# Impact of Dairy Cow Resting Area on Hygiene, Lying Behavior, and Milk Quality

**by**

**Ivelisse Robles**

**A Thesis**

**presented to**

**The University of Guelph**

**In partial fulfilment of requirements**
**for the degree of**

**Doctor of Philosophy**

**in**

**Animal Biosciences**

**Guelph, Ontario, Canada**

# ABSTRACT

IMPACT OF DAIRY COW RESTING AREA ON HYGIENE, LYING BEHAVIOR, AND MILK QUALITY

Ivelisse Robles
University of Guelph, 2018

Advisor:
Dr. Trevor DeVries

This thesis investigated the impact of dairy cow resting area on hygiene, lying behavior, and milk quality. The first cross-sectional study associated different bedding options (sand, straw, wood products, and recycled manure solids (**RMS**) and barn management practices with low bacterial count, improved cow hygiene, and milk quality. In that study it was demonstrated that elevated bacterial counts could be reported in any of the sampled used bedding types. At the cow level, factors associated with poor body hygiene for cows housed in free-stall barns were greater parity, while the opposite association was reported for cows housed in tie-stall barns. Regardless of housing system, cows in earlier stages of lactation were at higher risk of poor body hygiene when compared to later lactation cows. The second cross-sectional study aided in finding associations among barn management practices, lying behavior, and risk of elevated somatic cell count (**eSCC**). Lying duration was positively associated with deep-bedded stalls, negatively associated with cow body condition, and negatively associated with dirty stalls. Lameness prevalence was positively associated with dirty stalls. The last longitudinal, controlled study, investigated how the horizontal neck rail position in a free-stall was associated with stall cleanliness, cow hygiene, and the risk for intramamary infection (IMI) and lameness. Neck-rail position on the stall did not influence on SCC, nor in new cases of IMI. A greater percentage of cows had dirty udders when the neck-rail was positioned further from the stall during one of the treatment periods. Also, having a neck-rail positioned further from the curb tended to increase the risk for a new case of lameness in the second week of each treatment period. Finally, stalls were cleaner when bedding was added more frequently. Overall, this thesis provides evidence that bedding management can have a profound impact on cow milk quality, bacterial concentrations in the bedding substrates, and cow behavior, hygiene, and lameness risk.

For all the cows in the dairy industry and for those producers that set an example by loving, caring, and respecting these amazing creatures.

## ACKNOWLEDGEMENTS

I have been so blessed in many ways through this journey, and God is responsible for that, thank you for never leaving my side. Certainly, many humans and animals made this journey an unforgettable life experience and for that I am forever grateful.

Thank you to my advisor, Dr. Trevor DeVries, for accepting me to join the CowCrew and for being such a reliable mentor. My experience as your student was unique and has helped me to become the person that I am today. Thank you also for being the fastest proofreader that I have ever met, I admire you for that, you rock!!! Finally, thank you for remaining calm when research did not go according to plan (does it ever? Haha!) and always having an open ear for plan B. To my committee members Dr. Marina von Keyserlingk and Dr. David Kelton, thank you for all your support and guidance in this journey. Dave you are such a kind person, such a knowledgeable and positive mentor that I was never afraid to approach you for my many questions, thank you. Nina your expertise in animal behavior and welfare is so admirable, that I want to be like you when I grow up. Also, thank you Nina and Dave because when I lost my beloved dog Ike, you were both extremely supportive, Dave you had such a deep empathy, and Nina, I will never forget how you hugged me, and then held my shoulders while looking at me and said, “you did the right thing”.

Thank you to Dr. Stephen LeBlank, you are approachable and always willing to help with a smile. I would like to thank Dr. Renée Bergeron, for giving the opportunity to work with her as a TA, it was a great experience and very enjoyable, today she is here as my additional member on the exam and that makes me very happy. To Dr. Katerina Serlemitsos Jordan, I met you working with you as a TA and today I get to call you my friend, many thanks for everything. Thank you, Dr. Georgia Mason, I had your support when I needed the most, after my concussion and in other chapters of my life, and I am extremely grateful for that.

I am also grateful to my funding sources: the Dairy Research Cluster II Initiative, funded by the Dairy Farmers of Canada (Ottawa, ON, Canada), Agriculture and Agri-Food Canada (Ottawa, ON, Canada), the Canadian Dairy Network (Guelph, ON, Canada), and the Canadian Dairy Commission (Ottawa, ON, Canada) through the Canadian Bovine Mastitis and Milk Quality Research Network (St. Hyacinthe, QC, Canada). Also, thanks to the Natural Sciences and Engineering Research Council of Canada (NSERC) CREATE in Milk Quality Scholarship.

Thank you to the staff at Elora Dairy Research facility for all your assistance, and patience while completing my trial. Special thanks to Laura Right who is amazing, helpful, and always willing to help.

I would like to thank all the producers that allowed me to visit their farms, and to use their big mommas for my research, without you and without the cows, this research would have not been possible. Thank you to everyone in CowCrew, whether you were there for a summer or for years your presence counts! Dr. Meagan King (thank you for passing tips that Trevor taught to you and then you passed on to me, and a huge thank you for your help with moving!), to Rachael Coon (thank you for making me laugh, and for taking me with you to Norway and Denmark, such a great time), to Morgan Overvest (such a hard working lady, your farm looks amazing now) Emily Kaufman (thank you for being such a big help when you were the research tech, surely made everything go smoothly) to Robin Crossley (for being so bright and thoughtful), to Victoria (you were such a great help with data collection and your personality is amazing), Kaitlyn Dancy (great to have you in the lab), to Athena (you are bright and working with you was such an adventure, we even ended in the hospital with lovely marks from those couple of crazy cows), Kaitlin Sparkman (it was so much fun to have you working with me on those long days because your laughter and craziness are contagious), Megan Gannon (worked with you briefly and would love to have the opportunity to work with you in the future), Sarah McPherson (you were such a great help and detail oriented, thank you!), Stephanie Kamalanathan (I loved your enthusiasm, availability, and always with a smile!), Sarah Parsons (thank you for picking me up when it was still dark to go to Elora, for being such a hyper person, it is fun!), to Patrick Sudds (Kareoke with Taylor Swift were amazing times while in those long drives, and your help was great), Melissa Kuipers and Michelle Thompson (two sweethearts that were great as helpers), Casey Havekes (being in the lab with you was so much fun, you are so conversive), Kevin Foster (working with you was a great pleasure, amazing conversations, and great work ethics), Thijmen Miedema (such a goof ball, smart, helpful and fun), and Geísa Mainardes (I met you at the lab and now you are one of my closest friends, such a blessing in my life and Sam, *tu también por supuesto*!).

To my great friends, thank you for all your support! Gilma (my best friend, I have so much gratitude for having you in my life and being in yours, I love you), to Juan (thank you for your support at the beginning, and when I mentioned the new adventure), to Wilma and Sheehan (thank you for all of your support during this journey), Luz (your light always shines, and your positivism is like water in the desert). Catalina (I met you in behavior group, you are an amazing person and friend), Aitor (we have gone from TAing together, eating, dancing, and having a great time together, thank you, and when it is time to work, it gets done!), Peter (always ready to listen, and to give advice), Moran (I absolutely love that I met you through research as I volunteered Walkie and Snow on one of the trials, you are an angel, I am forever grateful to be your friend, see you in France), to Maria (who describes me as a hero, we met in first grade and every day counts, thank you).

Thank you, Mary and Lou, for all your support since I started this journey. Thank you, Melissa, and *Mami* some days you can be such amazing cheerleaders! To my nieces and nephews, thank you, *titi* loves you! To my beloved Ike that crossed the rainbow, I love you and miss you! To my Walkie and Snow White, thank you for being my company for 8 years, and laying on top of the papers that I need, to take naps, and for using my desk as your designated nap area, even when I must get creative to reach the keyboard! Papi and Danny, I love you, miss you, and I hope that you are both proud of me. To Billy, your love and support have made a world of difference in my life. Love you all!!!!

# STATEMENT OF WORK

Funding for this research was provided by the Dairy Research Cluster II Initiative, funded by the Dairy Farmers of Canada (Ottawa, ON, Canada), Agriculture and Agri-Food Canada (Ottawa, ON, Canada), the Canadian Dairy Network (Guelph, ON, Canada), and the Canadian Dairy Commission (Ottawa, ON, Canada) through the Canadian Bovine Mastitis and Milk Quality Research Network (St. Hyacinthe, QC, Canada).

Ivelisse Robles was responsible for study design implemented, protocol development, and data collection for chapters 2, 3, 4, and 5. Preparation of all thesis chapters was the primary responsibly of Ivelisse Robles, including data management, statistical analyses, and writing.

Athena Zambelis assisted in the data collection for chapter 4 and has provided assistance (revisions and editing) for the manuscript that will be submitted for that chapter.

Drs. Trevor DeVries, Marina von Keyserlingk, and David Kelton advised on the design and analyses of studies described in chapters 2, 3, 4, and 5, in addition to providing editing assistance for all chapters.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

# LIST OF ABBREVIATIONS

AM- morning

AMS- automated milking system

BC- bacterial count

BCS- body condition score

BTBC- bulk tank bacteria count

BTSCC- bulk tank somatic cell count

BW- body weight

CFU- colony forming unit

CNS- coagulase-negative staphylococci

DHI- dairy herd improvement

DIM- days in milk

DM- dry matter

DLL-dirty lower legs

DU-dirty udder

DULF- dirty upper legs and flank

IBC-individual bacterial count from bactoscan results

IMI- intra-mammary infection

EMCO- Edward's modified medium agar

eSCC- elevated somatic cell count

MAC-Mac Conkey's agarinositol-carbelillin agar

MALDI TOF-matrix assisted laser desorption ionization time-of-flight mass spectometry

mL-millilitre

NRS- numerical rating system

OR- odds ratio

PM-afternoon

SCC- somatic cell count

SD- standard deviation

SE- standard error

SS- soiled squares

RFM- rubber filled mattresses

RMS- recycled manure solids

TMR- total mixed ration

UK- United Kingdom

US-United States

95 % CI- 95 percentage confidence interval

## LIST OF APPENDICES

# CHAPTER 1: GENERAL INTRODUCTION

Providing indoor housed cattle access to a high quality resting area is important for cow welfare (Krawczel and Grant, 2009) and farm profitability (as suggested by van Gastelen et al., 2011). Indoor-housed mature dairy cattle spend on average 10 to 13 h/d lying down in a designated resting area (Vasseur et al., 2012), which in Canada is typically provided in the form of a cubicle (i.e. free-stall and tie-stall housing) or a bedded pack. Two primary factors affecting the decision of cows to lie down are the bedding surface (i.e. deep bedded, mattress) (Manninen et al., 2002; Tucker et al., 2003; Norring et al., 2008), and the stall dimensions (i.e. stall width and length, Tucker et al., 2004; 2005; 2006; neck rail position, Bernardi et al., 2009). The lying surface is typically covered with bedding (i.e. sand, sawdust, straw) to provide a soft, non-abrasive resting surface. Dairy cows have a preference for certain bedding types and will adapt their lying behavior based on their bedding type preference and previous experience (Norring et al., 2008). Ideally, the bedding material provides thermal comfort and softness, and helps maintain hygiene, and reduce labour costs associated with stall maintenance (Chaplin et al., 2000).

The bedding type can increase or decrease risk of exposure to mastitis pathogens (Rowbotham and Ruegg, 2015) given that bacteria can be transferred from the surface area of the stall to the teat end (Hogan et al., 1999). Environmental bacteria are chemotropic organisms and need organic materials to use as a food source (Hogan and Smith, 2012); thus, organic bedding supports greater bacterial growth (e.g. in Ruegg, 2012). The use of inorganic bedding materials, such as sand, have been reported to decrease teat-end exposure to environmental mastitis pathogens (Hogan et al., 1989; Husfeldt et al., 2012). As Husfeldt and Endres (2012) suggested, in many farms producers face challenges in obtaining, handling, and disposing of sand bedding. This, coupled with the increased cost of traditional bedding, has increased efforts to find efficient alternative bedding types (Hudsfeldt et al., 2012; Solano et al., 2015). Moreover, best farm management practices in areas such as bedding management, type of bedding (inorganic vs. organic), teats preparation before milking, play an important role in reducing pathogen exposure, increasing hygiene, and ensuring milk quality (Hogan and Smith, 2012; Rowbotham and Ruegg, 2015). Thus, it is imperative that we understand the effect of dairy cow resting area and management on hygiene, behavior, and milk quality, specifically by focusing on bedding choice, stall design, hygiene, and udder health.

### *1.1 Types of materials used as bedding*

Materials used as bedding for dairy cows are often classified as either organic (ie. straw and wood products) or inorganic (i.e. sand) (Hogan et al., 1989). Although straw is a common bedding material in some regions, weather conditions and location directly impact the availability of straw (Manninen et al., 2002). Sawdust and other wood products are also used as bedding; but similar to straw, availability and cost have forced producers to look for alternative materials (Hudsfeldt et al., 2012). Green box compost made from biodegradable waste from households is another bedding option that has been used by some producers (Groot Antink, 2009). Due to advanced technology in separation and anaerobic digestion, in addition to the large amounts of bedding that can be obtained from recycling of manure solids, recycled manure bedding is an alternative bedding type receiving much attention (Godden et al., 2008; Hudsfeldt et al., 2012). Another organic bedding recently introduced as a potentially viable alternative is switchgrass (Wolfe et al., 2018). Sand is an inorganic bedding type that is highly recommended (van Gastelen et al., 2011) and regarded as the most ideal bedding type for dairy cows; however, not all producers have access to this material nor the equipment and manure handling and storage to manage it properly (Hudsfeldt et al., 2012).

### *1.1.1 Impact of bedding type and amounts on lying time*

When given a choice, most cows prefer to lie down on a softer stall surface (Manninen et al., 2002; Tucker et al., 2003; Wolfe et al., 2018). Cows prefer concrete covered with ~10 cm of straw over soft rubber mats (Manninen et al., 2002). Likewise, cows show a preference for deep-bedded sand (after exposure to this bedding), sawdust (Tucker et al., 2003), and switch grass (Wolfe et al., 2018) over a mattress covered with 2-3 cm of sawdust (Tucker et al., 2003; Wolfe et al., 2018). Also, mattresses that are well-bedded increase cow lying time; for example, each additional kilogram of sawdust or straw on the mattress surface increased cow lying time per day by 12 min (Tucker and Weary, 2004; Tucker et al., 2009). Solano et al. (2016) reported a 70-min/d difference in lying time (mean ± SD) between 2 stall surfaces used in free-stall housing (mattress = 10.6 ± 0.8, sand or dirt = 11.3 ± 1.2 h/d). Some cows appear to be highly motivated to access a deep-bedded area, pushing a weighted gate (up to 40% of their body weight) to gain access (Tucker et al., 2018). Additional evidence of the importance of lying time was provided by Tucker et al. (2018), who reported that cows increased daily lying time by 5 h after being deprived of access to a deep-bedded rest area for 4 hours the day before (Tucker et al., 2018). Stall maintenance can also influence cows daily lying time; lying time is reduced about 10 min for every cm decline in sand bedding (Drissler et al., 2005). The percentage of cows lying down also decreases as the time since additional bedding added to the stalls is increased (Lombard et al., 2010).

In addition to affecting lying time, bedding materials can also impact teat end exposure to bacteria (Tucker et al., 2003; Norring et al., 2008). Manninen et al. (2002) provided evidence that cows, when given a choice, will avoid sand and spend more time lying on straw and soft rubber mattresses during winter (~13 h/d) and summer (~12 h/d). Similarly, cows reduced their lying time when forced to lie on sand (7.5 h/d winter; 1.1 h/d summer) compared to when forced to lie down on mattress or straw bedding t (~13 h/d winter; 11 h/d summer) (Manninen et al. (2002). However, it should be noted that the cows in the previous study had greater experience with the mattresses and straw than sand; a factor that others have argued can affect preference (Manninen et al., 2002; Tucker et al., 2003; Norring et al., 2008). For example, Norring et al. (2008) reported that cows lay down longer when provided straw when compared to sand (straw 749 ± 16 vs. sand 678 ± 19 min/d), but these authors also showed that previous experience mattered; cows housed previously with straw bedding preferred straw and those housed with sand showed no preference. Thus, preference for sand bedding can be improved with experience, but it is not consistent for all cows (Manninen et al., 2002; Tucker et al., 2003, Norring et al., 2008).

The studies mentioned above have limitations and potential biases. For instance, Manninen et al. (2002) used cows that had no previous experience with sand but did have experience with mattresses. Likewise, in Tucker et al. (2003) the cows had been housed for at least 2 lactations on sand, only 60 days on sawdust. Norring et al. (2008) tested cows after 21 wk. after being kept on either sand or sawdust to determine bedding preference and showed no preference. The issue of previous experience and its effect on stall bedding choice warrants additional investigation.

#### *1.1.2 Impact of bedding dry matter on cow behavior*

Bedding dry matter affects daily lying time of dairy cattle (Keys et al., 1976; Fregonesi et al., 2007; Reich et al., 2010). Keys et al. (1976) compared de-watered, pressed manure solids (29

% DM), sawdust (81% DM), and dehydrated manure solids (90% DM) in a free-choice test. Keys et al's (1976) work should be viewed with caution, given that cows were also forced to compete for access to stalls. These authors demonstrated that the preference of cows for drier bedding increased with increasing DM %. However, it is worthy to note that these authors also compared different bedding materials and DM %, with bedding material and DM % of bedding being confounded, thus the cows might have chosen the bedding based on the material itself, comfort, drainage capability, and or insulation provided, and not based partially or entirely on the bedding DM %.  Unfortunately, the authors also failed to provided information on whether the cows had previous experience with the bedding options, which could have biased cows to select a bedding type based on previous experience (Norring et al., 2008). Fregonesi et al. (2007) reported a difference of 5 h/d when cows were restricted to a mean of 26.5% DM in sawdust bedding compared to a mean of 86.5 % DM. Cows also stood longer outside of the stall, providing evidence that they prefer dry bedding to the point of forgoing lying down when "wet bedding" was the only option. Reich et al. (2010) examined 5 different moisture levels (89.8, 74.2, 62.2, 43.9, and 34.7 %) in sawdust bedding and reported that daily lying time ranged from 10 h/d in the wettest bedding treatment compared to 11.5 h/d in driest bedding treatment, and was not affected by temperature or day length. However, those authors did not report if Temperature Humidity Index varied over the course of the study and may have affected cow lying behavior (Ravagnolo et al., 2000; Herbut and Angreca, 2018). Reich et al. (2010) also noted that bedding containing 60 to 90% DM only caused a modest decline in lying time. Conversely, Chen et al. (2017) reported a dramatic difference of mean lying duration when cows were only provided a muddy lying area (3.5 h/d; 67% DM) compared to when they were provided dry soil (12.5 h/d; 90% DM). Overall, if appears that daily lying time is greater on drier bedding materials.

It would be interesting to see how the preference for different bedding materials may also change as DM % varies within the bedding options offered, since different bedding materials have different DM % based on the bedding or process used to obtain the bedding (e.g. digester vs. compost for RMS) (Hudsfeldt et al., 2012; Rowbowtham et al., 2016).

### *1.1.3 Impact of bedding types and stall surface on cow hygiene*

Little work has directly examined the effects of bedding type on cow hygiene. This is likely a consequence of the inherent confounding that arises when doing this type of work. It is almost impossible to disentangle the impact of bedding type on cow hygiene from the impact of stall base in combination with bedding type. Furthermore, experiments assessing cow hygiene have included comparisons of cow hygiene among different bedding types, treatments on bedding, and/or stall base within the same experiment. Additionally, researchers have used different tools/ methodologies to assess cleanliness, making comparisons among studies very challenging.

In a crossover study involving 16 cows that were rotated between deep-bedded stalls (40 cm) with sand or sawdust, cleaned 2x/d, cows lying in sand stalls had dirtier udders compared to when housed in sawdust stalls (Zdanowicz et al., 2004). Udder cleanliness was assessed using a 100-$cm^2$ metal grid and then counting how many of the 16 equal sized squares contained visible fecal material.  Thus, this tool assessed stall hygiene based on the total area of fecal contamination, and not the level/amount of fecal contamination. Fulwider et al. (2007) looked at the influence of stall base on cow hygiene in a study of commercial dairy herds. Pens with the most multiparous and early lactation cows were scored for cleanliness on each farm (n= 94) using a method developed by Reneau et al. (2005). Scores ranged from: 1-no visible manure stains or dried manure to 5-reserved for cows that had both manure stains and dried manure on the legs, udder, and ventral abdomen. Information from free-stall farms that used mattresses bedded with sawdust, rice hulls, chopped straw, or lime were combined into two groups: waterbeds and rubber filled mattresses (RFM), which were then compared to each other and to sand. A greater percentage of cows with hygiene score ≥3 were reported for farms with sand bedded stalls (26.3%) compared to those with rubber filled mattresses (15.6%) or waterbeds (19.2%); no difference between mattresses and waterbeds. Fulwider et al. (2007) also reported that management (frequency of bedding replenishment and stall cleaning) was different among studied farms, but unfortunately was not controlled for in the analysis, thus results should be viewed with caution given the possible interaction between bedding type and stall management.

Norring et al. (2008) reported results from a longitudinal study (at least 21 wk.) that cows using stalls with straw were dirtier than cows on sand stalls [median and interquartile range, straw 6.04 (5.39 to 6.28) vs. sand 4.19 (3.62 to 5.16)]. Norring et al. (2008) used an adaptation of a cleanliness scoring system (Haley et al., 2000), where various parts of the cow body (teats, other parts of the udder, belly sides of the belly, and legs) were scored separately and 1 point given for each area dirty (for a maximum of 10 pts). Schutz et al. (2014) tested the effects of short term exposure to different flooring surfaces on the behavior and physiology of dairy cattle. Results of the latter study indicated that hygiene score of cows on rubber mats were about 3 times as greater (dirtier) compared to cows provided woodchips. These results are not surprising, as we could expect wood bedding to have the ability to absorb some urine and manure, and perhaps the friction against the woodchips could have removed some of the caked manure on cow’s body compared to a potential smearing effect of a mattress.

In a cross-sectional study that evaluated hygiene of cows in free-stalls herds (n=297 herds), no associations among cow hygiene, stall bedding type, and quantity of bedding used were reported (Lombard et al., 2010). Van Gastelen et al. (2011) used the Dutch Udder Health Centre scoring system (score 1-clean- 4-dirty point scale; body areas include udder from lateral view, flank and upper rear legs, and lower rear legs) during a 1 d visit to each of 24 farms. Those authors reported no difference between the cleanliness of cows in stalls bedded with green box compost, sand, horse manure, or foam mattresses, nor associations with bedding characteristics, stocking density, or type of floor cleaning system. With so many different bedding types, Van Gastelen et al., 2011 clearly had no power to find differences among these bedding types that might exist. Although these authors accounted for farm management factors that are known to influence cow hygiene (floor type, type of floor cleaning system, and the presence of a brush), they failed to report their findings. In another study assessing associations between stall surface and animal measurements, including hygiene, no difference was reported in the hygiene of cows housed on mattresses versus deep recycled manure bedding (Husfeldt and Endres, 2012). Popescu et al. (2013) also reported no differences in percentage of cows with dirty lower legs, udder, upper legs and flanks between farms with access to pasture and those without access to pasture (40 with, 40 without access to pasture) using the Welfare Quality Assessment Protocol for Cattle from Netherlands. A very recent cow level study tested the suitability of alternative bedding (switchgrass, and switchgrass with a water and lime mixture) to rubber mats covered with chopped wheat straw (Wolfe et al., 2018) however, they only balanced groups (3 groups with n=8 cows) for body weight and DIM; but did not balance for parity. In another study, the effects of sand-bedded free-stalls were compared to a compost bedded pack farms (7 sand bedded free-stalls; 8 composted bedded packs; Eckelkamp et al., 2016). Collectively these studies (Popescu et al., 2013; Eckelkamp et al., 2016; Wolfe et al., 2018) reported no difference in cow hygiene between different bedding types, despite some (Popescu et al., 2013) noting overall poor cow cleanliness scores.

### *1.2. Impact of cow hygiene on somatic cell counts (SCC)*

Milk quality is determined using reference indicators, which include bulk milk cell SCC, and bulk milk total bacterial counts (Costello et al., 2003). Given that research has indicated an association of prevalence of IMI and SCC (Shukken et al., 2003) a cow revel relationship, bulk milk and milk is used as a reference indicator for premium payments for milk (Rowbotham, 2000; Jayarao et al., 2004). Thus, producers have an incentive to look for management practices that improve milk quality (as suggested by LeBlank et al., 2006). Herds with clean cows and clean housing have lower bulk tank SCC (BTSCC; Barkema et al., 1998; Schwarz et al., 2010) and rates of subclinical mastitis (Ward et al., 2002; Fulwider et al., 2007). Schreiner and Ruegg (2003) were the first to report a relationship between cow hygiene (n=1,250 cows) and intramammary infection (**IMI**; confirmed with bacterial culture of mastitis causing pathogens), noting a linear increase in SCC as hygiene scores of the udder increased (1-clean to 4-dirty) and for scores 2 and 4 of the legs. Further, those authors also reported associations between the prevalence of IMI environmental pathogens (*Escherichia coli, Klebsiella spp.*, *Streptococcus spp., Enterococcus spp.*), from 7.7 to 13.5%, with udder scores 1 to4 respectively, and a higher risk (1.5x) of isolation of major pathogens (*Staphylococcus aureus, Streptococcus agalactiae-contagious* and listed above environmental) from the milk culture for scores 3-4 compared to 1-2 (Schreiner and Ruegg, 2003). Cows with poor udder hygiene are more likely to have elevated SCC (**eSCC**) within 6 months of being hygiene scored (Cook, 2002), have large bacterial counts on teats (Zdanowicz et al., 2004), and a 47% chance of developing an IMI (de Pinho Manzi;

2011). Other researchers have reported an increase of SCC with dirtier udders and legs (Reneau et al., 2003; Dohmen et al., 2010; Santana et al., 2011), and an increased annual average herd SCC in farms with higher proportion of cows with dirty teats (Dohmen et al., 2010). Interestingly, Breen et al. (2009) reported dirty udders to be associated with lower risk of eSCC. Those researchers failed to explain their results, other than indicating that the primary IMI causing agents were *E. coli*, which causes a short SCC elevation.

It should be noted that other researchers have reported no associations between hygiene score and high SCC (DeVries et al., 2012; Eckelkamp et al., 2016), clinical mastitis incidence, or BTSCC (Eckelkamp et al., 2016). However, DeVries et al. (2012) noted the limitations of a small sample size for the study (n= 69 cows) and the overall poor cow cleanliness that restricted the ability to detect effect of a range of cleanliness on SCC. Inversely, Eckelkamp et al. (2016) stated that good management and housing environment should provide similar low SCC results among herds.

### *1.3 Bacterial content of bedding*

Bacterial content in dairy cattle bedding varies by material (Rendos et al., 1975; Zehner et al., 1986; Hogan et al., 1989; Zdanowicz et al., 2004; Hudsfeldt et al., 2012; Rowbotham and Ruegg et al., 2016 Bradley et al., 2018). Inorganic bedding is recommended over organic bedding since the latter (recycled manure, chopped straw, hardwood chips, chopped newspaper, and softwood sawdust) supports greater growth of mastitis inducing bacteria, such as *Klebsiella Pneumoniae*, *Escherichia coli*, *Streptococcus uberis, and Enterococcus faecium* (Zehner et al., 1986; Godden et al., 2008). Zehner et al. (1986) reported the greatest bacterial growth in recycled dried manure solids (RMS), followed by straw, and least in chopped newspaper and sawdust. Godden et al. (2008) reported that RMS, recycled sand, shavings, and clean sand supported growth of *Klebsiella pneumoniae*, with RMS supporting the greatest bacterial growth, and sand having the least bacteria compared to other bedding materials. In a study by Zehner et al. (1986) hardwood chips, sawdust, and RMS supported the growth of *Escherichia Coli* for at least a day. Environmental bacteria are chemotropic organisms and need organic materials to use as food source (Hogan & Smith 2012). Therefore, it is no surprise that organic bedding supports greater bacterial growth (Godden et al., 2008). The use of inorganic bedding materials decreases teat end exposure to environmental mastitis pathogens (Hogan et al., 1989)

During the past 20 years several researchers have compared commonly used bedding material types placed on stalls and quantified bacterial content (Hogan et al., 1989; Hogan et al., 1990; Zdanowicz, et al., 2004; Sorter et al., 2014; Rowbotham et al., 2016; Bradley et al., 2018). The current literature review is focused on describing trends of bacterial content for coliforms, gram negative bacteria *Klebsiella* spp., and *Streptococci* counts in different bedding types reported in these studies. In an earlier study that compared bedding types used in 9 commercial dairies (Hogan et al., 1989), *Klebsiella* spp. counts were reported greater in sawdust than straw, whereas *streptococci* counts were greater in straw than in sawdust. However, to my knowledge no recent study has provided bacterial counts of straw compared to other bedding types. A crossover study that compared deep-bedded sawdust and sand bedding (Zdanowicz et al., 2004) reported that coliform, *Klebsiella* spp. and *Streptococci* counts increased over the experimental weeks, but patterns of growth varied. Highest counts reported for coliforms in sand bedding (d 2) were similar to the lowest reported in sawdust (d 0), thus in sawdust bedding, bacteria count increased faster (Zdanowicz et al., 2004).

Sorter et al. (2014) analyzed bacterial counts in RMS bedding on mattresses, and manure solids deep bedding, and detected elevated counts of bacteria on both surfaces. Even when the back third of the free-stalls were replaced daily with recycled manure bedding coliforms were reduced, however, streptococci and all bacterial counts in bedding remained elevated throughout the study (Sorter et al. 2014). Gram-negative counts were not different between shallow and deep bedding, but coliform, *Klebsiella* spp. and *Streptococci* varied periodically (d 0, 1, 2 and 5 and 6 but not d 3 and 4) (Sorter et al., 2014). Rowbotham and Ruegg (2016) compared sand, recycled sand, RMS over a mattress, and deep bedded RMS, and reported gram-negative bacteria counts to be lower in sand. Interestingly they also reported large numbers of streptococci across all bedding types, with greatest numbers in deep bedded RMS; gram negative bacteria and streptococci were similar in deep bedded RMS. A very recent study compared bacteria counts in sand, sawdust, and RMS (Bradley et al., 2018). Counts of coliform were reported lower in sawdust, followed by sand and then RMS. *Streptococcus/Enterococcus* spp. counts were lower in sand farms when compared to sawdust and RMS herds (Bradley et al., 2018).

### *1.3.1 Impact of bedding on cow udder health*

Several researchers have reported associations between bacterial content in bedding and bacteria counts on teat ends (Hogan and Smith, 1997; Zdanowicz et al., 2004; Rowbowtham et al., 2016). Hogan et al. (1989) reported that rates of clinical mastitis, assessed retrospectively, were related to higher bacterial counts in bedding. Van Gastelen et al. (2011) reported a tendency for positive correlation between numbers of colony forming units per gram reported from the used bedding and number of colony forming units reported in the bulk tank milk. However, other studies (Rowbotham et al., 2015; Bradley et al., 2018) have reported no associations among total bacterial counts in bedding and bacterial count in milk and bedding type. It is important to note that in the latter study (Bradley et al., 2018) bedding samples were collected before bedding was changed and the days for bedding change varied by bedding type, thus, days since bedding was added, might have affected the total bacterial counts detected by bedding type. Bacterial counts have been demonstrated to vary by day (Rendos et al., 1975; Hogan and Smith 1997; Zdanowicz et al., 2004; Sorter et al., 2014). Further, the study by Bradley et al. (2018) was completed during the winter-early spring a time when bacterial growth is at a minimum (Hogan and Smith, 2012) and thus the effects may be different during periods of high bacterial growth (i.e. summer). Further research should also be encouraged to assess if there is a consistent relationship of udder health, bedding type, and milk quality.

### *1.4 Stall design*

Free-stall housing was created to promote stall cleanliness, reduce cost, while reducing bedding amounts used (Schmisseur et al., 1966), and stall maintenance time (Bramley, 1962). Free-stall lying cubicles often include partitions, neck rails, and brisket boards in effort to index the cow's position in the stall and keep the stall clean (Abade et al., 2015). However, the design of such stalls has brought two not ideal options for producers (Bernardi et al., 2009; Abade et al., 2015). Producers must decide whether the stalls should be more restrictive with limited space allowance in order to promote defecation and urination outside the stall, but this comes at the cost of increasing the risk for lameness (Bernardi et al., 2009; Abade et al., 2015; Bouffard et al., 2017). Alternatively, producers could have larger stalls that promote stall usage, thus, increasing the risk that the stalls become soiled (DeVries et al. 2012) and potentially decreasing stall and cow hygiene, increasing risk for mastitis (Breen et al., 2009; de Pinho Manzi et al., 2011), but reducing risk for lameness (Bernardi et al., 2009).

### *1.4.1 Effect of stall components on behavior*

Cow behavior is directly affected by the design and placement of various components of the stall (Tucker et al., 2004; 2005; 2006; Bernardi et al., 2009; Fregonesi et al., 2009; Abade et al., 2015). Lying time and bout duration have been reported to be important behaviors to assess stall usage (Mattachini et al., 2011). Bernardi et al. (2009) speculates that cows will likely spend more time standing on a hard surface when provided with a restrictive stall. Increased perching behavior, which is correlated to increased risk for lameness (Galindo et al., 2000), a behavior that increases in when neck-rails are positioned closer to the stall curb (Tucker et al., 2005; Fregonesi et al., 2009; Bernardi et al., 2009; Lombard et al., 2010; Sudolar et al., 2018). Inversely, perching behavior can be reduced by moving the neck rail to allow cows to stand fully in the stall (Bernardi et al., 2009). However, Sudolar et al. (2018) reported no differences in the stall preference or lying duration when heifers were exposed to two neck rail positions (160 cm from the curb at 124 cm vs. 150 cm from the curb at 122 cm height). The failure to note any differences may have been due to the small difference in neck rail position treatments (10 cm length) with respect to distance from the stall curb.

Cows prefer to enter and to lie down in a stall without brisket boards; lying time decreased by about 1 hour per day when cows were forced to lie down in stalls with brisket boards compared to when they were absent (Tucker et al., 2006). Similarly, close stall partitions width 112cm (narrow stalls) reduce lying duration in free-stalls (Tucker et al., 2004) and tie-stalls (Bouffard et al., 2017). Narrow stalls make it more difficult for cows to perform normal behaviors (Tucker et al., 2004; Bouffard et al., 2017). Cows in an alternative stall design (with no neck rail or stall dividers other than a wooden board protruding slightly (8 cm) above the lying surface) spent more time standing fully on the stall than lying (Abade et al., 2015). A similar effect was reported for cows standing on pasture, indicating that the surface in which they stand is also important and must not be overlooked (Hernandez-Mendo et al., 2007).

### *1.4.2 Impact of stall design on cow and stall hygiene*

Free-stalls with neck rails positioned further from the curb (i.e. longer stalls) are dirtier than those with restricted neck rails, as they are more likely to contain more fecal matter and urine due to increased cows standing in the stall (Tucker et al., 2005; Fregonesi et al., 2009; Bernardi et al., 2009; Ruud et al. 2011; Sudolar et al., 2018) and also take more time to clean (Fregonesi et al., 2009; Bernardi et al., 2009). Similarly, less restrictive stalls (with no neck rail or stall dividers, other than a wooden board 8 cm above the lying area) resulted in more fecal matter on the stall surface and on the cows' udders (Abade et al., 2015). Poor stall hygiene has been associated with poor udder hygiene (DeVries et al., 2012). Researchers have reported dirtier udders for cows in longer stalls (Fregonesi et al., 2009; Bernardi et al., 2009), with an interaction of cow size, where larger cows were more likely to have dirty udders when compared to smaller cows (Fregonesi et al., 2009). Zurbrigg et al. (2005) reported that stall length in tie-stall barns (n=317 farms) affected the risk for dirty legs in tie-stalls, but a more recent study (Bouffard et al., 2017) did not report any association of tie-stall length with cow hygiene (n=3,485 cows). Stall hygiene has been associated with SCC (Koster et al., 2006) and dirty udders to subclinical mastitis (Compton et al., 2007; Breen et al., 2009; de Pinho Manzi et al., 2011). The presence of a brisket board (Tucker et al., 2006), as well as closer partitions (i.e. narrow stalls; Tucker et al., 2004) and more restrictive neck rails (Sudolar et al., 2018) aid in keeping stalls clean, but at the expense of less stall usage (Tucker et al, 2004, 2005, 2006).

In a study by Husfeldt and Endres (2012) stall base (deep bedded vs. mattress) was not associated with cow hygiene when bedded with recycled manure solids. In contrast Fulwider et al., (2007) reported an association between stall base and cows hygiene, but it should be noted that differences in bedding types and management of bedding were not controlled. For example, rubber filled mattresses and waterbeds were bedded more frequently (3.9 times/wk.) than deep bedded stalls (sand: 1.9 times/wk.), thus without accounting for those factors, these results are not interpretable.

### *1.4.3 Impact of stall design on cow leg injuries and lameness*

Each stall component is designed to address a specific function (Abade et al., 2015). A cross-sectional study completed on 297 Holstein dairy herds across the United States reported severe hock lesions to be uncommon (~1.0%) on farms using sand as bedding, or dirt as a stall base (Lombard et al., 2010), it compared to prevalence's of 2.3% for herds that had rubber mats or mattresses as a stall base, compared to 1.8% on concrete stall base, and less than 1% for stalls using dirt as a stall base (Lombard et al., 2010). Other researchers (Weary and Taszkun, 2000; Mowbray et al., 2003; Fulwider et al., 2007; Hudsfeldt et al., 2012; Zaffino Heyerhoff et al., 2014) have reported a similar trend, with lower prevalence of hock lesions and severe lesions on cows housed in deep-bedded stalls compared to those housed on mattresses. A recent study (Cook et al., 2016) reported a 30% difference in cows with swollen or with injured (ulcerated) hocks, when the cows were housed on rubber mats or mattress stalls, compared to only ~ 5% when in deep-bedded stalls. Similarly, mattresses used with little bedding resulted in a doubling of the incidence of lameness (11-24%, respectively; Cook et al., 2004) or varied vastly in prevalence of lameness (~17 vs. 11% lameness) between the stall bases (deep-bedded, mattresses; Cook et al., 2016).

In tie-stall barns, cows have a higher risk for hock lesions when tied using a short neck chain (Zurbrigg et al., 2005; Nash et al., 2016; Bouffard et al., 2017). Bouffard et al. (2017)

reported that an increase in the chain length of 10 cm was associated with a decreased risk of up to 10% in hocks, knees, and neck lesions for cows housed in tie-stall barns. Results in that same study highlighted the unexpected result that an increase in the neck rail height would increase the risk for neck lesions and lameness. Zurbrigg et al. (2005) reported that a rail height above 117cm was associated with a lower risk of neck lesions in tie-stall herds.

Narrow stalls (decreased distance between stall partitions) have been associated with increased hock lesions (Nash et al., 2016), neck injuries for cow housed in tie stalls (Bouffard et al., 2017), and lameness in both free-stall and tie-stall herds (Solano et al., 2015; Bouffard et al., 2017). Lameness is also associated with knee lesions and hock lesions (Westin et al., 2016). The odds of being lame increased (1.4 x) for cows afflicted with hock injuries (Solano et al., 2015), potentially due to cows having difficulty of movement (getting up, lying down) and exacerbating the hock when they move (Zaffino Heyerhoff et al., 2014). It is also possible that the pain derived from a lesion may cause cows to become lame (Solano et al., 2015). Narrow stalls and more restrictive neck rails increase perching (standing with front two hooves in the stall). Cows that perform perching behavior are more likely to become lame (Galindo et al., 2000; Proudfoot et al., 2010) and experience claw lesions (Dippel et al., 2011). Narrow stalls fitted with mattresses were moderately correlated negatively to prevalence of severe tarsal joint lesions (Fulwider et al., 2007). Neck-rails that are closer to the stall curb in free-stalls increase perching behavior (Tucker et al., 2005; Fregonesi et al., 2009; Bernardi et al., 2009; Lombard et al., 2010; Sudolar et al., 2018). Inversely, greater stall width is associated with reduced risk of neck lesions, hind claw rotation (Zurbrigg et al., 2005) and lameness in tie-stalls, but at the expense of reduced cow hygiene for legs and flanks (Bouffard et al., 2017). However, Bouffard et al. (2017) argue that the minimal (4% dirty udders) prevalence of dirtier udders expense is not justifiable to lameness (25%) and lesions (30-60%) with reduced width space. Longer stall length has been associated with less lameness (Solano et al., 2015), less knee injuries (Bouffard et al., 2017), and with increased SCC only for rubber filled mattresses (Fulwider et al., 2007). However, Bernardi et al. (2009) found no effect of neck rail position on clinical or subclinical mastitis but highlighted that their study was not designed to assess the effects of udder health due to neck rail movement. Thus, research on the effect of neck rail placement on clinical and subclinical mastitis is needed.

### *1.5 Justification for research*

Most studies above mentioned, have focused on the comparison between inorganic versus organic bedding. As discussed in the literature, we do know that not all producers have access to inorganic bedding. Those producers that only have access or can only utilize organic bedding sources, would be better informed if there was updated information on which bedding types had greater bacterial counts and bedding management practices that are associated with cow hygiene and milk quality . This valuable information could help producers on selecting a bedding type that they would consider best suited for their farm. The last study that compared bacterial content in used bedding sources such as straw, sawdust, and RMS was published in 1989. Recent work has looked at bedding sources that promote bacterial growth and/ or have not included a popular bedding source- straw. Further, to date no study has made direct connections of stall hygiene, with cow hygiene and risk of elevated somatic cell count, as well as intra-mammary infection cause by an environmental pathogen with regards to stall dimensions.

*1.6 Thesis Objectives*

The overall objective of the work described in this thesis was to identify the primary aspects of the cow's resting area, and its management, that are associated with hygiene, standing and lying behavior, and milk quality (measured by individual cow SCC), specifically focusing on bedding choice, stall design, hygiene, and cow health.

The objective of the first study (Chapter 2) was to determine which bedding options and bedding management practices are associated with low bacterial count, and improved cow hygiene and milk quality. It was hypothesized that organic-based bedding, particularly manure-based bedding options, would be associated with a higher bacterial count and higher cow SCC. Additionally, we predicted that management practices, such as frequency of addition of new dry bedding and frequency of manure removal, would be associated with improved cow hygiene and milk quality.

The objective of the second study (Chapter 3) was to identify cow-level factors associated with poor body hygiene. We predicted that cows in early stage of lactation would be at greater risk of poorer hygiene and that cows in first parity would be a lower risk of poor hygiene when compared to older cows. We also predicted that cows in tie-stalls would have better lower leg and udder hygiene than cows housed in free-stall barns.

The objective of the third study (Chapter 4) was to investigate the associations among cow behavior and housing management, and the risk of eSCC in lactating dairy cows housed in commercial free-stall barns. In particular, to determine how stall design and usage interacts with hygiene to affect the risk of eSCC and lameness. It was hypothesized that cows lying in more restrictive free-stalls, with harder lying surfaces, will have higher gait scores (i.e. more lameness), but potentially lower hygiene scores (cleaner) and risk of eSCC, due to defecation and urination in alleyways, and less usage of the stalls.

The objective of the fourth study (Chapter 5) was to determine how neck-rail position impacts stall cleanliness, cow hygiene, and the risk for IMI and lameness. It was hypothesized that cows lying in stalls with neck rail positioned closer to the stall curb (155 cm) will have higher gait scores (i.e. more lameness), but potentially lower hygiene scores (cleaner), and risk of IMI, due to defecation and urination in alleyways instead of the stalls.

## 1.7 REFERENCES


Abade, C.C., Fregonesi, J.A., von Keyserlingk, M.A.G., Weary, D.M. 2015. Dairy cow preference and usage of an alternative freestall design. J. Dairy Sci. 98, 960-965.

Barkema, H. W., Y. H. Schukken, T. J. G. M. Lam, M. L. Beiboer, H. Wilmink, G. Benedictus, and A. Brand. 1998. Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts. J. Dairy Sci. 81:411–419.

Bernardi, F., J. Fregonesi, C. Winckler, D. M. Veira, M. A. G. von Keyserlingk, and D. M. Weary. 2009. The stall-design paradox: Neck rails increase lameness but improve udder and stall hygiene. J. Dairy Sci. 92:3074–3080.

Bouffard, V., A. M. de Passillé, J. Rushen, E. Vasseur, C. G. R. Nash, D. B. Haley, and D. Pellerin. 2017. Effect of following recommendations for tie-stall configuration on neck and leg lesions, lameness, cleanliness, and lying time in dairy cows." J. Dairy Sci.100:2935-2943.

Bradley, A. J., K. A. Leach, M. J. Green, J. Gibbons, I. C. Ohnstad, D. H. Black, B. Payne, V. E. Prout, and J. E. Breen. 2018. The impact of dairy cows' bedding material and its microbial content on the quality and safety of milk–A cross sectional study of UK farms. Inter. J. Food. Microbiol. 269:36-45.

Bramley, M.1962. Kow kubicles. Farm Buildings Association, Stowmarket, Suffolk, UK, pp. 69–73.

Breen, J. E., A. J. Bradley, and M. J. Green. 2009a. Quarter and cow risk factors associated with a somatic cell count greater than 199,000 cells per milliliter in United Kingdom dairy cows. J. Dairy Sci. 92:3106–3115.

Breen, J.E., M. J. Green, A. J. Bradley. 2009b. Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the United Kingdom. J. Dairy Sci. 92:2551-2561.

Chaplin, S. J., G. Tierney, C. Stockwell, D. N. Logue, and M. Kelly. 2000. An evaluation of mattresses and mats in two dairy units. App. Anim. Behav. Sci. 66: 263-272.

Chen, J. M., Stull, C. L., Ledgerwood, D. N., & Tucker, C. B. (2017). Muddy conditions reduce hygiene and lying time in dairy cattle and increase time spent on concrete. J. Dairy Sci. 100:2090-2103.

Compton, C. W. R., C. Heuer, K. I. Parker, and S. McDougall. 2007b. Risk factors for peripartum mastitis in pasture-grazed dairy heifers. J. Dairy Sci. 90:4171–4180.

Cook, N. B., T. B. Bennett, and K. V. Nordlund. 2004. Effect of free-stall surface on daily activity patterns in dairy cows with relevance to lameness prevalence. J. Dairy Sci. 87:2912–2922.

Cook, N. B., J. P. Hess, M. R. Foy, T. B. Bennett, and R. L. Brotzman. 2016. Management characteristics, lameness, and body injuries of dairy cattle housed in high-performance dairy herds in Wisconsin. J. Dairy Sci. 99:5879-5891.

Cook, N. B. 2002. The influence of barn design on dairy cow hygiene, lameness and udder health. Pages 97–103 in Proc. 35th Annu. Conf. Am. Assoc. Bovine. Pract., Stillwater, OK. Am. Assoc. Bovine. Pract., Auburn, AL.

Costello, M., M. Rhee, M. P. Bates, S. Clark, L. O. Luedecke, and D. Kang. 2003. Eleven year trends of microbiological quality in bulk tank milk. Food Prot. Trends 23:393–400.

de Pinho Manzi, M., Nóbrega, D.B., Faccioli, P.Y., Troncarelli, M.Z., Menozzi, B.D., Langoni, H., 2011. Relationship between teat-end condition, udder cleanliness and bovine subclinical mastitis. Res. Vet. Sci. 93, 430-434.

Devries, T. J., M. G. Aarnoudse, H. W. Barkema, K. E. Leslie, and M. A. Von Keyserlink. 2012. Associations of dairy cow behavior, barn hygiene, cow hygiene, and risk of elevated somatic cell count. J. Dairy Sci. 95:5730–5739.

Dippel, S., Tucker, C.B., Winckler, C. and Weary, D.M., 2011. Effects of behavior on the development of claw lesions in early lactation dairy cows. App. Anim. Behav. Sci. 134:16-22.

Dohmen, W., F. Neijenhuis, and H. Hogeveen. 2010. Relationship between udder health and hygiene on farms with an automatic milking system. J. Dairy Sci. 93:4019–4033.

Drissler, M., M. Gaworski, C. B. Tucker, and D. M. Weary. 2005. Free-stall maintenance: effects on lying behavior of dairy cattle. J. Dairy Sci. 88:2381- 2387.

Eckelkamp, E. A., J. L. Taraba, K. A. Akers, R. J. Harmon, and J. M. Bewley. 2016. Sand bedded free-stall and compost bedded pack effects on cow hygiene, locomotion, and mastitis indicators." Livest. Sci. 190:48-57.

Fregonesi, J. A., D. M. Veira, M. A. G. von Keyserlingk, and D. M. Weary. 2007. Effects of bedding quality on lying behavior of dairy cows. J. Dairy Sci. 90:5468–5472.

Fregonesi, J. A., M. A. G. von Keyserlingk, C. B. Tucker, D. M. Veira, and D. M. Weary. 2009. Neck-rail position in the free-stall affects standing behavior and udder and stall cleanliness. J. Dairy Sci. 92:1979-1985.

Fulwider, W. K., Grandin, T., Garrick, D. J., Engle, T. E., Lamm, W. D., Dalsted, N. L. and Rollin, B.E., 2007. Influence of free-stall base on tarsal joint lesions and hygiene in dairy cows. J. Dairy Sci. 90:3559-3566.

Galindo, F., Broom, D. M., Jackson, P. G. G., 2000. A note on possible link between behavior and the occurrence of lameness in dairy cows. Appl. Anim. Behav. Sci. 67:335–341.

Godden, S., R. Bey, K. Lorch, R. Farnsworth, and P. Rapnicki. 2008. Ability of organic and inorganic bedding materials to promote growth of environmental bacteria. J. Dairy Sci. 91:151–159.

Groot Antink, M. 2009. Boxcompost voor koe in opmars. Veldpost 24:17.

Haley, D. B., J. Rushen, and A. M. de Passille´. 2000. Behavioral indicators of cow comfort: Activity and resting behavior of dairy cows in two types of housing. Can. J. Anim. Sci. 80:257–263.

Hernandez-Mendo, O., M. A. G. Von Keyserlingk, D. M. Veira, and D. M. Weary. 2007. Effects of pasture on lameness in dairy cows. J. Dairy Sci. 90: 1209-1214.

Hogan, J. S., and Smith, K. L., 1997. Bacteria counts in sawdust bedding. J. Dairy Sci. 80:1600–1605.

Hogan, J. and Smith, K. L., 2012. Managing environmental mastitis. Vet. Clin. Anim. Food. Pract. 28:217-224.

Hogan, J. S., K. L. Smith, K. H. Hoblet, D. A. Todhunter, P. S. Schoenberger, W. D. Hueston, D. E. Pritchard, G. L. Bowman, L. E. Heider, B. L. Brockett, and H. R. Conrad. 1989. Bacterial counts in bedding materials used on nine commercial dairies. J. Dairy Sci. 72:250–258.

Hogan, J. S., V. L. Bogacz, L. M. Thompson, S. Romig, P. S. Schoenberger, W. P. Weiss, and K. L. Smith. 1999. Bacterial counts associated with sawdust and recycled manure bedding treated with commercial conditioners. J. Dairy Sci. 82:1690– 1695.

Husfeldt, A.W, and M. I. Endres. 2012. Association between stall surface and some animal welfare measurements in free-stall dairy herds using recycled manure solids for bedding. J. Dairy Sci. 95:5626–5634.

Husfeldt, A.W, M. I. Endres, J. A. Salfer, and K. A. Janni. 2012. Management and characteristics of recycled manure solids used for bedding in Midwest free-stall dairy herds. J. Dairy Sci. 95:2195–2203.

Jayarao, B. M., S. R. Pillai, A. A. Sawant, D. R. Wolfgang, and N. V. Hegde. 2004. Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. J. Dairy Sci. 87:3561–3573.

Keys, Jr., J. E., L. W. Smith, and B. T Weinland. 1976. Response of dairy cattle given a free choice of free-stall location and three bedding materials. J. Dairy Sci. 59:1157-1162.

Köster, G., B-A. Tenhagen, and W. Heuwieser. 2006. Factors associated with high milk test day somatic cell counts in large dairy herds in Brandenburg. I: Housing conditions. J. Vet.Med. 53: 134-139.

Krawczel and Grant, 2009. P. Krawczel, R. Grant. Effects of cow comfort on milk quality, productivity, and behavior National Mastitis Council, Verona, WI (2009) Pages 15–24 in Proc. 48th Annu. Meet. Natl. Mastit. Counc., Charlotte, NC.

LeBlanc, S. J., K. D. Lissemore, D. F. Kelton, T. F. Duffield, and K. E. Leslie (2006). Major advances in disease prevention in dairy cattle. J. Dairy Sci. 89: 1267-1279.

Lombard, J. E., C. B. Tucker, M. A. G. von Keyserlingk, C. A. Kopral, and D. M. Weary. 2010. Associations between cow hygiene, hock injuries, and free-stall usage on U.S. dairy farms. J. Dairy Sci. 93:4668–4676.

Manninen, E., A. M. de Passille´, J. Rushen, M. Norring, and H. Saloniemi. 2002. Preferences of dairy cows kept in unheated buildings for different kind of cubicle flooring. Appl. Anim. Behav. Sci. 75:281–292.

Mattachini, G., Riva, E., Provolo, G., 2011. The lying and standing activity indices of dairy cows in free-stall housing. Appl. Anim. Behav. Sci. 129:18-27.

Mowbray, L., T. Vittie, and D. M. Weary. 2003. Hock lesions and free-stall design: Effects of stall surface. Pages 288–295. In Proc. 5th Intern. Dairy Housing Conf. American Society of Agriculture Engineers, St. Joseph, MI.

Nash, C. G. R., D. F. Kelton, T. J. DeVries, E. Vasseur, J. Coe, J. C. Z. Heyerhoff, V. Bouffard, D. Pellerin, J. Rushen, A. M. de Passillé, and D. B. Haley. 2016. Prevalence of and risk factors for hock and knee injuries on dairy cows in tie-stall housing in Canada. J. Dairy Sci. 99:6494–6506.

Norring, M., E. Manninen, A. M. de Passille, J. Rushen, L. Munksgaard, and H. Saloniemi. 2008. Effects of sand and straw bedding on the lying behavior, cleanliness, and hoof and hock injuries of dairy cows. J. Dairy Sci. 91:570–576.

Piotr Herbut & Sabina Angrecka. 2018. Relationship between THI level and dairy cows' behavior during summer period, Ital. J. Anim. Sci. 17:226-223.

Popescu, S., C. Borda, E. A. Diugan, M. Spinu, I. S. Groza, and C. D. Sandru. 2013. Dairy cows' welfare quality in tie-stall housing system with or without access to exercise. Acta Vet. Scand. 55:43.

Proudfoot, K.L., Weary, D.M., von Keyserlingk, M.A.G., 2010. Behavior during transition differs for cows diagnosed with claw horn lesions in mid lactation. J. Dairy Sci. 93:3970–3978.

Ravagnolo, O., I. Misztal, and G. Hoogenboom. 2000. Genetic component of heat stress in dairy cattle, development of heat index function. J. Dairy Sci. 83:2120-2125.

Reich, L. J., D. M. Weary, D. M. Veira, and M. A. G. von Keyserlingk. 2010. Effects of sawdust bedding dry matter on lying behavior of dairy cows: A dose-dependent response. J. Dairy Sci. 93:1561–1565.

Rendos, J. J., R. J. Eberhart, and E. M. Kesler. 1975. Microbial populations of teat ends of dairy cows, and bedding materials. J. Dairy Sci. 58:1492–1500.

Reneau, J. K., A. J. Seykora, and B. J. Heins. 2003. Relationship of cow hygiene scores and SCC. Pages 362–363 in Proc. Natl. Mast. Coun., Madison, WI.

Reneau, J. K., A. J. Seykora, B. J. Heins, M. I. Endres, R. J. Farnsworth, and R. F. Bey. 2005. Association between hygiene scores and somatic cell scores in dairy cattle. J. Am. Vet. Med. Assoc. 227:1297–1301.

Rowbotham, R. F. 2000. The value of quality milk to the processor. Pages 1–6 in Natl. Mastitis Counc. Mtg. Proc., Cleveland, OH. Natl. Mastitis Counc. Inc., Verona, WI.

Rowbotham, R. F., and P. L. Ruegg. 2015. Association of bedding types with management practices and indicators of milk quality on larger Wisconsin dairy farms. J. Dairy Sci. 98:7865 7885.

Rowbotham, R. F., and P. L. Ruegg. 2016. Bacterial counts on teat and skin in new sand, recycled sand, and recycled manure solids used as bedding in free-stalls. J. Dairy Sci. 99:6594 6608.

Ruud, L. E., Kielland, C., Østerås, O., Bøe, K. E. 2011. Free-stall cleanliness is affected by stall design. Livest. Sci. 135:265–273.

Sant'Anna, A. C., and M. J. R. Paranhos da Costa. 2011. The relationship between dairy cow hygiene and somatic cell count in milk. J. Dairy Sci. 94: 3835-3844.

Schmisseur, W. E., Albright, J. L., Brown, C. M., Dillon, W. M., Kehrberg, E.W., Morris, W. H. M.1966. Comparison of free-stall to conventional loose housing. J. Dairy Sci. 49:730.

Schreiner, D. A. and P. L. Ruegg. 2003. Relationship between udder and leg hygiene scores and subclinical mastitis. J. Dairy Sci. 86:3460–3465.

Schütz, K. E., and N. R. Cox. 2014. Effects of short-term repeated exposure to different flooring surfaces on the behavior and physiology of dairy cattle. J. Dairy Sci. 97:2753–2762.

Schukken, Y. H., D. J. Wilson, F. Welcome, L. Garrison-Tinofsky, and R. N. Gonzales. 2003. Monitoring udder health and milk quality using somatic cell counts. Vet. Res. 34:579–596.

Schwarz, D., U. S. Diesterbeck, K. Failing, S. König, K. Brügemann, M. Zschöck, W. Wolter, and

C. P. Czerny. 2010. Somatic cell counts and bacteriological status in quarter foremilk samples of cows in Hesse, Germany—A longitudinal study. J. Dairy Sci. 93:5716–5728.

Solano, L., H. W. Barkema, E. A. Pajor, S. Mason, S. J. LeBlanc, J. C. Zaffino Heyerhoff, C. G. R. Nash, D. B. Haley, D. Pellerin, J. Rushen, A. M. de Passillé, E. Vasseur, and K. Orsel. 2015. Prevalence of lameness and associated risk factors in Canadian Holstein- Friesian cows housed in free-stall barns. J. Dairy Sci. 98:6978–6991.

Solano, L., H. W. Barkema, E. A. Pajor, S. Mason, S. J. LeBlanc, C. G. R. Nash, D. B. Haley, D. Pellerin, J. Rushen, A. M. de Passille, E. Vasseur, and K. Orsel. 2016. Associations between lying behavior and lameness in Canadian Holstein-Friesian cows housed in free-stall barns. J. Dairy Sci. 99:2086–2101.

Sorter, D.E., H. J. Kester, and J. S. Hogan. 2014. Short communication: Bacterial counts in recycled manure solids bedding replaced daily or deep packed in free-stalls. J. Dairy Sci, 97:2965–2968.

Sudolar, N. R., Panivivat, R., and Sopannarath, P. 2017. Effects of two neck rail positions on heifer behavior and stall cleanliness in free-stall barn. Agric. Nat. Resour. Sci. 51:432-435.

Tucker, C. B., D. M. Weary, and D. Fraser. 2003. Effects of three types of free-stall surfaces on preferences and stall usage by dairy cows. J. Dairy Sci. 86:521–529.

Tucker, C. B., D. M. Weary, and D. Fraser. 2004. Free-stall dimensions: Effects on preference and stall usage. J. Dairy Sci. 87:1208–1216.

Tucker, C. B., D. M. Weary, and D. Fraser. 2005. Influence of neck-rail placement on free-stall preference, use, and cleanliness. J. Dairy Sci. 88:2730–2737.

Tucker, C. B., G. Zdanowicz, and D. M. Weary. 2006. Brisket boards reduce free-stall use. J. Dairy Sci. 89:2603–2607.

Tucker, C. B., Munksgaard, L., Mintline, E. M., & Jensen, M. B. (2018). Use of a pneumatic push gate to measure dairy cattle motivation to lie down in a deep-bedded area. Appl. Anim. Behav. Sci. 201:15-24.

van Gastelen, S., B. Westerlaan, D. J. Houwers, and F. J. C. M. van Eerdenburg. 2011. A study on cow comfort and risk for lameness and mastitis in relation to different types of bedding materials. J. Dairy Sci. 94:4878–4888.

Vasseur, E., J. Rushen, D. B. Haley, and A. M. de Passillé. 2012. Sampling cows to assess lying time for on-farm animal welfare assessment. J. Dairy Sci. 95:4968–4977.

Ward, W. R., J. W. Hughes, W. B. Faull, P. J. Cripps, J. P. Sutherland, and J. E. Sutherst. 2002.

Observational study of temperature, moisture, pH and bacteria in straw bedding, and faecal consistency, cleanliness and mastitis in cows in four dairy herds. Vet. Rec. 151:199–206.

Weary, D. M., and I. Taszkun. 2000. Hock lesions and free-stall design. J. Dairy Sci. 83:697–702.

Westin, R., A. Vaughan, A. M. de Passillé, T. J. DeVries, E. A. Pajor, D. Pellerin, J. M. Siegford, E. Vasseur, and J. Rushen. 2016. Lying times of lactating cows on dairy farms with automatic milking systems and the relation to lameness, leg lesions, and body condition score. J. Dairy Sci. 99:551–561.

Wolfe, T., E. Vasseur, T. J. DeVries, and R. Bergeron. 2018. Effects of alternative deep bedding options on dairy cow preference, lying behavior, cleanliness, and teat end contamination. J. Dairy Sci. 101:530-536.

Zaffino Heyerhoff, J. C., S. J. LeBlanc, T. J. DeVries, C. G. R. Nash, J. Gibbons, K. Orsel, H. W. Barkema, L. Solano, J. Rushen, A. M. de Passillé, and D. B. Haley. 2014. Prevalence of and factors associated with hock, knee, and neck injuries on dairy cows in free-stall housing in Canada. J. Dairy Sci. 97:173–184.

Zdanowicz, M., J. A. Shelford, C. B. Tucker, D. M. Weary, and M. A. G. von Keyserlingk. 2004. Bacterial populations on teat ends of dairy cows housed in freestalls and bedded with either or sawdust. J. Dairy Sci. 87:1694–1701.

Zurbrigg, K., D. Kelton, N. Anderson, and S. Millman. 2005. Tie-stall design and its relationship to lameness, injury, and cleanliness on 317 Ontario dairy farms. J. Dairy Sci. 88:3201–3210.

# CHAPTER 2: BACTERIAL CONCENTRATIONS IN BEDDING AND THEIR ASSOCIATION WITH COW HYGIENE AND MILK QUALITY

## 2.1 INTRODUCTION

Environmental bacteria, such as *Escherichia coli*, *Klebsiella* spp, and *Streptococcus uberis,* are a common cause of clinical mastitis in dairy cows (Lago et al., 2011; Levington et al., 2016; Pinzon-Sanchez and Ruegg 2011; Oliviera et al., 2013), particularly on farms with a low bulk tank SCC (**BTSCC**; Barkema et al., 1998; Olde Riekerink et al., 2008). Dirty or wet bedding can become heavily contaminated with these bacteria and act as a source of intramammary infections (IMI) that may result in (sub)clinical mastitis (e.g. Hogan and Smith, 2012). It is therefore important to keep bedding clean and, as a result, reduce exposure to primary agents that may cause environmental mastitis (Hogan and Smith, 2012). In a study at a single facility, following 1,234 quarters from 309 cows, all reported cases of clinical mastitis cases (n = 73), with the exception of a single case, were caused by environmental pathogens (Rowbotham and Ruegg, 2016a). Furthermore, these same authors note that environmental Streptococci, *Escherichia coli,* and *Klebsiella* spp. were identified in 50% of culture-positive cases of clinical mastitis and were also found in cultures of bedding and teat swab samples (Rowbotham and Ruegg, 2016a).

Dairy cattle spend approximately 11 h/d lying down (Ito et al., 2009) and during that time bacteria can be transferred from the lying surface to the teat (Hogan et al., 1999). Direct exposure to pathogens may therefore occur as cows rest (Rowbotham and Ruegg, 2015). Reducing the bacterial content of the bedding surface is therefore a key step in mastitis control. Many studies (e.g. Zdanowicz et al., 2004; Kristula et al., 2005; Rowbotham et al., 2016a) have demonstrated the advantage of using clean and dry sand (inorganic bedding) as a way of reducing the growth of bacteria associated with environmental mastitis. However, producers face numerous challenges when attempting to handle, use and dispose of sand, and given the high costs of traditional organic bedding sources efforts have focused on identifying alternative bedding sources (Husfeldt et al., 2012). Recycled manure solids (**RMS**) are an alternative bedding option that has received some attention. Like sand, and other organic bedding sources, RMS may be placed in deep-bedded stalls; large amounts of bedding are known to maximize stall usage, reduce lameness and hock lesions (Tucker and Weary, 2004), and could increase cow longevity given that udder health of cows is less compromised. However, RMS has a higher bacterial count (**BC**) compared to sand (Rowbotham and Ruegg, 2016b) and shavings (Godden et al., 2008). To our knowledge no recent study has included a comparison between the bacterial content of RMS and straw, which is also commonly used as bedding on dairy farms.

The objective of this study was to determine which bedding options and bedding management practices are associated with low BC, and improved cow hygiene and milk quality. It was hypothesized that organic-based bedding compared to inorganic bedding, particularly manure-based bedding options, would be associated with a higher BC and higher cow SCC. Additionally, we predicted that management practices, such as frequency of addition of new dry bedding and frequency of manure removal, would be associated with cow hygiene and milk quality.

## 2.2 MATERIALS AND METHODS

### *2.2.1 Farm Selection*

A cross-sectional study was conducted on 75 dairy farms in Ontario, Canada. Farms were visited 3 times, 7 d apart, from October 2014 to February 2015. Farms were recruited through an email sent by CanWest DHI (Guelph, ON, Canada) to producers located within 150 km of the University of Guelph, Kemptville Campus (Kemptville, ON, Canada). Producers were asked to contact the principle investigator directly, to obtain more information about the project and then confirm interest in enrolling in the study. Specific criteria for selection of farms included: DHI participation and having lactating cows housed in a free-stall, tie-stall or bedded-pack barn with any of various types of bedding and lying surfaces. Cows were primarily of the Holstein breed. Animal use and study design were approved by the University of Guelph's Animal Care Committee (AUP#3140) and Research Ethics Board (REB#14JN019), respectively, and animal use complied with the guidelines of the Canadian Council on Animal Care (2009).

### *2.2.2 Farm Data Collection*

During the first visit, a graduate student with previous experience conducting surveys conducted an oral questionnaire designed to collect information about the bedding, housing management practices, and udder health. The questionnaire (Appendix 1; modified from Dufour et al. (2010)), consisted of 30 questions that requested information on stocking density, stall base, bedding type, amount and frequency of bedding applied including date of the last time additional bedding was added, time of last raking/cleaning, type of bedding application (whole stall, back half), and whether bedding conditioners were applied to the bedding. At the second and third visit, producers were asked when additional bedding was last added to the stalls.

Stall dimensions (including stall width, length, and neck rail position) were recorded in one of the three visits, using methodology adapted from von Keyserlingk et al. (2012). For free-stall barns, 2 stalls from each row in each pen were measured. For tie-stall barns, 2 (but not consecutive) stalls of every 20 stalls in each row of stalls were measured (minimum of 4 stalls per farm). Overall stall dimensions (mean and SD) for each farm were calculated. Temperature and humidity in the 7 d prior to each farm visit were obtained from Environment Canada (Ottawa, ON, Canada) recordings at the closest local weather station (Schüller et al., 2013).

### *2.2.3 Bedding Samples*

At each farm visit, duplicate composite samples of the unused and used bedding material were collected to determine %DM and perform bacterial culture. Unused bedding samples were collected from separate sections of the storage piles on each farm. Each composite sample taken from the pile contained 9 grab samples, with approximately half representing the surface and the remaining from ~12 cm into the pile. When more than one pile of new bedding was available, the same collection procedure was repeated on all piles. Samples were then thoroughly mixed, and 2 smaller composite samples, each ~500 cc (2 cups of volume), were labeled and stored. Used bedding samples from the cow lying surfaces were collected from the back 1/3 of every $10^{th}$ stall in each lactating cow pen (free-stall) or row of stalls (tie-stalls). Nine grab samples of bedding were collected from the back 1/3 of each selected stall. All samples were mixed thoroughly and 2 composite samples, each ~500 mL (2 cups of volume), were labelled and stored. All composite samples were immediately placed into a cooler with ice packs and later transported to the University of Guelph, Kemptville Campus and stored at -20°C until analysis.

Of the duplicate bedding samples, one set was dried at the University of Guelph, Kemptville Campus. Bedding samples were removed from the freezer and thawed, then oven-dried at 55°C for 48 h. The second set of samples were shipped frozen to the University of Prince Edward Island (Charlottetown, PEI, Canada) for bacterial culture of major pathogens (*Streptococcus* spp., all Gram-negative bacteria, and *Klebsiella* spp.).

In the case of the unused and used cultured bedding samples, 100 mL of thawed bedding was placed in a filter bag with 30 mL of sterile water (1:10). The entire 100 mL sample was mixed by repeatedly squeezing the bag 20-30 times, then allowing it to stand for 15-20 minutes, and then repeating the squeezing procedure once more. Once completed, 100 µL of the bedding liquid was added to a 9.9 mL dilution tube (1:1000) and vortexed. In the case of the used bedding material, 1 mL of the 1:100 tube was added to the 9.0 mL dilution tube (1:1000).

For unused and used bedding samples, 50 µL samples of the 1:100 and 1:1000 dilution, respectively, were plated onto each of the media plates and incubated for 24 h (MacConkey's agar, **MAC**, and MacConkey's agarinositol-carbenicillin agar, **MAC IC**) and 48 h (Edward's modified medium agar, **EMCO**) at 35°C. Bacterial groups were identified as Gram-negative bacteria (total growth on MAC), *Klebsiella* spp. (red to pink colonies on MAC IC), and *Streptococcus* spp. (total growth on EMCO). Bacterial count was expressed as cfu/mL.

### *2.2.4 Animal Selection*

At each farm, 25% of the cows in each lactating pen (free-stalls) or row of stalls (tie-stalls) in herds with > 160 cows, or a minimum of 40 cows/herd in those herds with < 160 cows, were randomly selected (from each area of the pen including stalls) and scored for hygiene. The same cows were scored on each of the 3 visits (n = 2770 cows). All lactating cows were scored at the 8 farms with < 40 lactating cows at the first visit. Percentage of cows selected was based on recommendations made by Cook and Reinemann (2007) regarding the percentage of animals sampled from a given herd that would be representative of the herd. Given the longitudinal nature of the study, selection of individual cows for hygiene scoring on the first farm visit was restricted to those cows expected to stay in the lactating pens for the duration of the study.

Udder, lower legs, and upper legs/flank were each scored on a 4-point hygiene scale (1 = very clean to 4 = very dirty) (Cook and Reinemann, 2007), and the percentage of cows with poor hygiene (scores 3 and 4) was calculated per farm (Schreiner and Ruegg, 2003). Two trained researchers scored the cows, with each researcher scoring the same cows at each visit. Inter-observer reliability was evaluated by comparing hygiene scores from a farm assessed independently by the 2 observers.

### *2.2.5 Milk Quality Data*

Data on milk quality (herd average SCC) and cow demographics (parity, DIM) were retrieved from CanWest DHI (Guelph, ON, Canada) reports using the test closest to the first visit and another test closest to the last visit for all cows in each herd. Herd average SCC (average SCC for the herd, weighted by each cow's milk production) from the 2 DHI tests was then used for analysis to assess milk quality at the herd level. Bulk tank SCC and BC (**BTBC**) using Bactoscan test (results in individual bacteria count (IBC/mL)) were also obtained, for a week prior to each farm visit, from the Dairy Farmers of Ontario (Mississauga, ON, Canada).

### *2.2.6 Statistical Analyses*

Bedding substrates were classified by farm: sand (n = 12), straw (straw and hay; n = 33), wood products (shavings and sawdust; n = 17), and RMS (recycled manure solids-compost and digestate; n = 8). In total data from 70 dairy farms were analyzed. Farms with bedding packs were excluded from analysis due to small sample (n = 2), and 3 other farms were removed because they were the only farms using a specific bedding type (straw with lime and water, peat moss, and organic compost, respectively). Further exclusion of farms from specific analyses was performed if information for a specific outcome variable was not available (see Table 1).

Prior to analyses, all data were screened for normality by assessing the distribution of data and any outliers using the UNIVARIATE procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC). Mean cow SCC, BTSCC, BTBC and all bacterial culture results were right skewed and were therefore transformed by taking their natural logarithm. Data were summarized per farm per visit.

Associations between outcomes and bedding types and management, stall design, and bedding %DM, were analyzed with multivariable linear mixed models using the MIXED procedure of SAS, treating visit as a repeated measure. Farm was included as a random effect. Outcome variables averaged at the herd level were milk quality (herd weighted average SCC), percentage of dirty cows per farm (body areas: lower legs, udder, upper legs and flanks); those expressed at the farm level were %DM of used bedding, bacterial content (*Streptococcus* spp., all Gram-negatives, *Klebsiella* spp.), and milk quality (BTSCC and BTBC). Association among hygiene of cow body parts was assessed using the Pearson Correlation (using the CORR procedure of SAS).

To build the multivariable linear mixed models, first, all individual explanatory variables were tested in univariable models. Covariance structures were selected on the basis of best fit according to Schwarz's Bayesian information criterion. Compound symmetry covariance structure was selected for all outcome variables, except for all Gram-negative bacteria as a dependent variable in which the autoregressive (1) structure was selected. Explanatory variables with $P < 0.25$ in the univariable analysis were included in the multivariable models (Dohoo et al., 2009). The Pearson correlation test from CORR of SAS was then used to check for correlations among the retained explanatory variables. If 2 variables were correlated ($r > 0.60$), the one with the lower $P$-value in the univariable analysis was kept. Manual backward elimination was used to remove any variables with $P > 0.10$; those variables retained were considered significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. Mean separation for bedding type differences were adjusted using the Tukey-Kramer procedure. If housing type was associated with an outcome variable, additional housing-specific aspects were investigated in a separate multivariable analysis, by housing type, using the procedure described above. Housing-specific aspects for free-stall barns included: surface of cow walking alleyways, frequency and device used to clean alleyways, and position of brisket board on the lactating cow stalls. Housing-specific aspects for tie-stall barns included: herd access to pasture and product added to bedding.

## 2.3 RESULTS

### *2.3.1 Inter-Observer Reliabilities*

Kappa values were: lower leg = 0.81, udder = 0.82, and upper leg/flank = 0.79. High levels of agreement were found between the observers when scores were categorized as good or poor hygiene (Kappa values for lower leg = 1.00; 95% CI = 1.00-1.00, udder = 0.79; 95% CI = 0.59-0.98, upper leg/ flank = 1.00; 95% CI = 1.00-1.00)

### *2.3.2 Characteristics of Surveyed Farms*

In total, data from 70 dairy farms were analyzed. Data on cow demographics, milk yield and quality, and stall size and maintenance are summarized in Table 2.1. Lactating cows were housed in free-stalls (n = 44) and tie-stalls (n = 26) barns. In average, the frequency of alleyway cleaning in free-stall farms was 12.4 ± 11.0 times/d; mean ± SD). At 24 (55%) of 44 free-stall farms the milking system used was an automatic milking system (AMS), while the 20 (45%) other farms used a milking parlor. At 30 of the free-stall barns, mattresses were used as a stall surface whereas 14 barns had deep bedding. At 11 (42%) of the 26 tie-stall farms, cows had access to pasture in the summer, the remaining 15 herds stayed in the tie-stall year-round. At 3

(7%) of the 44 free-stall farms, cows had access to pasture in the summer, the remaining 41 herds stayed in the free-stall year-round. Producers added a lime product to the bedding after placing on the stall in 12 (46%) of the 26 tie-stall farms. Producers added a lime product to the bedding after placing on the stall in (21%) of the 44 free-stall farms.

### *2.3.3 Cow and Milking Information*

Average size of the 70 herds was 100 lactating cows ranging from 25 to 404, with a mean parity of 2.3 and mean DIM of 170 (Table 1). Average milk production was 32.7 kg of milk/d. Producers used 1 of 3 milk systems: AMS (n = 25; 36%), parlor (n = 21; 30%), or milked in the tie-stall (n = 24; 34%). On farms with a milking parlor or tie-stall, cows were milked 2 (n = 37; 82%) or 3 (n = 8; 18%) times/d. Cows in AMS farms were milked on average 2.6 times/d.

### *2.3.4 Hygiene Score*

All 3 body hygiene scores (lower legs, udder, upper legs and flanks) were analyzed in a separate multivariable analysis by housing, since housing type was associated ($P < 0.001$) with both the percentage of cows with dirty lower legs and with dirty upper legs and flanks ($P < 0.001$). Cows in free-stall barns more often had dirty lower legs, upper legs, and flanks compared to tie-stall barns ($P < 0.001$; Table 2.2).

In the 24 free-stall AMS farms, 11.4% more cows had dirty lower legs compared to cows of farms with a milking parlour (n=20; Table 2.3). In free-stall barns, proportion of cows with dirty udders, upper legs and flank increased with a decreased frequency of alley scraper cleaning (12.4 ± 11 cleanings/d; mean ± SD; Table 2.3). Proportion of cows with dirty udders and upper legs and flanks was higher in herds with a higher average DIM. Farms with mattresses tended to have more cows with dirty upper legs and flank compared to farms with deep bedding (Table 2. 3). At the herd level, poor hygiene of the upper legs and flanks was associated with having dirty udders (r = 0.83; $P < 0.001$). Proportion of cows with poor udder hygiene (percentage of cows per farm with a score of ≥ 3 for udder hygiene) among farms varied per visit ($P = 0.04$; Table 4), being highest at the third visit (Table 2.3).

Herds housed in a tie-stall with access to pasture had 17.7% more cows with dirty legs compared to herds without access to pasture (Table 2.4). Farms that utilized a lime product in their bedding tended to have 16.2% more cows with dirty legs. Farms that added additional bedding every other day, tended to have 7.0% more cows with dirty udders compared to those that added bedding every day. Proportion of cows with dirty upper legs and flanks in tie-stall farms was not associated with explanatory variables tested. Farms with a high proportion of cows with poor udder hygiene also had a higher proportion of cows with poor hygiene of the lower legs (r = 0.56; $P < 0.001$) and of the upper legs and flanks (r = 0.87; $P < 0.001$). Similarly, there was a moderate association between having poor hygiene of lower legs and upper legs and flanks (r = 0.45; $P < 0.001$).

### *2.3.5 Milk Quality*

Weighted average herd SCC of the 64 farms participating in DHI was 184,000 cells/mL (median: 161,000 cells/mL), whereas BTSCC (n = 70) averaged 186,000 cells/mL (median: 180,000 cells/mL), and BTBC averaged 18,000 IBC/mL (median: 10,000 IBC/ml) (Table 2.1). Mean SCC was higher in herds with higher average DIM (intercept = 12.5; $\beta = 0.005$; SE = 0.002; $P = 0.03$); for every 10-d increase in DIM, average weighted herd SCC increased by 10,000 cells/mL. Stall width tended to be associated with average SCC (intercept = 12.5; $\beta$ = -

0.011; SE = 0.006; $P = 0.09$); for every 5-cm increase in stall width, average SCC tended to decrease by 5,000 cells/mL. A weak positive correlation ($r= 0.23$; $P < 0.001$) was reported for stall width and frequency/d of scraping stalls. However, stall length and frequency of scraping stalls were not correlated ($r=-0.11$; $P=0.27$).

Bulk tank bacteria count was higher for farms using RMS bedding, followed by wood products, straw, and sand (Table 2.5). Wider stalls were associated with lower BTBC; for every 5 cm increase in stall width, average BTBC decreased by 5,000 IBC/mL. Lower %DM of used bedding was associated with higher BTBC; for every 10% decrease in used bedding %DM, average BTBC increased by 10,000 IBC/mL. An interaction ($P < 0.01$) was observed between stall width and distance (length) from the back of the stall to the neck rail for BTBC, indicating that BTBC was lower on farms with both longer and wider stalls.

### *2.3.6 Bedding Types and Management*

Organic bedding was used most commonly (71% of farms), followed by inorganic bedding (17%) and RMS (11%). Surface of the milking cow stalls consisted either of mattresses (n = 56) or deep bedding (n = 14). On average, farms added additional bedding to stalls every 3.0 ± 5.0 d (range: 0 to 28 d) (Table 2.1). Frequency of stall cleaning varied greatly among farms ranging from 1 to 8 cleanings per d (Table 2.1).

### *2.3.7 Bedding Samples*

Unused RMS bedding types had lower %DM and higher *Streptococcus* spp. count compared to all other bedding types (Table 2.6). Unused straw bedding was higher than all other bedding types in Gram-negative and *Klebsiella* spp counts. In used bedding, sand was driest, followed by straw and wood products, whereas RMS types were wettest (Table 2.6). Higher relative humidity of the environment tended to be associated with lower %DM (intercept = 87.7; $\beta = -0.162$; SE = 0.09; $P = 0.07$). In used bedding, *Streptococcus* spp. count did not differ among used bedding types (Table 2.6; $P = 0.15$). Higher %DM in used bedding was associated with lower *Streptococcus* spp. count (intercept= 17.58; $\beta = -0.03$; SE = 0.01; $P < 0.01$). *Streptococcus* spp. and all Gram-negative count increased with increasing days since additional bedding was added (intercept = 17.58; $\beta = 0.11$; SE = 0.01; $P < 0.001$; and intercept = 9.85; $\beta = 0.15$; SE = 0.01; $P < 0.001$), respectively). In used bedding, Gram-negative count was highest in RMS, followed by straw and sand, whereas Gram-negative count was lower in wood products (Table 6). *Klebsiella* spp. count in used bedding was similar for sand, straw, and RMS types, whereas this count was lower in wood products (Table 2.6).

## 2.4 DISCUSSION

In this study, bedding types were classified as sand, wood products, straw, and RMS. As expected, unused sand (inorganic bedding) was very dry (92 %DM), which also likely explained the drier used sand bedding samples (95 %DM); good drainage properties of sand help maintain this high DM%. The findings for used sand DM content are similar to those results found in previous studies (Hogan et al., 1989: 95 %DM; Zdanowicz et al., 2004: 95%DM; Kristula et al., 2005; 95 %DM). Likewise, the results for RMS DM content are in agreement with other research that reported this material to be quite wet (~ 40 %DM or lower), but also that RMS dries, albeit only slightly, over time once placed into the stalls (Husfeldt et al., 2012; Sorter et al., 2014; Cole and Hogan, 2016). In the current study, %DM of straw and wood products were similar, in both unused as well as in used bedding. Used straw and wood bedding material had lower %DM compared to the unused material, whereas used RMS bedding %DM was greater than unused RMS and sand %DM did not differ between unused and used bedding. Zdanowicz et al. (2004) reported a similar trend, with %DM in used sawdust bedding decreasing over time after bedding was added to the stall, but %DM of sand remained the same between sampling days. An increase in %DM content once RMS was used as stall bedding was also reported by others (Husfeldt et al., 2012; Sorter et al., 2014; Cole and Hogan, 2016).

As can be expected, relative humidity of the environment (values obtained from the nearest weather station) and %DM in used bedding were negatively associated. Lower %DM in used bedding was also associated with a higher *Streptococcus* spp. count. These results are in agreement with Godden et al. (2008) who demonstrated that bacteria growth is promoted by moisture/humidity. Similarly, Zehner et al. (1986) and Hogan et al. (1990) reported a positive association between relative humidity of the environment and BC in used bedding. Zdanowicz et al. (2004) also reported higher BC (coliforms, *Klebsiella* spp., and *Streptococcus* spp.) in bedding samples of used sawdust with lower %DM. Interestingly, for used sand bedding, Zdanowicz et al. (2004) reported a positive association between %DM and coliform counts. Unfortunately, these researchers did not offer any explanation for this result and thus should be viewed with caution, particularly since their finding contradicts our own and others who have reported that BC are typically lower in drier samples.

Several researchers have demonstrated that BC varies among bedding types, with inorganic bedding containing fewer bacteria than organic bedding types (Hogan et al. 1989; Zdanowicz et al., 2004; Godden et al., 2008). Zehner et al. (1986) tested the ability of different sterilized bedding types to promote growth of environmental bacteria (*E. coli*, *Klebsiella* *pneumoniae*, and *Streptococcus uberis*) under controlled conditions and reported that growth of all bacteria was highest in recycled dried manure, second highest in straw, third in hardwood chips, and lowest in paper and sawdust. Our results indicate that *Streptococcus* spp. count in unused bedding was higher in RMS when compared to sand, straw and wood products, whereas Gram-negative bacteria counts were higher in straw, followed by RMS, and similar in sand and wood. Comparably, *Klebsiella* spp. BC was higher in straw and similar in RMS, wood products, and sand. Similarly, Godden et al. (2008) found that *K. pneumoniae* count was similar among clean sand, recycled sand, digested manure solids and shavings bedding; these researchers did not report the bacterial content of straw bedding.

For used bedding samples, our results are similar to previous studies (e.g. Hogan et al., 1989; Zdanowicz et al., 2004). For example, in the current study Gram-negative count in used bedding was highest in RMS, followed by straw and sand, while it was lower in wood products. Robowtham and Ruegg (2016a) also reported that total Gram-negative bacteria counts were 100 to 1000 times lower in used sand bedding versus used RMS bedding. It is noteworthy that wood-based bedding contained the least Gram-negative bacteria, similar to reported by Zehner et al. (1986) for sterilized unused bedding samples. Comparably, counts of *Klebsiella* spp. in used bedding were similar for sand, straw, and RMS, while lower in wood products. Contrary to our results, Zdanowicz et al. (2004) reported that sawdust had higher coliform and *Klebsiella* spp. counts compared with sand; these results were positively correlated with BC on teat ends. The disagreement in results could be due to the grouping of wood types bedding (sawdust, shavings, wood chips) in our study versus just sawdust used in their study. For example, in an experimental study by Zehner et al. (1986), fine hardwood chips resulted in more *Klebsiella* spp. growth than did softwood sawdust. Furthermore, *Klebsiella* spp. growth was much higher in both recycled manure and straw than in softwood sawdust (Zehner et al., 1986). Similar to Rowbotham et al. (2016b), we detected high levels of *Klebsiella* spp. in used RMS. However, when compared to other bedding types, only RMS differed from wood products, whereas Rowbotham et al. (2016b) detected a difference between sand and RMS. These differences between studies may be due to differences in study design. In their experimental study, Rowbotham et al. (2016b) added new bedding twice weekly whereas in our observational study sand bedding was added primarily on either a weekly or biweekly basis, depending on the participating farms. The longer time interval in our study may have promoted the growth of bacteria on sand, which may have prevented any observable differences in the current study between bedding types (with the exception of the RMS). Additionally, the longer time interval in our study may have increased the proportion of fecal matter in the bedding, and thus also increasing the bacterial counts in the bedding. A study in which stall cleanliness and bacterial counts in used bedding types (sand, sawdust) were examined, it was reported that dirtier stalls (i.e. greater number of squares containing any visible fecal matter) contained greater bacteria counts (Coliforms- lactose- positive colonies on MC agar, *Streptococci,* and *Klebsiella spp.)* (Zdanowicz et al., 2004). However, stall cleanliness was not assessed in the current study, thus, although plausible, bacterial count increase with increased stall dirtiness cannot be confirmed.

*Streptococcus* spp. were high in all used bedding types, but no differences between bedding types were present. This is consistent with previous work showing environmental bacteria numbers are high for all bedding types typically used for cows, including sand and RMS, feces, and feed (Bramley, 1982; Hogan and Smith, 2012; Rowbotham and Ruegg,2016b).

Management plays a key role in reducing environmental pathogen exposure to dairy cows. The number of days since each bedding substrate is placed upon the lying surface can affect bacterial growth (Zdanowicz et al., 2004; Kristula et al., 2005; Cole and Hogan, 2016). For instance, in the present study, *Streptococcus* spp. and Gram-negative BC increased with days since additional bedding was added. Further, low %DM of used bedding was associated with higher BTBC. It is possible that higher BC in low %DM bedding resulted in greater transfer of bacteria from the bedding to cow teat ends. Evidence has been reported of a strong association between bacteria in bedding and on cow teats and higher bacteria counts for bedding samples with low %DM (Zdanowicz et al., 2004; Rowbotham et al., 2016b). Collectively, other research and the results of our general linear model for bedding addition support the recommendation that bedding be added and replaced frequently to reduce exposure to environmental mastitis pathogens.

Farms with RMS bedding had the highest BTBC, followed by wood, straw and sand, which were all similar. In contrast, Rowbotham and Ruegg (2015) did not report an association between total BC in milk and bedding type. Conversely, in our study BTSCC was not associated with bedding type, wheras Robowtham and Ruegg (2015) reported that farms using inorganic bedding had lower BTSCC. The differences between these two studies may due to study design. Our study was a cross-sectional study, while Robowtham and Ruegg (2015) was a longitudinal study, with farms followed over a 2-yr period. Given that SCC (in response to environmental pathogens) can elevate for short period of time (Harmon, 1994; De Haas et al., 2004), the longitudinal observation period would have captured more of those potential elevations, whereas our own study design would not. Another possible explanation could be a lack of power, given the inherent large variability in BTSCC among farms.

Dairy cows lie down longer in wider stalls compared to narrower stalls and, not surprisingly, wider stalls are also more likely to get dirty (Tucker et al., 2004). Longer stalls (as determined by neck rail position relative to the rear curb) also result in increased time spent standing with all 4 feet in the stall, as well as higher fecal contamination compared to a restrictive neck rail position (Bernardi et al., 2009). Therefore, we predicted stall width and length to be associated with BTSCC and BTBC, based on bacteria exposure in the lying stall. Contrary to this hypothesis, wider stalls were associated with lower BTSCC. Further, BTBC was lower on those farms with both wider stalls and longer stall space (i.e. greater distance from neck rail to the back of the stall). It is possible that farms with larger (wider and longer) stalls were more diligent in bedding management, as a weak positive correlation was reported for stall width and how often stalls were scraped. However, no correlation was found between stall length and how often stalls were scraped.

The myriad of differences in both facility design and management practices between tie-stall barns and free-stall barns likely contributed to the differences in percentage of cows with dirty upper legs and flanks between these two types of systems (Cook, 2002). However, system comparisons of this type are challenging and must be viewed with caution (von Keyserlingk and Weary, 2017).

For free-stall farms, the use of an AMS, as compared to a milking parlor, was associated with a higher proportion cows with dirty lower legs. DeVries et al. (2012) reported that nearly 100% of cows in an AMS farm had poor hygiene scores (n = 69 cows). To our knowledge, our findings are the first empirical evidence comparing hygiene in cows milked AMS versus milking parlor. Further research is needed to determine if management factors (e.g. cow cleaning associated with milking in a parlour) and differences between these milking systems could account for the difference in poorer hygiene reported for AMS farms. A higher proportion of cows with dirty udders and upper legs and flanks were also present in free-stall barns with less frequent alley scraper cleaning. These results are in agreement with DeVries et al. (2012), who reported an association between less frequent alley scraping and proportion of cows with poorer hygiene. In the present study, the proportion of cows per farm with poor hygiene of the upper legs and flanks was associated with the proportion with dirty udders. Other researchers have reported similar findings (Sant'anna and Paranhos da Costa 2011; Popescu et al. 2013; Zurbrigg et al., 2005).

For tie-stall farms, a higher proportion of cows with dirty lower legs was observed on farms with access to pasture. Contrary to our results, Popescu et al. (2013) reported no differences in proportion of cows with dirty lower legs, udder, upper legs and flanks between farms where cows had access to pasture, compared to those farms without pasture access. Although to our knowledge no work has investigated the effects of walking conditions on cow hygiene, it is possible for cows to get dirty while walking on pasture, especially if pasture conditions and the path to the pasture are not ideal. Further, it is possible that there is a seasonal impact (i.e. early versus end of grazing season due to weather patterns and path integrity, and potential impact on cow hygiene); however no associations were reported between hygiene of lower legs and average temperature nor hygiene of lower legs and relative humidity of the environment. Tie-stall farms that utilized a product (lime) in their bedding tended to have a higher percentage of cows with dirty lower legs. It is possible that farms with dirtier cows had identified cleanliness as a problem and, thus, had made the conscious decision to use a product potentially to reduce the risk of increased bacteria exposure to cows due to manure accumulation on cow bodies; our results indicate, however, that this solution appears not to work. Moreover, Lombard et al. (2010) also found an association between the proportion of dirty cows and use of tail docking. Again, this suggests that producers are aware that they have a cleanliness problem but may implement a solution (e.g. tail docking) that does not work (Lombard et al., 2010).

## 2.5 CONCLUSIONS

Bedding BC differed between unused and used bedding types sampled on Ontario dairy farms. Unused RMS bedding types had lower DM content and higher *Streptococcus* spp. count compared to all other bedding types. Unused straw bedding had a higher Gram-negative and *Klebsiella* spp. count than all other types. Sand was driest, followed by straw and wood products, in used bedding, whereas RMS types were wettest. RMS had the highest *Klebsiella* spp. and all Gram-negative count, followed by straw and sand, whereas these counts were lowest in wood products. In used bedding, higher %DM was associated with lower *Streptococcus* spp. count regardless of bedding type. Lower %DM in used bedding was associated with higher BTBC. Greater number of days since adding bedding was the primary management practice associated with greater BC in used bedding. Although cow hygiene was different between housing types, management factors were associated with poorer hygiene within each housing type. For example, proportion of cows with dirty udders and upper legs and flanks was higher in free-stall barns with less frequent alley scraper cleaning. Tie-stall farms that utilized a lime product in their bedding tended to have a higher percentage cows with dirty lower legs. The results of this study suggest that elevated BC can be found in samples of used bedding across bedding types. Further, it provides evidence that bedding management can have a profound impact on cow milk quality, bacterial concentrations in the bedding substrates, and cow hygiene, which in turn may affect mastitis risk.

## 2.6 ACKNOWLEDGEMENTS

We are grateful to the participating producers for the use of their animals, facilities, and access to their data. We thank Dr. Meagan King and Patrick Sudds of the University of Guelph for their technical assistance with data collection. This project was financially supported by a contribution from the Dairy Research Cluster II Initiative, funded by the Dairy Farmers of Canada (Ottawa, ON, Canada), Agriculture and Agri-Food Canada (Ottawa, ON, Canada), the Canadian Dairy Network (Guelph, ON, Canada), and the Canadian Dairy Commission (Ottawa, ON, Canada), through the Canadian Bovine Mastitis and Milk Quality Research Network (Montreal, QC, Canada).

## 2.7 REFERENCES

Bernardi, F., J. Fregonosi, D. M. Veira, C. Winkler, M. A. G. von Keyserlingk, and D. M. Weary. 2009. The stall design paradox: Neck rails increase lameness but improve udder and stall hygiene. J. Dairy Sci. 92:3074–3080.

Bramley, A. J. 1982. Sources of *Streptococcus uberis* in the dairy herd I. Isolation from bovine faeces and from straw bedding of cattle. J. Dairy Res. 49:369–373.

CCAC. 2009. Guidelines on: The care and use of farm animals in research, teaching and testing.

Canadian Council on Animal Care, Ottawa, ON, Canada. Available at: https://www.ccac.ca/Documents/Standards/Guidelines/Farm_Animals.pdf

Cole, K. J. and Hogan, J. S. 2016. Short communication : Environmental mastitis pathogen counts in free-stalls bedded with composted and fresh recycled manure solids. J. Dairy Sci. 99:1–5.

Cook, N. 2002. The influence of barn design on dairy cow hygiene, lameness, and udder health. Page 1-19 in Proc. 35th Ann. Conv. Am. Assoc. Bov. Pract. Available at: http://www.vetmed.wisc.edu/dms/fapm/publicats/proceeds/THE_INFLUENCE_OF_BARN_DESIGN_ON_DAIRY_COW_HYGIENE.pdf.

Cook, N. B. and D. J. Reinemann. 2007. A toolbox for assessing cow, udder and teat hygiene. Page 31-43 in Proc. NMC. Annu. Mtg, NMC. Inc., Verona, WI, USA. Available at http://www.vetmed.wisc.edu/dms/fapm/fapmtools/4hygiene/hygiene.pdf.

De Haas, Y., R.F. Veerkamp, H.W. Barkema, Y.T. Gröhn, and Y.H. Schukken. 2004. Associations between pathogen-specific cases of clinical mastitis and somatic cell count patterns. J. Dairy Sci. 87:.95-105.

DeVries, T. J., M. G. Aarnoudse, H. W. Barkema, K. E. Leslie, and M. A. G. von Keyserlingk. 2012. Associations of dairy cow behavior, barn hygiene, cow hygiene, and risk of elevated somatic cell count. J. Dairy Sci. 95:5730–5739.

Dohoo, I., S. W. Martin, and H. Stryhn. 2009. Veterinary Epidemiologic Research. VER Inc., Charlottetown, PE, Canada.

Dufour, S., H. W. Barkema, L. DesCôteaux, T. J. DeVries, I. R. Dohoo, K. Reyher, J. P. Roy, and D. T. Scholl. 2010. Development and validation of a bilingual questionnaire for measuring udder health related management practices on dairy farms. Prev. Vet. Med. 95:74-85

Godden, S., R. Bey, K. Lorch, R. Farnsworth, and P. Rapnicki. 2008. Ability of organic and inorganic bedding materials to promote growth of environmental bacteria. J. Dairy Sci. 91:151–159.

Harmon, R. J. 1994. Symposium: Mastitis and genetic evaluation forsomatic cell count—Physiology of mastitis and factors affecting somatic cell counts. J. Dairy Sci. 77:2103–2112

Hogan, J. and K. L. Smith. 2012. Managing environmental mastitis. Vet. Clin. North Am. Food Anim. Pract. 28:217–224.

Hogan, J. S., K. L. Smith, K. H. Hoblet, D. A. Todhunter, P. S. Schoenberger, W. D. Hueston, D. E. Pritchard, G. L. Bowman, L. E. Heider, B. L. Brockett, and H. R. Conrad. 1989. Bacterial counts in bedding materials used on nine commercial dairies. J. Dairy Sci. 72:250–258.

Hogan, J. S., K. L. Smith, D. A. Todhunter, and P. S. Schoenberger. 1990. Bacterial counts associated with recycled newspaper bedding. J. Dairy Sci. 73:1756–1761

Hogan, J. S., V. L. Bogacz, L. M. Thompson, S. Romig, P. S. Schoenberger, W. P. Weiss, and K. L. Smith. 1999. Bacterial counts associated with sawdust and recycled manure bedding treated with commercial conditioners. J. Dairy Sci. 82:1690–1695.

Husfeldt, A. W., M. I. Endres, J. A. Salfer, and K. A. Janni. 2012. Management and characteristics of recycled manure solids used for bedding in Midwest free-stall dairy herds. J. Dairy Sci. 95:2195– 2203.

Ito, K., D. M. Weary, and M. A. G. von Keyserlingk. 2009. Lying behavior: assessing within- and between-herd variation in free-stall-housed dairy cows. J. Dairy Sci. 92:4412–4420.

Kristula, M.A., W. Rogers, J. S. Hogan, and M. Sabo. 2005. Comparison of bacteria populations in clean and recycled sand used for bedding in dairy facilities. J. Dairy Sci. 88:4317–4325.

Lago, A., S. M. Godden, R. Bey, P. L. Ruegg, and K. Leslie. 2011. The selective treatment of clinical mastitis based on on-farm culture results: I. Effects on antibiotic use, milk withholding time, and short-term clinical and bacteriological outcomes. J. Dairy Sci. 94:4441–4456.

Levison, L. J., E. K. Miller-Cushon, A. L. Tucker, R. Bergeron, K. E. Leslie, H. W. Barkema, and T. J. DeVries. 2016. Incidence rate of pathogen-specific clinical mastitis on conventional and organic Canadian dairy farms. J. Dairy Sci. 99:1341–1350.

Lombard, J. E., C. B. Tucker, M. A. G. Von Keyserlingk, C. A. Kopral, and D. M. Weary. 2010. Associations between cow hygiene, hock injuries, and free-stall usage on US dairy farms. J. Dairy Sci. 93:4668–4676.

Popescu S., C. Borda, E. A. Diugan, M. Spinu, I. S. Groza, D. Sandru. 2013. Dairy cows' welfare quality in tie-stall housing system with or without access to exercise. Act Vet Scand 55: 43.

Pinzón-Sánchez, C. and P. L. Ruegg. 2011. Risk factors associated with short-term post-treatment outcomes of clinical mastitis. J. Dairy Sci. 94:3397–3410.

Oliveira, L., C. Hulland, and P. L. Ruegg. 2013. Characterization of clinical mastitis occurring in cows on 50 large dairy herds in Wisconsin. J. Dairy Sci. 96:7538–7549.

Reyher, K. K., I. R. Dohoo, D. T. Scholl, and G.P. Keefe. 2012. Evaluation of minor pathogen intramammary infection, susceptibility parameters, and somatic cell counts on the development of new intramammary infections with major mastitis pathogens. J. Dairy Sci. 95:3766–3780

Rowbotham, R.F. and P. L. Ruegg. 2015. Association of bedding types with management practices and indicators of milk quality on larger Wisconsin dairy farms. J. Dairy Sci, 98:7865–7885.

Rowbotham, R.F. and P. L. Ruegg. 2016a. Associations of selected bedding types with incidence rates of subclinical and clinical mastitis in primiparous Holstein dairy cows. J. Dairy Sci. 99:4707–4717.

Rowbotham, R. F. and P. L. Ruegg. 2016b. Bacterial counts on teat and skin in new sand, recycled sand, and recycled manure solids used as bedding in free-stalls. J. Dairy Sci, 99:6594–6608.

Sant’anna, A. C. and M. J. R. Paranhos da Costa. 2011. The relationship between dairy cow hygiene and somatic cell count in milk. J. Dairy Sci. 94:3835–3844.

SAS Institute. 2013. SAS version 9.4. SAS Institute Inc., Cary, NC.

Schreiner, D. A. and P. L. Ruegg. 2003. Relationship between udder and leg hygiene scores and subclinical mastitis. J. Dairy Sci. 86:3460–3465.

Schüller, L. K., O. Burfeind, and W. Heuwieser. 2013. Short communication: Comparison of ambient temperature, relative humidity, and temperature-humidity index between on-farm measurements and official meteorological data. J. Dairy Sci. 96:7731–7738.

Sorter, D.E., H. J. Kester, and J. S. Hogan. 2014. Short communication: Bacterial counts in recycled manure solids bedding replaced daily or deep packed in free-stalls. J. Dairy Sci, 97:2965–2968.

Tucker, C.B. and D. M. Weary. 2004. Bedding on geotextile mattresses: how much is needed to improve cow comfort? J. Dairy Sci. 87:2889–2895.

Tucker, C.B., D. M. Weary, and D. Fraser. 2004. Free-stall dimensions: Effects on preference and stall usage. J. Dairy Sci. 87:1208–1216.

von Keyserlingk, M. A. G., A. Barrientos, K. Ito, E. Galo, and D. M. Weary. 2012. Benchmarking cow comfort on North American free-stall dairies: lameness, leg injuries, lying time, facility design, and management for high-producing Holstein dairy cows. J. Dairy Sci. 95:7399–7408.

Zdanowicz, M., J. A. Shelford, C. B. Tucker, D. M. Weary, and M. A. G. von Keyserlingk. 2004. Bacterial populations on teat ends of dairy cows housed in free-stalls and bedded with either or sawdust. J. Dairy Sci. 87:1694–1701.

Zehner, M. M., R. J. Farnsworth, R. D. Appleman, K. Larntz, and J. A. Springer. 1986. Growth of environmental mastitis pathogens in various bedding materials. J. Dairy Sci. 69:1932–1941.

Zurbrigg, K., D. Kelton, N. Anderson, and S. Millman. 2005. Stall dimensions and the prevalence of lameness, injury, and cleanliness on 317 tie-stall dairy farms in Ontario. Can. Vet. J. 46:902–909.

**Table 2. 1** Descriptive statistics of herd information, housing, and management of 70 Ontario, Canada, dairy farms

| Variable | Farms[1] | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|---|
| Herd size (# lactating cows)[2] | 70 | 100 | 70 | 25 | 404 |
| DIM[3] | 66* | 170 | 21 | 127 | 226 |
| Lactation number[3] | 66* | 2.3 | 0.3 | 21.6 | 3.2 |
| Milk yield (kg/d) [3] | 65* | 32.7 | 3.9 | 21.8 | 40.9 |
| Herd average SCC (x 1,000 cells/ml)[3] | 64* | 184 | 75 | 71 | 465 |
| Bulk tank SCC (x 1,000 cells/ml)[4] | 70 | 186 | 73 | 52 | 515 |
| Bulk tank bacteria count (x 1,000 IBC/ml)[5] | 70 | 18 | 26 | 3 | 229 |
| Stall dimensions[6] | 70 | | | | |
| Curb height (cm) | | 17 | 5 | 3 | 29 |
| Width (cm) | | 119 | 8 | 104 | 148 |
| Length (cm) | | 221 | 37 | 166 | 310 |
| Neck rail distance from rear curb (cm) | | 182 | 18 | 152 | 230 |
| Neck rail height (cm) | | 120 | 10 | 94 | 141 |
| Frequency of stall cleaning (#/d)[7] | 70 | 3.2 | 1.3 | 1.0 | 8.0 |
| Frequency of adding bedding (#/d)[7] | 70 | 3.0 | 5.3 | 0 | 28 |
| Daily environmental temperature (ºC)[8] | 70 | 0.38 | 5.0 | -15.0 | 16 |
| Relative humidity daily average (%)[8] | 70 | 76.5 | 12.1 | 63.0 | 92.0 |

[1]Number of farms with data available for each measure.
[2]Data from cows at the beginning of the study period.
[3]SCC = individual cow SCC; data from 2 DHI tests: one closest to the first visit and one closest to the last visit.
[4]Data from a week prior to each of the 3 farm visits; 7 d apart.
[5] BTBC= bulk tank bacteria count (IBC/mL) assessed 2x/wk for a week prior to each of the 3 farm visits; 7 d apart.
[6]Dimensions were measured by research personnel during one of the visits.
[7]Management practices described by producers.
[8]Average temperature in the 7 d before each farm visit from the closest weather station of Environment Canada.
*Data presented for farms that had DHI recordings 10-12 tests/yr.

**Table 2. 2** Proportion of cows with poor hygiene scores in 70 Ontario, Canada, dairy herds, by housing type and body area[1]

| | Tie-stall[2] | | | Free-stall[3] | | |
|---|---|---|---|---|---|---|
| Area | Mean (±SD) | Minimum | Maximum | Mean (±SD) | Minimum | Maximum |
| Upper leg-flank | $40^a \pm 4.6$ | 2.5 | 100 | $59^b \pm 3.6$ | 7.5 | 100 |
| Lower legs | $26^a \pm 3.0$ | 0.0 | 98 | $89^b \pm 2.4$ | 43 | 100 |
| Udder | 31 ± 4.0 | 0.0 | 81 | 36 ± 3.1 | 5.0 | 95 |

[a-b]Means within a row with different superscripts differ ($P < 0.01$).

[1]Hygiene score was assessed on a 4-point scale (1 = very clean to 4 = very dirty) during each of the 3 visits; 7 d apart. Hygiene scores ≥ 3 were classified as poor.

[2]Tie-stall barns (n=26), a total of 986 cows scored.

[3]Free-stall barns (n=44), a total of 1784 cows scored.

**Table 2. 3** Final general linear model for factors associated with proportion of cows with poor hygiene of the upper legs-flank, udder, and lower legs[1] in free-stall barns[2]

| | Upper legs-flank | | | Udder | | | Lower legs | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | β[3] | SE | *P*-value | β | SE | *P*-value | β | SE | *P*-value |
| Intercept | -16.5 | 34.14 | 0.63 | -26.4 | 27.96 | 0.35 | 81.6 | 2.72 | <0.001 |
| Milking system | | | - | | | - | | | 0.005 |
| Automated milking system | - | - | - | - | - | - | 12.4 | 3.65 | |
| Milking parlor | - | - | - | - | - | - | Ref[4] | - | |
| Alley cleaning frequency (#/d)[5] | -0.9 | 0.35 | 0.03 | -0.6 | 0.29 | 0.04 | - | - | - |
| Days in milk | 0.5 | 0.20 | 0.02 | 0.4 | 0.17 | 0.02 | - | - | - |
| Stall base | | | 0.10 | | | - | | | - |
| Deep bedding | -14.7 | 8.82 | 0.06 | - | - | | - | - | |
| Mattress | Ref | - | | - | - | | - | - | |
| Visit | | | - | | | 0.04 | | | - |
| First | - | - | - | -5.89 | 2.84 | | - | - | - |
| Second | - | - | - | -6.76 | 2.84 | | - | - | - |
| Third | - | - | - | Ref | - | | - | - | - |

[1]Hygiene score was assessed on a 4-point scale (1 = very clean to 4 = very dirty) and classified as poor if ≥ 3. Proportion of cows with poor hygiene per farm was calculated during each of the 3 visits; 7 d apart.
[2]Free-stall barns (n=44), n=1784 cows scored.
[3] $\beta$ = estimated regression coefficient.
[4]Ref = Reference category.

[5]Management practices described by producers.

**Table 2. 4** Final general linear model for factors associated with proportion of cows with poor hygiene of the upper legs-flank, udder, and lower legs[1] in tie-stall barns[2]

| | Upper legs-flank | | | Udder | | | Lower legs | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | β[3] | SE | *P*-value | β | SE | *P*-value | β | SE | *P*-value |
| Intercept | - | - | - | 29.1 | 3.93 | <0.001 | 45.8 | 8.95 | <0.001 |
| Access to pasture | | | - | | | - | | | 0.04 |
| No | - | - | - | - | - | - | -17.7 | 8.31 | |
| Yes | - | - | - | - | - | - | Ref[4] | - | |
| Product added to bedding | | | - | | | - | | | 0.07 |
| No | - | - | - | - | - | - | -16.2 | 8.41 | |
| Yes | - | - | - | - | - | - | Ref[4] | - | |
| Days since additional bedding was added (#/d)[5] | - | - | - | 7.0 | 4.36 | 0.10 | - | - | - |

[1]Hygiene score was assessed on a 4-point scale (1 = very clean to 4 = very dirty) and classified as poor if ≥ 3. Proportion of cows with poor hygiene per farm was calculated during each of the 3 visits; 7 d apart.

[2]Tie-stall barns (n=26), a total of 986 cows scored.

[3] $\beta$ = estimated regression coefficient.

[4]Ref = Reference category.

[5]Management practices described by producer.

**Table 2. 5** Bulk tank bacteria count of the 70 participating farms in Ontario, Canada, assessed 2x/wk for a week prior to each of the 3 farm visits; 7 d apart

| Bedding type | Bacterial count[1,2] (IBC/mL) |
| --- | --- |
| Sand | 7,708 [bc] (141, 2944) |
| Straw | 11,968[b] (5996, 11166) |
| Wood products | 12,836[ac] (9518, 14234) |
| RMS | 20,333[a] ± (9873, 16421) [3] |

[a-c]Means within the column with different superscripts differ ($P$ < 0.01).

[1]Accounted for stall width and neck rail distance from back of the stall in milking cow stalls per farm.

[2]Bacterial count was analyzed using natural logarithm (ln) values of bactoscan test results in IBC/mL; geometric mean and 95% CI (lower, upper) are reported as back-transformed values for presentation purposes.

**[3]**Tendency for difference between Straw and RMS ($P$= 0.09).

**Table 2. 6** Dry matter and bacterial counts for unused and used bedding types for farms (n=70) visited 3 times, 7d apart

| Content | Sand | Straw | Wood products | RMS[1] |
|---|---|---|---|---|
| Unused bedding[2] | | | | |
| %DM | 92.1[a] ± 1.75 | 88.3[b] ± 0.76[3] | 85.3[b] ± 1.09[3] | 37.9[c] ± 1.50 |
| Bacterial counts (cfu/mL)[4] | | | | |
| *Streptococcus* spp. | 3.7[b] (0.6, 23.3) | 11.8[b] (3.9, 35.3) | 6.3[b] (1.3, 30.0) | 161.8[a] (16.5, 1586) |
| All Gram-negative | 12.5[c] (2.3, 67.3) | 419,534[a] (154,369, 1140184) | 160.9[c] (38.2, 678) | 26142.0[b] (3068, 22277) |
| *Klebsiella* spp. | 13.7[b] (1.8, 103) | 129,832[a] (39293, 428995) | 80.1[b] (14.4, 447) | 103.3[b] (8.1, 1319) |
| Used bedding | | | | |
| %DM | 95.2[a] ± 1.75 | 77.9[b] ± 1.06 | 75.4[b] ± 1.48 | 44.7[c] ± 2.03 |
| Bacterial counts (cfu/mL)[4] | | | | |
| *Streptococcus* spp. | 1,9454,715 (7198621, 5258220) | 5,942,260 (3738893, 9443148) | 9,519,948.5 (5045968,17960761) | 1,772,320 (461252, 6809982) |
| All Gram-negative | 736,526[b] (208668, 2599943) | 968,302.7[b] (478734,1958325) | 35,391[c] (13691, 91473) | 12,480,775[a] (3122049, 49893436) |
| *Klebsiella* spp. | 48,650[a] (7342, 322384) | 124,020[a] (39510, 389298) | 359[b] (73, 1759) | 54,819[a] (5408, 555653) |

[a-c]Means within the row with different superscripts differ ($P < 0.01$).

[1]RMS= Recycled manure solids from composted manure or digestate.
[2]Adjusted for temperature, humidity, and visit.

[3]Tendency for difference between straw and wood ($P$= 0.07).

[4]Bacterial counts were analyzed after natural logarithm transformation; geometric mean and 95% CI (lower, upper) are reported for presentation purposes.

**Appendix 2. 1:** Producer Questionnaire

1) What type of housing system do you have for your cows? *(Check all that apply)*

(a) **Lactating cows**
- ☐ Free-stall
- ☐ Tie-stall
- ☐ Bedding pack

(b) **Dry cows**
- ☐ Free-stall
- ☐ Tie-stall
- ☐ Bedding pack

(c) **Bred heifers:**
- ☐ Free-stall
- ☐ Tie-Stall
- ☐ Bedding pack

2) Do lactating (milking) cows in your herd have access to pasture?

- ☐ Yes (#hours/day)____________ Months (e.g. June-Sept.)___________
- ☐ Not applicable

3) What breed of cattle do your milk?___________(if mixed please state proportions)

4) How many lactating cows do you milk, currently? Milking #__________ Dry # ___________

5) Where are cows milked? *(Check all that apply)*

- ☐ In their stall
- ☐ In a parlour
- ☐ In a robotic milking system
- ☐ Other *(Please specify):*_____________________________

6) What type of material is the **base** of your cows' stalls made of? *(Check all that apply and please provide proportions in percentage of each material)*

(a) **Lactating cows:**
____ Concrete
____ Deep bedding
____ Mattress-type : _____________
____ Other: *(Please specify)* _________________

(b) **Dry cows**
____ Concrete
____ Deep bedding
____ Mattress-type : ______________
____ Other: *(Please specify)* _________________

(c) **Bred heifers:**
____ Concrete
____ Deep bedding
____ Mattress-type : _____________
_____Other: *(Please specify):* _________________

7) What type of **bedding** is used for your cows' stalls? *(Check all that apply please provide proportions of each material)*

(a) **Lactating cows:**

- [ ] Straw (if straw, is it chopped ___; to what length________)
- [ ] Sawdust
- [ ] Shavings
- [ ] Sand
- [ ] No bedding
- [ ] Other *(Please specify)*: _______________________

(b) **Dry cows**

- [ ] Straw (if straw, is it chopped ___; to what length________)
- [ ] Sawdust
- [ ] Shavings
- [ ] Sand
- [ ] No bedding
- [ ] Other *(Please specify)*: _______________________

(c) **Bred heifers:**

- [ ] Straw (if straw, is it chopped ___; to what length________)
- [ ] Sawdust
- [ ] Shavings
- [ ] Sand
- [ ] No bedding
- [ ] Other *(Please specify)*: _______________________

8) How often do you source new bedding? *(Check all that apply)*

- [ ] Once a week
- [ ] Once every 2 weeks
- [ ] Once a month
- [ ] Other *(Please specify)*: _______________________

9) What type of **bedding management** do you use in milking cows' stalls?

- [ ] I do not use any bedding
- [ ] Less than 2 cm deep
- [ ] Between 2 cm- 8 cm deep
- [ ] More than 8 cm (i.e. deep bedding)

10) How often do you **clean out manure** from your **milking** cows' stalls?

- [ ] Never
- [ ] ______times/day

*(Number)*

☐ ______times/week
*(Number)*

☐ Not applicable

11) How often do you **scrape out the dirty bedding** from your **milking** cows' stalls?

☐ Never

☐ _______times/day
*(Number)*

☐ _______times/week
*(Number)*

☐ Not applicable

12) How often do you maintain (i.e. level the bedding, or pull back sand…) the stalls?

☐ Never

☐ _______times/day
*(Number)*

☐ _______times/week
*(Number)*

☐ Not applicable

13) If you do **scrape out dirty bedding** from your **milking** cows' stalls, **what part of the stall** do you scrape off?

☐ Back third of the stall
☐ Back half of the stall
☐ The whole stall
☐ Other *(Please specify)*:_________________________________________
☐ Not applicable

14) How often do you **add new bedding** to your **milking** cows' stalls?

☐ Never

☐ _______times/day
*(Number)*

☐ _______times/week
*(Number)*
☐ Not applicable

15) What is the last date you added new bedding to your stalls? ______________

16) How do you apply bedding to stalls? *(Check all that apply)*

☐ By hand (shovel, pitchfork, etc)
☐ Bedding chopper
☐ Front-end loader/skid steer
☐ Slinger attachment
☐ Other *(Please specify)*: ________________________

17) When you add bedding to your stalls, where do you add it to?

☐ Back third of the stall
☐ Back half of the stall
☐ The whole stall
☐ Other *(Please specify)*:____________________________________________
☐ Not applicable

18) Do you add any type of conditioner (or product) to the bedding/stall?

☐ Yes. If yes, please specify ___________________
☐ No

19) How often do you completely **replace the bedding** in your **milking** cows' stalls?
☐ Never
☐ _______times/month
*(Number)*

☐ Other *(Please specify)*: ________________________
☐ Not applicable

20) How often do you clean the stalls (or pens or both) completely?

☐ Never
☐ _______times/month
*(Number)*

☐

Other *(Please specify)*: ________________________

☐ Not applicable

21) When you clean the stalls (or pens or both) completely, do you **wash or disinfect** them? *(Check all that apply)*

☐ Yes, pressure washed or brushed

☐ Yes, disinfected with ________________________________
*(Name of the product)*

☐ Yes, other *(Please specify)*: __________________________

☐ No

☐ Not applicable

22) How many **stalls** are available per cow for your **milking cows** today?

**Pen #1:** _______ stalls for ______ cows
*(Number)* *(Number)*

**Pen #2:** _______ stalls for ______ cows
*(Number)* *(Number)*

**Pen #3:** _______ stalls for ______ cows
*(Number)* *(Number)*

**Pen #4:** _______ stalls for ______ cows
*(Number)* *(Number)*

☐ Not applicable

*(Use back of the page if needed)*

23) What is the surface of the **alley ways** in the barn?

☐ Concrete

☐ Grooved/scratched concrete

☐ Rubber coveredconcrete

☐ Other *(Please specify)*: __________________________________________

24) How are the alleyways in the barn cleaned?

☐ Slatted floor

☐ Scraped (alley scrapers or tractor/skid steer)

☐ Flushed with water

☐

☐ Other *(Please specify)*: ________________________________________
☐ Not applicable

25) How many times a day are the alley ways cleaned?

☐ _______ times/day.
*(Number)*
☐ Not applicable

26) How often are cows milked?

☐ Two times per day
☐ Three times per day
☐ Other (robotic milker):______

27) Milking and post milking practices:

(a) Gloves:
☐ All milkers wear gloves ☐ some milkers wear gloves ☐ do not wear gloves

(b) Post-dip:
☐ Apply post-dip using a dipper ☐ apply post-dip using a sprayer ☐ do not post-dip

(c) Other – additional milking/post milking procedures not covered by above methods:

___________________________________________________________________

28) (a) How many cases of mastitis (abnormal milk, with or without swelling in the quarter) has your farm had in the last 6 months?

_____per month **OR if < 1 per month** _____ cases per year.

(b) **Of the above total cases**, how many severe cases of mastitis (abnormal milk **AND** the cow is sick) has your farm had in the last 6 months?
_____per month **OR if < 1 per month** _____ cases per year.

29) What is the average bulk tank somatic cell count ________
Last bulk tank somatic cell count __________

30) May we contact CanWest DHI for your farm production data?

# CHAPTER 3: SHORT COMMUNICATION: ASSOCIATIONS OF COW-LEVEL FACTORS WITH THE RISK OF POOR HYGIENE IN COWS HOUSED IN FREE-STALL AND TIE-STALL BARNS

## 3.1 INTRODUCTION

Indoor housed dairy cattle spend an average of 10-13 h/d lying down (Vasseur et al., 2012) and prefer surfaces that are clean and dry (Tucker and Weary, 2004; Fregonesi et al., 2007). However, when forced to lie down on wet surfaces cows will reduce their daily lying time (Reich et al., 2010). Lying on soiled areas potentially increases the risk that cows will become dirty, which in turn increases the risk of intramammary infection (IMI) (Schreiner and Ruegg, 2003; Watters et al., 2013). Although many management factors have been associated with poor cow hygiene (DeVries et al., 2012, Zdanowicz et al., 2004; Barkema et al., 1999), little work has looked at which cow-level factors contribute to increased risk of poor hygiene (Watters et al., 2013; Popescu et al. 2013). Watters et al. (2013) demonstrated that for cows housed in a free-stall barn, older cows, and those with greater milk production, were dirtier and that poorer hygiene was associated with increased odds of an elevated somatic cell count (eSCC).

The myriad of differences in both facility design and management practices among tie-stall barns and free-stall barns may contribute to differences in the degree to which body parts get dirty on cows (Cook, 2002). Although, system comparisons of this type are challenging and must be viewed with caution (von Keyserlingk and Weary, 2017). No previous research has focused on identifying cow-level risk factors for poor hygiene in either tie-stall or free-stall barns. Thus, the objective of this study was to identify the association of cow-level factors with the risk of having poor body hygiene in tie-stall and free-stall barns.

Primiparous cows are more active than multiparous cows (Roelofs et al., 2005), and as DIM increases, standing time decreases (Watters et al., 2014). Thus, we predicted that cows in early stage of lactation would be at increased risk of poorer hygiene while cows in first parity would be at reduced risk for being dirty compared to other parity cows. We also predicted that cows in tie-stalls would have improved lower leg and udder hygiene compared to cows housed in free-stall barns.

## 3.2 MATERIALS AND METHODS

This research was part of a larger study aimed at evaluating which bedding options and bedding management practices are associated with low bacterial counts, improved cow hygiene, and milk quality. As such, detailed descriptions of the methodology are presented in Robles et al. (submitted). In brief, farms (n=43 free-stall; n=25 tie-stall) in Ontario, Canada were visited 3 times, 7 d apart, during the months from October 2014 to February 2015. Farms were recruited by contacting them through an email sent by CanWest DHI (Guelph, Ontario, Canada) to producers located within 150 km of the University of Guelph, Kemptville Campus (Kemptville, Ontario, Canada). The producers were requested to contact the researchers if interested in participating in, or wanting more information about, the study. Participation was confirmed when farmers signed a written consent form. Specific criteria for herd selection included: participation in regular DHI testing, conformation that the lactating cows were housed in either free-stalls or tie-stalls. Animal use and study design were approved by the University of Guelph's Animal Care Committee (AUP#3140) and Research Ethics Board (REB#14JN019), respectively.

Lactating Holstein dairy cows (n = 2644 parity = 2.3 ± 1.5 (Mean ± SD); DIM = 160.0 ± 91.8) were enrolled in a cross-sectional study. At each farm 25% of the cows in each lactating pen (free-stalls) or row of stalls (tie-stalls) in herds with > 160 cows, or a minimum of 40 cows/herd in those herds with < 160 cows, were randomly selected for hygiene scoring; these same cows were scored at each of the 3 visits. All lactating cows were scored on farms that had less than 40 cows lactating during the first visit (n = 8). The percentage of cows scored was based on a recommended percentage of cows to score in order to quantify of hygiene on a farm, and as a monitor - to assess improvements in hygiene management as described by Cook and Reinemann (2007). Selection of individual cows for hygiene scoring on the first farm visit was restricted to those cows expected to stay (based on approx. 305 DIM) in the lactating pens for at least 2 wk after the beginning of the study to increase the likelihood that they would be available for the remaining two hygiene scorings events.

The udder, back lower legs, and upper legs/flank of each cow were hygiene scored on a 4-point scale (1 = very clean to 4 = very dirty) (Cook and Reinemann, 2007). Cows were classified as having good (≤ 2) or poor (≥ 3) hygiene score for each body part. Hygiene scoring was performed at each visit by 2 trained researchers. Inter-observer reliability was evaluated by comparing hygiene scores from one farm assessed independently by the 2 observers

Parity and DIM for all study cows in each herd were retrieved from CanWest Dairy Herd Improvement (DHI) (Guelph, Ontario, Canada) reports from the test date closest to the first visit and a subsequent test date closest to the last visit.

### *3.2.1 Statistical Analysis*

Data were analyzed using multivariable logistic regression models (using the GLIMMIX procedure of SAS; SAS Institute, 2013), treating visit as a repeated measure. The models included the random intercept of farm and cow within farm. Binary outcome (yes/no) variables expressed, on a per cow basis, were dirty lower legs (**DLL**), dirty udder (**DU**), and dirty upper legs and flanks (**DULF**). Prior to analyses, all data were screened for normality using the UNIVARIATE procedure of SAS (SAS Institute, 2013). First, all single explanatory variables were tested in univariable models. Covariance structures were selected on the basis of best fit according to Schwarz's Bayesian information criterion. Compound symmetry covariance structure was selected for all outcome variables. Only variables with $P < 0.25$ in this initial screening were considered for inclusion in the multivariable models (Dohoo et al., 2009). For the multivariable model, effects were considered significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. Manual backward elimination of non-significant and non-trending effects was used to construct the final multivariable models. When housing type (tie- or free-stall) was associated with an outcome variable, then housing specific aspects were included and investigated by housing type in a separate multivariable analysis using the same approach as described above.

## 3.3 RESULTS AND DISCUSSION

Kappa values for lower leg = 0.81, udder = 0.82, and upper leg/flank = 0.79. High levels of agreement were found between the observers when scores were categorized as good or poor hygiene (Kappa values for lower leg = 1.00; 95% CI = 1.00-1.00, udder = 0.79; 95% CI = 0.59-0.98, upper leg/ flank = 1.00; 95% CI = 1.00-1.00).

Summary descriptive statistics of data for cows housed in both free-stall and tie-stall barns are presented in Table 3.1. Proportion of dirty cows varied by body area and between the two housing types (*P*<0.001; Figure 3.1). The results in the current study for proportion of dirty cows, by body area, in tie-stall herds are similar to those found by Popescu et al. (2013), who studied dairy cows in tie-stall housing systems with (DLL= 31.1%; DU= 26.0%; DULF=31.1%), and without (DLL= 35.1%; DU= 29.0%; DULF=43.0%) access to exercise. In agreement with previous reports (Cook, 2002; Popescu et al., 2013), the upper legs and flanks were the dirtiest body area for cows housed in tie-stall barns. Similar to the results reported here, DeVries et al. (2012) found a very high proportion of cows (99.7%) with poor hygiene scores of the lower legs in free-stall housing. Similarly, Watters et al. (2013) reported a mean score of 4 (dirtiest on a 4-point scale) for the hygiene of the lower legs of 40 focal cows from each of the 5 free-stall herds observed.

In free-stall barns, parity 1 and parity 2 cows were at 1.5× and 1.4×, respectively, greater risk of having DLL compared to ≥3 parity cows (Table 3.2). Also in free-stall barns, parity 1 cows had a 2.3× lower risk of having a DU, as well a 1.2× lower risk of DULF when compared to ≥3 parity cows. Others (DeVries et al., 2011; Nielsen et al., 2011) have reported that poorer hygiene scores observed in older cows might be a consequence of the high nutritional demands causing the cows to spend more time feeding, thus increasing the risk that they come into contact with manure in the alley. Unfortunately we did not monitor feed alley cleanliness in the current study, as it is a herd level factor, but suggest future research should consider this factor. In free-stall barns ≥3 parity cows were more likely to have poorer udder hygiene (Reneau et al., 2005); moreover, greater parity has also been associated with cases of elevated somatic cell counts

(Watters et al., 2013). Our findings demonstrate a similar pattern where younger cows (i.e. 1 parity) were at lower risk for DU compared to parity ≥3 cows. This finding may be attributed to older cows often having more pendulous udders compared to younger cows (Watters et al., 2013). Compton et al. (2007) found that heifers with teats closer to the ground were more likely to have poor udder hygiene, and increased bacterial exposure due to manure exposure compared to heifers with teats further from the ground.

In tie-stall barns, parity 1 cows had a 2.3× greater risk of having DLL compared to ≥3 parity cows (Table 3.2). Parity 1 and parity 2 cows in tie-stall barns had greater risk (OR=1.9 and 1.3, respectively) of having DULF compared to parity ≥3. Popescu et al. (2013) indicated that poor hygiene in tie-stall herds is inevitable given that cows are constantly tied in their stall where they both stand and lie, thereby, exposing them to dirt and manure. Primiparous cows are more active than multiparous cows (Roelofs et al., 2005), and have higher lying bout frequency and shorter lying bouts compared to multiparous cows, regardless of whether they are housed in tie or free-stalls (Vasseur et al., 2012). The increased activity and altered lying behavior observed in primiparious cows may potentially increase their risk for DLL and DULF. Alternatively decreasing feed intake as DIM increases may reduce manure output, thus reducing the risk of cows becoming dirty.

Each 20 d increment in DIM was associated with lower risk of having DLL (OR=0.86; 95% CI=0.83-0.88; $P$=0.01), DU (OR=0.92; 95% CI= 0.90-0.94; $P$<0.001), and DULF (OR=0.91; 95% CI= 0.89-0.94; $P$<0.01) for cows in tie-stall barns. Similarly, each 20 d increment in DIM was tended to be associated with lower risk of having a DU (OR= 0.98; 95% CI= 0.97-1.00; $P$=0.09) and DULF (OR=0.97; CI= 0.95-0.99; $P$=0.01) for cows housed in free-stall barns. These results agree with the findings of DeVries et al. (2012), who observed that higher milk yield was associated with reduced cleanliness of the udder and lower legs. Interestingly, Reneau et al. (2005) reported that cow hygiene improved with increasing DIM which can be associated with a reduced milk production based on dairy lactation stage (Nielsen et al., 2011). DeVries et al. (2011) hypothesized that increased metabolic and production demands would cause increases in the time spent eating, thus increasing standing time, a factor previously associated with poor hygiene (Nielsen et al. 2011). Further, given that cows alter their lying bout behavior (frequency decreases and duration increases) as DIM increases in both tie- and free-stall housing systems (Vasseur et al., 2012), cows may potentially be at lower risk for poor hygiene as DIM increases. Poor udder hygiene has been associated with poor stall hygiene; thus, the effect of activity on cow hygiene may be dependent on the cleanliness of the cow's environment (DeVries et al., 2012).

## 3.4 CONCLUSION

In summary, our results suggest that cow hygiene varies by parity and stage of lactation in cows housed in both free-stall and tie-stall barns. This study might can aid producers to have an idea of how cow housing, parity, and DIM might influence the risk of poor hygiene in cows. Older cows were at increased risk of poor hygiene when housed in free-stall barns, while younger cows where at increased risk of poor hygiene when housed in tie-stall barns. Cows earlier in lactation were also at increased risk of poor hygiene in both housing types. Overall, these differences in hygiene observed among cows with different housing, parity, and DIM may be related to the behavior of cows, in terms of how they use the environment made available to them.

## 3.5 ACKNOWLEDGEMENTS

Special thanks to the participating producers, for allowing the use of their animals, facilities, and access to their data. We thank Meagan King and Patrick Sudds of the University of Guelph for their technical assistance with data collection. This project was financially supported by a contribution from the Dairy Research Cluster II Initiative, funded by the Dairy Farmers of Canada (Ottawa, ON, Canada), Agriculture and Agri-Food Canada (Ottawa, ON, Canada), the Canadian Dairy Network (Guelph, ON, Canada), and the Canadian Dairy Commission (Ottawa, ON, Canada); these funds were made available through the Canadian Bovine Mastitis and Milk Quality Research Network (Saint-Hyacinthe, QC, Canada)

### 3.6 REFERENCES

Barkema, H.W., Y. H. Schukken, T. J. G. M. Lam, M. L. Beiboer, G. Benedictus, A. Brand. 1999. Management practices associated with the incidence rate of clinical mastitis. J. Dairy Sci. 82:1643–1654.

Compton, C. W. R., C. Heuer, K. I. Parker, and S. McDougall. 2007. Risk factors for peripartum mastitis in pasture-grazed dairy heifers. J. Dairy Sci. 90:4171–4180.

Cook, N., 2002. The influence of barn design on dairy cow hygiene, lameness, and udder health. *Proc. of the 35 th Ann. Conv. Amer. Assoc. Bov. Pract., …*, pp.1–19. Available at: http://www.vetmed.wisc.edu/dms/fapm/publicats/proceeds/THE_INFLUENCE_OF_BARN_DESIGN_ON_DAIRY_COW_HYGIENE.pdf.

Cook, N. B., and D. J. Reinemann. 2007. A toolbox for assessing cow, udder and teat hygiene. Pages 31-43 in Proc. NMC. Annu. Mtg, NMC. Inc., Verona, WI, USA. http://www.vetmed.wisc.edu/dms/fapm/fapmtools/4hygiene/hygiene.pdf.

DeVries, T. J., J. A. Deming, J. Rodenburg, G. Seguin, K. E. Leslie, and H. W. Barkema. 2011. Association of standing and lying behavior patterns and incidence of intramammary infection in dairy cows milked with an automatic milking system. J. Dairy Sci. 94:3845–3855.

DeVries, T. J., M. G. Aarnoudse, H. W. Barkema, K. E. Leslie, M. A. G. and von Keyserlingk. 2012. Associations of dairy cow behavior, barn hygiene, cow hygiene, and risk of elevated somatic cell count. J. Dairy Sci. 95:5730–5739.

Dohoo, I., W. Martin, and H. Stryhn. 2009. Veterinary Epidemiologic Research. VER Inc., Charlottetown, Prince Edward Island, Canada.

Fregonesi, J. A., D. M. Veira, M. A. G. von Keyserlingk, and D. M. Weary. 2007. Effects of bedding quality on lying behavior of dairy cows. J. Dairy Sci. 90:5468–5472.

Hultgren, J., and C. Bergsten, 2001. Effects of a rubber-slatted flooring system on cleanliness and foot health in tied dairy cows. Prev. Vet. Med. 52:75–89.

Ito, K., D. M. Weary, and von Keyserlingk, M. A G., 2009. Lying behavior: assessing within- and between-herd variation in free-stall-housed dairy cows. J. Dairy Sci. 92:4412–4420.

Nielsen, B. H., P. T. Thomsen, J. T. and Sørensen, 2011. Identifying risk factors for poor hind limb cleanliness in Danish loose-housed dairy cows. Animal. 5:1613–1619.

Popescu S, C. Borda, E. A. Diugan, M. Spinu, I. S. Groza, D. Sandru. 2013. Dairy cows' welfare quality in tie-stall housing system with or without access to exercise. Act Vet Scand 55: 43.

SAS Institute. 2013. SAS version 9.4. SAS Institute Inc., Cary, NC.

Reich, L.J., Weary, D.M., Veira, D.M. and von Keyserlingk, M.A.G. 2010. Effects of sawdust bedding dry matter on lying behavior of dairy cows: A dose-dependent response. J. Dairy Sci, *93*:1561-1565.

Reneau, J. K., Seykora, A. J., Heins, B. H., Endres, M. I., Farnsworth, R. J., and Bey, R. F., 2005. Association between hygiene scores and subclinical mastitis. J. Am. Vet. Med. Assoc. 227:1297–1301

Robles, I., D. F. Kelton, H.W. Barkema, G. P. Keefe, J. P. Roy, M. A. G. von Keyserlingk, and T.J. DeVries. 2018. Bacterial concentrations in bedding and their association with cow hygiene and milk quality. J. Dairy Sci (submitted).

Roelofs, J. B., van Eerdenburg, F. J. C. M., Soede, N. M., Kemp, B., 2005. Various behavioral signs of estrus and their relationship with time of ovulation in dairy cattle. Theriogenology 63:1366–1377.

Schreiner, D. and Ruegg, P. L., 2003. Relationship between udder and leg hygiene scores and subclinical mastitis. J. Dairy Sci, 86:3460–3465.

Tucker, C. B. and Weary, D. M., 2004. Bedding on geotextile mattresses: how much is needed to improve cow comfort? J. Dairy Sci. 87:2889–2895.

Vasseur, E., Rushen, J. Haley, D. B., and de Passillé, A. M., 2012. Sampling cows to assess lying time for on-farm animal welfare assessment. J. Dairy Sci. 95:4968–4977.

Watters, M. E. A., Barkema, H. W., Leslie, K. E, von Keyserlingk, M. A. G., and DeVries, T. J., 2013. Associations of herd- and cow-level factors, cow lying behavior, and risk of elevated somatic cell count in free-stall housed lactating dairy cows. Prev. Vet. Med. 111:245-255.

Watters, M.E., Barkema, H.W., Leslie, K. E., Keyserlingk, M. A. G., and DeVries, T. J. 2014. Relationship between postmilking standing duration and risk of intramammary infection in free-stall housed dairy cows milked 3 times per day. J. Dairy Sci. 97:3456–3471.

Welfare Quality Assessment Protocol for Cattle. 2009. Lelystad, Netherlands: Welfare Quality Consortium.

**Table 3. 1** Descriptive summary of data from cows housed in free-stall (n=1716 cows) and tie-stall (n= 928 cows) barns, across all 68 herds (n= 43 free-stall; n = 25 tie-stall).

| | Free-Stall | | | | Tie-Stall | | | |
|---|---|---|---|---|---|---|---|---|
| Variable | Mean | SD | Min | Max | Mean | SD | Min | Max |
| DIM | 159.3 | 89.9 | 5 | 369 | 160.5 | 93.6 | 6 | 369 |
| Parity | 2.2 | 1.3 | 1 | 12 | 2.4 | 1.6 | 1.0 | 12.0 |

**Table 3. 2** Comparison of body areas (lower legs, udder, upper legs and flank) at risk for poor hygiene from cows in parities 1, 2 and ≥ 3 housed in 25 tie-stall (928 cows) and 43 free-stall (1716 cows) barns.

| Housing | Body Area | Variable | Coefficient | SE | Odds Ratio (95% CI) | *P*-value |
|---|---|---|---|---|---|---|
| Free-stall | Lower | Intercept | 2.30 | 0.19 | - | <0.001 |
| | | Parity | | | | |
| | | 1 | 0.38 | 0.14 | 1.5 (1.1-1.9) | 0.005 |
| | | 2 | 0.34 | 0.14 | 1.4 (1.1-1.9) | 0.02 |
| | | 3 | - | - | Ref | - |
| | Udder | Intercept | 0.03 | 0.19 | - | 0.87 |
| | | Parity | | | | |
| | | 1 | -0.83 | 0.10 | 0.44 (0.36-0.53) | <0.001 |
| | | 2 | -0.15 | 0.10 | 0.86 (0.70-1.05) | 0.13 |
| | | 3 | - | - | Ref | - |
| | Upper | Intercept | 1.64 | 0.40 | - | <0.001 |
| | Flanks | Parity | | | | |
| | | 1 | -0.28 | 0.10 | 0.75 (0.62-0.91) | 0.002 |
| | | 2 | -0.18 | 0.10 | 0.84 (0.68-1.03) | 0.09 |
| | | 3 | - | - | Ref | - |
| Tie-stall | Lower | Intercept | -0.48 | 0.30 | - | 0.12 |
| | | Parity | | | | |
| | | 1 | 0.85 | 0.10 | 2.3 (1.69-3.26) | <0.001 |
| | | 2 | 0.27 | 0.10 | 0.84 (0.92-1.87) | 0.14 |
| | | 3 | - | - | Ref | - |
| | Udder | Intercept | -0.79 | 0.22 | - | <0.001 |
| | | Parity | | | | |
| | | 1 | 0.01 | 0.13 | 0.99 (0.76-1.29) | 0.94 |
| | | 2 | 0.11 | 0.14 | 1.11 (0.84-1.48) | 0.46 |
| | | 3 | - | - | - | - |
| | Upper | Intercept | 0.32 | 0.28 | - | <0.001 |
| | Flanks | Parity | | | | |
| | | 1 | -0.31 | 0.09 | 1.9 (1.50-2.6) | <0.001 |
| | | 2 | -0.07 | 0.09 | 1.3 (0.97-1.8) | 0.40 |
| | | 3 | - | - | Ref | - |

*Hygiene score was assessed on a 4-point scale (1 = very clean to 4 = very dirty). Hygiene scores classified as poor if ≥ 3.

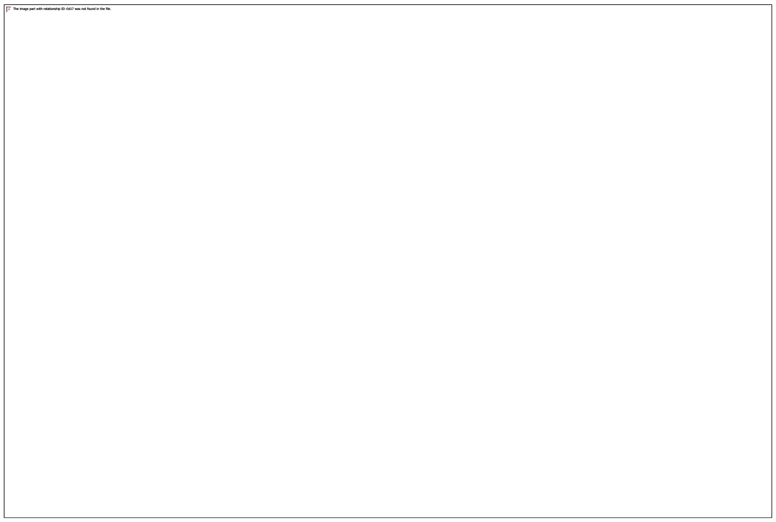


**Figure 3. 1** Proportion of cows with poor hygiene scores in 68 dairy herds, by housing type; free-stall (n=43 herds; 1716 cows scored) or tie-stall (n=25 herds; 928 cows scored) barns and body area. Hygiene score was assessed on a 4-point scale (1 = very clean to 4 = very dirty). Hygiene scores classified as poor (dirty) if ≥ 3.

# CHAPTER 4: ASSOCIATIONS AMONG STALL DESIGN FEATURES AND CLEANLINESS, COW LYING BEHAVIOR, HYGIENE, AND THE RISK OF ELEVATED SOMATIC CELL COUNT

## 4.1 INTRODUCTION

The resting area in any dairy facility should provide a clean, comfortable area for cows to lie down. Factors such as the stall surface (base, bedding type, amount (Tucker and Weary 2004) and quality (Reich et al., 2010), and stall dimensions (Tucker et al., 2004; 2005; 2006) have all been reported to affect how cows will rest in free-stall barns. Cows prefer softer stalls with more bedding, and spend more time lying down in well-bedded, dry stalls (Tucker and Weary, 2004; Reich et al., 2010. It is well established that the configuration of lying stalls (e.g. stall width, length, position of neck rail) can have a major effect lying behavior (Tucker et al., 2004; 2005; 2006; Bernardi et al., 2009). The position of the neck rail seems to be particularly important in determining how the cow uses the stall for standing but there is an interaction with cow size where larger cows spend more time with front hooves in the stalls compared to smaller cows (Fregonesi et al., 2009). For instance, Sudolar et al. (2018) reported that pregnant dairy heifers did not show any clear preferences for either of 2 neck rail positions: 160 cm from the curb at a height of 124 cm versus 150 cm from the curb at a height of 122 cm. Not surprising, these latter authors did report that stalls were cleaner in the more restrictive neck rail position treatment. Keeping cows out of the stalls will keep stalls cleaner(not recommended)– both narrow free-stalls and the more restrictive neck rail placements (lower and closer to the rear of the stall) reduce the amount of fecal matter in the stall (Tucker et al., 2005; Bernardi et al., 2009; Sudolar et al., 2018). In a cross sectional study of 224 free-stall herds by Ruud et al. (2011), the most important factors in improving stall cleanliness (reduced fecal contamination) in ranked order, were amount of bedding >1.0 L, diagonal stall length ≤1.96 m, absence of lower head rail, stall length < 2.30 m, brisket locator distance ≤1.83 m, stall width >1.13 m and upper head rail 0.70 m.

Stall cleanliness alone is, however, a poor measure of how well specific stall design features work from the cows perspective. Less restrictive stall designs, which allow cows to stand fully in the stall, increase four foot standing in the stall which has been shown to improve locomotion in lactating cows (Bernardi et al., 2009). Stalls with higher occupancy rates are, however, more likely to contain feces. DeVries et al. (2012) reported a positive association between lying duration and poorer hygiene of upper legs-flank and udder and speculated that when the stall surface is soiled, increased lying duration might increase exposure to manure and moisture. Thus, as described by Bernardi et al. (2009), there appears to be a paradox whereas stalls are either designed to promote lying and 4 foot standing and thus less lameness or alternatively reduce lying and 4 foot standing but improve cow hygiene. Unfortunately, Bernardi et al. (2009)research did not address the effects of stall design on udder health.

The objective of this study was to investigate the associations among cow behavior and housing management, and the risk of elevated SCC (**eSCC**) in lactating dairy cows housed in commercial free-stall barns. In particular, to determine how stall design and usage interacts with hygiene to affect the risk of eSCC and lameness. It was predicted that cows lying in more restrictive free-stalls, with harder lying surfaces, would have lower lying times, higher gait scores (i.e. more lameness), but lower hygiene scores (cleaner) and thus reduced risk of eSCC.

## 4.2 MATERIALS AND METHODS

### *4.2.1 Herd Selection*

A longitudinal study of 400 cows was conducted on 18 commercial dairy herds in Ontario. Initially during the spring of 2015, 6 farms were recruited through an email sent by the researchers to producers located within 150 km of the University of Guelph-Kemptville Campus (Kemptville, ON, Canada). During the fall of 2016, an additional 12 farms were recruited through an email sent by Progressive Dairy Operators (Guelph, ON) to producers located within 150 km of the University of Guelph. Producers were asked to contact the researchers directly, to obtain more information about the project and to confirm their interest in enrolling in the study. Specific criteria for herd selection included: milking Holstein cows, free-stall housing, parlor-milking, participation in milk recording (Dairy Herd Improvement (**DHI**)) (CanWest DHI, Guelph, Ontario, Canada), and farms proportionally divided between those that milked 2x/d versus 3x/d, and used sand, sawdust or straw as bedding in the free-stalls available to the lactating cows Producers willing to participate in the study were asked to authorize the principle investigator to access their online DHI reports. This information was used to select focal cows (i.e. those under a 100,000 cells/mL SCC threshold at the first DHI sampling) immediately after the testing results became available. At each farm all eligible cows/herd (400 cows in total across herds) were selected and enrolled based on the following criteria: DIM (<120 d), absence of mastitis treatment in the last 3 months, and SCC (<100,000 cells/mL).

### *4.2.2 Data Collection*

Each farm was visited 5 ± 3 d (mean ± SD) after a DHI test, and this continued until 3 tests were completed (~105 d), for a total of 3-observation periods/cow (at ~5-wk intervals) and 2 visits per period (7 d apart) (6 visits per farm in total).

During the first visit to each farm, an oral questionnaire was administered to each producer by the principal investigator. The questionnaire (Appendix 1; modified from Dufour et al. (2010)) was designed to collect information about bedding and housing management practices, and measures of udder health. The questionnaire was designed to gain information on stall stocking density, stall base, bedding type, amounts applied, type of application (the entire stall or only partial), frequency of application (including date of last new bedding added, time of last raking/ cleaning), and use of bedding conditioners. The date of the last new bedding application was requested directly from the farmer in each of the 6 visits during the 3 periods.

Standing and lying behavior of the focal cows were collected for 6 d per period, using data loggers (HOBO Pendant G Data Logger, Onset, Pocasset, MA; validated by Ledgerwood et al., 2010). A data logger was attached to the most accessible hind leg of each cow in the parlour after milking of each cow using veterinary bandaging (CoFlex, Andover Coated products Inc., Salisbury, MA, USA), and programmed to record the position of the cow (lying or standing) at 1-min intervals for 6 consecutive days. Recording was set to begin at 0000 h on the day following the first farm visit, in each of the 3 periods. The average numbers of days between DHI test and data logger attachment was 5 ± 3 d (mean ± SD).

On the same day that the data loggers were attached (1x per period=3 scores in total), cows were gait scored when leaving the milking parlor, using a 5-point numerical rating system, where 1 = sound and 5 = severely lame (Flower and Weary, 2006). On the days when data loggers were attached and removed (2x per period=6 scores in total), cow hygiene was recorded using a hygiene scoring system (Cook and Reinemann (2007): http://www.vetmed.wisc.edu/dms/fapm/fapmtools/4hygiene/hygiene.pdf; where the lower legs, udder, and upper leg and flank are all assigned a cleanliness score (ranging from 1 = clean to 4 =

dirty). On the days when data loggers were removed (7 d after attachment) cow body condition was scored (1x per period=3 scores in total) based on the Wildman et al. (1982) system where body condition score (BCS) 1 describes animals that are extremely thin and BCS 5 describes animals that are extremely fat, including half point scores. The BCS were based on visual observation and when necessary palpation. Data were left skewed so cows were classified into each of the scoring categories for hygiene (clean: ≤2, dirty: ≥3), BCS (over-conditioned: ≥4, normal: 3-3.5, under-conditioned: ≤2.5), gait (sound: ≤2, lame: ≥3) and parity (parity 1, 2, and ≥3). Inter-observer reliability was evaluated by comparing scores made independently by 2 trained observers for 34 cows evaluated for all measures over 2 consecutive visits at the same farm.

***4.2.3 Stall Cleanliness and Dimensions***

Stall hygiene was assessed at each visit (previously described by Zdanowicz et al., 2004; Bernardi et al., 2009; Fregonesi et al., 2009). In brief, 10% of milking cow stalls were scored using a 1.20 × 1.65 m wire grid, containing 88 equally sized squares (0.15 × 0.15 m). The grid was placed on the rear section of the stall, closest to the alley, and centered between the stall partitions. A hygiene score was determined by counting the total number of grid squares containing any visible fecal material or urine (defined as wet bedding). The grid was disinfected (Virkon; LANXESS Corporation; Pittsburgh PA, USA) in between farms for biosecurity purposes. Stalls were labeled on the first farm visit using black permanent markers, to ensure the same stalls were assessed for hygiene on each visit. Stall dimensions (including stall width, length, and neck rail position) were recorded, using methodology adapted from von Keyserlingk et al. (2012), during 1 of the 6 visits. In total, dimensions of 10 stalls per herd were recorded (from the 10% of stalls of milking cows, previously selected for stall hygiene scoring). Average stall dimensions (mean and SD) for each farm were calculated and used for further analysis.

### *4.2.4 Calculations and Statistical Analyses*

Elevated SCC has been used as an indicator of subclinical mastitis (Dufour et al., 2011). An incident eSCC is defined as a cow having a SCC >200,000 cells/mL at the end of defined period in which she was <100,000 cells/mL at the beginning of that period (Dohoo and Leslie, 1991; Schepers et al., 1997; Schukken et al., 2003). Incidence rate of eSCC was calculated as: number of new eSCC/# of cows at risk/d at risk × 365 d/year (Dohoo et al., 2009).

Explanatory variables of interest included herd-level measures of bedding type and management (frequency of adding bedding, frequency of stall cleaning), stall hygiene, stall dimensions, season, herd management (stocking density, milking practices) and cow-level measures (Days in milk (DIM), SCC, BCS, milk yield, and parity). Factors associated with categorical outcome variables gait score, eSCC, and hygiene (body areas: lower legs, udder, upper legs and flanks) were analyzed using multivariable logistic regression models. Factors associated with continuous outcome variables cow lying behavior variables (lying time, bout length), were analyzed using multivariable mixed-effect linear regression models, treating period as a repeated measure. These models included the random intercept of farm within season, and cow within farm.

Prior to analysis, all data were screened for normality and identification of outliers using the UNIVARIATE procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC). Cow SCC and stall hygiene results were right skewed, and were transformed by taking their natural logarithm. Covariance structures were selected based on best fit according to Schwarz's Bayesian information criterion. Compound symmetry covariance structure was selected for all outcome variables. As a first step in model building, all individual explanatory variables were tested in univariable models. Variables with $P < 0.25$ in the univariable analysis were included in the multivariable models (Dohoo et al., 2009). If 2 variables were correlated (r > 0.60), the one with the lower $P$-value in the univariable analysis was kept. Manual backward elimination was used to remove any variables with $P > 0.10$; those variables retained were considered significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. The Pearson correlation test, using Proc CORR of SAS, was then used to determine associations among hygiene of cow body parts.

## 4.3 RESULTS AND DISCUSSION

All of the measures assessed had very high inter-observer reliability with a Cohen's kappa (± SE) of 0.89 ± 0.07 for lower leg hygiene, 0.94 ± 0.06 for udder hygiene, 0.94 ± 0.06 for upper leg/flank hygiene, 0.89 ± 0.07 for gait score, and 0.83 ± 0.09 for BCS.

Table 4.1 contains a descriptive summary of the data for all the cows (n=400 cows) collected across the study periods. Lying behavior measures for the current study were all within the range of previous reports (Ito et al., 2009; Gomez and Cook (2010); Solano et al., 2016).

In this observational study, 9 of the18 farms (50%) used deep bedding (8 using sand and 1 using wood shavings) and the other 9 farms used mattresses, covered in sand, sawdust, or straw. Stocking density across herds was similar (1.0 ± 0.16 cows/stall mean ± SD; min=0.6, max=1.4) to that of Gomez and Cook (2010), who reported the stocking density across free-stall herds (n=16) in Wisconsin ranged from 0.83 to 1.26 cows per stall (mean = 1.05 ± 0.10 cows/stall).

Stall hygiene (based on the mean proportion of soiled squares (SS)/stall) was 20.1 ± 0.50% (mean ± SD) (range= 3.0 to 81%) with an average of 18 contaminated grid squares, which is equivalent to 4,050 $cm^2$ (= 225 $cm^2$ per square x 18 SS of stall contamination). Previous controlled studies reported a mean stall contamination across sampling days for stalls bedded with sand of 700 $cm^2$ (100 $cm^2$/square x 7 SS), 1,400 $cm^2$ (100 $cm^2$/square x 14 SS) for stalls bedded with sawdust (Zdanowicz et al., 2004), 320 $cm^2$ (80 $cm^2$/square x 4 SS) for less restrictive neck rail stalls (Bernardi et al., 2009), and 320 $cm^2$ (64 $cm^2$/square x 5 SS) (Fregonesi et al., 2009). DeVries at al. (2012) using similar methodology to the current study, reported a stall contamination area of 1,125 $cm^2$. Although it appears that the stalls in the current study were dirtier than those in DeVries et al. (2012) the latter authors only accounted for visible fecal matter when scoring stall contamination, while in this study both fecal and visible urine matter were accounted for.

Lying time was associated with BCS, stall surface, and proportion of soiled squares (SS)/stall (Table 4.2). On average, under-conditioned cows (BCS $\leq$ 2.5) spent 37 $\pm$ 16.6 min/d less time lying down than over-conditioned cows (BCS $\geq$ 4.0; Table 4.2). King et al. (2016) reported almost identical results for cows milked in automatic milking systems (AMS), with under-conditioned cows (BCS $\leq$ 2.5) spending 35 min/d more time standing compared to cows with $\geq$ 3.5 BCS. Similarly, Westin et al. (2016) reported cows milked in AMS with $\geq$ 3.5 BCS lying down on average 1 h/d longer than cows with a BCS $\leq$ 2.5. Westin et al. (2016) speculated that under-conditioned cows have to spend more time standing and eating, than over-conditioned cows. A reduced lying time in high producing cows producing that are under-conditioned (Fregonesi and Lever 2001) may be due to increased time spent standing to ingest feed, to meet their higher nutritional requirements for production (Bewley et al., 2010).

In the current study, we noted an association between stall surface type and daily lying time. On average, cows tended to spend 35.9 min/d more time (Table 4.2) lying down in deep-bedded vs. mattress-based stalls. Mattress surfaces have consistently been associated with reduced lying times in comparison to deep bedded sand (Ito et al, 2009; Cook et al., 2004). Tucker et al. (2003) reported a 1-h/d difference in lying time between cows lying in deep bedded sawdust (15.0 ± 0.40 h/d) or sand (14.9 ± 0.62 h/d) compared to when lying on a mattress surface covered with 2 to 3 cm of sawdust (13.3 ± 0.54 h/d). Further, Solano et al. (2016) reported a 70-min/d difference in lying time (mean ± SD) when doing a multiple farm comparison between the 2 stall surfaces (mattress = 10.6 ± 0.8, sand or dirt = 11.3 ± 1.2 h/d).

As the proportion of dirty stalls increased across farms, lying time decreased (Table 4.2). The model predicted a difference in daily lying time of ~80 min/d between the farms with the cleanest stalls as compared to those with the dirtiest stalls. As the proportion of SS/stall increased by 10%, cow lying time decreased by 16.6 min/d ($P$=0.03; Figure 4.1). These results are not surprising, as others have previously shown lying time to vary with bedding DM %, averaging 10.4 ± 0.4 h/d on dry bedding (34.7 ± 3.8 % DM (and increasing to 11.5 ± 0.4 h/d on very wet bedding (89.8 ± 3.7 % DM) (Reich et al., 2010). Although the moisture content of bedding was not measured in the current study, the protocol for assessing stall hygiene was to count squares containing any visible fecal material or urine (defined as wet bedding), thus our measure of stall hygiene could serve as a proxy for stall moisture content. Chen et al. (2017) also reported a reduction of mean lying time for cows lying down in the muddiest treatment (3.5 h/d) compared with cows housed in a dry lot covered in dry soil (12.5 h/d). These latter authors also reported that cows chose to lie down on concrete in the muddiest treatment, whereas, no cows chose to lie on the concrete in the dry treatment. Further, when cattle chose to lie down on wetter soil, they limited the surface area exposed to their surroundings by tucking their legs beneath their bodies (Chen et al., 2017). Collectively there is a growing body of evidence suggesting that cows do not want to lie down in a dirty, wet area.

In the present study, dirtier stalls were not only associated with decreased lying duration, but did have greater odds of cows being lame at time of observation (Table 4.3); for every SD increase in SS/stall, the odds of a cow of a cow being lame increased by 1.3×. This relationship between stall hygiene and likelihood of lameness is likely a consequence of increased standing duration on harder surfaces, which increases the risk for lameness (Bernardi et al., 2009).

Cow factors associated with prevalence of lameness at time of observation were BCS and parity (Table 4.3; $P<0.001$). Cows with BCS ≥ 4 were 2.7× more likely to be lame when compared to sound cows (Table 4.3). Gearhart et al. (1990) reported that cows with BCS > 4 in the dry period were 7× more likely to develop foot problems in the subsequent lactation, although an actual definition of foot problems was not provided. Olechnowicz and Jásckowski (2014) reported that non-lame cows scored on average 0.2 units lower in BCS compared to lame cows. Whether the development of foot problems is due to the body condition or the association between laminitis and excessive feeding during the dry period remains highly debated (Newsome et al., 2017). However, the current study provides additional evidence that BCS may be linked with lameness given that cows with BCS ≤ 2.5 were 2.4× more likely to be lame compared to sound cows (Table 4.3).

Cows in parity 1, 2, and 3 were 6.6, 2.3 and 2.1 times less likely, respectively, than those cows in parity > 3 to be lame (Table 4.3). King et al. (2017) reported greater parity and lower BCS to be associated with lameness. The authors speculate that the older cows, which generally produce more milk, have a greater impact on body mass loss that comes accompanied with the increased milk production. However, both King et al. (2017) and the present study were cross sectional studies, thus not able to assess long term effect or relationship direction between lameness and parity and lameness and BCS. However, previous longitudinal studies have reported that the order in which lameness happens with regards to BCS starts with increased milk production, followed by body condition loss, and finally results in greater rates of lameness (Green et al., 2014; Lim et al., 2015)

Over the study period, 50 eSCC were detected; an incidence rate of 0.45 eSCC/cow-year at risk. Contrary to our hypothesis, none of the factors evaluated as part of this study were associated with risk of new eSCC; likely explained by the low frequency of new cases of eSCC across the study period. Watters et al. (2013), using the same definition as in the present study for eSCC, reported a mean herd eSCC incidence rate of 0.91 ± 0.49 eSCC/cow-year (mean ±SD). However, Watters et al. (2013) used a different animal inclusion criteria and study type, which may explain some of these differences. Our animal inclusion criteria only included cows that were <120 DIM, absence of mastitis treatment in the last 3 mo, as well as SCC <100,000 cells/mL. In the Watters et al. (2013) study, eligible cows were <200 DIM, with an SCC <100,000 cells/mL at the most recent DHI test, and no requirement regarding previous mastitis treatments. The more flexible inclusion criteria implemented by Watters et al., (2013) may have resulted in an increased detection rate of eSCC, but potentially also increase the false positives. Additionally, our study used 18 herds with some management factors differences, that had to be accounted for, were used in our study, while Watters et al. (2013) included 40 focal cows from each of 5 herds. Thus, inflexibility in inclusion criteria for cows (cows with low SCC, no mastitis treatment in past 3 mo.) and herds variable management factors could have affected the incidence rate of eSCC detected during this study.

## 4.4 CONCLUSIONS

In our cross-sectional study of cows kept on commercial free-stall herds, factors associated with cow lying time were stall surface, stall hygiene, and body condition. Lying duration of cows was longer in deep-bedded stalls compared to mattress based stalls. Poor stall hygiene was associated with reduced lying time. Further cows exposed to soiled stalls were more likely to be experiencing lameness. Under-conditioned cows spent less time lying down than over-conditioned cows. Additionally, cows of both low and high body condition were more likely to be lame, as well as older cows. Overall, these results confirm that cows prefer to lie down in cleaner and more comfortable environments. Further, our results highlight the importance of the need for improved stall hygiene to potentially reduce lameness and to optimize lying time, especially for older cows and as a preventative measure for younger cows.

## 4.5 ACKNOWLEDGEMENTS

We are grateful to the participating producers for the use of their animals, facilities, and access to their data. We thank Athena Zambelis, Victoria Asselstine, Kaitlyn Dancy, Megan Gannon, Melissa Kuipers and Kaitlin Sparkman of the University of Guelph for their technical assistance with data collection. This project was financially supported by a contribution from the Dairy Research Cluster II Initiative, funded by the Dairy Farmers of Canada (Ottawa, ON, Canada), Agriculture and Agri-Food Canada (Ottawa, ON, Canada), the Canadian Dairy Network (Guelph, ON, Canada), and the Canadian Dairy Commission (Ottawa, ON, Canada), through the Canadian Bovine Mastitis and Milk Quality Research Network (Montreal, QC, Canada).

## 4.6 REFERENCES

Bernardi, F., J. Fregonesi, D. M. Veira, C. Winkler, M. A. G. von Keyserlingk, and D. M. Weary. 2009. The stall design paradox: Neck rails increase lameness but improve udder and stall hygiene. J. Dairy Sci. 92:3074–3080.

Bewley, J. M., R. E. Boyce, J. Hockin, L. Munksgaard, S. D. Eicher, M. E. Einstein, and M. M. Schutz. 2010. Influence of milk Yield, stage of lactation and body condition on dairy cattle lying behavior using an automated activity monitoring sensor. J. Dairy Res.77:1-6.

Chen, J. M., C. L. Stull, D. N. Ledgerwood, and C. B. Tucker. 2017. Muddy conditions reduce hygiene and lying time in dairy cattle and increase time spent on concrete. J. Dairy Sci.100: 2090-2103.

Cook, N. B. and D. J. Reinemann. 2007. A toolbox for assessing cow, udder and teat hygiene. Page 31-43 in Proc. NMC. Annu. Mtg, NMC. Inc., Verona, WI, USA. Available at http://www.vetmed.wisc.edu/dms/fapm/fapmtools/4hygiene/hygiene.pdf.

Cook, N. B., T. B. Bennett, and K. V. Nordlund. 2004. Effect of free-stall surface on daily activity patterns in dairy cows with relevance to lameness prevalence. J. Dairy Sci. 87:2912–2922.

DeVries, T. J., M. G. Aarnoudse, H. W. Barkema, K. E. Leslie, and M. A. G. von Keyserlingk 2012. Associations of dairy cow behavior, barn hygiene, cow hygiene, and risk of elevated somatic cell count. J. Dairy Sci. 95:5730–5739.

Dohoo, I. R., and Leslie, K. E. 1991. Evaluation of changes in somatic cell counts as indicators of new intramammary infections. Prev. Vet. Med. 10: 225–237.

Dohoo, I., W. Martin, H. Stryhn. 2009. Veterinary Epidemiologic Research, 2nd ed. VER Inc., Charlottetown, PEI, Canada.

Dufour, S., H. W. Barkema, L. DesCôteaux, T. J. DeVries, I. R. Dohoo, K. Reyher, J. P. Roy, and D. T. Scholl. 2010. Development and validation of a bilingual questionnaire for measuring udder health related management practices on dairy farms. Prev. Vet. Med. 95:74-85.

Dufour, S., A. Fréchette, H. W. Barkema, A. Mussell, D.T. Scholl. 2011. Effect of udder health management practices on herd somatic cell count. J. Dairy Sci. 94:563–579.

Flower, F.C., D.M. Weary. 2006. Effect of hoof pathologies on subjective assessments of dairy cow gait. J. Dairy Sci. 89:139–146.

Fregonesi, J. A. and Leaver, J. D. 2001 Behavior, performance, and health indicators of welfare for dairy cows housed in strawyard or cubicle systems. Livestock Prod. Sci. 68:205-216.

Fregonesi, J. A., C. B. Tucker, and D. M. Weary. 2007. Overstocking reduces lying time in dairy cows. J. Dairy Sci. 90:3349–3354.

Fregonesi, J. A., M. A. G. von Keyserlingk, C. B. Tucker, D. M. Veira, and D. M. Weary. 2009. Neck-rail position in the free-stall affects standing behavior and udder and stall cleanliness. J. Dairy Sci. 92:1979–1985.

Galindo, F., and D. M. Broom. 2000. The relationships between so- cial behavior of dairy cows and the occurrence of lameness in three herds. Res. Vet. Sci. 69:75–79.

Gearhart M. A., Curtis C. R., Erb H. N., Smith R. D., Sniffen C. J., Chase L. E., Cooper M. D. 1990. Relationship of changes in condition score to cow health in Holsteins. J. Dairy Sci.73:3132-3140.

Green, L. E., J. N. Huxley, C. Banks, and M. J. Green. 2014. Temporal associations between low body condition, lameness and milk yield in a UK dairy herd. Prev. Vet. Med. 113:63–71

Gomez, A., and N. B. Cook. 2010. Time budgets of lactating dairy cattle in commercial free-stall herds. J. Dairy Sci. 93:5772–5781.

Ito, K., D. M. Weary, and M. A. G. von Keyserlingk. 2009. Lying behavior: Assessing within- and between-herd variation in free-stall- housed dairy cows. J. Dairy Sci. 92:4412–4420.

King, M. T. M., E. A. Pajor, S J. LeBlanc, T. J. DeVries. 2016. Associations of herd-level housing, management, and lameness prevalence with productivity and cow behavior in herds with automated milking systems. J. Dairy Sci. 99:9069-9079

Corrigendum to King, M. T. M., S. J. LeBlanc, E. A. Pajor, and T. J. DeVries. 2017. Cow-level associations of lameness, behavior, and milk yield of cows milked in automated systems. J. Dairy Sci. 100:4818-4828.

Lim, P. Y., J. N. Huxley, J. A. Willshire, M. J. Green, A. R. Othman, and J. Kaler. 2015. Unravelling the temporal association between lameness and body condition score in dairy cattle using a multi state modelling approach. Prev. Vet. Med. 118:370–377.

Ledgerwood, D. N., C. Winckler, and C. B. Tucker. 2010. Evaluation of data loggers, sampling intervals, and editing techniques for measuring the lying behavior of dairy cattle. J. Dairy Sci. 93:5129–5139.

Newsome, R .F., M. J. Green, N. J. Bell, N. J. Bollard, C. S. Mason, H. R.Whay, and J. N. Huxley. 2017. A prospective cohort study of digital cushion and corium thickness. Part 2: Does thinning of the digital cushion and corium lead to lameness and claw horn disruption lesions?. J. Dairy Sci.100:4759-4771.

Olechnowicz, J. and Jaskowski, J.M., 2014. Body condition related to lameness in dairy cows. Med. Weter. 70:353-356.

Reich, L.J., D. M. Weary, D.M. Veira, and M. A. G Von Keyserlingk. 2010. Effects of sawdust bedding dry matter on lying behavior of dairy cows: A dose-dependent response. J. Dairy Sci. 93:1561-1565.

Ruud, L.E., C. Kielland, O. Østerås, and K. E. Bøe. 2011. Free-stall cleanliness is affected by stall design. Livestock Science. 135:265-273.

Schepers, A. J., T. J. Lam, Y. H. Schukken, J. B. Wilmink, and W. J. Hanekamp. 1997. Estimation of variance components for somatic cell counts to determine thresholds for uninfected quarters. J. Dairy Sci. 80:1833–1840.

Schukken, Y. H., D. J. Wilson, F. Welcome, L. Garrison-Tikofsky, R. N. Gonzalez. 2003. Monitoring udder health and milk quality using somatic cell counts Vet. Res. 34:579–596.

Solano, L., H. W. Barkema, E. A. Pajor, S. Mason, S. J. LeBlanc, C. G. R. Nash, D. B. Haley, D. Pellerin, J. Rushen, A. M. de Pas- sille, E. Vasseur, and K. Orsel. 2016. Associations between lying behavior and lameness in Canadian Holstein-Friesian cows housed in free-stall barns. J. Dairy Sci. 99:2086–2101.

Solano, L., H. W. Barkema, E. A. Pajor, S. Mason, S. J. LeBlanc, J. C. Zaffino Heyerhoff, C. G. R. Nash, D. B. Haley, E. Vasseur, D. Pellerin, J. Rushen, A. M. de Passillé, and K. Orsel. 2015. Prevalence of lameness and associated risk factors in Canadian Holstein- Friesian cows housed in free-stall barns. J. Dairy Sci. 98:6978–6991.

Sudolar, N. R., Panivivat, R., and Sopannarath, P. 2018. Effects of two neck rail positions on heifer behavior and stall cleanliness in free-stall barn. Agric. Nat. Resour. Sci. 51:432-435.

Tucker, C.B., D. M. Weary, and D. Fraser. 2003. Effects of three types of free-stall surfaces on preferences and stall usage by dairy cows. J. Dairy Sci. 86:521-529.

Tucker, C. B., D. M. Weary, J. Rushen, and A. M. de Passillé. 2004. Designing better environments for cattle to rest. Pages 39–53 in Adv. Dairy Technol. Vol. 16. J. Kennelly, ed. Univ. Alberta, Red Deer, Alberta, Canada.Schreiner, D. A., & Ruegg, P. L. (2002). Effects of tail docking on milk quality and cow cleanliness. J. Dairy Sci. 85:2503–2511.

Tucker, C. B., and D. M. Weary. 2004. Bedding on geotextile mattresses: how much is needed to improve cow comfort? J. Dairy Sci. 87:2889–2895.

Tucker, C. B., D.M. Weary, and D. Fraser. 2003. Effects of three types of free-stall surfaces on preferences and stall usage by dairy cows. J. Dairy Sci. 86:521–9.

Tucker, C. B., D. M. Weary, and D. Fraser, 2005. Influence of neck-rail placement on free-stall preference, use, and cleanliness. J. Dairy Sci. 88:2730–2737.

Tucker, C. B., G. Zdanowicz, and D. M. Weary. 2006. Brisket boards reduce free-stall use. J. Dairy Sci. 89:2603–2607.

von Keyserlingk, M. A. G., A. Barrientos, K. Ito, E. Galo, and D. M. Weary. 2012. Benchmarking cow comfort on North American free-stall dairies: lameness, leg injuries, lying time, facility design, and management for high-producing Holstein dairy cows. J. Dairy Sci. 95:7399–7408.

Watters, M.A., K. Meijer, H. W. Barkema, K. E. Leslie, M. A. von Keyserlingk, and T.J. DeVries. 2013. Associations of herd-and cow-level factors, cow lying behavior, and risk of elevated somatic cell count in free-stall housed lactating dairy cows. Pre. Vet. Med. 111:245-255.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt, and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495–501.

Westin, R., A. Vaughan, A. M. De Passille, T. J. DeVries, E. A. Pajor, D. Pellerin, J. M. Siegford, E. Vasseur, J. Rushen. 2016. Lying times of lactating cows on dairy farms with automatic milking systems and the relation to lameness, leg lesions, and body condition score. J. Dairy Sci. 99:551-561.

Wolfe, T., E. Vasseur, T. J. DeVries, and R. Bergeron. 2018. Effects of alternative deep bedding options on dairy cow preference, lying behavior, cleanliness, and teat end contamination. J. Dairy Sci. 101:530-536.

Zdanowicz, M., J. A. Shelford, C. B. Tucker, D. M. Weary, and von Keyserlingk, M. A. G. 2004. Bacterial populations on teat ends of dairy cows housed in free=stalls and bedded with either sand or sawdust. J. Dairy Sci. 87:1694–1701.

**Table 4. 1** Descriptive summary of data for all the cows (n=400 cows), from 18 herds, collected across 3 study periods.

| Variable | Mean | SD | Min | Max | Median |
|---|---|---|---|---|---|
| DIM | 99 | 45.2 | 3 | 222 | 98 |
| Parity | 2.4 | 1.3 | 1 | 5 | 2 |
| SCC (× 1000 cells/mL) | 32.7 | 23.3 | 3 | 385 | 27 |
| Milk yield (kg/d) | 43.4 | 10.8 | 12.3 | 75.5 | 43.0 |
| Lying time (h/d) | 10.9 | 1.9 | 4.4 | 16.9 | 11.0 |
| Lying bouts (#/d) | 9.3 | 2.6 | 3 | 22 | 9 |
| Lying bout duration (h/bout) | 1.3 | 0.3 | 0.4 | 3.2 | 1.2 |

**Table 4. 2** Final multivariable mixed-effect linear regression model for those factors associated with lying time.

| Variables | Estimate | SE | *P*-value |
|---|---|---|---|
| Intercept | 731.2 | 33.29 | <0.001 |
| Body condition score (BCS) [1] | | | |
| Under-conditioned | -37.2 | 16.58 | 0.03 |
| Normal | -39.6 | 15.34 | 0.01 |
| Over-conditioned | Ref[2] | - | - |
| Stall surface | | | |
| Deep bedding | 35.9 | 18.32 | 0.06 |
| Mattress | Ref[2] | - | - |
| Ln SS/stall[3] | -26.3 | 8.56 | 0.003 |

[1] Body Condition score= BCS (under-conditioned: ≤ 2.5, normal: 3-3.5, over-conditioned: ≥ 4).

[2] Ref=Reference category.

[3] Ln SS/stall = natural log of proportion of fecal or urine contaminated squares (soiled squares; [out of 88 equally sized squares (0.15 x 15 m) in a 1.20 x 1.65-m wire grid]), averaged over a sample of 12.8 ± 5.2 (mean ± SD) stalls from each of 18 farms.

**Table 4. 3** Final multivariable logistic regression model for factors associated with prevalence of lameness at time of observation.

| Variable | Coefficient | SE | Odds Ratio (95% CI) | *P*-value |
|---|---|---|---|---|
| Intercept | -0.56 | 0.49 | - | 0.26 |
| Parity | | | | |
| 1 | -1.89 | 0. 27 | 6.6 (0.09, 0.26) | <0.001 |
| 2 | -0.82 | 0.24 | 2.3 (0.27, 0.71) | <0.001 |
| 3 | -0.74 | 0.28 | 2.1 (0.28, 0.82) | <0.001 |
| >3 | - | - | Ref[1] | - |
| BCS[2] | | | | |
| ≥ 4.0 | -0.97 | 0.39 | 2.7 (0.18, 0.81) | 0.01 |
| ≤ 2.5 | 0.88 | 0.22 | 2.4 (1.57, 3.72) | <0.001 |
| 3.0-3.5 | - | - | Ref[1] | - |
| Ln SS/stall[3] | 0.38 | 0.16 | 1.3 (1.04, 1.61) | 0.02 |

[1] Ref=Reference category.

[2] Body Condition score= BCS (under-conditioned: ≤ 2.5, normal: 3-3.5, over-conditioned: ≥ 4).

[3] Ln SS/stall = natural log of proportion of fecal or urine contaminated squares (soiled squares; [out of 88 equally sized squares (0.15 x 15 m) in a 1.20 x 1.65-m wire grid]), averaged over a sample of 12.8 ± 5.2 (mean ± SD) stalls from each of 18 farms.

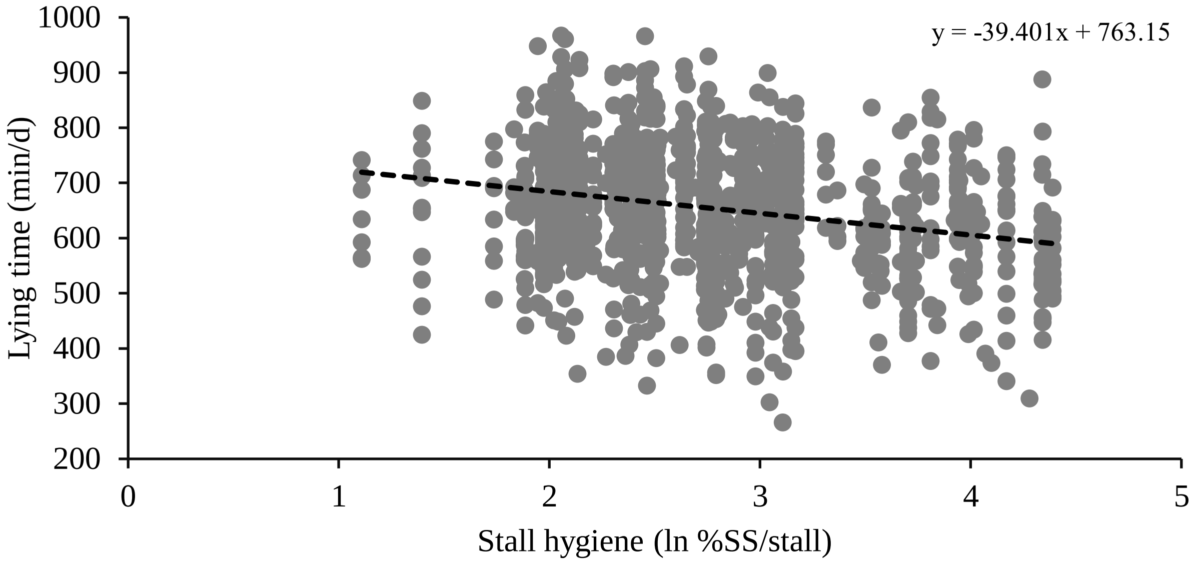


**Figure 4. 1** Associations of lying time with stall hygiene for dairy cows housed in free-stalls (n=400 cows). Lying time was measured over 3 periods with data logger attached to one of the hind legs of each cow and programmed to record the position of the cow (lying or standing) at 1-min intervals for 6 consecutive days. Stall hygiene was recorded in 12.8 ± 5.2 SD stalls from each of 18 farms per farm. Ln Proportion of soiled squares (SS)/stalls= natural log of average number of fecal or urine contaminated squares in free-stalls pens, x 100% [out of 88 equally sized squares (0.15 x 15 m) in a 1.20 x 1.65-m wire grid].

**Appendix 4. 1** Producer Questionnaire

1) Housing for:
   a) Dry cows: ☐ bedding pack ☐ free-stall ☐ tie-stall

   b) Bred heifers: ☐bedding pack ☐free-stall ☐ tie-stall
2) What breed of cattle do your milk? __________(*if mixed please state proportions*)

3) Do lactating (milking) cows in your herd have access to pasture?

   ☐Yes (#hours/day)___________ Months (e.g. June-Sept.)__________
   ☐Not applicable

4) What type of ventilation do you use?

   ☐ Natural ☐ Forced ☐ Other: ________________

5) What type of lighting do you use in the barn?

   a) ☐ Natural ☐Continuous ☐Photoperiod: __h light + __ dark ☐Other:_______

   b) ☐ Full barn is lit ☐ Only parts are lid: (*please specify*)_____________________

6) How many lactating cows do you milk, currently?

   a) Milking #_________ b) Dry # __________

7) How often are cows milked and at what times?

   ☐ Two times per day: _____ _______
   ☐ Three times per day: _______ ________ _______
   ☐ Other: _______________

8) Approximately, how long does it take to milk all the cows? _______ hrs.

9) In which pen order are your cows milked? a) Does this order vary? ☐Yes ☐No

   Pen __________, Pen __________, Pen ____________, Pen _____________,
   Pen, ____________

10) Do you have a maternity pen for your close up cows? ☐ Yes ☐No

    a) How many days/weeks before calving are cow's moved into this area? __________

    b) After how many days/weeks after calving do they move to the main pen? _______

    c) Housing type for maternity area: ☐ bedding pack ☐ free-stall ☐tie-stall

11) **Trimming**

    a) Who hoof trims your cows?

    ☐ In-house ☐ Trimmer ☐ Both

    b) How often? ___________ c) Date when last hoof trimmed? (Approx.)_______________

12) How often do you do foot baths?

a) ☐ 2Xweek ☐ 1Xweek ☐ Other:___________ ☐ Not Applicable

b) Which product/s do you use? _______________ ☐ Not Applicable

13) **Milking and post milking practices**:

(a) Gloves:

☐ All milkers wear gloves ☐ some milkers wear gloves ☐ do not wear

of ___ milkers gloves

(b) Pre and Post-dip: (*add product used*)

☐ Apply pre-post-dip using a dipper: ___________________
☐ Pre-wash and post dip: ____________________________
☐ No pre-wash and apply post-dip only:_________________
☐ Other: ________________________

(c) What do you use to clean cow's udders?

☐ Microfiber towel
☐ Regular towel
☐ Napkins
☐ Other:_________________

(d) Do you use individual towel/napkin for each cow?

☐ Yes
☐ No
☐ Not Applicable

14) **Feeding Practices** (milking cows only)

a) How many times do you feed your cows a day?

☐ 1xd ☐ 2xd ☐ Other: _________

b) At what time/s do you feed? ______ _______

c) How many push-ups per day? ________ ☐ Not Applicable

d) At which time/s is the feed pushed? _____ ____ ☐ Continuous ☐ Not Applicable

e) What equipment do you use to push feed?

☐ By hand ☐ Tractor/skid steer ☐ Automatic pusher (Juno) ☐ Not Applicable

15) What type of material is the **base** of your cows' stalls made of? *(Check all that apply and please provide proportions in percentage of each material)*

| (a) **Lactating cows:** | (b) **Dry cows** | (c) **Bred heifers:** |
|---|---|---|
| ____ Concrete | ____ Concrete | ____ Concrete |
| ____ Deep bedding | ____ Deep bedding | ____ Deep bedding |
| ____ Mattress-type : ____________ | ____ Mattress-type : _____________ | ____ Mattress-type : ____________ |

____ Other: *(Please specify)* ____ Other: *(Please specify)* ____Other: *(Please specify):*

_______________ _______________ ____________________

16) What type of **bedding** is used for your cows' stalls? *(Check all that apply please provide proportions of each material)*

(a) **Lactating cows:**

- ☐ Straw (if straw, is it chopped ___; to what length________)
- ☐ Sawdust
- ☐ Shavings
- ☐ Sand
- ☐ No bedding
- ☐ Other *(Please specify)*: ____________________

b) **Dry cows**

- ☐ Straw (if straw, is it chopped ___; to what length________)
- ☐ Sawdust
- ☐ Shavings
- ☐ Sand
- ☐ No bedding
- ☐ Other *(Please specify)*: ____________________

(c) **Maternity Pen (Close ups___ Fresh cows ____ Not applicable___)**

- ☐ Straw (if straw, is it chopped ___; to what length________)
- ☐ Sawdust
- ☐ Shavings
- ☐ Sand
- ☐ No bedding
- ☐ Other *(Please specify)*: ____________________

(d) **Bred heifers:**

- ☐ Straw (if straw, is it chopped ___; to what length________)
- ☐ Sawdust
- ☐ Shavings
- ☐ Sand
- ☐ No bedding
- ☐ Other *(Please specify)*: ____________________

17) How often do you source new bedding? *(Check all that apply)*

- ☐ Once a week
- ☐ Once every 2 weeks
- ☐ Once a month
- ☐ Other *(Please specify)*: ____________________

18) What type of **bedding management** do you use in milking cows' stalls?

- ☐ I do not use any bedding
- ☐ Less than 2 cm deep
- ☐ Between 2 cm- 8 cm deep
- ☐ More than 8 cm (i.e. deep bedding)

19) How often do you **clean out manure** from your **milking** cows' stalls?

☐ ☐ ☐ ☐

Never ______times/day ______times/week Not applicable

20) How often do you **scrape out the dirty bedding** from your **milking** cows' stalls?

☐ Never ☐ ______times/day *(Number)* ☐ ______times/week *(Number)* ☐ Not applicable

21) How often do you maintain (i.e. level the bedding, or pull back sand…) the stalls?

☐ Never ☐ ______times/day *(Number)* ☐ ______times/week *(Number)* ☐ Not applicable

22) If you do **scrape out dirty bedding** from your **milking** cows' stalls, **what part of the stall** do you scrape off?

☐ Back third of the stall ☐ Back half of the stall ☐ The whole stall
☐ Other *(Please specify)*:______________________ ☐ Not applicable

23) How often do you **add new bedding** to your **milking** cows' stalls?

☐ Never ☐ ______times/day *(Number)* ☐ ______times/week *(Number)* ☐ Other:___ ☐ Not applicable

24) What is the last date you added new bedding to your stalls? _____________

25) Do you add any type of conditioner (or product) to the bedding/stall?

☐ Yes. a) If yes, please specify product and frequency __________________
☐ No

26) How often do you completely **replace the bedding** in your **milking** cows' stalls?

☐ Never ☐ ______times/month *(Number)* ☐ Other: ____________ ☐ Not applicable

27) How often do you clean the stalls (or pens or both) completely?

☐ Never ☐ ______times/month ☐ Other: ____________ ☐ Not applicable

28) When you clean the stalls (or pens or both) completely, do you **wash or disinfect** them? *(Check all that apply)*

☐ Yes, pressure washed or brushed
☐ Yes, disinfected with ______________________________ *(Name of the product)*
☐ Yes, other *(Please specify)*: _________________
☐ No
☐ Not applicable

29) How many **stalls** are available per cow for your **milking cows** today?

| Pen # | Number of Cows | Number of Stalls |
|---|---|---|
| 1- | | |
| 2- | | |
| 3- | | |

| | | |
|---|---|---|
| 4- | | |
| 5- | | |
| 6- | | |

30) What is the surface of the **alley ways** in the barn?

- ☐ Concrete
- ☐ Slatted floor
- ☐ Grooved/scratched concrete
- ☐ Rubber covered concrete
- ☐ Other *(Please specify):* ________________________________________

31) What is the surface of the **parlor area** in the barn? (i.e. where cows wait before milking)

- ☐ Concrete
- ☐ Slatted floor
- ☐ Grooved/scratched concrete
- ☐ Rubber covered concrete
- ☐ Other *(Please specify):* ________________________________________

32) What is the surface by the **feed bunk** in the barn?

- ☐ Concrete
- ☐ Slatted floor
- ☐ Grooved/scratched concrete
- ☐ Rubber covered concrete
- ☐ Other *(Please specify):* _______________________________________

33) How are the alley ways in the barn cleaned?

- ☐ Scraped (alley scrapers or tractor/skid steer)
- ☐ Flushed with water
- ☐ Other *(Please specify):* ________________________________________

34) How many times a day are the alley ways cleaned?

☐ _______ times/day.
*(Number)*

35) Have you treated any of the cows to be used in the study in the past three months?

a) If Yes, When? ___________________ What for? __________________

36) May we contact CanWest DHI for your farm production data?

☐ Yes ☐ No If yes, DHI Herd number : _________
Contact number : __________

PIN number :______

# CHAPTER 5: IMPACT OF FREE-STALL NECK RAIL POSITION ON STALL AND COW HYGIENE, RISK OF INTRAMAMMARY INFECTION AND LAMENESS

## 5.1 INTRODUCTION

Configuration of dairy cow free stalls (e.g. stall size, position of neck rail) can have a major effect on stall usage and cow health (Tucker et al., 2004; 2005; 2006). Increased perching behavior, which is associated with increased risk of lameness (Galindo et al., 2000), has been well documented when free-stall neck rails are placed closer to the stall curb (Tucker et al., 2005; Fregonesi et al., 2009; Bernardi et al., 2009). Inversely, perching behavior declines when cows are provided sufficient space to stand fully in the stall (Bernardi et al., 2009) However, more comfortable stalls have higher occupancy rates (Sudolar et al 2018) and are, therefore, more likely to contain feces (Weary et al., 2008; DeVries et al., 2012). Greater lying duration in a soiled stall may, however, increase dirtiness of the upper legs-flank and udder of lactating dairy cows (DeVries et al., 2012), which may in turn increase the risk of mastitis (Breen et al., 2009; de Pihno Manzi et al., 2011). Since both narrow free-stalls and more restrictive neck rail placements (lower and closer to the rear of the stall) reduce the amount of fecal matter that ends up in the stall and thus may be viewed as improving cow hygiene (Tucker et al., 2005; Anderson, 2007; Bernardi et al., 2009; Sudolar et al., 2018), producers may elect to provide their animals more restrictive free stalls, particularly given that these types of stalls are associated with less time cleaning (Bernardi et al., 2009 Fregonesi et al, 2009).

While researchers have previously investigated the impact of neck rail position on cow hygiene, stall usage, stall cleanliness, and perching behavior, to our knowledge no work has looked at the impact of neck-rail position on risk of environmental mastitis. Thus, the objective of this study was to determine how free-stall neck-rail position impacts stall cleanliness, cow hygiene, and the risk of intrammamary infection (IMI) and lameness. It was hypothesized that cows lying in restrictive stalls will have higher gait scores (i.e. more lameness), but potentially lower hygiene scores (cleaner), and reduced risk of IMI.

## 5.2 MATERIALS AND METHODS

This study was conducted at the University of Guelph Livestock Research and Innovation Centre Dairy Facility (Elora, ON, Canada) from February to April 2017. Animal use and study design were approved by the University of Guelph's Animal Care Committee (AUP#3140) who follow the guidelines set out by the Canadian Council on Animal Care (2009).

### *5.2.1 Animals, Housing and Management*

A focal group of 120 cows (182.7 ± 92.8 days in milk (DIM); parity = 2.5 ± 1.2) was selected for inclusion in the trial. Cows were split into 4 groups of 30. Groups were balanced for DIM, parity, body weight (BW), gait score, hock injury score, and body condition score (BCS). Groups were housed in 4 pens, with each group at 100% stocking density (i.e.1 cow per stall). Each pen had a total of 30 stalls, configured in 2 rows that faced one another, were open at the front, and had a length of 259 cm. All stalls were 125 cm wide (center to center), and the neck rail was positioned 127 cm above the stall surface, with no brisket locator. Stalls were cleaned twice a day at 0500 and 1700 h. Stall bases were mattresses (Pasture Mat, PROMAT; Ontario, Canada) covered with chopped straw (~2 cm of depth at all times), with additional bedding added 1x/wk (on d 4, 11, 18, 25 of each treatment period). Crossover alleyways were scraped manually 2 ×/d and all other alleys were cleaned 10 ×/d with automatic scrapers. With the exception of the freestalls, all standing surfaces were rubber covered concrete. Cows were fed a

TMR 2 ×/d, at 0930 and 1330 h, and feed was pushed 10 ×/d with an automatic feed pusher (Juno, Lely N.V., Maassluis, the Netherlands). Cows were milked twice daily at 0500 and 1700 h in a 24-stall herringbone internal rotary parlor (DeLaval, Tumba, Sweden). Daily milk production and clinical disease events were recorded for all cows as per current farm protocols.

### *5.2.2 Treatment and Experimental Design*

Each pen of 30 cows was exposed to each of 2 stall design treatments, in a crossover design, with 28-d treatment periods (February 6 - March 5; March 13 - April 9, 2017). There was a washout period in between both treatments that lasted 7 d. The 2 treatments were: 1) adequate stall space (neck rail positioned 175 cm from the vertical plane above the rear curb), and 2) restrictive stall space (neck rail positioned 20 cm closer to the rear of the stall; 155 cm). Treatment for pen was randomly selected as long as it was alternating from the adjacent pen.

### *5.2.3 Milk Sampling*

Composite milk samples from each cow were collected 2 x/wk during the AM milking on d 1, 4, 8, 11, 15, 18, 22, and 25 of each treatment period, using milk meters (model: mm27bc) and samplers (DeLaval, Hashofet, Israel) attached to the parlour (for a total of 8 composite milk samples/cow/period). Samples were immediately transported by the first author to CanWest DHI (Guelph, ON, Canada) for SCC determination, using a Fossomatic cell counter (Fossomatic 5000 series, Foss Electric, Hillerød, Denmark).

Upon receipt of results from DHI (same day), cows with elevated SCC (i.e. ≥ 200,000 cell/mL) were selected for composite milk sampling, at the next AM milking, for bacterial culture. Whole udder, composite milk samples were collected aseptically (see Appendix 5.1), 2x/wk, on d 2, 5, 9, 12, 16, 19, 23, and 26 of each treatment period. Immediately after collection, all samples were stored (-20$^0$C) in a freezer located at the research facility. Frozen samples were kept in a cooler filled with ice packs and transported to the University of Guelph and stored frozen (-20$^0$C) until analyzed. Bacterial culture and identification were completed at the University of Guelph, Animal Health Laboratory (Guelph, ON, Canada) using laboratory standard operating procedures, according to National Mastitis Council (NMC) guidelines. Bacterial identification was completed using MALDI-TOF (matrix assisted laser desorption/ ionization time-of-flight mass spectrometry; MALDI Biotyper, Bruker Daltonics). In brief, 10 uL of milk was streaked onto Oxoid Columbia Blood Agar plates (Thermo Scientific; Nepean, Ontario, Canada) using an isoplater (Isoplater 180 Petri Dish Streaker, Vista Technologies Inc.; Edmonton, Alberta, Canada) to achieve optimum isolation of individual colonies. Inoculated Blood Agar plates were incubated for 24 h at 35$^0$C in $CO_2$ atmosphere. Cultures were read 24 h following incubation and any bacterial growth identified by MALDI-TOF. Primary culture plates were re-incubated for another 24 h (using the same process described previously) any new growth was identified in the MALDI-TOF. The milk (remainder of vial sample) was incubated for 24 h in 35$^0$C $O_2$ atmosphere. The incubated milk was re-plated (streaked) onto a blood agar plate and incubated for 24 h at 35$^0$C in $CO_2$ atmosphere. Re-plates were read at 24 h only - looking for *Staphylococcus aureus* and enteric pathogens only like *Escherichia coli.*

To ensure accuracy for gait scoring, hock injury scoring, body condition scoring and hygiene scoring, inter-observer reliability testing was conducted between 2 individuals. One of these individuals conducted the scoring for the entirety of the trial.

### *5.2.4 Gait Scoring*

Cows were individually scored for gait, by one observer, at the initial assessment (d -5 prior to treatment allocation) as they returned from the milk parlour (Ito et al., 2010), and on d 3, 10, 17, and 24 of each treatment period while in their pen when they were individually released from the headlock (for a total of 4 scores/per period), using a 5-point Numerical Rating System (**NRS**), where 1= sound and 5= severely lame (Flower and Weary, 2006). Percentage of clinically lame cows (gait score ≥ 3; von Keyserlingk et al., 2012) in each pen per week was calculated for further analysis. New cases of clinically lame cows were also calculated for further analysis. A new case of clinical lameness was defined as a cow with a gait score ≤ 2 for one wk observation followed by a score of ≥ 3 for the following wk.

### *5.2.5 Hock Injury Scoring*

All cows were individually scored by one observer for hock (tarsal joint) condition at the initial assessment (d -5 prior to treatment allocation), and on d 3, 10, 17, and 24 of each treatment period, on a 3-point scale, where 1 = healthy hock and 3 = swollen hock, open wound, or both, according to the Hock Assessment Chart for Cattle developed by the Cornell Cooperative Extension (http:// www.ansci.cornell.edu/prodairy/pdf/hockscore.pdf). Before the study, all groups were balanced for hock injury scores based on initial assessment scores. The percentage of cows with severe hock injury (=3; von Keyserlingk et al., 2012) per pen per week was calculated for further analysis.

### *5.2.6 Cow Weighing and Body Condition Scoring*

Cows were weighed individually using a scale (I-20 W scale Oahus, Dundas, ON, Canada), located in a chute next to the milking parlor, at an initial assessment d -7 and -6 prior to treatment allocation, and at the end of the study (d 57-58) to account for average BW of cows during the treatment period. Body condition was recorded by one observer, after each cow was weighed, using a five-point scale, as described by Wildman et al. (1982). All groups were balanced for body condition score (BCS) and body weight (BW) based on initial assessment score results.

### *5.2.7 Hygiene Scoring*

Cow hygiene was scored by one observer 2 x/wk, on d 1, 4, 8, 11, 15, 18, 22, and 25 of each treatment period. Udder, lower legs, and upper legs/flank were each scored on a 4-point hygiene scale (1 = very clean to 4 = very dirty) (Cook and Reinemann, 2007), and the percentage of cows with poor hygiene scores (3 and 4) per sampling day (by pen) are reported (Schreiner and Ruegg, 2003).

### *5.2.8 Stall Hygiene Scoring*

Stall hygiene was evaluated at the initial assessment, 2 times/wk., on d 1, 4, 8, 11, 15, 18, 22, and 25 of each treatment period (Zdanowicz et al., 2004; Bernardi et al., 2009; Fregonesi et al., 2009). In brief, 20% of milking cow stalls were scored (every 5$^{th}$ stall for a total of 6 stalls per pen) using a 1.20 × 1.65 m wire grid, containing 88 equally sized squares (0.15 × 0.15 m). The grid was placed on the rear section of the stall, closest to the alley, and centered between the stall partitions. A hygiene score was determined by counting the total number of grid squares containing any visible fecal material or urine (defined as wet bedding).  Stalls were labeled on the first farm visit using black permanent markers, the same stalls on each visit were scored. Mean proportion of soiled squares (SS)/ stall (number of dirty squares/total number of squares*100) was calculated per pen, per day, and was used for further analyses.

### *5.2.7 Calculations and Statistical Analyses*

Elevated SCC (eSCC ≥ 200,000 cell/mL) was used as an indicator of subclinical mastitis (Dufour et al., 2011). A new case of IMI with an environmental pathogen was defined as a cow milk sample SCC < 200,000 cells/ mL, followed by a eSCC at the subsequent milk sample, and a confirmed environmental mastitis pathogen identified in the composite milk sample taken on the day after the eSCC sample. Incidence of environmental mastitis in each treatment group was calculated by dividing the number of new cases by the total number of at risk cows. A cow was considered "at risk" if SCC was <200,000 cell/mL at the previous SCC test. Cows that were confirmed to be infected with *Staphylococcus aureus* based on composite culture were excluded (n=31 cows) from this analysis. A *Staphylococcus aureus* cow was defined as a cow that had two consecutive eSCC in either treatment period, followed by confirmed *Staphylococcus aureus* presence in both composite milk samples. This definition was put in place to work with sampling scheme to define cows with *Staphylococcus aureus*, as the farm used for this study is a high *Staphylococcus aureus* prevalence herd.

All continuous variables were initially screened for normality by assessing the distribution of the data and any outliers using PROC UNIVARIATE or PROC FREQ (version 9.3, SAS Institute Inc., Cary, NC). Somatic cell counts were right skewed and were, therefore, transformed by taking their natural logarithm. As treatment was applied at the pen level, pen was considered the experimental unit for all analyses and data were summarized at that level. Data for proportion of stall and percentage of cows with poor hygiene were summarized per pen and day. Data for percentage of gait and hock scores were summarized per pen and week.

Due to the low number of cases of environmental IMI (22 in total), statistical analyses were limited to the following: 1) Chi-square test was used to determine if the number of IMI cases caused by environmental pathogens varied between the two neck rail position treatments, by period. 2) A *T* test was used to determine if the SCC in cows with confirmed environmental IMI cases varied with neck rail position. We used the Cochran option (SAS version 9.3) to account for unequal variance. Backtransformed mean and 95% CI (lower, upper) for SCC from cows with confirmed IMI cases are reported.

Predictor variables of interest for the outcome variable stall hygiene included treatment, period, number of days since additional bedding was added, temperature, and relative humidity of the environment. Predictor variables of interest for percentage of dirty cows (body areas: lower legs, udder, upper legs and flanks) per pen, percentage clinically lame, percentage swollen hocks (left, and right, separately), and stall poor hygiene (Mean proportion of soiled squares (SS)/ stall per pen) were treatment, period, number of days since additional bedding was added, and local external temperature of the environment. Temperature and humidity of the environment were obtained retroactively from Environment Canada (Elora station, ON, Canada) and summarized based on the number of days since additional bedding was placed on the stall.

Data were analyzed with multivariable mixed effect linear regression models using the MIXED procedure of SAS, treating day as a repeated measure for the models for stall and cows hygiene, and week as a repeated measure for the models for gait and hock scores.

To build the multivariable linear mixed effect models, first, all individual explanatory variables were tested in univariable models. The MIXED procedure was used, with the fixed effects of treatment, period, and treatment x period interaction. Covariance structures were selected on the basis of best fit according to Schwarz's Bayesian information criterion. Compound symmetry covariance structure was selected for all outcome variables. Explanatory variables with $P < 0.25$ in the univariable analysis were included in the multivariable models (Dohoo et al., 2009). Manual backward elimination was used to remove any variables with $P > 0.10$; those variables retained were considered significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

To test the hypothesis that risk of new cases of clinical lameness would be higher in the restrictive neck rail position treatment than the adequate neck rail position treatment, a logistic regression model (using the GLIMMIX procedure of SAS; distribution=binomial, link = logit; version 9.3, SAS Institute Inc., Cary, NC) was used. Fixed effects included treatment, week, period, treatment*week and treatment*period interactions. Degrees of freedom for fixed effects were estimated using the Kenward-Roger option in the MODEL statement. The pen was considered a random effect and residual subject was pen within treatment and period. Since there was a significant interaction of treatment*week, the analysis was subsequently run for each week separately.

## 5.3 RESULTS

### *5.3.1 Inter-observer Reliabilities*

Kappa values for hygiene of lower leg = 0.92; 95% CI = 0.76-1.00, udder = 0.80; 95% CI = 0.57-0.93, upper leg/ flank = 0.83; 95% CI = 0.70-0.96; BCS=0.86; 95% CI = 0.73-0.99, gait scoring = 0.89; CI = 0.81-1.0.

Baselines scores for all treatment groups are presented on Table 5.1. Prevalence of clinical lameness was 37.5 % and prevalence of severe hock injuries were 3.0% and 0 % for left and right hocks, respectively.

### *5.3.2. Stall Hygiene*

Mean proportion of SS/stall, across treatment and periods, was 37.8 ± 7.7 % (range = 31 to 56%). No association between neck rail treatment and stall cleanliness were found ($P = 0.49$). The only factor associated with stall hygiene was days since additional bedding was added ($P < 0.001$). Mean number of days since additional bedding was added (2 ± 2 d) was associated with proportion of SS/stall; for every 2 d since additional bedding was added there was a 5-percentage point increase in mean proportion of SS/stall.

### *5.3.3. Cow Hygiene, Lameness, and Hocks Data*

A summary of cow hygiene, lameness, and hock injury data by treatment and period is presented in Table 5.2. No effect of neck rail position on the percentage of cows with dirty lower legs and upper legs and flanks was detected (Table 5.2). There was a tendency for an interaction between period and treatment for percentage of cows with dirty udders (Table 5.2). In the first period, there were a lower percentage of cows with dirty udders when the neck rail was closer to the rear of the stall (9.8 ± 1.9%) as compared to having the neck rail position further away (13.3

± 2.1%; Table 5.2). A greater percentage of cows with dirty udders were reported in the second period as compared to the first period (Table 5.2). Mean environmental temperature varied by period (Period 1: -4.3 ± 2.8 $^{0}$C, Min = -8.0, Max = 1.0; Period 2: -2.9 ± 5.4 $^{0}$C, Min = -10.0, Max = 5.0).

Mean percentage of clinically lame cows (NRS ≥ 3) across treatments and periods was 41.8 ± 9.5 % but did not vary by treatment (Table 5.2). A tendency for greater odds of experiencing a new case of lameness was identified, only in wk. 2, when the neck rail was positioned further from the stall curb as compared to having the neck rail positioned closer to the rear of the stalls (Estimate=1.05 ± 0.8; OR = 2.0, 95% CI = 0.92, 4.47; $P$=0.08). Treatment did not affect the percentage of cows with left ($P$=0.32) or right ($P$=0.49; Table 5.2) hock injuries. Percentage of cows with left and right hock injuries varied per period (Table 5.2). Percentage of cows with left hock injuries was 3.1 ± 1.2% and 2.0 ± 2.0% for period 1 and 2, respectively. Percentage of cows with right hock injuries was 0.8 ± 1.3 % and 0.95 ± 0.7 % for period 1 and 2, respectively.

### *5.3.4. Cases of IMI Caused by an Environmental Pathogens*

There were a total of 22 IMI that are classified as environmental pathogens. Incidence rate of IMI caused by an environmental pathogen was 0.02 per cow at risk/period, for both short and long stall treatment. The number of cases of IMI did not vary between treatments ($P$= 0.99; Table 5.2). Specific pathogens cultured in the composite milk samples from IMI cases are presented in Table 5.3. Coagulase-negative staphylococci (CNS) and Staphylococci were the most prevalent species isolated (13 and 11 cases, respectively; Table 5.3). Mean SCC for cows with IMI for both treatments and periods was 268,337 cells/mL (Median: 239,000; 95% CI =

217,510 and 392,385 cells/mL). Somatic cell count did not vary by treatment ($P$=0.59) or period ($P$=0.29).

## 5.4 DISCUSSION

In this study free-stall neck rail position did not affect stall cleanliness, although in previous research it has been reported that positioning the neck-rail further from the rear curb negatively affected stall cleanliness (Fregonesi et al., 2009; Bernardi et al., 2009; Sudolar et al., 2018). The 20 cm distance between neck-rail positioning from the curb between the treatments used in the current study may have been insufficient to detect a difference between the treatment groups. For instance, Bernardi et al. (2009) used an extreme range of neck rail positions (130 and 190 cm from the curb). Conversely, Fregonesi et al. (2009) used 5 different neck-rail positions (130, 145, 160, 175, 175, and 190 cm from the curb) and reported that when neck-rail was positioned further from the curb, stalls were dirtier, as cows were more likely to defecate in the stalls. However, it is worth mentioning that Tucker et al. (2005) and Fregonesi et al. (2009) noted that defecation in the stall was rare, but still made an impact on stall hygiene.

Cow size has also been shown to affect stall standing behavior (Fregonesi et al., 2009); unfortunately, we did not monitor this behavior.

Stall bedding may have also affected how the cows in this study used the lying stalls. In Fregonesi et al. (2009), stalls were covered with 10 cm of sand, while in the present study stalls had ~2 cm depth of straw at all times. Zdanowicz et al. (2004) demonstrated improved stall hygiene for sand versus sawdust. In contrast, Norring et al. (2008) reported no difference in stall cleanliness between deep bedded stalls with sawdust and sand. The fact that the farm only used a about 2 cm straw bedding may have also been a contributing factor. This can only be speculated since no previous study has assessed stall cleanliness by bedding depth and the current study was not designed to assess that hypothesis. In a very recent study by Sudolar et al. (2018), differences in stall hygiene were also reported between two neck rail treatments (160 cm from the curb at 124 cm height vs. 150 cm from the curb at 122 cm height). The differences noted between our findings and those of Sudolar et al. (2018) may be a consequence of how stall hygiene was measured; in our case it was the entire stall whereas Sudolar et al., (2018) partitioned the stall into sections.

The only explanatory factor in the current study was greater days since additional bedding which was negatively associated with stall hygiene. Zdanowicz et al. (2004) reported a similar finding, with stalls becoming increasingly contaminated with feces over 7 days for both sand and sawdust bedding types. Bacterial content of used bedding has been demonstrated to increase as the number of days since bedding was added increased (Chapter 2). Thus, the results of these studies provide evidence for supporting more frequent addition of bedding to the stall as a means to improving stall cleanliness (current study) and reducing bacterial counts in the bedding (Chapter 2).

There was an interaction between udder hygiene and treatment periods. Cows' udders tended to be dirtier when exposed to the more permissive neck rail position in the stall, but only in one of the treatment periods. Bernardi et al. (2009) similarly reported that udders were dirtier in the more permissive neck rail treatment compared to the restrictive treatment (190 and 130 cm from the curb). Assessing the linear response to treatment, Fregonesi et al. (2009) reported that cows in stalls with the neck rail positioned further from the curb were more likely to have dirty udders, with an interaction with cow size, where larger cows were more likely to have dirty udders as compared to smaller cows. Cow size was not measured in the current study, however, pens were balanced by cow BW and BCS, and both are strongly correlated to body size measurement (Yan et al., 2009). Seasonal variation for cow hygiene has been reported, with a greater proportion of clean cows observed in the dry season compared to the lowest in the wet season for cows from 2 herds in Sao Paulo State, Brazil (Sant'Anna and Paranhos da Costa et al., 2011). Thus, it may not be surprising to have an interaction of period and treatment in the current study, with more cows having dirty udders during the second period, when there were warmer temperatures and when rain was more likely, as compared to the first period. Bacterial growth increases during the wet season (as discussed in Hogan and Smith, 2012), but it can be speculated that humidity may also facilitate the adhesion of bedding and manure to the teat, thus potentially decreasing cleanliness.

Clinical lameness prevalence reported in this study (41.7 % across treatments and periods) is within the range of herd-level prevalence reported in free-stall herds in North America (28-55%; von Keyserlingk et al., 2012). Higher proportions of lame cows have been reported for farms with more restrictive neck rail positions (Fulwider et al., 2007). Bernardi et al. (2009) reported that more clinically lame cases developed in their more restrictive neck rail treatment. However, contradicting results were reported in the current study, where in week 2 when the neck rail was positioned further from the stall curb as compared to having the neck rail positioned closer to the rear of the stalls, cows were at greater odds for becoming clinically lame. The more restrictive neck rail may have prevented the large cows from standing four feet in the stall. Thus, they may have spent more time standing on a harder surface, which increases the risk for lameness.

Brenninkmeyer et al. (2013), in a cross-sectional study, reported that length of lying area in a cubicle had a strong relationship with the prevalence of hock lesions, and that increasing the length of lying area by 20 cm would decrease hock lesions prevalence by 6 %. However, in the current study, no differences were noted in prevalence of hock injuries by treatment in our longitudinal study. It is well established that mattresses with little amounts of beddings greatly increases the risk for hock injuries (Weary and Taszkun, 2000; Fulwilder et al., 2007). However, severe hock injury prevalence in our study (1.5 % across treatments and periods) was lower than previous reports (US: 1.8-5.4%; von Keyserlingk et al., 2012). The only factor associated with hock injury prevalence in our study was period, with period 2 having 0.05% percent more cows with severe hock injuries compared to period 1. To our knowledge, in no study to date has it been reported that seasonal/temperature changes affect hock injury prevalence. However, Lombard et al. (2010) reported greater numbers of hock lesions on farms in EasternUnited States when compared to the west, thus, part of that variance might be due to temperature differences between the two regions, which would in part explain the current study results in terms of differences of percentage of hock injured based on the two different periods where temperature varied significantly.

The lack of effect of neck rail position on SCC in the current study is in agreement with Bernardi et al. (2009). There was also no difference in environmental IMI, with 10 cases in one group and 12 in the other. In terms of pathogen variety reported in the current study, Rowbotham et al., (2016) described that the major environmental pathogens, environmental Streptococcus, *Escherichia coli, and Klebsiella spp.* were detected in 50% cultures of milk samples with an IMI, while in this study *Escherichia coli* was detected in only 1 of the milk cultures of IMI cases (respectively). Conversely, coagulase-negative staphylococci (CNS; Breen et al., 2009), were the most prevalent isolates in the current study milk composite samples from cows with an eSCC. Coagulase-negative staphylococci species, such as *Staphylococcus chromogenes, Staphylococcus haemolyticus, and Staphylococcus xylosus* detected in composite milk samples in this study are among the prevalent CNS species (Dolder et al., 2016). Coagulase-negative staphylococci species are the focus of much recent research, as the efforts to control minor IMI causative agents is increasing (Dufour et al., 2012; Supré et al., 2011; Tomazi et al., 2015). Dufour et al. (2012) also reported greater odds of acquiring a new CNS IMI in straw bedding as compared to sand and wood-based bedding (Dufour et al. 2012). Thus, the increased risk of IMI in straw bedding could partly explain CNS prominence in the IMI cases in the current study. Given that CNS have been described as some of the most common group of pathogens causing IMI (Tenhagen et al., 2006; Pyörälä and Taponen, 2009; Sampimon et al., 2009), close attention to these minor pathogens might help control IMI, as the agents causing the cases continue to emerge and their potential effect in udder health is studied (Dufour et al., 2012).

## 5.5 CONCLUSIONS

Despite free-stall neck rail position not having an impact on SCC, new cases of IMI caused by environmental pathogens, hock injuries, and stall cleanliness, a greater percentage of cows with dirty udders were reported when the neck rail was positioned further from the stall curb, but only during the second treatment period. Thus, this result must be viewed with caution. Further research into this area is needed to disentangle the interaction between treatments of neck rail position and external factors that influence the hygiene of cows.. Additionally, stall hygiene was improved when bedding was added more frequently. Positioning the neck rail further from the stall curb also tended to increase the risk for a new case of lameness in the second week of each treatment period. Overall, the results of this study provide evidence stall cleanliness is improved with frequent addition of bedding and it warrants additional research to confirm that

that cow udders hygiene, and risk for a case of clinical lameness, can be affected by neck rail position in a fre-stall,

## 5.5 ACKNOWLEDGEMENTS

We thank Kevin Foster, Sarah McPherson, Stephanie Kamalanathan, and Sarah Parsons of the University of Guelph (Guelph, ON, Canada) for their assistance with data collection. Thank you to the staff and management of the University of Guelph Livestock Research and Innovation Centre (Elora, ON, Canada). This project was financially supported by a contribution from the Dairy Research Cluster II Initiative, funded by the Dairy Farmers of Canada (Ottawa, ON, Canada), Agriculture and Agri-Food Canada (Ottawa, ON, Canada), the Canadian Dairy Network (Guelph, ON, Canada), and the Canadian Dairy Commission (Ottawa, ON, Canada), through the Canadian Bovine Mastitis and Milk Quality Research Network (St. Hyacinthe, QC, Canada).

## 5.6 REFERENCES


Anderson, N., 2007. Free Stall Dimensions. Info Sheet January 2007. Ministry of Agriculture, Food and Rural Affairs, Ontario, Canada.

Breen, J. E., M. J. Green, A. J. Bradley. 2009. Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the United Kingdom. J. Dairy Sci. 92:2551-2561.

Bernardi, F., J. Fregonesi, D. M. Veira, C. Winkler, M. A. G. von Keyserlingk, and D. M. Weary. 2009. The stall design paradox: Neck rails increase lameness but improve udder and stall hygiene. J. Dairy Sci. 92:3074–3080.

Bewley, J., 2008. Improving Cow Comfort through Proper Neck Rail Placement. Kentucky Dairy Notes September 2008, 3 September 2015. http://www.uky.edu/Ag/AnimalSciences/dairy/dairysystems/jb092008.pdf.

Brenninkmeyer, C., S. Dippel, J. Brinkmann, S. March, C. Winckler, and U., Knierim. 2013. Hock lesion epidemiology in cubicle housed dairy cows across two breeds, farming systems and countries. Prev. vet. Med.109: 236-245.

Cook, N. B. and D. J. Reinemann. 2007. A toolbox for assessing cow, udder and teat hygiene.Page 31-43 in Proc. NMC. Annu. Mtg, NMC. Inc., Verona, WI, USA. Available at http://www.vetmed.wisc.edu/dms/fapm/fapmtools/4hygiene/hygiene.pdf.

de Pinho Manzi, M., N. Obrega, D. B. Faccioli, P. Y. Troncarelli, M. Z. Menozzi, B. D. Langoni, H. 2011. Relationship between teat-end condition, udder cleanliness and bovine subclinical mastitis. Res. Vet. Sci. 93: 430-434.

DeVries, T. J., M. G. Aarnoudse, H. W. Barkema, K. E. Leslie, and M. A. G. von Keyserlingk. 2012. Associations of dairy cow behavior, barn hygiene, cow hygiene, and risk of elevated somatic cell count. J. Dairy Sci. 95:5730–9.

Dohoo, I., W. Martin, H. Stryhn. 2009. Veterinary Epidemiologic Research, 2nd ed. VER Inc., Charlottetown, PEI, Canada.

Dolder, C., van den Borne, B. H. P. Traversari, J. Thomann, A. Perreten, V. and M. Bodmer. 2017. Quarter-and cow-level risk factors for intramammary infection with coagulase-negative staphylococci species in Swiss dairy cows. J. Dairy Sci. 100: 5653-5663.

Dufour, S., I. R. Dohoo, H. W. Barkema, L. DesCôteaux, T. J. DeVries, K. K. Reyher, J-P. Roy, and D. T. Scholl. 2012. "Epidemiology of coagulase-negative staphylococci intramammary infection in dairy cattle and the effect of bacteriological culture misclassification." J. Dairy Sci. 95: 3110-3124.

Flower, F. C., D. M. Weary. 2006. Effect of hoof pathologies on subjective assessments of dairy cow gait. J. Dairy Sci. 89:139–146.

Fregonesi, J. A., M. A. G. von Keyserlingk, C. B. Tucker, D.M. Viera, D.M. Weary. 2009. Neck rail position in the free stall affects standing behavior and udder and stall cleanliness. J. Dairy Sci. 92: 1979-1985.

Fulwider, W. K., T. Grandin, D. J. Garrick, T. E. Engle, W. D. Lamm, N. L. Dalsted, and B. E. Rollin. 2007. Influence of free-stall base on tarsal joint lesions and hygiene in dairy cows. J. Dairy Sci. 90:3559–3566.

Galindo, F., and D. M. Broom. 2000. The relationships between social behavior of dairy cows and the occurrence of lameness in three herds. Res. Vet. Sci. 69:75–79.

Lombard, J. E., C. B. Tucker, M. A. G. Von Keyserlingk, C. A. Kopral, and D. M. Weary. 2010. Associations between cow hygiene, hock injuries, and free stall usage on US dairy farms. J. Dairy Sci. 93: 4668-4676.

Ito, K., M. A. G. von Keyserlingk, S. J. LeBlanc, and D. M. Weary. 2010. Lying behavior as an indicator of lameness in dairy cows. J. Dairy Sci. 93:3553–3560.

Norring, M., E. Manninen, A.M. De Passillé, J. Rushen, L. Munksgaard, and H. Saloniemi. 2008. Effects of sand and straw bedding on the lying behavior, cleanliness, and hoof and hock injuries of dairy cows. J. Dairy Sci. 92: 570-576.

Piessens, V., E. Van Coillie, B. Verbist, K. Supré, G. Braem, A. Van Nuffel, L. De Vuyst, M. Heyndrickx, and S. De Vliegher. 2011. Distribution of coagulase-negative Staphylococcus species from milk and environment of dairy cows differs between herds. J. Dairy Sci. 94:2933–2944.

Pyörälä, S., and S. Taponen. 2009. Coagulase-negative staphylococci emerging mastitis pathogens. Vet. Microbiol. 134:3–8.

Rowbotham, R.F. and P. L. Ruegg. 2016. Associations of selected bedding types with incidence rates of subclinical and clinical mastitis in primiparous Holstein dairy cows. J. Dairy Sci.99:4707–4717.

Sampimon, O., H. W. Barkema, I. Berends, J. Sol, and T. Lam. 2009. Prevalence of intramammary infection in Dutch dairy herds. J. Dairy Res. 76:129–136.

Smid, A. M. C., D. M. Weary, J. H. Costa, M. A. von Keyserlingk, M.A., 2018. Dairy cow preference for different types of outdoor access. J. Dairy Sci. 101:1448-1455.

Sant'Anna, A. C., and M. J. R. Paranhos da Costa. 2011. The relationship between dairy cow hygiene and somatic cell count in milk. 94: 3835-3844.

Schreiner, D. A. and P. L. Ruegg. 2003. Relationship between udder and leg hygiene scores and subclinical mastitis. J. Dairy Sci. 86:3460–3465.

Schukken, Y. H., R. N. Gonzalez, L. L. Tikofsky, H. F. Schulte, C. G. Santisteban, F. L. Welcome, G. J. Bennett, M. J. Zurakowski, and R. N. Zadoks. 2009. CNS mastitis: Nothing to worry about? Vet. Microbiol. 134:9–14.

Sudolar, N. R., Panivivat, R., and Sopannarath, P. 2018. Effects of two neck rail positions on heifer behavior and stall cleanliness in free stall barn. Agric. Nat. Resour. Sci. 51:432-435.

Supré, K., F. Haesebrouck, R. N. Zadoks, M. Vaneechoutte, S. Piepers, and S. De Vliegher. 2011. Some coagulase-negative Staphylococcus species affect udder health more than others. J. Dairy Sci. 94:2329–2340.

Tenhagen, B.-A., G. Köster, J. Wallmann, and W. Heuwieser. 2006. Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. J. Dairy Sci. 89:2542–2551.

Thorberg, B. M., M. L. Danielsson-Tham, U. Emanuelson, and K. Persson Waller. 2009. Bovine subclinical mastitis caused by different types of coagulase-negative staphylococci. J. Dairy Sci. 92:4962–4970.

Tomazi, T., J. L. Gonçalves, J. R. Barreiro, M. a. Arcari, and M. V. dos Santos. 2015. Bovine subclinical intramammary infection caused by coagulase-negative staphylococci increases somatic cell count but has no effect on milk yield or composition. J. Dairy Sci. 98:3071–3078.

Tucker, C.B., D. M. Weary, and D. Fraser. 2004. Free-stall dimensions: Effects on preference and stall usage. J. Dairy Sci. 87:1208–1216.

Tucker, C. B., D.M. Weary, D. Fraser. 2005. Influence of neck rail placement on free stall preference, use and cleanliness. J. Dairy Sci. 88, 2730-2737.

Tucker, C. B., G. Zdanowicz, and D. M. Weary. 2006. Brisket boards reduce freestall use. J. Dairy Sci. 89:2603–2607.

von Keyserlingk, M. A. G., A. Barrientos, K. Ito, E. Galo, and D. M. Weary. 2012. Benchmarking cow comfort on North American freestall dairies: lameness, leg injuries, lying time, facility design, and management for high-producing Holstein dairy cows. J. Dairy Sci. 95:7399–7408.

Weary, D. M., and I. Taszkun. "Hock lesions and free-stall design." 2000. J. Dairy Sci. 83: 697-702.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt, and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495–501.

Yan, T., C. S. Mayne, D. C. Patterson, and R. E. Agnew. 2009. Prediction of body weight and empty body composition using body size measurements in lactating dairy cows. Livest. Sci. 124: 233-241.

Zdanowicz, M., J. A. Shelford, C. B. Tucker, D. M. Weary, and von Keyserlingk, M. A. G. 2004. Bacterial populations on teat ends of dairy cows housed in free stalls and bedded with either sand or sawdust. J. Dairy Sci. 87:1694–1701.

**Table 5. 1** Baseline scores (1 wk. prior to study start) for cows (n=120) used in trial to study effects of neck rail position on stall and cow hygiene, the risk intrammamary infection and lameness.

| Variable | Mean ± SD | Prevalence number of cows (%) out of 120 cows |
|---|---|---|
| DIM | 182.7 ± 92.8 | - |
| Parity | 2.5±1.2 | - |
| Body weight | 758.4 ± 340.1 kg | - |
| Gait score | 2.4 ± 0.6 | 45 (37.5%) were clinically lame (NRS ≥ 3) |
| Hock scores | left hind leg=1.1 ± 0.5 | 3 (2.5%) had severe left hind leg injuries (= 3) |
| | right hind leg=1.1 ± 0.6 | 0 (0%) had severe right hind leg injuries (= 3) |
| Body condition score | 2.4 ±.6 | 8 (6.7%) were under-conditioned (≤ 2.5) |

**Table 5. 2** Effects of neck rail position[1] on the percentage of dirty cows by body area[2] (udder, lower legs, upper legs and flank), clinically lame[3], and swollen hocks[4], cases of IMI[5] and stall hygiene[6] per treatment per period.

| Variable | | Treatment[1] | | | | SE[7] | Effects | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Restrictive stall space | | Adequate stall space | | | Treatment | Period | T x P |
| | | Period 1 | Period 2 | Period 1 | Period 2 | | *P* | *P* | *P* |
| Hygiene | | | | | | | | | |
| | Udder | 9.8 | 21.8 | 13.3 | 18.3 | 2.22 | 1.00 | <0.001 | 0.07 |
| | Lower legs | 95.5 | 96.6 | 95.0 | 93.9 | 1.07 | 0.14 | 0.99 | 0.66 |
| | Upper legs and flank | 8.8 | 8.3 | 8.2 | 13.6 | 1.83 | 0.20 | 0.20 | 0.37 |
| Lameness | | 44.5 | 37.5 | 40.1 | 45.0 | 1.96 | 0.43 | 0.59 | 0.48 |
| Hock injuries | | | | | | | | | |
| | Left hocks | 2.5 | 2.0 | 3.5 | 1.9 | 0.44 | 0.32 | 0.02 | 0.86 |
| | Right hocks | 0.8 | 0.4 | 0.8 | 1.5 | 0.27 | 0.49 | 0.04 | 0.42 |
| Cases of IMI | | 8 | 4 | 2 | 8 | ---- | 0.99 | 0.61 | 1.00 |
| Stall Hygiene | | 36.4 | 37.6 | 36.3 | 40.2 | 1.7 | 0.49 | 0.16 | 0.38 |

[1] Four pens, including 30 cows per pen, were exposed to 2 stall design treatments, in a crossover design, with 28-d treatment periods each (in total 2 periods). The 2 treatments, were: 1) adequate stall space (Neck rail positioned 175 cm from the vertical plane above the rear curb), and 2) restrictive stall space (neck rail positioned 20 cm closer to the rear of the stall; 155 cm).

[2] Udder, lower legs, and upper legs/flank were each scored on a 4-point hygiene scale (1 = very clean to 4 = very dirty) 2x/wk. on each of the 28-d periods. Percentage of dirty cows per body area was calculated as number of cows with ≥3 for hygiene on the body area scored/ 30 cows for each pen (n=4), per day.

[3]Gait was scored 1x/wk. (total of 4 scores/per period) on each of the 28-d periods, using a 5-point Numerical Rating System (**NRS**), where 1= sound and 5= severely lame. Percentage of clinically lame cows was calculated as number of cows with NRS≥ 3 for each pen (n=4) per wk.

[4]Each hock in the hind legs was scored 1x/wk. (total of 4 scores/per period) on each of the 28-d periods, using a 3-point scale, where 1 = healthy hock and 3 = swollen hock, open wound, or both. Percentage of cows with swollen hocks was calculated as number of cows with =3 for hock's pen (n=4) per wk. Left and right hocks were analyzed separately.

[5]Composite milk samples per cow (n=120 cows) per pen (n=4) were taken 2x/wk.  A case of intrammamary infection (IMI) by an environmental pathogen was defined as a cow milk sample SCC < 200,000 cells/ mL followed by an elevated SCC (eSCC; ≥ 200,000 cell/mL) milk sample, plus a confirmed environmental pathogen in the bacteria cultured milk sample taken on the milking after the eSCC sample. Number of IMI cases were summarized per treatment per each 28-d period.

[6]Stall hygiene was scored 2x/wk. on each of the 28-d periods. Proportion of fecal or urine contaminated squares (soiled squares; [out of 88 equally sized squares (0.15 x 15 m) in a 1.20 x 1.65-m wire grid]), averaged over a sample of 6 stalls (every 5th stall per pen out of 30 stalls) per pen (n=4), per day.

Standard error of the mean.

[7]Tx P = interaction of treatment and period.

**Table 5. 3** Pathogens isolated from dairy composite milk samples[1] that contributed to 22 IMI[2] cases

| Pathogens isolated from IMI cases | Total number of isolates[3] |
|---|---|
| Coagulase Negative Staphylococci (CNS) | 13 |
| *Staphylococcus epidermidis* | 6 |
| *Staphylococcus cromogenes* | 3 |
| *Staphylococcus xylosus* | 1 |
| *Staphylococcus haemolyticus* | 1 |
| *Staphylococcus A* | 2 |
| *Staphylococcus B* | 2 |
| Staphylococci | 11 |
| *Enterococcus* spp. | 3 |
| *Aerococcus* spp. | 4 |
| *Escherichia Coli* | 1 |
| *Lactococcus* spp. | 2 |

[1]Composite milk samples were collected aseptically, 2x per week during two 28 d periods with 1 wk. between periods.

[2]A case of intrammamary infection (IMI) by an environmental pathogen was defined as a cow milk sample SCC < 200,000 cells/ mL followed by an elevated SCC (eSCC; ≥ 200,000 cell/mL) milk sample, plus a confirmed environmental pathogen in the bacteria cultured milk sample taken on the milking after the eSCC sample.

[3] All cases had more than one pathogen isolated

**Appendix 5. 1** Milk Sample Collection Protocol

The protocol is provided to reproduce and give to researchers who will be taking milk samples. The goal of the protocol is to ensure sterile technique in milk sampling in order to limit the number of contaminated samples submitted.

**Milk Sample Collection Protocol Guide**

(Details marked in **bold** are essential for sampling for the NCDF)

Materials

1. Sterile vials, **45 ml**
2. Permanent ink marker
3. Microfiber towels
4. Teat dip
5. **Gauze swabs in 70% alcohol**
6. Gloves

Method

1. **Samples should be collected before milking.**
2. **Vials should be pre-labeled with cow numbers.**
   A paper record of cows to be sampled should be available and the individual numbers can be matched up with cow number
3. Don gloves for sampling cows
4. Clean teats with a microfiber towel to remove gross contamination if it is present.
5. **Forestrip a few streams of milk into a strip cup and observe for signs of clinical mastitis. Record mastitis clinical score.**
6. Pre-dip teats. Wait 30 seconds, then wipe dry with a microfiber towel.
7. Beginning with the teats on the farthest from you, **scrub vigorously with a cotton gauze moistened with alcohol (not saturated - squeeze out any excess)**. **Scrub until the gauze, when applied to the teat end, comes back clean**, using a new gauze (or side) each time one becomes soiled. Then clean the teats nearest to you. **Avoid ALL contact with cleaned teat ends. If the teat end comes into contact with your hand or the cow's leg, that teat end must be sanitized again.**
8. To collect a composite sample (milk from all four quarters in the same tube), begin sample collection with the nearest teats and progress to the teats on the far side of the udder. One to 2 ml of milk should be collected from each quarter of the udder. Remove the cap from the vial. **Do not touch the inner surface of the cap or set the cap down.** Hold the vial at a 45 degree angle, with its cap partially obscuring the opening if possible. This will **keep contaminants from falling from the udder and teat into the sample vial.** Squirt milk into the sterile vial sideways, just under the cap. Do **not allow the vial to touch the teat end.** Immediately replace and tightly secure the cap. **Each vial must contain at least 30 ml of milk (greater than halfway full).**
9. **Post-sampling teat dipping with the conventional on-farm product should be performed at request of the farmer if the sampled cow is not being milked straightaway.**

Storage

1. **All samples should be placed on ice or refrigerated as soon as possible after collection.**
2. **Samples should then be transferred to DHI lab as soon as possible**

# CHAPTER 6. GENERAL DISCUSSION

The overall objective of the work described in this thesis was to identify important aspects of the cow's resting area, and its management, that are associated with hygiene, cow behavior, and milk quality, with specific focus on bedding choice, stall design, hygiene, and cow health. This research provides practical information that the dairy industry, veterinary practitioners, extension agents, and particularly producers may use to improve management practices and thus provide a suitable resting environment for dairy cows.

## 6.1 IMPORTANT FINDINGS

The results of the work described in Chapter 2 demonstrate that although elevated bacterial counts are present in used bedding types, *Streptococcus* spp. counts were not different among used bedding types. Gram-negative bacterial counts in used bedding were greatest in recycled manure solids (RMS), followed by straw and sand, and least in wood. Robowtham and Ruegg (2016a) similarly reported that total Gram-negative bacteria counts were 100 to 1000 times lower in used sand bedding versus used RMS bedding. It is noteworthy that used wood-based bedding contained the least Gram-negative bacteria, similar to findings reported by Zehner et al. (1986) for sterilized unused bedding samples. Comparably, wood bedding types had the lowest *Klebsiella* spp. counts, while the other bedding types had similar consistently higher counts.  Across all used bedding samples, sand was driest, followed by straw and wood, while RMS was wettest. Further, higher % DM was associated with lower *Streptococcus* spp. count. The results for RMS % DM content are in agreement with previous research that reported this material to be quite wet (~ 40 % DM or lower), but also that RMS dries, albeit only slightly, over time once placed into the stalls (Husfeldt et al., 2012; Sorter et al., 2014; Cole and Hogan, 2016).

Lower % DM of used bedding was associated with higher bulk tank bacteria count. These results are in agreement with Godden et al. (2008), who demonstrated that bacteria growth is promoted by moisture/humidity. Bulk tank bacteria counts were highest on farms using RMS bedding, than on other farms using wood products, straw, or sand, which were all similar. There was no association between total bacterial counts in milk and bedding type. In the current study BTSCC was not associated with bedding type; whereas, Robowtham and Ruegg (2015) reported that farms using inorganic bedding had lower BTSCC.

Results reported in Chapter 2 provide evidence that bedding management may have a profound impact on milk quality, bacterial concentrations in bedding, and cow hygiene, which in turn may affect mastitis risk. *Streptococcus* spp. and all Gram-negative bacteria counts increased with increasing days since additional bedding was added. The number of days since bedding substrates are placed upon the lying surface may affect bacterial growth (Zdanowicz et al., 2004; Kristula et al., 2005; Cole and Hogan, 2016). Contrary to my original hypothesis that wider stalls would lead to increased risk of subclinical mastitis, wider stalls were associated with lower bulk tank SCC. Further, bulk tank bacterial counts were lower on those farms with both wider stalls and longer stall space (i.e. greater distance from neck rail to the back of the stall). Geometrical mean cow SCC tended to be higher on farms with narrower stalls. It is possible that farms with larger (wider and longer) stalls were more diligent in bedding management, as there was a weak positive correlation between stall width and how often stalls were scraped. However, no correlation was found between stall length and how often stalls were scraped.

Findings reported in Chapter 2 are the first empirical evidence to suggest a difference in hygiene between cows milked by AMS versus those milked in a parlor. For free-stall farms, the use of an AMS, as compared to a milking parlor, was associated with a higher proportion cows with dirty lower legs. DeVries et al. (2012) reported that nearly 100% of cows in an AMS farm had poor hygiene scores (n = 69 cows). For tie-stall farms, a higher proportion of cows with dirty lower legs were observed on farms with access to pasture. Contrary to our results, Popescu et al. (2013) reported no differences in proportion of cows with dirty lower legs, udder, upper legs and flanks between farms where cows had access to pasture, compared to those farms without pasture access.

Although cow hygiene was different among housing types, management factors were associated with poorer hygiene within each housing type (Chapter 2). For example, the proportion of cows with dirty udders and upper legs and flanks was higher in free-stall barns with less frequent alley scraper cleaning. Tie-stall farms that utilized a lime product in their bedding tended to have a higher percentage cows with dirty lower legs. It is plausible that those farmers recognized that they had a hygiene problem but implemented a perceived solution that did not work.

In agreement with previous reports (Cook, 2002; Popescu et al., 2013), the work summarized in Chapter 3 indicated that the upper legs and flanks were the dirtiest body areas for cows housed in tie-stall barns. Similarly, DeVries et al. (2012) also found a very high proportion of cows (99.7%) with poor hygiene scores of the lower legs in free-stall housing. Likewise, Watters et al. (2013) reported a mean hygiene score of 4 (dirtiest on a 4-point scale) on the lower legs of 40 focal cows from each of the 5 free-stall herds.

The proportion of dirty cows varied by body area and between the two housing types (see Chapter 3). Older cows were at greater risk of being dirtier when housed in free-stall barns, while younger cows where at greater risk of being dirty when housed in tie-stall barns. Watters et al.

(2013) demonstrated that for cows housed in a free-stall barn, older cows, and those with greater milk production, were dirtier; those authors also reported that poorer hygiene was associated with increased odds of an elevated somatic cell count (**eSCC**). Primiparous cows are more active than multiparous cows (Roelofs et al., 2005), thus, the increased activity and altered lying behavior may potentially increase their risk for dirty lower and upper legs and flank. Popescu et al. (2013) indicated that poor hygiene in tie-stall herds is inevitable given that cows are often continuously tied in their stall exposing them to dirt and manure.

Irrespective of housing type, cows in earlier stages of lactation were at increased risk of being dirty (Chapter 3). These results agree with the findings of DeVries et al. (2012), who observed that higher milk yield was associated with reduced cleanliness of the udder and lower legs. Each 20-d increment in DIM was associated with lower risk of having dirty lower legs and dirty udder for cows housed in either tie- and free-stall barns. Overall, these differences in hygiene observed among cows with different housing, parity, and DIM may be related to the behavior of cows, in terms of how they use the environment made available to them.

The objective of the work described in Chapter 4 was to determine how stall design and usage links with hygiene to affect the risk of eSCC and lameness. In a longitudinal study of 18 herds (400 cows in total) stall cleanliness was assessed using a 1-$m^2$ metal grid, containing 88 squares, centered between stall partitions of every $10^{th}$ stall in each farm, and then counting the squares containing visible urine and/or fecal matter. Overall, we observed a mean stall contamination area of 4,050 $cm^2$. DeVries et al. (2012), using a similar methodology, reported a stall contamination area of 1,125 $cm^2$. The discrepancy between the two studies may be a consequence of how the DeVries et al (2012) study only scored fecal matter whereas in the current study both fecal and visible urine matter were scored. Stall cleanliness gives an insight on the area of contamination on which cows lie down during the day (Chapter 4 and 5).

In Chapter 4, lying time was associated positively to body condition score (BCS), stall surface, and stall hygiene. These results are in agreement with other work from our laboratory (King et al. (2016) and Westin et al. (2016)) showing that under-conditioned cows spent less time lying down than over conditioned cows. Chapter 4 results provide more evidence that cows spend more time lying down in deep-bedded vs. mattress based stalls (Cook et al., 2004; Ito et al, 2009; Solano et al., 2015; Wolfe et al., 2018). Additionally, cows have improved hygiene when they lie down on deep-bedded stalls compared to mattress-based stalls (Chapter 2). Lastly, results from Chapter 4 confirm that lying time declines as stalls become more soiled. Reich et al. (2010) reported decreased lying time on very wet bedding. Although the moisture content of bedding in our study was not measured, the protocol for assessing stall hygiene was to count squares containing any visible fecal material or urine (defined as wet bedding); thus our measure of stall hygiene could serve as a proxy for stall moisture content. Chen et al. (2017) also reported a trend of reduced lying time for cows in their muddiest treatment vs. cows housed on dry soil. Collectively, there is a growing body of evidence suggesting that cows do not want to lie down in a dirty, wet area. Furthermore, cows that are housed in facilities with dirtier stalls are more likely to be lame (Chapter 4). This relationship between stall hygiene and likelihood of lameness is likely a consequence of increased standing duration on harder surfaces, which increases the /hoof health problems (Somers et al., 2003). The results from Chapter 4 highlight the importance of improved stall hygiene to reduce lameness and increase resting time.

Other factors associated with prevalence of lameness at time of observation were BCS and parity (Chapter 4). BCS may also be linked with lameness, given that under-conditioned cows were more likely to be lame compared to sound cows. Greater parity cows were more likely to be lame, a finding consistent with King et al. (2016). Contrary to what was hypothesized in Chapter 4, none of the factors evaluated as part of this study were associated with risk of new eSCC; likely explained by the low frequency of new cases of eSCC across the study period.

Chapter 5 was the first experimental study to investigate the impact of neck-rail position on risk of intrammamary infection (**IMI**). We set out to determine how neck-rail position impacts stall cleanliness, cow hygiene, and the risk for IMI and lameness. Our findings indicate that neck rail position treatment was not associated with stall cleanliness; a finding that is in contrast to previous work that reported a neck-rail positioned further from the rear curb reduces stall cleanliness (Fregonesi et al., 2009; Bernardi et al., 2009; Sudolar et al., 2018). It is plausible that the bedding type used in the current study, in addition to the very low amount of bedding used, could have contributed to the elevated mean proportion of soiled stalls and our failure to show a treatment effect on stall cleanliness, Particularly given that cows in stalls with less bedding have a higher risk of being dirty (Hauge et al., 2012).

The only factor reported in Chapter 5 to be negatively associated with stall hygiene was greater days since additional bedding was added. Zdanowicz et al. (2004) reported a similar trend, with stalls becoming increasingly contaminated with feces over the course of the week for both sand and sawdust bedding types. Thus, collectively Chapter 2 and 5 provided evidence supporting the need for frequent addition of bedding to the stall to improve stall cleanliness (Chapter 5) and reduce bacterial counts in the bedding (Chapter 2).

In Chapter 5, cow udders tended to be dirtier when housed in stalls where the neck rail was positioned 175 cm from the stall curb (permissive neck rail treatment); however, this was only found in the first treatment period. A possible explanation may be the variance in temperature/season between periods, but more work is required to disentangle these factors. Bernardi et al. (2009) also found dirtier udders in the more permissive neck rail treatment compared to a more restrictive neck rail position. Fregonesi et al. (2009) also reported that cows in the stalls with neck rail positioned further from the rear curb were more likely to have dirty udder. Both of these latter studies did note that their findings were confounded by cow size; larger cows tended to have dirtier udders when compared to small cows. Unfortunately, we did not measure cow size, however, pens were balanced by cow BW and BCS, both of which are strongly correlated to body size measurement (Yan et al., 2009). Seasonal variation in cow hygiene has been reported, with a greater proportion of clean cows observed in winter compared to the lowest in summer for cows (Sant'Anna and Paranhos da Costa, 2011). Thus, it was not surprising to have an interaction of period and treatment, with more cows having dirty udders during the second period, when more rain can fall during the second period versus the first period.

Clinical lameness prevalence reported in Chapter 5 (42%) is comparable to herd-level prevalence reported in free-stall herds in North America (30-55%; von Keyserlingk et al., 2012). Bernardi et al. (2009) reported that more clinically lame cases developed in the more restrictive neck rail treatment, while we noted that cases reduced over time in the less restrictive neck rail position but only during period 2, a warmer temperature when compared to period 1.

In Chapter 5, the second period that had significantly with warmer temperatures associated with lower prevalence of swollen/injured hocks. To our knowledge, this is the first empirical evidence for seasonal/temperature changes associated with hock lesion prevalence. Lombard et al. (2010) reported higher numbers for hock lesions on farms in the eastern United States when compared to the west, thus, part of that variance might be due to potential temperature differences between the two regions, which would, in part, explain the current study results.

Our failure to detect an effect of neck rail position on SCC (see Chapter 5) is similar to that reported by Bernardi et al. (2009). Although our study included a larger size as it was suggested by Bernardi et al. (2009) no effects of neck rail position on udder health were detected. Only 22 cases of IMI, caused by environmental pathogens, were detected during this study and did not vary across treatments. The environmental bacterial species most prevalent in the cases of IMI were coagulase negative staphylococcus (CNS). Although a minor mastitis pathogen CNS seems to have veterinarians actively searching for ways to effectively address infections caused by it, as it is a frequently isolated species (e.g. in Ruegg, 2012).

## 6.2 LIMITATIONS

Chapter 2 was a cross-sectional study, which comes with inherent limitations, as it is a snapshot in time. This type of study cannot inform us of how long the problem was present. For instance, if a cow has frequent infections but was in recovery during the “snapshot” we could not have detected it. For example, Robowtham and Ruegg (2015) conducted a longitudinal study, with farms followed over a 2-yr period. Given that SCC (in response to environmental pathogens) can be elevated for a short period of time (Harmon, 1994; De Haas et al., 2004), a longitudinal observation period would have captured more of those potential elevations; which our study design may not have detected. Cross-sectional studies can give a good picture of what is investigated at a given time. In Bradley et al. (2018), a cross-sectional study design was used to examine bedding material microbial content in farms in Uk. Finfings in both Bradley et al. (2018) study and thethe work reported in Chapter 2 have produced valuable results that allow for practical recommendations to producers regarding bedding type and maintenance.

Unfortunately we were limited, due to time constraints and manpower to only sampling 70 farms in Chapter 2, which forced grouping of similar materials for presentation of bedding bacterial analysis with respect to sample size, thus limiting our interpretations. Contrary to our results, Zdanowicz et al. (2004) reported that sawdust had higher coliform and *Klebsiella* spp. counts compared with sand; in that study, these results were positively correlated with bacteria counts on teat ends. The disagreement in results between these two studies could be due to the source of wood, and or grouping of wood types bedding (sawdust, shavings, wood chips) versus just sawdust used in the Zdanowicz et al. (2004) study. For example, in an experimental study by Zehner et al. (1986), fine hardwood chips resulted in more *Klebsiella* spp. growth than did softwood sawdust.

Currently there is no standardized method of evaluating bacterial content in bedding; thus, the choice of methodology can be controversial. In Chapter 2, we evaluated bacterial content of bedding based on volume of the sample, as opposed to the weight, which has been used by others (Bradley et al., 2018). Given the very different densities of the various bedding materials analyzed in my study, I argue that measuring samples based on volume instead of weight was a more suitable (preferred) method when comparing different bedding materials with different densities (Harrison et al., 2008; Godden et al., 2008; Husfeldt et al., 2012). Additionally, in my study we collected samples of both unused and used bedding. Thus, given that for some bedding materials like manure based ones, the % DM of unused bedding was very different from that of used bedding; another argument justifying the use of a sample based on volume rather than weight. Harrison et al(2008) stated than "the difference between bacterial concentrations made on a wet weight basis is much greater than the difference on a dry weight basis or on a volume basis". In my study, bedding culture methods were based on procedures used by the University of Minnesota Laboratory of Udder Health, with some modifications (Godden et al., 2008; Husfeldt et al., 2012). Nevertheless, using the volume vs. weight approach is still a limitation given that it makes it difficult to compare results of studies when different methodologies are used (and thus have different units of measure).

Stall cleanliness was not assessed in Chapter 2; limiting my ability to make inferences regarding the relationship between greater bacterial and stall dirtiness. However, overall the findings presented in Chapter 2 and 5 provide support for the frequent addition of bedding to the stall because it can improve stall cleanliness (Chapter 5) and reduce bacterial counts in the bedding (Chapter 2).

Chapter 3 results indicated that greater parity cows had higher risk of poor hygiene, but we could not provide any causal context for these results. Other researchers (e.g. DeVries et al., 2011; Nielsen et al., 2011) have reported that poorer hygiene scores observed in older cows might be a consequence of the high nutritional demands causing the cows to spend more time feeding, thus increasing the risk that they come into contact with manure in the alley. Unfortunately, we did not monitor feed alley cleanliness, nor bunk attendance but recommend future research to consider this factor.

In Chapter 4, I assessed lameness through locomotion scoring, but did not examine hooves for the presence of lesions, the presence of skin lesions/injuries. Hoof trimming records were collected from producers, but these were not standardized between trimmers and thus were not useable as a potential covariate. Locomotion scoring (Chapter 4 and 5) might also not fully capture the degree of lameness of a cow; as this scoring system does not discriminate pain associated with different hoof lesions (Flower and Weary, 2006; Dyer et al., 2007; Tadich et al., 2010). Despite the limitations of locomotion scoring, numerous researchers support the utility of this method to identify sole ulcers (Flower and Weary, 2006; Chapinal et al., 2009; Tadich et al., 2010), interdigital inflammation (Tadich et al., 2010), various claw disorders (Sogstad et al., 2012), and hyperalgesia (Tadich et al., 2013).

There are several identifiable limitations in Chapter 5 that could have accounted for null results with regards to neck-rail treatment effect on IMI. Although the study was more longitudinal in nature with cows monitored over two, 28 d-treatment periods, the duration of the study may have not allowed for detection of differences of IMI. Further, I was unable to collect and analyze quarter level milk samples and had to rely on composite samples. Composite samples could dilute results in terms of bacteria detected, in addition to different pathogens detected due to the nature of a composite sample. Composite samples have decreased sensitivity, which in turn would bias results towards the null (Nyman et al., 2008) Given that this study was completed on a farm where I was unable to systematically weigh the cows throughout the study, however, we did have an initial weight which was one factor used balance the two treatments. Also, washing cows prior to the study start was not feasible, thus, differences might have not been observed on cow cleanliness based on treatment, as cows had some level of dirtiness prior to the study start. However, these differences were accounted for in the analysis.

The bedding type and quantity used in Chapter 5 may have contributed to the elevated mean proportion of soiled stalls and our failure to detect any stall cleanliness differences for the neck rail placement treatments. Hauge et al. (2012) reported an increased risk of being dirty for cows that have less bedding. However, I speculate since no previous study has assessed stall cleanliness in a controlled study by bedding depth and my study was not designed to assess that hypothesis.

**6.3 FUTURE RESEARCH**

Collectively, other research and the results of this thesis support the recommendation that adequate amounts of bedding be added and replaced frequently to reduce exposure to environmental mastitis pathogens. It would be very interesting to analyze the longitudinal benefits of specific frequencies of stall cleaning (e.g. 2, 4, or 6 ×/d) or bedding addition with regards to stall cleanliness, cow cleanliness, and IMI risk. A controlled experiment, with different deep-bedding types, would be beneficial to assess the hygiene of cows in different bedding types and the impact on IMI risk. My finding that there was an association between stall dirtiness and lameness should also be followed up with work that examines the longitudinal effect of stall hygiene on lameness and likely cause (e.g. infection, injury).

Further research is needed to determine if management factors (e.g. cleaning associated with milking in a parlour) and differences between milking systems could account for the difference in poorer hygiene reported for AMS farms in Chapter 2. To our knowledge, no work has investigated the effects of walking conditions on cow hygiene, thus it may be possible for cows to get dirty while walking on pasture, especially if pasture conditions and the path to the pasture are not ideal. Further, it is possible that there is a seasonal impact (i.e. early versus end of grazing season due to weather patterns and path integrity, and potential impact on cow hygiene); thus, more research on how pasture access (and condition) affects cow hygiene is warranted.

No associations were reported between hygiene of lower legs and average temperature, or hygiene of lower legs and relative humidity of the environment (see Chapter 2). Relative humidity of the environment and % DM in used bedding were, however, negatively associated with hygiene. There is a need for further research to assess which management practices need to be improved to reduce the effect of environmental humidity.

Lastly, it would be beneficial to assess the impact of free-stall neck rail position on stall and cow hygiene, the risk of intrammamary infection and lameness with a large sample of primiparous cows that do not have a history of elevated SCC, and for a longer period of time.

## 6.4 THESIS IMPLICATIONS

This thesis provides relevant and practical information that may be used by the dairy industry, including farmers, veterinary practitioners, and extension agents. I have demonstrated that elevated bacterial counts are present in most used bedding types. Thus, the focus should be two fold: 1) type of bedding used, and 2) management of the bedding type. Bedding should be added and maintained frequently to reduce bacterial content in bedding, reduce bulk tank milk bacteria counts, and to keep stalls clean.  Further, stalls should be kept clean to maximize resting time and to minimize the risk for lameness. Stall cleanliness was not affected by neck rail treatment, therefore, the perception that a more restrictive neck rail position to maintain stall hygiene should be questioned. Also, improved milk quality may be obtained from having wider and longer stalls, with good bedding management practices. Further, cows will increase their resting duration and be cleaner when provided well-maintained deep-bedded stalls. Additionally, other barn management practices, such as recurrent use of alley scrapers, will aid to keep cow legs clean. Overall, this thesis provides evidence that bedding management can have a profound impact on cow milk quality, bacterial concentrations in the bedding substrates, and cow behavior, hygiene, and lameness risk.

## 6.5 REFERENCES


Bernardi, F., J. Fregonesi, D. M. Veira, C. Winkler, M. A. G. von Keyserlingk, and D. M. Weary. 2009. The stall design paradox: Neck rails increase lameness but improve udder and stall hygiene. J. Dairy Sci. 92:3074–3080

Bradley, A. J., K. A. Leach, M. J. Green, J. Gibbons, I. C. Ohnstad, D. H. Black, B. Payne, V. E. Prout, and J. E. Breen. 2018. The impact of dairy cows' bedding material and its microbial content on the quality and safety of milk–A cross sectional study of UK farms. Inter. J. Food. Microbiol. 269:36-45.

Chapinal, N., A. M. de Passillé, D. M. Weary, M. A. G. von Keyserlingk, and J. Rushen. 2009. Using gait score, walking speed, and lying behavior to detect hoof lesions in dairy cows. J. Dairy Sci. 92:4365–4374.

Chen, J. M., C. L. Stull, D. N. Ledgerwood, and C. B. Tucker. 2017. Muddy conditions reduce hygiene and lying time in dairy cattle and increase time spent on concrete. J. Dairy Sci.100: 2090-2103.

Cole, K. J. and Hogan, J. S. 2016. Short communication : Environmental mastitis pathogen counts in free-stalls bedded with composted and fresh recycled manure solids. J. Dairy Sci. 99:1–5.

Cook, N. 2002. The influence of barn design on dairy cow hygiene, lameness, and udder health. Page 1-19 in Proc. 35th Ann. Conv. Am. Assoc. Bov. Pract. Available at: http://www.vetmed.wisc.edu/dms/fapm/publicats/proceeds/THE_INFLUENCE_OF_BARN_DESIGN_ON_DAIRY_COW_HYGIENE.pdf.

Cook, N. B., T. B. Bennett, and K. V. Nordlund. 2004. Effect of freestall surface on daily activity patterns in dairy cows with relevance to lameness prevalence. J. Dairy Sci. 87:2912–2922.

De Haas, Y., R.F. Veerkamp, H.W. Barkema, Y.T. Gröhn, and Y.H. Schukken. 2004. Associations between pathogen-specific cases of clinical mastitis and somatic cell count patterns. J. Dairy Sci. 87:.95-105.

DeVries, T. J., M. G. Aarnoudse, H. W. Barkema, K. E. Leslie, and M. A. G. von Keyserlingk. 2012. Associations of dairy cow behavior, barn hygiene, cow hygiene, and risk of elevated somatic cell count. J. Dairy Sci. 95:5730–5739.

Dyer, R. M., N. K. Neerchal, U. Tasch, Y. Wu, P. Dyer, and P. G. Rajkondawar. 2007. Objective determination of claw pain and its relationship to limb locomotion score in dairy cattle. J. Dairy Sci. 90:4592–4602.

Flower, F. C., D. M. Weary. 2006. Effect of hoof pathologies on subjective assessments of dairy cow gait. J. Dairy Sci. 89:139–146.

Fregonesi, J. A., M. A. G. von Keyserlingk, C. B. Tucker, D. M. Veira, and D. M. Weary. 2009. Neck-rail position in the free-stall affects standing behavior and udder and stall cleanliness. J. Dairy Sci. 92:1979–1985.

Godden, S., R. Bey, K. Lorch, R. Farnsworth, and P. Rapnicki. 2008. Ability of organic and inorganic bedding materials to promote growth of environmental bacteria. J. Dairy Sci. 91:151–159.

Harrison, E., Bonhotal, J., Schwarz, M., 2008. Using Manure Solids as Bedding. Final Report. Cornell Waste Management Institute, Ithaca, NY.

Harmon, R. J. 1994. Symposium: Mastitis and genetic evaluation forsomatic cell count Physiology of mastitis and factors affecting somatic cell counts. J. Dairy Sci. 77:2103–2112

Hauge S. J, C. Kielland, G. Ringdal, E. Skjerve, O. and Nafstad. 2012. Factors associated with cattle cleanliness on Norwegian dairy farms. J. Dairy Sci. 95:2485-2496.

Husfeldt, A. W., M. I. Endres, J. A. Salfer, and K. A. Janni. 2012. Management and characteristics of recycled manure solids used for bedding in Midwest free-stall dairy herds. J. Dairy Sci. 95:2195– 2203.

Ito, K., D. M. Weary, and M. A. G. von Keyserlingk. 2009. Lying behavior: Assessing within and between-herd variation in free-stall- housed dairy cows. J. Dairy Sci. 92:4412–4420.

King, M. T. M., E. A. Pajor, S J. LeBlanc, T. J. DeVries. 2016. Associations of herd-level housing, management, and lameness prevalence with productivity and cow behavior in herds with automated milking systems. J. Dairy Sci. 99:9069-9079.

Kristula, M.A., W. Rogers, J. S. Hogan, and M. Sabo. 2005. Comparison of bacteria populations in clean and recycled sand used for bedding in dairy facilities. J. Dairy Sci. 88:4317–4325.

Lombard, J. E., C. B. Tucker, M. A. G. Von Keyserlingk, C. A. Kopral, and D. M. Weary. 2010. Associations between cow hygiene, hock injuries, and free-stall usage on US dairy farms. J. Dairy Sci. 93: 4668-4676.

Nielsen, B. H., Thomsen, P. T., and Sørensen, J. T., 2011. Identifying risk factors for poor hind limb cleanliness in Danish loose-housed dairy cows. Animal. 5:1613–1619.

Popescu S., C. Borda, E. A. Diugan, M. Spinu, I. S. Groza, D. Sandru. 2013. Dairy cows' welfare quality in tie-stall housing system with or without access to exercise. Act Vet Scand 55: 43.

Reksen, O., L. Sølverød, and O. Østerås. 2008. Relationships between milk culture results and composite milk somatic cell counts in Norwegian dairy cattle. J. Dairy Sci. 91:3102–3113.

Reich, L.J., D. M. Weary, D.M. Veira, and M. A. G Von Keyserlingk. 2010. Effects of sawdust bedding dry matter on lying behavior of dairy cows: A dose-dependent response. J. Dairy Sci. 93:1561-1565.

Roelofs, J. B., F. J. C. M. van Eerdenburg, N. M. Soede, and B. Kemp. 2005. Various behavioral signs of estrus and their relationship with time of ovulation in dairy cattle. Theriogenology 63:1366–1377.

Rowbotham, R.F. and P. L. Ruegg. 2015. Association of bedding types with management practices and indicators of milk quality on larger Wisconsin dairy farms. J. Dairy Sci, 98:7865–7885.

Ruegg, P. L. 2012. New perspectives in udder health management.Vet. Clin. North Am. Food Anim. Pract. 28:149–163.

Sant'Anna, A. C., and M. J. R. Paranhos da Costa. 2011. The relationship between dairy cow hygiene and somatic cell count in milk. 94: 3835-3844.

Sogstad, Å. M., T. Fjeldaas, and O. Østerås. 2012. Locomotion score and claw disorders in Norwegian dairy cows, assessed by claw trimmers. Livest. Sci. 144:157–162.

Somers, J. G. C. J., K. Frankena, E. N. Noordhuizen-Stassen, and J. H. M. Metz. 2003. Prevalence of claw disorders in Dutch dairy cows exposed to several floor systems. J. Dairy Sci. 86:2082–2093.

Solano, L., H. W. Barkema, E. A. Pajor, S. Mason, S. J. LeBlanc, C. G. R. Nash, D. B. Haley, D. Pellerin, J. Rushen, A. M. de Pas- sille, E. Vasseur, and K. Orsel. 2016. Associations between lying behavior and lameness in Canadian Holstein-Friesian cows housed in free-stall barns. J. Dairy Sci. 99:2086–2101.

Sorter, D.E., H. J. Kester, and J. S. Hogan. 2014. Short communication: Bacterial counts in recycled manure solids bedding replaced daily or deep packed in free-stalls. J. Dairy Sci, 97:2965–2968.

Sudolar, N. R., Panivivat, R., and Sopannarath, P. 2018. Effects of two neck rail positions on heifer behavior and stall cleanliness in free-stall barn. Agric. Nat. Resour. Sci. 51:432-435.

Tadich, N., C. Tejeda, S. Bastias, C. Rosenfeld, and L. E. Green. 2013. Nociceptive threshold, blood constituents and physiological values in 213 cows with locomotion scores ranging from normal to severely lame. Vet. J. 197:401–405.

Tadich, N., E. Flor, and L. Green. 2010. Associations between hoof lesions and locomotion score in 1098 unsound dairy cows 184:60–65.

von Keyserlingk, M. A. G., A. Barrientos, K. Ito, E. Galo, and D. M. Weary. 2012.

Benchmarking cow comfort on North American free-stall dairies: lameness, leg injuries, lying time, facility design, and management for high-producing Holstein dairy cows. J. Dairy Sci. 95:7399–7408.

Watters, M. E. A., H. W. Barkema, K. E. Leslie, M. A. G. von Keyserlingk, and T. DeVries. J. 2013. Associations of herd- and cow-level factors, cow lying behavior, and risk of elevated somatic cell count in free-stall housed lactating dairy cows. Prev. Vet. Med. 111:245-255.

Westin, R., A. Vaughan, A. M. De Passille, T. J. DeVries, E. A. Pajor, D. Pellerin, J. M. Siegford, E. Vasseur, J. Rushen. 2016. Lying times of lactating cows on dairy farms with automatic milking systems and the relation to lameness, leg lesions, and body condition score. J. Dairy Sci. 99:551-561.

Wolfe, T., E. Vasseur, T. J. DeVries, and R. Bergeron. 2018. Effects of alternative deep bedding options on dairy cow preference, lying behavior, cleanliness, and teat end contamination. J. Dairy Sci. 101:530-536.

Yan, T., C. S. Mayne, D. C. Patterson, and R. E. Agnew. 2009. Prediction of body weight and empty body composition using body size measurements in lactating dairy cows. Livest. Sci. 124: 233-241.

Zdanowicz, M., J. A. Shelford, C. B. Tucker, D. M. Weary, and M. A. G. von Keyserlingk. 2004. Bacterial populations on teat ends of dairy cows housed in free-stalls and bedded with either or sawdust. J. Dairy Sci. 87:1694–1701.

Zehner, M. M., R. J. Farnsworth, R. D. Appleman, K. Larntz, and J. A. Springer. 1986. Growth of environmental mastitis pathogens in various bedding materials. J. Dairy Sci. 69:1932–1941.