobial use reduced disease in both populations. For dairy calves that received a long acting antibiotic upon arrival at a heifer raising facility, the incidence of otitis and diarrhea were reduced in the first 8 weeks of life. For this reason, the efficacy criterion for
strategic antimicrobial use was met. However, otitis, the targeted disease, had minimal effect on growth, indicating that on this farm, otitis did not have substantial long-term impacts. In addition, otitis could not be definitively linked with *M. bovis*, an organism previously associated with severe BRD, arthritis and otitis media. Furthermore, the incidence of *M. bovis* was not different between antibiotic and placebo treatment groups. Although culture methods used in this study limited interpretations, the minimal impact of both diarrhea and otitis on growth suggest that this disease does not meet our stated criteria for responsible prophylactic antimicrobial use of a severe disease with poor recovery rates. In addition, the small difference in ADG (0.02 kg) between calves that received the antibiotic compared to the control group also indicates a small impact. When prophylactic antimicrobial use was evaluated in 8 week old calves after movement to group housing, the incidence of BRD was reduced and calves that received TUL had 0.12 kg higher ADG compared to control animals, provided there was no prior history of BRD (Chapter 6). The lack of growth effect in calves with a prior history of BRD provided important information for the efficacy of this approach and indicated that the benefits of prophylactic antimicrobial use may be reduced in calves with a history of BRD. An additional benefit was that the strategic use of a long-acting antibiotic improved the probability of surviving to first lactation for calves treated for BRD after movement to group housing.

Long-term effects of disease are important to evaluate not just for productivity, but to identify long-term implications which may compromise animal welfare. The final objective of this thesis program was to update and identify the long-term implications of this disease (Chapter 7). BRD was found to decrease ADG for almost 6 months after the initial disease period, resulting in a 15 kg difference in body weight between affected and unaffected animals at 9
months of age. The lower growth rate indicates that calves recover very slowly from BRD, and that BRD has potentially altered the calves’ physiology or behavior either through decreased feed efficiency or decreased feed intake. The long-term effects of BRD were further demonstrated by the lower probability that calves with BRD would survive to first calving, the greater age at first calving and the higher risk of some level of dystocia at first calving, relative to calves that did not experience BRD during the 60 days following movement to group housing. The effect of BRD on milk production was less clear due to a likely healthy worker bias resulting from the high rate of removal of BRD heifers prior to calving. To avoid this bias, a different approach may be required in that the animal’s genetic potential for milk production should be accounted for in the model to determine if BRD decreases milk production. This evaluation would require a larger sample size than was available in this study since it would require many daughters sharing the same sire.

The results of these three thesis objectives have demonstrated that a multi-disciplinary approach to improving management of dairy calf health and the identification of long-term impacts of disease are beneficial. Future research should continue to address the issues of early disease identification, risk management and animal health in order to improve the productivity and welfare of dairy calves.

8.2 FUTURE RESEARCH

Although several management procedures that are associated with reducing the risk of dairy calf disease, and some behaviors associated with poor growth were investigated in this thesis, these findings also illustrated many other areas of investigation that need to be considered.
Specifically,

**How can disease severity be appropriately identified to improve animal care?**

Bovine respiratory disease complex and neonatal calf diarrhea have been associated with many long-term impacts on calf health and welfare which brings into question the recovery rate of these diseases and raises the potential for altered management to improve recovery. However, for this to be best utilized, the calves that are most severely affected need to be identified. Currently there is a scoring system reported in literature which attempts to categorize animals based on clinical signs (Lago et al., 2006). However, this scoring system has not been formally validated. The signs selected include nasal and ocular discharge, rectal temperature, and ear droop. Behavioral indicators of illness are not included in this score. Defined behavioral indicators may be useful for identifying animals that are in the greatest discomfort and most severely affected, which is not provided in the current scoring system. In order to validate an effective scoring system and to determine cut points for mortality risk, a cohort study in which calves are scored during clinical disease and the number of re-treatments are recorded should be conducted. Animals would then be monitored until calving, in order to determine which cut-points and signs are the most sensitive and specific for identifying severely affected animals. From this information, recovery information for different cut-points can be collected and improved treatment and management decisions may be made.

**Why is the growth of dairy calves affected for a prolonged time period?**

There are two potential mechanisms for the poor growth of heifers with BRD: decreased feed intake and feed conversion. These two factors may also be affected by social factors including the ability of heifers affected by BRD to compete at the feed bunk. In order to improve
the management of heifers recovering from BRD, there is a need to determine which factors impair their growth. There are four hypotheses for this decreased weight gain observed in heifers with BRD after recovery from clinical disease;

1) decreased feed efficiency,

2) decreased feed intake,

3) fewer, shorter feeding bouts,

4) more displacements at the feed bunk.

Each of these hypotheses should be tested at different stocking densities and social mixing to determine if BRD heifers at high stocking densities show greater reductions in feed efficiency and intake, greater alterations in feeding behavior, and more feed bunk displacements relative to healthy heifers and to BRD heifers housed at lower stocker density.

If the outcomes of feeding behavior or feed efficiency are altered by stocking density, there is a potential to alter the management of dairy calves during convalescence based on this knowledge. The impact of different proportions of calves with a prior history of BRD within a group on disease spread, relapse rates and growth was recently investigated at a commercial heifer rearing facility in Spain (Bach et al., 2011). These researchers did not find a difference in ADG depending on group composition. However, their analysis was completed at the pen level, and using a small sample size. Further research to determine the impact of group composition on the growth of sick calves would be beneficial to identify improved methods of managing animals during convalescence.
Does transportation, initial social mixing and a novel environment in conjunction with weaning result in additive stressors, and how far apart in time must these stressors occur to reduce stress and avoid the additive effect?

This thesis began to investigate the impact of a graduated weaning practice on the behavior of dairy calves. However, this was a pilot study and many more variables need to be understood before this system can be recommended or discouraged on commercial farms. Three main questions must be addressed for this program to be understood: 1) which stressors result in additive stress?, 2) what is the duration of response to the individual stressors?, 3) what is minimum length of time required between each of the stressors to avoid inflicting additive stress? These questions would best be answered using controlled experiments that incorporate behavior, together with physiological responses, specifically heart rate, cortisol levels with an ACTH challenge to detect stress induced immunosuppression, and weight gain. The stressors investigated should include transportation stress in weaned calves, first social mixing, removal of milk from the diet, dehorning, and change in environment. Although removal of milk from diet and dehorning have been extensively studied in dairy calves, the effect of change in environments, transportation, and first social mixing need further investigation to determine how they interact with the above stated stressors. In addition, there is some suggestion from the results of Chapter 3 that housing may play a role in adaption to a novel environment. As such, the stress responses of calves reared in hutches versus nursery pens should be compared when subjected to the above stressors.
8.3 REFERENCES


