BETTER UNDERSTANDING OF DAIRY COW MASTITIS: INCIDENCE OF CLINICAL MASTITIS AND PATHOGEN-SPECIFIC MILK LOSSES, BEDDING BACTERIAL COUNTS, AND E-LEARNING TRAINING METHODS

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

Valeria Maria Alanis Gallardo

August 2021

BETTER UNDERSTANDING OF DAIRY COW MASTITIS: INCIDENCE OF CLINICAL MASTITIS AND PATHOGEN-SPECIFIC MILK LOSSES, BEDDING BACTERIAL COUNTS, AND E-LEARNING TRAINING METHODS

Valeria Maria Alanis Gallardo, D.V.M., M.V.M., Ph. D.

Cornell University 2021

Bovine clinical mastitis is a frequent, complex, and costly disease in dairy cattle, where environmental mastitis represents major a challenge through production losses, hence understanding the environment is critical to improving udder health programs. On the other hand, achieving or maintaining high standards still relies heavily on dairy employees and remains a constant challenge for many farms. The objectives herein were to 1) understand clinical mastitis incidence and the associated pathogen-specific milk losses, 2) describe bacterial counts in bedding material and their associations with milk quality, and 3) test the use of e-learning training systems as an approach for dairy farm workers on milking equipment. Clear reporting about the methods used to report mastitis incidence can improve our ability to discuss and learn about the differences and similarities across regions and worldwide. In terms of applicability, incidence risk could be the easiest to calculate method to measure the frequency at the farm level. However, for research describing the mastitis dynamics, incidence rate at quarter level may be a more complete and granular evaluation. Although farms may have similar mastitis incidence, the pathogen distribution was different. Regarding milk yield losses, the most important pathogen causing losses both in primiparous and multiparous was *E. coli*, with losses that lasted longer than any other pathogen in the analysis. Cases with a no-growth result caused fewer milk losses and more rapid recovery compared to other pathogens. No associations between bulk tank bacteria counts and bedding bacterial counts were observed. No association

between bulk tank somatic cell counts based on bedding type was observed. Despite using a standardized procedure to consistently collect samples, we still observed a large amount of variability, both within and among bedding samples. This variability may have obscured any potential association between BT milk quality and bedding type. Finally, milking equipment malfunctions, which milkers could detect, are common on dairy farms and reinforces the need for additional milker training in this area. Practical logistics of on-farm training are a limiting factor, so the use of online training is a reasonable alternative for dairy farms.

BIOGRAPHICAL SKETCH

Valeria Maria Alanis Gallardo was born in Mexico City, Mexico in 1986. She graduated as a veterinarian in 2009 from the Universidad Nacional Autónoma de México (UNAM). Thereafter she started to work as a clinical assistant for 30 private dairy farms, each having about 300 cows. That veterinary position gave her enough background to pursue a position for the Mexican Government to aid a group of 15 indigenous dairy cow farms to develop capacities, innovation, and improve their production practices. After that, she was in charge of the udder health and milking practices, rearing heifer replacements, and artificial insemination on a 500-cow dairy farm. This job proved to her that the parlor is the place where the labor of veterinarians, owners, and farmworkers are put to the test, so this challenging experience made her realize that neither her veterinary studies nor previous job positions provided her with the necessary tools required to effectively handle milk quality. For this reason, she decided to continue her studies and returned to her Alma mater to pursue a Master's in the Program for Animal Production and Health, specifically in the milk quality area. Due to her interest in milk quality, she pursued the opportunity to intern at Cornell University, Quality Milk Production Services (QMPS); and she was lucky to contact Dr. Paula Ospina and coordinated a six-month internship, and this experience was extremely valuable. Through various talks with Dr. Paula Ospina and Dr. Daryl Nydam, she became aware of the opportunity to pursue a Ph.D. in the Field of Animal Science. Valeria will defend her thesis in July 2021 and then will return to her beloved country to continue working in the public health area for UNAM.

A mis padres,
Raymundo y Ma. Lourdes

ACKNOWLEDGMENTS

I could not have completed this dissertation without the support of my family: my father Raymundo, up there in heaven, my mother Ma. Lourdes, my sister Lourdes, my nephew Arthur Jr, my niece Roma, my brother-in-law Arturo, and my cousin Daniela, and my pet family. Thank you all for your love and for being there.

I wish to express my deepest gratitude to Dr. Daryl V. Nydam, who accepted me as a graduate student, for all of the opportunities I was given for my research, and whose expertise was many times overwhelming, but also invaluable in reformulating my thinking when it was most needed. Thanks again for this once-in-a-lifetime opportunity. I would like to express my sincere gratitude to Dr. Paula Ospina, for the opportunity to collaborate with her; first as an intern and later as her graduate student. It was life-changing. For the continuous support during my time here in Ithaca, for her patience and motivation. You provided me with the tools that I needed to complete every step as a graduate student.

I would also like to thank my committee members, Dr. Sabine Mann and Dr. Julio Giordano, for their valuable knowledge and understanding throughout my studies.

I would particularly like to thank all the amazing experts in Quality Milk Productions Services: Dr. Welcome, Dr. Moroni, Dr. Virkler, Dr. Wieland, Dr. Zurakowski, Dr. Sipka, Dr. Goia, Dr. Heuwieser, and to all the amazing crew: Belinda Gross, Kerry Case, Deb Pawloski, and Will Recker. Thank you for sharing your knowledge and for teaching me new skills while working with cows. I would like to acknowledge Dr. Tiago Tomazi for his wonderful support, guidance, and insightful feedback while writing the manuscripts.

A special thanks to my colleagues and friends back in Mexico, Esmeralda Sandoval and Miguel Ortega for their daily words of encouragement in the most difficult times, and to my Cornellian friend Matilde Portnoy, who provided support as well as happy distractions to rest my mind outside of my research.

Finally, my gratitude extends to Consejo Nacional de Ciencia y Tecnología (CONACYT) for the funding opportunity to carry out my studies at the Department of Animal Science.

TABLE OF CONTENTS

BIOGRAPHICAL SKETCH v
ACKNOWLEDGMENTS vii
TABLE OF CONTENTS viii
LIST OF FIGURES xi
LIST OF TABLES xiii
LIST OF ABBREVIATIONS xiv
CHAPTER ONE 1
LITERATURE REVIEW
INTRODUCTION 2
DEFINITION OF CLINICAL MASTITIS IN LACTATING DAIRY COWS 2
Milk losses 7
Herd parameters 10
Industry 12
MASTITIS FREQUENCY MEASURES 12
DIAGNOSTIC METHODS 14
PREVENTION MANAGEMENT 17
Cow environment: bedding management and environmental mastitis 17
Managing training on the dairy 25
SUMMARY 30
REFERENCES 31
CHAPTER TWO 51
SHORT COMMUNICATION: COMPARISON AMONG THREE METHODS FOR EVALUATING CLINICAL MASTITIS FREQUENCY IN DAIRY COWS: INCIDENCE RISK AT THE COW LEVEL, INCIDENCE RATE AT THE COW LEVEL, AND INCIDENCE RATE AT THE QUARTER LEVEL
ABSTRACT 52
INTRODUCTION 53
MATERIALS AND METHODS 54
RESULTS AND DISCUSSION 59
CONCLUSIONS 63
REFERENCES 63

CHAPTER THREE 65
CLINICAL MASTITIS AND PATHOGEN SPECIFIC MILK LOSSES IN NEW YORK DAIRY HERDS
ABSTRACT 66
INTRODUCTION 67
MATERIALS AND METHODS 70
Population description 70
Clinical mastitis sample collection and processing 70
Laboratory analysis 71
Data collection 72
Selection of mammary quarter CM cases 73
Incidence of Clinical Mastitis 73
Diseases other than mastitis 74
Statistical analysis 74
RESULTS 77
Milk losses among primiparous cows by pathogen 78
Milk losses among multiparous cows by pathogen 80
DISCUSSION 100
CONCLUSIONS 106
REFERENCES 108
CHAPTER FOUR 108
DESCRIPTION OF THE CHARACTERISTICS OF 5 BEDDING MATERIALS AND ASSOCIATION WITH BULK TANK MILK QUALITY ON 5 NEW YORK DAIRY HERDS
ABSTRACT 115
INTRODUCTION 116
MATERIALS AND METHODS 119
Herd selection and sample collection 119
Herd bedding practices 120
Bedding samples 120
Bulk tank milk samples 121
Laboratory analysis and bacteria quantification 121
Bedding samples 121

BT samples 122
Plate Inoculation and Incubation Parameters (bedding and BT samples) 122
Bacteria Counts calculation 123
Moisture Content (Dry matter content) estimation 124
pH estimation 124
Somatic cell count 125
Statistical analysis 125
RESULTS 126
Study herds 126
Bacterial counts in bedding samples 127
Detection of specific bacteria in bedding 128
Dry matter content and pH 128
Bulk tank bacterial counts 129
Detection of specific bacteria in BT 129
Bulk tank Somatic Cell Linear Score 129
DISCUSSION 140
STRENGTHS AND LIMITATIONS 143
CONCLUSIONS 144
REFERENCES 145
CHAPTER FIVE 149
SHORT COMMUNICATION: DAIRY FARM WORKER MILKING EQUIPMENT TRAINING WITH AN E-LEARNING SYSTEM
ABSTRACT 150
INTRODUCTION 151
MATERIALS AND METHODS 153
RESULTS AND DISCUSSION 157
CHAPTER SIX 168
OVERALL CONCLUSIONS AND FUTURE DIRECTIONS

LIST OF FIGURES

Figure 2. 1 Workflow in SAS using Macros and SQL to create tables to evaluate clinical mastitis frequency in dairy cows 62

Figure 3. 1 Flow-chart showing the enrollment of herds and cows in a cohort study evaluating the CM incidence, pathogen distribution, and daily milk losses related to CM events. 83
Figure 3. 2 Overall pathogen-specific distribution of all clinical mastitis cases (n=7,513) occurred in dairy cows from eight commercial herds from Central New York, United States over a year. 85
Figure 3. 3 Pathogen distribution by herd of all clinical mastitis cases (n=7,513) occurred in dairy cows from eight commercial herds from Central New York, United States over a year. 86
Figure 3. 4 Average daily milk production (kg/d) of primiparous cows (parity =1), in four New York commercial dairy herds[1] followed for a year. Error bars represent SD. Each graph represents a pathogen-specific and controls (No Mastitis)[2]. Each point represents one day: 49 d before and 49 d after CM detection (Day 0).....88
Figure 3. 5 Average daily milk production prediction based on the model for primiparous cows (parity =1) infected with pathogens versus non-clinical controls [1], in 4 dairy commercial herds[2]. 90
Figure 3. 6 Average daily milk production (kg/d) of multiparous cows (parity ≥2), in four New York commercial dairy herds[1] followed for a year. Error bars represent SD. Each graph represents a pathogen-specific and controls (No Mastitis)[2]. Each point represents one day: 49 d before and 49 d after CM detection (Day 0).....93
Figure 3. 7 Average daily milk production prediction based on the mixed model for multiparous cows (parity >1) infected with pathogens versus non-clinical controls[1], in 4 commercial dairy herds[2].....95

Figure 4. 1 Average bacterial counts (log10 CFU/g) for fresh and used bedding samples collected from July 2018 to July 2019 from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens. One fresh bedding sample and 3-5 used bedding samples were collected monthly following a Standard Operating Procedure at each visit (unless there was no bedding available due to lack of supply or equipment malfunction on the follow-up visit). Error bars represent SD. The same letters are not different at P≤0.01 (P-values adjusted for multiple contrasts). SBC (Sum Bacterial Count) = Streptococcus spp, Coliforms and Non-coliforms summed.....131
Figure 4. 2 Boxplots showing 25th, 50th (median), and 75th percentiles of the distribution of bacteria counts (log10 CFU/g) for used bedding samples collected from July 2018 to July 2019 from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens. Used bedding samples (3-5) were collected monthly following a Standard Operating Procedure at each visit (unless there was no bedding available due to lack of supply or equipment malfunction on the follow-up visit). 132
Figure 4. 3 Proportion of used or fresh bedding samples with detectable organisms of mastitis importance. Fresh and used bedding samples collected from July 2018 to July 2019 from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens. One fresh bedding sample and 3-5 used bedding samples were collected monthly following a

Standard Operating Procedure at each visit (unless there was no bedding available due to lack of supply or equipment malfunction on the follow-up visit). 133
Figure 4. 4 Average Dry Matter content (% DM) and pH values (Error bars represent SD) for fresh and used bedding samples collected from July 2018 to July 2019 from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens. One fresh bedding sample and 3-5 used bedding samples were collected monthly following a Standard Operating Procedure at each visit (unless there was no bedding available due to lack of supply or equipment malfunction on the follow-up visit). 134
Figure 4. 5 Scatter plot of dry matter content (% DM) vs bacteria counts (log10 CFU/g) by bacteria group from fresh samples collected from July 2018 to July 2019 from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens. One fresh bedding sample was collected monthly following a Standard Operating Procedure at each visit (unless there was no bedding available due to lack of supply or equipment malfunction on the follow-up visit). When no bacteria were identified, a value of $\log_{10}$ + 1 CFU/g was given, assuming that at least 10 CFU were present. SBC (Sum Bacterial Count) = *Streptococcus* spp, Coliforms and Non-coliforms summed. 135
Figure 4. 6 Scatter plot of dry matter content (% DM) vs bacteria counts (log10 CFU/g) by bacteria group and bedding type in used bedding samples collected from July 2018 to July 2019 from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens. Three to five used bedding samples were collected monthly following a Standard Operating Procedure at each visit (unless there was no bedding available due to lack of supply or equipment malfunction on the follow-up visit). When no bacteria were identified, a value of $\log_{10}$ + 1 CFU/g was given, assuming that at least 10 CFU were present. SBC (Sum Bacterial Count) = *Streptococcus* spp, Coliforms and Non-coliforms summed. 136
Figure 4. 7 Average bacteria counts (log10 CFU/ml) in milk samples monthly collected (unless milk had been picked up prior to arrival for follow up visit) from the bulk tank after mechanically agitating the milk for at least 5 min until sufficient homogeneity is obtained, from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens. Error bars represent SD. TBC = Total Bacteria count. When no bacteria was identified, a value of $\log_{10}$ + 1 CFU/ml was given, assuming that at least 10 CFU were present. 137
Figure 4. 8 Displayed only the proportion from BT milk samples with detectable organisms of mastitis importance. Milk samples monthly collected (unless milk had been picked up prior to arrival for follow up visit) from the bulk tank after mechanically agitating the milk for at least 5 min until sufficient homogeneity is obtained, from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens. 138
Figure 4. 9 Boxplots showing 25th, 50th (median), and 75th percentiles of somatic cell score in milk samples monthly collected (unless milk had been picked up prior to arrival for follow up visit) from the bulk tank after mechanically agitating the milk for at least 5 min until sufficient homogeneity is obtained, from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens. Milk samples analyzed using DeLaval cell counter (DCC) to get Somatic Cell Counts and transformed into somatic cell scores (BTSLS) by applying the following equation: BTSLS = log2 (BTSCC/100) + 3. 139

LIST OF TABLES

Table 2. 1 Example arrangement of the Dairy Comp 305 data of 1 farm extracted and put into the main Excel (Microsoft Corp; Redmond, WA) file used in Clinical Mastitis (CM) incidence analysis (May-June 2016 as an example).....62
Table 2. 2 Descriptive characteristics of eight herds from Central New York, United States; evaluated for average monthly clinical mastitis (CM) incidence risk (IRiC) at cow-level, incidence rate at cow-level (IRaC), and incidence rate at quarter-level (IRaQ) from May 2016 to May 2017 (SD in parentheses) 61

Table 3. 1 Descriptive characteristics of eight herds from Central New York, United States, average monthly clinical mastitis for incidence risk (IRiC) at cow-level and incidence rate at quarter-level (IRaQ) evaluated over 1 year (SD in parentheses).....84
Table 3. 2 Effects of pathogen-specific clinical mastitis on milk yield of primiparous cows in 4 New York State dairy herds. Estimates obtained from the mixed model with autoregressive (AR1) covariance structure. Values in Kg/d, C.I= Confidence interval. Each cow was matched with a control based on herd, DIM (±7d), and parity, and those were considered as no mastitis cows and used as the reference category in each model. 89
Table 3. 3 Effects of pathogen-specific clinical mastitis on milk yield of multiparous cows in 4 New York State dairy herds. Estimates obtained from the mixed model with autoregressive (AR1) covariance structure. Values in Kg/d, C.I= Confidence interval. Each cow was matched with a control based on herd, DIM (±7d), and parity, and those were considered as no mastitis cows and used as the reference category in each model. 94

Table 4. 1 Herd characteristics from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens.....130

Table 5. 1 Priorities based on extension surveys1 used to identify mastitis risk factors in 4 main areas: milking equipment malfunction, equipment malfunctions that could be detected by milker, milking routine errors, and other2 in 15 commercial dairy farms in Northern New York State.161
Table 5. 2 Initial questions to 95 milkers and 15 herd managers in 15 commercial dairy farms in Northern New York State prior training and milking equipment.....162
Table 5. 3 Anonymous responses (no.; % in parentheses[1]) from 95 milkers in 15 commercial dairy farms in Northern New York State concerning training and milking equipment.....163
Table 5. 4 Responses (no.; % in parentheses[1]) from 57 participants who completed a survey embedded in an E-learning module on milking equipment in 15 commercial dairy farms in Northern New York State.....164

LIST OF ABBREVIATIONS

AIC	Akaike's information criterion
BT	Bulk tank
BTSCC	Bulk tank somatic cell count
BTSLS	Bulk milk somatic cell linear score
CFU	Colony-forming units
CI	Confidence interval
CM	Clinical mastitis
CMT	California Mastitis Test
DC305	DairyComp 305
DCC	DeLaval cell counter
DHIA	Dairy Herd Information Association
DIM	Days in milk
DM	Dry matter
EC	Electric conductivity
iLOOP	Internet loop
IMI	Intramammary infection
IRaC	Incidence rate at the cow level
IRaQ	Incidence rate at the quarter level
IRiC	Incidence risk at the cow level
LF	Left front
LH	Left hind
MALDI-TOF	Matrix-assisted laser desorption/ionization time-of-flight
NAS	Non-aureus *Staphylococcus*
NMC	National Masittis Council
QDAR	Quarters days at risk
QMPS	Quality Milk Production Services
RF	Right front
RH	Right hind
SCC	Somatic cell count
SCM	Subclinical mastitis
SD	Standard deviation
Se	Sensitivity
SOP	Standard Operating Procedures
Sp	Specificity
SQL	Structured Query Language
TBC	Total bacteria count
USDA	U.S. Department of Agriculture

CHAPTER ONE

LITERATURE REVIEW

INTRODUCTION

The following literature review provides a brief background on mastitis; mastitis epidemiology and epidemiology of mastitis pathogens, milk production losses, and the effect of bedding material on clinical mastitis in dairy herds along with a discussion of training opportunities on dairy farms. This provides the background and most recent knowledge as the basis for the objectives of this dissertation and the application of this information in regards to the challenge of on-going mastitis control.

DEFINITION OF CLINICAL MASTITIS IN LACTATING DAIRY COWS

Defined as the inflammatory disorder of the mammary gland often associated in lactating animals, mastitis is frequently related to an intramammary infection (IMI) by several groups of pathogenic microorganisms (Norcross and Stark, 1970; Watts, 1988). Bovine mastitis is the most common and costly disease in dairy cattle around the world. Mastitis research shows its earliest work by Bardy de Brassac (1814), and Vatel (1828) as the first to describe its pathology. In those times, it was referred to as “gelber Galt”, which was later discovered was more related to streptococcic mastitis. As noted by (Helmboldt et al., 1953), these initial works on mastitis have no bibliographic notes or references, hence are difficult to locate. One of the first mentions of mastitis research work in a scientific journal described the microscopic method to classify dairy herds based on bacteria load in milk and the public health risk associated with high counts (Breed and Brew, 1917). Ever since, mastitis has been one of the most cited and investigated diseases in dairy cattle, mainly because of the cost and profitability loss that it brings to dairy farmers, and the negative effects on the welfare of cows reflected on behavioral changes due to pain and discomfort.

Dependent on a complex web of interactions of cow and pathogen within the mammary

gland, the outcome can be variable; resulting in acute or chronic symptomatology that can range from subclinical (SCM) to clinical mastitis (CM), with different duration and magnitude of immune responses conditional in part to specific pathogen virulence factors (Aitken et al., 2011). Diagnosis of SCM is essentially based on Somatic Cell Count (SCC), where the acceptable threshold of 200 000 cells/ mL (Sensitivity 72% and Specificity 85%) has been used as a diagnostic indicator for the presence of an IMI (Dohoo and Leslie, 1991). On the other hand, CM is detected visually as abnormal consistency or appearance of milk (clotted, watery, pink, or red-tinged), swelling and pain of the affected quarters, and in some cases, various systemic signs such as fever, depression, weakness, and lack of appetite, and no reference to SCC is necessary (Smith, 1999), although milk from quarters affected with CM holds SCC higher than this cut point.

Different cow-level and environmental factors can increase the risk of mastitis. For example, cow-level factors include age, changes in recruitment of leukocytes into milk during lactation stages; being lower after parturition and in early lactation than in mid-lactation (Burton and Erskine, 2003; Burvenich et al., 2003; Wellnitz and Bruckmaier, 2012), and certain udder and teat conformational features. It has been reported that the odds of a CM event in cows with flat teat ends is 1.5 times higher and 2.8 times higher than those with low rear udder height than those with round teat ends and intermediate udder height, respectively (Miles et al., 2019). Additionally, the odds of a CM event were 1.2 higher for those teats ends with longer diameter during pre milking process (Guarín and Ruegg, 2016)

MASTITIS EPIDEMIOLOGY AND MASTITIS PATHOGENS

Considered as a multi-etiological disease, with up to 137 microbial species identified and cultured from the mammary gland (Watts, 1988), the majority of those are from the kingdom

Bacteria, being mainly grouped in species from the genus *Staphylococcus*, *Streptococcus,* and coliforms. Generally, a CM case is caused by one main pathogen, which is usually isolated from the milk samples from affected udders, although some may be the result of two different pathogens at the same time. Typically, pathogens are divided into two big categories: contagious and environmental. This classification is based on the reservoir: contagious pathogens are host-adapted and establish inside the mammary gland, and the environment in numerous surroundings in the cow's environment: bedding materials, soil, rumen, feces, vulva, lips, nares, and feed samples (Bramley, 1982; Kruze and Bramley, 1982; Petersson-Wolfe et al., 2008; Paduch et al., 2013). The transmission of contagious pathogens occurs during milking while transmission of environmental pathogens generally happens between milking.

Contagious pathogens include *Staphylococcus aureus*, *Streptococcus agalactiae,* and *Mycoplasma* spp. The most common of this category, *Staphylococcus aureus* is often related to subclinical and chronic mastitis, mostly due to its capability to avoid immune response (Contreras and Rodríguez, 2011), being responsible for between 3-21% of mastitis cases worldwide. However, in North America is been reported in 3.3% of the CM in 50 herds in Wisconsin (Oliveira et al., 2013); in 7% in herds in Minnesota, Wisconsin, and Ontario (Lago et al., 2011); and in 6.3% of the cases in a research facility in Wisconsin (Rowbotham and Ruegg, 2016a). These values are lower than the reported in other countries, where the incidence of this pathogen is higher, ranging from 9.6 to 21.7% (Olde Riekerink et al., 2008; Levison et al., 2016; Gao et al., 2017; Tomazi et al., 2018).

Although environmental pathogens include a wider diversity, there are a few which are most commonly involved in mastitis include *E. coli*, *Klebsiella* spp, environmental *Streptococcus,* as well as Non-aureus *Staphylococcus* (NAS). This type of mastitis is more

difficult to control because these pathogens are in the cow's environment and it is difficult to reduce the level of exposure at the teat level. As farms do a better job of identifying mastitis and have better pre and post milking procedures, several studies show that the percent of cows in USA herds with contagious forms of mastitis have decreased but environmental and opportunistic forms of mastitis are the predominant challenges (Makovec and Ruegg, 2003; Lago et al., 2011; Pinzón-Sánchez and Ruegg, 2011; Oliveira et al., 2013). In these U.S studies, gram-negative pathogens range from 29.6 to 35.6%, gram-positive from 27.5to 36.7%, and negative for pathogen or also called no growth from 27.3 to 42.1%. Within these results; contagious pathogens such as *Staphylococcus aureus* ranged from 0.7 to 7% and *Streptococcus agalactiae* from zero to 0.7%. On the other hand, environmental pathogens such as *E. coli* ranged from 14 to 18%, *Klebsiella* spp from 7.7 to 22.5%, and environmental streptococci from 12.8 to 24.5%.

Despite these distribution changes, according to the USDA, the reported incidence of CM increased from 13% in 1996 to 25% in 2016 (USDA, 2016). This was stated by Hogan et al., 1989, indicating that successful control of contagious pathogens (mainly characterized with low SCC) could bring higher rates of CM (Hogan et al., 1989). This was also observed in Canadian herds, where *E. coli* incidence was highest and *Staphylococcus aureus* incidence lowest in farms where bulk tank somatic cell counts were ≤250,000 cells/mL (Olde Riekerink et al., 2008).

Mycoplasma spp, a growing concern and important objective in screening programs in large dairies, is found in the respiratory and urogenital tracts of healthy animals, where precipitating factors can initiate their role as pathogens (Bushnell, 1984). Out of the 25 species, only a few are related to CM, particularly *Mycoplasma bovis*. This pathogen can be spread not only during milking but also by aerosols and by blood (Fox, 2012), with the possibility to isolate multiple species from the same herd; which can be of importance when studying the relevance in

milk production losses and management practices (Gioia et al., 2021). Animal culling remains the recommended practice to control this pathogen, resulting in significant animal replacement costs to the producer, so an early diagnosis and separation of affected cows are essential to prevent spread in the herd (Nicholas et al., 2016).

In addition to the effects that a CM event causes a cow, the fact that mastitis can be a recurrent event during the same lactation also needs to be considered. A study in 5 New York dairy herds, found that the etiology of repeated cases was the same pathogen of the first case in less than half of the CM cases, and associated with less severe milk losses observed than those caused by the first CM case (Bar et al., 2007). Another study showed that a multiparous cow with a first CM case caused by gram-negative bacteria was 2.3 times more likely to die in the same month than a cow without CM, and 2.2 times more with a second case caused by gram-negative bacteria. Likewise for primiparous cows, in which each recurrent case in the current month had a greater effect on the risk of mortality: 8.2 times in the second case and 17.1 in a third case (Hertl et al., 2011). This increased risk of mortality was also observed in another study, especially in cows with a second or third CM case caused by *Klebsiella* spp or *E. coli* (Cha et al., 2013).

However, according to a recent meta-analysis whose objective was to compute risk ratio comparing the risk of CM in cows that already had one CM event in the current lactation with risk of CM in healthy cows (i.e., no mastitis event yet), this can vary depending on the definition of recurrent case. If the recurrent case is defined within a lax period of only 24 h this risk can be 1.54 times greater, compared to a no significant change if the definition is ≥5 d. At the end of the analysis, they showed that CM risk is not increased or reduced, but maintained after a first CM event (Jamali et al., 2018). In this analysis, they also stated the most relevant factors associated

with recurrent events: parity (higher for as older cows), high milk production, pathogen involved, and bacteriological cure in the preceding case.

MASTITIS IMPACTS ON DAIRY PRODUCTION

Milk losses

One of the main costs related to CM is milk production loss. Frequently associated with acute CM, resolution depends on the health status of the cow, as well as the pathogen. The immune response in the udder is pathogen-involved dependent (Bannerman et al., 2004; Rainard and Riollet, 2006; Petzl et al., 2008), so different immunological responses result in tissue damage, which leads to a decrease in milk production during and after a CM case (Zhao and Lacasse, 2008; Sharma and Jeong, 2013). Previous studies have shown how cows failed to gain a complete recovery, showing a long loss pattern (Coulon et al., 2002; Gröhn et al., 2004; Heikkilä et al., 2018) or even a complete cessation of milk production (Blum et al., 2014).

Pathological changes in udders of slaughtered cows affected with mastitis were classified in a study into seven patterns based on the histopathological findings. The most prevalent patterns were associated with gram-positive pathogens and included, lymphoplasmacytic, and suppurative (including combinations of these two patterns). Other patterns included: pyogranulomatous, mostly caused by *S. aureus* and *Nocardia* spp, abscedative mastitis, mainly caused by *Trueperella pyogenes,* and necrosuppurative characterized by severe parenchyma necrosis and caused by *Escherichia coli* (Bianchi et al., 2019). These pathological changes showed the destruction of the secretory mammary epithelium producing a decrease in milk production.

Several research groups around the world have evaluated the effect of CM cases on milk production. These effects have been investigated in generic cases (Rajala-Schultz et al., 1999;

Bar et al., 2007; Hagnestam et al., 2007); in pathogens-specific (Coulon et al., 2002; Gröhn et al., 2004; Reksen et al., 2007; Heikkilä et al., 2018; Kayano et al., 2018), as well in repeated cases (Schukken et al., 2009; Hertl et al., 2011, 2014). The estimated effect on milk production varies across studies, mostly attributable to a different definition and inclusion criteria of CM case, production level, cattle breeds, estimation methods, controls selection, and milk production measurement methods.

In these studies, major milk losses have been observed due to *Staphylococcus aureus* and gram-negative pathogens, especially coliforms such as *E.coli* and *Klebsiella* spp, with the greatest losses immediately following the CM diagnosis, but often never recovering the initial yield, showing a continuing effect during the same lactation. Also, the magnitude of the yield losses has been observed to change according to time of lactation at clinical onset, being greater during early lactation (≤50 DIM) in both primiparous and multiparous cows (Gröhn et al., 2004; Hertl et al., 2014; Heikkilä et al., 2018).

It has been observed that cows that develop a CM case tend to be higher producers compared to the non-mastitic cows before diagnosis (Gröhn et al., 2004; Hagnestam et al., 2007; Hertl et al., 2014). Different pathogens may have longer or shorter periods of subclinical infection before the CM occurrence. For example, a cow affected with a CM caused by *Staphylococcus aureus*, *Staphylococcus* spp, *Klebsiella* spp or No pathogen isolated, their milk yield was reduced in the week before diagnosis; or even further losses, such as those produced by *Truperella pyogenes*, that was observed 4 weeks before diagnosis (Gröhn et al., 2004).

The effect of recurrent or repeated cases on milk yield has also been studied, reporting a loss in the first 2 months following a second and a third CM event of 141 kg and 119 kg, respectively (Bar et al., 2007). Cows affected with CM had milk yield losses of 93 to 193 kg for recurrent

gram-positive CM compared with 128 kg for a first case, and 276 to 295 kg for recurrent gram-negative CM compared with 304 kg following a first gram-negative CM case (Schukken et al., 2009).

E.coli causes inflammation that ranges from subacute to peracute, and necrosis of the mammary epithelium occurs during severe natural and experimental mastitis. However, in moderate cases, minimal alveolar tissue damage has been reported (Zhao and Lacasse, 2008). Changes at the histological level show hyperplasia and degeneration of epithelial cells and proliferation of fibro vascular tissue (Bianchi et al., 2019), similar to findings in an experimental intra-mammary challenge, where increased interlobular collagen-rich areas with fibrous stroma and fat were observed (Blum et al., 2020). This study also suggested that *E.coli* could have two possible phases. The first one should be acute, with rapid immune and inflammatory responses and in which the endothelial-mammary barriers are open, allowing cellular and humoral factors infiltration. The second should be a chronic phase, where tight junctions are closed and infiltration is limited to areas of deep damage in the gland, which may endure for a prolonged time, regardless of bacteria elimination. This characterization can explain milk losses and alterations in composition. *E. coli* CM cases are of economical significance, due to the important milk losses in both multiparous and primiparous cows, with a reduced milk yield of 2 kg /d even 10 weeks after CM onset (Gröhn et al., 2004) or up to 10.6% less of the 305-d milk yield (Heikkilä et al., 2018).

Compared to *E.coli* cases, cows affected by *Klebsiella* spp are more likely to have less survival on herd after a CM case and greater milk losses(Erskine et al., 2002; Gröhn et al., 2004). This pathogen is also more likely to have an increased incidence in well-managed herds that keep the SCC under <150,000 cells/mL (Barkema et al., 1998), which makes it a current

challenge in commercial dairy herds. In addition *Klebsiella* spp has a more severe innate response compared to other coliforms; with a higher concentration of TNF-α and cytokine IL-10 (Schukken et al., 2012), low spontaneous cure rate, and low rates of response to antibiotic therapy (Fuenzalida and Ruegg, 2019a; Nobrega et al., 2020).

Milk losses produced by environmental streptococcus have been established in previous studies as a general bacterial group (Coulon et al., 2002; Gröhn et al., 2004; Hertl et al., 2014). In 2018, Heikkilä et al., described that milk yield between cows without a CM and cows with *Streptococcus dysgalactiae* and *Streptococcus uberis* seems to be similar with a moderate loss of 1.2 and 1.3 kg/d, respectively (Heikkilä et al., 2018).

Estimated milk losses due to cases with no pathogen detected or no growth have been considered and presented in 1 study, showing losses similar to those caused by gram negatives pathogens, but lasting less time, being no longer different from non-mastitic cows at 43 d after CM onset (Gröhn et al., 2004).

A study in one research facility showed that based on a 305-d milk yield loss, after CM resolution cows given antibiotics along with support therapy returned to normal, whereas the cows receiving only support therapy had long-term losses (Shim et al., 2004). This post-treatment milk yield should be interpreted cautiously, considering that a recent review mentioned that in 6 control trials evaluating this outcome, only one showed a relationship between bacteriological cure among treatments and milk yield (Ruegg, 2021).

Herd parameters

According to the 2014 USDA Milk Quality, Milking Procedures, and Mastitis on U.S. Dairies Studies, producers on almost all operations (99.7%) reported having at least one case of mastitis in a year, and was detected in 24.7% of all cows at some point during 2013; with ¼ of

the cows removed or sold because of this reason (USDA APHIS | NAHMS Dairy Studies). The monetary impact on a dairy herd has been widely evaluated based on different methods and different inputs in the models, having diverse ranges of economic losses. As an example, the average cost within the first 30 days in milk (DIM) is estimated to $444, and 71% is comprised of indirect costs such as future milk production loss, premature culling, and replacement loss, and future reproductive loss, in addition to the commonly seen direct costs: reduced milk quality, additional labor, and treatment costs (Rollin et al., 2015). This was also stated in a Finnish study, where they also found a large variation in the cost of CM, depending on the lactation stage and the timing of the disposal of a cow, where elimination of primiparous cows at the end of her first lactation month caused the highest costs (Heikkilä et al., 2012).

Along with the monetary impact, mastitis also affects different parameters in the herd which include negative effects on reproductive efficiency and welfare. Mastitis has been associated to increase services per conception, affected interval from calving to the first service, and reduced pregnancy rate (Barker et al., 1998; Santos et al., 2004; Ahmadzadeh et al., 2009; Campos et al., 2020), with a possible link either with anovulation at estrus, fertilization failure or embryonic mortality. This suggests that reactions happening in a mastitis case might have an impact in other systems too, considering IMI activates inflammatory and immune responses, including an increase in cytokine synthesis from the mammary gland, lymph nodes draining the mammary and that these cytokines can have a direct inhibitory effect to oocyte and embryonic function (Hansen et al., 2004).

Meanwhile, cow's welfare is also affected; with several veterinarians and researchers declaring the need for correct use of drugs to treat pain and discomfort; for instance the use of nonsteroidal anti-inflammatory drugs (Hillerton, 1998; Leslie et al., 2010; Petersson-Wolfe et al.,

2018). Cows tend to show early signs of distress and changes in behavior days before and after a mastitis case detection; exemplified by reduced food intake (Sepúlveda-Varas et al., 2016), increase kicking at milking associated with discomfort (Medrano-Galarza et al., 2012), and a reduction in lying, drinking and rumination time compared to healthy cows (Siivonen et al., 2011; Stangaferro et al., 2016).

Industry

Although mastitis also affects the processing in the dairy value chain, these effects are more related to SCM. These are closely linked to the short shelf life of fluid milk and a reduction in the yield of manufactured milk products such as cheese (Barbano et al., 2006). The impact is also notable in the content of protein and fat. Changes in protein profile include an increase in the level of whey proteins and changes in the casein profile, with a decrease in β and α-caseins, and an increase in γ-casein (Le Maréchal et al., 2011). On the other hand, fat levels have a reduction due to a decrease in secretory capacity (Auldist and Hubble, 1998).

Cooperatives that purchase milk may offer premiums or penalties for low SCC or high SCC, respectively. Standards for bulk tank somatic cell counts (BTSCC) to be used as fluid milk for human consumption differ in different countries, ranging from a legal maximum of 750,000 cells/mL in the United States to a limit of 400,000/ml in Europe, New Zealand and Australia, and a limit of 500,000/ml in Canada. However, in 2018, the geometric BTSCC mean in the United States was 172,000 cells/mL (APHIS, USDA, 2018).

MASTITIS FREQUENCY MEASURES

CM incidence measurements are often obtained using longitudinal study designs involving repeated cow or quarter bacteriological milk culture (Barkema et al., 1998; Olde Riekerink et al., 2008). At the herd level, monitoring incidence provides a deeper understanding

of their situation compared with other farms, which may motivate to improve udder health (Santman-Berends et al., 2015). Due to the long at-risk period, research that evaluates CM must incorporate time at risk to accurately evaluate the association between CM and relevant epidemiologic outcomes (Dohoo et al., 2003).

Defined as the probability that an individual animal will contract or develop a disease in a defined period; incidence risk, also referred to as cumulative incidence, is a measure of frequency that should be restricted to closed populations; except for the open populations considered stable, where the rate of additions and withdrawals are relatively constant over time. On the other hand, incidence rate also referred to as incidence density, is the number of new cases of a disease in a population per unit of animal time at-risk during a given time period (Dohoo et al., 2003).

Incidence rate and incidence risk represent the epidemiological measures of disease frequency that can translate data into valuable clinical knowledge to monitor spreads, trends in time and enable early detection and management's adjustments on an udder health program. For this purpose, a guideline to standardize how to record and calculate the incidence of specific diseases in dairy cattle was suggested (Kelton et al., 1998). Nonetheless, CM is not uniformly registered on a routine basis on dairy herds or research papers. In addition, the new case definition also varies between studies, and that makes comparisons of incidences even more difficult, (i.e. the time interval between cases of mastitis or using only the first case). Therefore, a lot of information is lost due to the inability to compare results across different studies.

Although some research reported mastitis frequency as incidence risk, this varying between 14.1% to 31% per year (Kelton et al., 1998; Sargeant et al., 1998; Oliveira et al., 2015), the majority report incidence rates, evaluating the at-risk period at the cow-level; either using

cow-days (Barkema et al., 1998; Petrovski et al., 2009; Verbeke et al., 2014), cows-months (Gao et al., 2017; Naqvi et al., 2018), or cow-years (Olde Riekerink et al., 2008; Santman-Berends et al., 2015; Levison et al., 2016). The use of different denominators when evaluating incidence rate makes it difficult to compare magnitude across studies. Additionally, even in studies using the same denominator, results are difficult to compare due to the different multipliers selected. For example, the ones using cow-days at risk reported rates of 31.6 cases per 10,000 cow-days at risk (Verbeke et al., 2014), 0.26 cases per 365 cow-days at risk (Barkema et al., 1998), and 0.19 cases per 305 cows-days at risk (Petrovski et al., 2009).

Another and more granular approach has also been reported: use quarter-days as denominator by Rowbotham and Ruegg, 2016; and Tomazi et al., 2018, with 0.26 cases per 1,000 quarter-days at risk and 9.7 cases per 10,000 quarter-days at risk, respectively(Rowbotham and Ruegg, 2016a; Tomazi et al., 2018). Evaluation of CM at the quarter level seems a more reasonable methodology because quarters are grouped within udder (cow), thus cow level factors (e.g., lactation, DIM, teat features) can influence the risk of disease. Recent studies suggest that a single quarter with CM can affect the overall immune status of the udder (Barkema et al., 1998; Berry and Meaney, 2006; Paixão et al., 2017); so susceptibility of the quarter during each time period is at risk of becoming a new CM case might differ in these quarters compared to those in cows with no CM. Evaluation of the incidence of CM should incorporate this structure, and control for both cow level factors and time at risk at the quarter level.

DIAGNOSTIC METHODS

An essential step to control and choose better treatments for mastitis is to have early detection and accurately identify the causal pathogen. Diagnostic methods vary according to the type of mastitis: CM is usually detected based on visual examination of the milk and the udder,

while the SCM relies on indirect techniques. Among the indirect techniques, the most common at the farm level is the California Mastitis Test (CMT), developed in 1957 (Schalm and Noorlander, 1957) modified from the Whiteside test (Whiteside, 1939). This is considered a cow-side indicator test by qualitatively estimating concentrations of DNA and white blood cells. Quantitative devices are used in diagnostic laboratories, such as the Fossomatic cell counter (Miller et al., 1986), or even some commercial counters that can be used on farm level, including on-line sensors (Dalen et al., 2019), portable system (Moon et al., 2007); and automated cell counter (Sarikaya and Bruckmaier, 2006). The Sp and Se varied among these devices, depending on others factors including from when the sample was taken, either in foremilk or the total cisternal fraction, physiological fluctuations (DIM, lactation), and even milk sample temperature (Sarikaya and Bruckmaier, 2006; Kandeel et al., 2018; Nørstebø et al., 2019). Another indicator trait that has been considered for mastitis is electric conductivity (EC). The EC is a measure of the concentration of ions. In mastitic cows, the concentration of Na^+ and Cl^- ions increases, therefore a rise in the EC of the milk produced in the affected quarter (Kitchen, 1981). The average conductivity obtained for healthy animals has been reported between 9.4 and 10.9 mS/cm (Gáspárdy et al., 2012; Jensen et al., 2016; Paudyal et al., 2020), but also reported that parity and stage of lactation were associated with different levels of EC (greater in early and late lactation). Inter-quarter ratios of EC improved classification between clinical and subclinically infected cows compared to using absolute conductivity (Norberg et al., 2004). Nonetheless, this trait has shown a debatable ability to separate subclinically infected cows from healthy ones (Norberg et al., 2004; Fosgate et al., 2013). These studies suggested using the already in-line milking systems capacity for measuring EC along with data to improve herd manager decisions on these cows.

Despite the wide number of methods to detect changes in SCC, the methods to identify mastitis etiology are fewer. A true gold standard would be a test (or combination of tests) that would correctly classify both infected and noninfected quarters 100% of the time (Dohoo et al., 2011). However, in the absence of a perfect gold standard, microbiological culture (using broth culture, plate count, serial dilution, enrichment, and microbial identification) is still considered the best diagnostic available for IMI. The criteria for considering a quarter positive in microbiological culture is ≥1 CFU/10 μL for all pathogens and >2 CFU/10 μL for Non-aureus *staphylococcus* (Dohoo et al., 2011).

Bacterial growth in the laboratory depends on the choice of growth media, incubation temperature, and duration, and atmosphere, (i.e. aerobic, anaerobic, or microaerophilic). Another important aspect to consider when using microbiological culture is to have a good sample. Sample quality affects the likelihood of detecting a pathogen if present, so a proper milk sampling technique is critical, especially if we consider the potential contamination from the teat skin and the parlor environment. One of the main disadvantages of culture-based methods is that they rely on phenotypic biochemical characterization, so the time required to have results increased considerably. To minimize this time, new methods with a rapid turnaround time are necessary to have a rapid diagnosis so the farmers can make adequate treatment decisions. One example of an accurate and rapid method is Matrix-assisted laser desorption-ionization time of flight mass spectrometry (MALDI-TOF MS). MALDI-TOF MS is an analytical technique in which particles are ionized, separated according to their mass-to-charge ratio, and measured by determining the time it takes for the ions to travel to a detector at the end of a time-of-flight tube. The resulting spectrum, with mass-to-charge values along the x-axis and intensity along the y-axis, is compared to a database of spectra from known organisms to provide identifications, with

confidence to species or genus-level (Rychert, 2019). Its use in veterinary medicine has been evaluated, showing reliable results at the genus and species level that can be compared to biochemical tests and 16S rDNA sequencing (Randall et al., 2015). However, the main weakness in this method is that it relies completely on the database, so an updated database for microorganisms of interest is critical to achieving accurate results.

Another on-farm method includes On-farm culture systems. Among these, the Minnesota Easy® Culture plates (University of Minnesota Laboratory for Udder Health, St. Paul), with an overall high Sp (>80%) and intermediate or high Se depending on the pathogen category (Royster et al., 2014); and Accumast colorimetric media (FERA Animal Health, Ithaca, NY), with an overall Se and Sp of 82.3% and 89.9% (Ganda et al., 2016) are the most common ones used in the USA. These systems allow farmers to run their own bacteriological cultures on their farm, and still make more informed decisions regarding CM treatment. Nevertheless, adequate training for the personnel that will be in charge of this task is crucial to facilitate appropriate management decisions (Sipka et al., 2021).

PREVENTION MANAGEMENT

Cow environment: bedding management and environmental mastitis

Traditionally, housing options in dairy herds have consisted of conventional bedded-pack, tie stall, free stall, drylots, and compost bedded-pack barns (Bewley et al., 2017). Based on the National Animal Health Monitoring Services in 2014 in 17 major US dairy states, (USDA APHIS | NAHMS Dairy Studies) tie-stall was the primary housing type used for lactating cows on 38.9 % and 20.0 % in free stalls, and about a half (51.5%) of large operations housed lactating cows with no outside access. Several research has demonstrated that cows and heifers in the stages of pregnancy demand 12-14 hours of lying daily and that they prioritize it over other

activities (Phillips and Schofield, 1994; Jensen et al., 2005; Munksgaard et al., 2005). Considering this strong behavioral need for adequate rest, a proper stall design is necessary to provide longer resting times and to reduce the standing time on the concrete. Current studies showed that cows preferred deep bedding materials that provide a more comfortable lying surface compared with mattresses, based on the time to lie down and duration of the lying, but is also associated with cow (udders and legs) cleanliness (van Gastelen et al., 2011; Wolfe et al., 2018; Gieseke et al., 2020). In addition, cows spend more time lying down in wider stalls and with no brisket board (Tucker et al., 2003, 2006). Increased lying time can be increased with management changes, for example, if wet bedding is changed to dry one, showing a clear preference for a dry lying surface (Fregonesi et al., 2007). Therefore, cow lying behavior is considered a comfort and welfare indicator (Shepley et al., 2019; Villettaz Robichaud et al., 2019).

One of the most difficult management practices in a dairy farm is to minimize the level of exposure to mastitis pathogens at the teat level between milkings and maintain good udder hygiene. Subjective scoring has been used to evaluate udder based on a 4-point possible scale, ranging from 1) completely free of dirt to 4) completely covered, caked-on dirt (Schreiner and Ruegg, 2002). This scoring system showed that udders scoring 3 and 4 were 1.5 times more likely to have major pathogens isolated from milk samples compared with those scored 1 and 2 (Schreiner and Ruegg, 2003) and 1.5 times more likely to have a clinical mastitis event (Breen et al., 2009). As previously stated, teats may be in direct contact with bedding materials for up to 12-14 hours per day, and bedding is one of the reservoirs for environmental pathogens. The type of bedding and how that bedding is kept clean are critical issues for control in an udder health program, due to several studies show that bacteria can be transferred between the lying surface

and the teats (Zdanowicz et al., 2004; Guarín and Ruegg, 2016; Guarín et al., 2017; Wolfe et al., 2018).

Sources of bedding material: advantages and disadvantages

A good bedding material must be comfortable, provide thermal comfort, durable, and should keep udders clean while absorbing moisture. Another important aspect to consider is that the selected material should match with the housing, handling, storage, and disposal system of the farm. Bedding material itself has physical and biochemical properties that support bacterial growth along with external factors that influence it, such as temperature, humidity, management practices; that allow the greatest amounts of growth for specific bacterial groups (Godden et al., 2008). Bedding material can be classified as organic and inorganic. Organic bedding materials are typically plant byproducts and consist of straw, hay, saw dust, wood shavings, crop residues (corn stalks, cobs, etc.) shredded paper, composted manure, or dried manure solids. Within organic materials, non-manure materials are the most commonly used worldwide, either as topper of the mattresses or in deep–bedded stalls (Oliveira et al., 2019; Patel et al., 2019; Robles et al., 2019; Ferraz et al., 2020). On the other hand, inorganic materials include sand and recycled sand. Historically, some of the organic materials had been reported to have greater bacterial counts compared to inorganic materials.

Sawdust bedding showed higher coliforms counts, specifically *Klebsiella* spp (Newman and Kowalski, 1973; Hogan et al., 1989; Hogan and Smith, 1997), in addition, research reported that this type of bedding material increased *Klebsiella* spp and *E. coli* mastitis, if coliform counts exceeded 10^6 CFU/g of wet bedding (Carroll and Jasper, 1980; Bramley, 1985). Sawdust was also identified as being a reservoir of some species of NAS (Piessens et al., 2011). In another study *Klebsiella* spp counts were lowest immediately after fresh bedding was added to stalls, and

slowly increased reaching maximum levels by day two. Also in this study, cows housed on sawdust showed 2 times more coliforms and 6 times more *Klebsiella* spp counts on teat ends compared with those housed on sand (Zdanowicz et al., 2004).

Another typical organic bedding material is straw, a material that shows similar bacterial count patterns to sawdust, both being wood byproducts (Ward et al., 2002). A study showed that cows using straw stalls were dirtier than those using sand, although, in behavior, cows tended to prefer straw-bedded stalls with a higher frequency of lying periods (Norring et al., 2008), behavior that was also observed in another experimental study (Phillips and Schofield, 1994)). As for the effect on CM, Hogan et al., 1989 reported a linear relationship between total rates of CM during lactation and both gram-negative bacterial and *Klebsiella* spp counts in cows housed with straw and sawdust bedding.

As for manure materials, these have been used bedding sources since the 1970s (Keys et al., 1976), looking for an appropriate and practical use of an increased amount of manure produced by the increasing number of dairy farms in the US during that time. There are several methods to separate and use the solid fraction of manure, being freshly recycled manure solids and composting freshly recycled manure solids, the most commonly used in dairy herds in North America. However, composted forms cannot be used in the European Union and United Kingdom (The European Parliament and The Council of the European Union, 2009; APHA, 2016), due to concerns that composting has been associated with counts of spore-forming bacteria in bulk tank milk reported in some studies (Miller et al., 2015; Murphy et al., 2019). A study characterizing manure solids in 38 Midwest dairy operations from Wisconsin, Minnesota, South Dakota, and Iowa found that digested manure solids had lower total bacteria counts compared to drum-composted and separated raw manure solids, which had similar counts.

Additionally digested solids contained fewer environmental streptococci than drum-composted and separated raw solids, but coliform counts were similar for all 3 bedding sources (Husfeldt et al., 2012). Drum-composting treatments had shown an effect on reducing bacterial counts of *E. coli* and *Klebsiella* (Okamoto et al., 2018; Fournel et al., 2019a). As for separation methods, none of the physical separation techniques had demonstrated superior efficiency to reduce slurry initial levels of *E. coli* or *Klebsiella* spp (Liu et al., 2017; Fournel et al., 2019b).

In regards to management practices, daily replacement of recycled manure bedding from the back one-third of the stalls appeared to be an effective approach to reducing exposure to coliforms, specifically *Klebsiella* spp, but with no effect over streptococci-like counts (Sorter et al., 2014). Comparing manure solids with other organic materials showed that even if fresh bedding bacterial counts were higher, other materials can have similar counts once in use (Leach et al., 2015; Patel et al., 2019; Robles et al., 2019).

At teat level, streptococci-like organisms have been shown to be higher in cows housed in hallow-bedded manure solids over foam core mattresses, and that gram-negative bacteria were higher for those cows bedded with deep-bedded manure solids, showing significant correlations between bacterial counts of bedding samples and teat skin swabs were observed for several types of bacteria (Rowbotham and Ruegg, 2016b). Smith et al., 1985 found a maximal CM rate during summer and coincided with maximum exposure to coliforms in cows housed with recycled manure bedding (Smith et al., 1985). In New York farms, an increase in environmental mastitis caused by *E. coli* and *Klebsiella* spp was seen in farms using manure solids (Ostrum et al., 2008).

Following these research results, the effect of manure solids materials on CM was investigated in a cohort study in primiparous Holstein cows in a research facility in Wisconsin. The results showed greater incidence rates in quarters of cows housed with shallow-bedded

manure solids over foam-core mattresses than those housed with deep-bedded manure solids (Rowbotham and Ruegg, 2016c). However, in the same study population, bacterial counts of teat skin of cows in pens containing shallow-bedded manure solids over foam-core were lower compared to those in deep-bedded manure solids. This can indicate that risk factors other than bacterial counts of teat skin may be involved in the development of CM (Guarín et al., 2017). Buelow, and Patel et al., also reported this lack of association between bacterial counts and CM (Buelow, 2008; Patel et al., 2019). In other countries, a Dutch study showed no relationship between *Klebsiella* spp CM, even though *Klebsiella* spp counts were higher in manure solids compared to sawdust (Feiken and van Laarhoven, 2012). Therefore, no consistent evidence on the use of manure solids and their effect on CM has been observed.

Over the years, inorganic bedding, i.e., new and recycled sand has been associated with lower bacterial counts compared to organic materials (Hogan et al., 1989; Zdanowicz et al., 2004; Godden et al., 2008; Rowbotham and Ruegg, 2016b; Murphy et al., 2019; Robles et al., 2019). This might be related to the dry matter content in this type of material, with reported values ranging between 83.6–100% in different studies (Zdanowicz et al., 2004; Kristula et al., 2005; Patel et al., 2019). Higher moisture content in different bedding materials was associated with higher counts of gram-negative bacteria, coliforms, *Klebsiella* spp, and *Streptococcus* spp (Hogan et al., 1989). This characteristic of keeping a dry stall environment has been also reported resulting in higher udder cleanliness compared to other organic materials (Norring et al., 2008; van Gastelen et al., 2011; Esser et al., 2019; Patel et al., 2019). In a comparison of sand and recycled sand, both showed similar bacterial population counts during summer and winter, but *Streptococcus* spp counts were higher in sand during winter (Kristula et al., 2005). This finding agrees with Zdanowicz et al., 2004, who also reported higher counts of *Streptococcus*

spp compared to sawdust as well as 10 times higher levels of this bacterial group at teat level when cows were housed on sand. As for gram-negative bacteria counts, sand and recycled sand showed being lower when compared to organic materials (Rowbotham and Ruegg, 2016b), but at the teat skin level, bacterial counts from front teats of cows housed in recycled sand and shallow-bedded manure solids over foam mattresses were lower than those found in rear teats of cows housed with sand and deep-bedded manure solids (Guarín et al., 2017). The effect of inorganic material in CM occurrence was described in primiparous cows, showing that cows housed with sand as bedding had a greater survival time to CM occurrence than cows with recycled sand or manure solids (Rowbotham and Ruegg, 2016c). Another 3-yr period cohort study in a research facility in Wisconsin found that cows bedded with organic materials had a greater incidence of CM than those bedded with sand (Esser et al., 2019).

Bedding material and associations with milk quality

In the US, the Grade "A" Pasteurized Milk Ordinance (PMO) sets the standards established by the Food and Drug Administration (FDA) for regulating the production, processing, and packaging of grade A milk, and to have this classification farms need to have a total bacteria count (TBC) <100,000 CFU/mL and bulk tank SCC <750,000 cells/mL (Pasteurized Milk Ordinance, 2019). Generally, bulk milk quality (meaning SCC and bacterial load) is a reflection of what is happening in a herd, including animal hygiene in housing and milking parlor, and is frequently used as a tool to monitor udder health status; including the presence of contagious pathogens (Farnsworth, 1993; Jayarao and Wolfgang, 2003; Olde Riekerink et al., 2006)). Another use is for defining milk quality premiums (Jayarao and Wolfgang, 2003; Pantoja et al., 2009).

Certain management practices related to the level of hygiene of the parlor and housing has been associated with bacterial load in the bulk tank, such as clipping udder hair, udder preparation, and cleaning (manual or automated) of the bulk tank (Kelly et al., 2009; Elmoslemany et al., 2010). Associations between bedding material and milk quality have been studied with different results: some authors reporting a strong association and others that could not find any in their study populations, so findings are not consistent across literature.

One prospective study using data from BT test results from 325 dairy herds in Wisconsin using the same bedding in all pens during the 2-yr study period showed that total bacterial counts in the bulk tank were not associated with bedding type, but bulk milk somatic cell score was lower for farms using inorganic materials (Rowbotham and Ruegg, 2015). A cross-sectional study using data from 125 herds in the United Kingdom showed no significant differences between bedding material in bacterial counts in milk for any of the organisms studied, and no significant correlations between bacterial load in used bedding and milk (Bradley et al., 2018). Another cross-sectional study that was conducted on 75 dairy farms in Ontario, Canada found that bulk milk bacteria count was higher for farms using manure solids bedding, followed by wood products, straw, and sand (Robles et al., 2019).

Looking at another aspect of milk quality, research focused on food safety showed that bedding management practices (e.g., re-bedding frequency, raking frequency) were associated with mesophilic and thermophilic spore levels, and used organic bedding spore levels were positively related to those in bulk tank milk (Miller et al., 2015; Murphy et al., 2019). Another study looking for the microbiological implications on cheese quality found that bulk milk from herds with straw had higher levels of thermophilic and mesophilic spores compared to farms with manure solids as bedding material. However, milk from farms using manure solids bedding

had an increased risk of having thermoresistant streptococci and enterococci that might cause organoleptic defects during cheese maturing (Gagnon et al., 2020).

Managing training on the dairy

History, definition, and value of training in business

Training research has a long history within applied psychology, with the first training article published in the early 1900s, discussing the demand for technically trained consulting psychologists (Geissler, 1918). Since then, training research has focused on describing and evaluating training methods. These research objectives changed over the years; from describing training success in military settings in the 1940s, evaluate factors associated with training success in the 1970s, increase interest in the study of transfer to improve the application of workplace training in the 1980s and 1990s; to finally in the 2000s to start to evaluate online training and learning that happens through work experiences (Bell et al., 2017).

Fully trained and competent workers know what to do and how to do it, and have the skills and abilities to do the work. However, competent workers will often fail to perform when conflict or lack of satisfaction, motivation, and/or communication occurs, resulting in lower work performance. A high level of job satisfaction means that the employee will have positive feelings towards their work, so is directly related to the attitudes and their conditions of work (Gil-Lacruz et al., 2019). According to the "two-factor theory" by Herzberg, extrinsic factors cause job dissatisfaction whereas intrinsic factors cause job satisfaction. Extrinsic factors are related to supervisor, work conditions, salary, relationship with peers, and personal life. Intrinsic or motivating factors are growth, development, recognition, and achievement (Herzberg, 1968). To increase job satisfaction the called job enrichment helps to make jobs more motivating, including increasing skill variety and giving feedback.

There is no doubt that strategic management of human capital is a necessity in any productive organization. This management could lead to either success or failure in any business, so it is crucial to invest in ongoing training, which is considered an integral part of the employer-employee relationship (Truitt, 2011). It has also been stated that both training and education are necessary components for a successful conflict management system (Costantino and Merchant, 1995).

By definition, learning is the absorption of information aimed to increase knowledge, skills, and behaviors by employees to apply it in real-world situations. On the other hand, training is the planned intervention that facilitates learning and depends on the quality of the transfer of the desired information (Noe, 2017). To have successful training, the skills and behaviors learned have to be transferred to real-life situations and maintain over time. Relationships between training experiences and attitudes about perceived job expertise showed that employees who had updated training had the most positive attitudes toward training (Truitt, 2011). Furthermore, a key factor related to training effectiveness is a continuous learning culture, where supervisor support seems to play an important role in training motivation (Chiaburu and Tekleab, 2005). Moreover should be always supported by other interventions, such as performance expectations communicated to workers, feedback mechanisms, and employee incentives (Berge, 2008).

Importance of defined training needs

It is important to define training needs and ensure that the learning objectives align with the business strategy for appropriateness (is training the best approach for addressing the need?) and feasibility (is it likely to be successful?). There are reasons why need analysis must be done before develop training materials: to identify specific problems or gap, to determine the root

cause of the gap (work environment, motivation, incentives, or skills and knowledge), to obtain supervisor or manager support, to develop data for evaluation and finally to determine the costs and benefits of the training (Brown, 2002).

Human resources challenges in a dairy business: Demographic changes, language, recruitment, satisfaction, engagement, and retention

The dairy industry has grown worldwide and become increasingly dynamic as advances in nutrition, genetics, understanding of disease pathogenesis, and technologies associated with feeding, milking, reproduction, and labor efficiency have increased productivity. This growth is also diverse in terms of employment, and many dairy owners and managers have not had formal training in employee management (Hagevoort et al., 2013).

The Economic Research Service of USDA (Economic Research Service, 2018) has estimated that nearly half of U.S. farm employees are immigrants of Hispanic origin. With this increasing number also seen in the dairy farms often employed in milking and livestock care (Grusenmeyer and Maloney, 2004; Jenkins et al., 2009), the availability of employees is a common challenge. Another issue is the great variation in terms of the level of education, language and literacy skills, legal status, and job backgrounds. Only looking at language, the primary native language of twenty-five percent of these immigrant Latino workers is an indigenous language, and sometimes this is not in written form (Arcury et al., 2010). As for the educational level, the distribution is not homogenous, ranging from zero years of education to individuals with professional degrees (Rodriguez et al., 2018; Sischo et al., 2019; Maloney et al.).

Additionally, several sources of stress were found in immigrant dairy workers focus groups, where long work hours, sleep-disruptive shift-work, distance from family, language

barriers, and insecurity of immigration status were the most common ones (Griffin et al., 2020).

All these barriers complicate management-employee relationships and result in a rapid employee turnover (Barkema et al., 2015; Erskine et al., 2015), with rates ranging from 8 to 144% in Michigan, Pennsylvania, New York, and Connecticut farms (Durst et al., 2018). The estimated turnover cost can be as low as 150% and as much as 250% of an employee's annual wages (Billikopf and González, 2012; Center of Economic and Policy Research, 2012). To develop effectively and appropriately training materials it is essential to understand the diverse realities of the immigrant Latino/a worker, together with bilingual content, as it demonstrated that this benefits the employees (Chase et al., 2006; Raymond et al., 2006; Rovai et al., 2016).

Insufficient training of farm workers has been argued by some to be the cause of lower detection of health problems and poor animal handling, calving management, and milking technique (Gutierrez-Solano et al., 2011; Schuenemann et al., 2013). Achieving or maintaining high standards of milk quality still relies heavily on dairy employees. Hence, milkers should be well trained and conscientious about milking routines and milking equipment. Herds with frequent training of milking personnel achieve faster milking speeds and lower rates of clinical mastitis (Rodrigues et al., 2005).

Previous works with training materials

A recent study found that because farmers feel confident in their abilities, they reported low interest in seeking out information and training for themselves. Conversely, they do believe that training is critical for their employees (Wilmes and Swenson, 2019). Although most dairy producers and industry professionals would agree that both initial hire and ongoing employee training are essential to assure proper adherence to protocols (Jansen et al., 2010; Erskine et al., 2015; Belage et al., 2019), the practical logistics of on-farm training are a limiting factor. Dairy

farms choose several training methods for their employees; including traditional University extension programs or training through big pharmaceutical companies.

Some of the training methods stated in literature in dairy farms include the use of guided talks, videos visualization using mobile technology, online, hands-on or combination, and micro learning courses online (Rodrigues et al., 2005; Rodriguez et al., 2018; Winder et al., 2018; Hesse et al., 2019). For example, a study aimed to evaluate safety awareness training using mobile technology (videos displayed on tablets) in dairy farm employees. This showed that 90% of employees found the device easy to use, and that safe practices improved when comparing test scores before and after the use of these videos, with a change in behavior related to reducing safety risks in the workplace in 98% of the trainees (Rodriguez et al., 2018).

All the same, evaluation has also been performed in online approaches. An online disbud with corneal nerve block training module showed to be 75% effective when performed by veterinary students, although participants reported lower confidence and took more time to perform the technique compared to the hands-on group (Winder et al., 2017). When this same module was tested on producers it was 91% effective but again, being the self-reported confidence lower in the online group (Winder et al., 2018). This was opposite to the results shown in another study on micro learning online courses on colostrum management, which was effective at creating a feeling of confidence and accuracy in work performance (Hesse et al., 2019).

Use of e-learning as a training instruction

According to the 2018 State of the Industry report, currently, e-learning accounts for about 41% of all formal training hours in organizations, and about 60% of all e-learning is self-paced compared to instructor-led training (ASTD, 2018).

A meta-analysis was conducted from 96 studies involving 19,331 trainees comparing e-learning to classroom instruction, showing that e-learning was 6% more effective for declarative knowledge (i.e., facts and principles) when courses were longer and when had the opportunity to practice and received feedback during the training. Both instructions were equally effective for procedural knowledge (i.e., rules and procedures), however, trainees were equally satisfied with the two delivery methods (Sitzmann et al., 2006). These results confirm the need to analyze the desired learning outcomes and training conditions before designing a training program.

Managerial support has also been identified as one key factor to increase the degree to which an employee believes that using the e-learning system is beneficial to their job. In the same way, if coworkers adopted a positive attitude toward the use of the e-learning system, other employees became more engaged in the e-learning activities, which led to increased levels of job satisfaction (Cheng et al., 2012; Lin et al., 2019).

SUMMARY

Dairy farms continue experiencing economic losses through the damage associated with mastitis. Even though many improvements have been made controlling contagious pathogens, the inner complexity and associated risk factors demand more research to better understand and control this disease. Research involving multiple areas of expertise is required to identify opportunity areas, develop strategies and make recommendations to develop customized udder health plans.

In the following chapters, I present the results of my research organized on three main components: clinical mastitis incidence and associated milk losses, bacteria counts in bedding, and employee training.

The objectives of these studies are focused on improving our ability to 1) compare

clinical mastitis incidence results across other studies; 2) understand pathogen epidemiology in modern dairy farms; 3) obtain more precise pathogen-specific milk losses estimates; 4) understand the characteristics of bedding material and the associations with milk quality, and 5) to improve our ability to train people by detecting training needs.

REFERENCES

Ahmadzadeh, A., F. Frago, B. Shafii, J.C. Dalton, W.J. Price, and M.A. McGuire. 2009. Effect of clinical mastitis and other diseases on reproductive performance of Holstein cows. Anim Reprod Sci 112:273–282. doi:10.1016/j.anireprosci.2008.04.024.

Aitken, S.L., C.M. Corl, and L.M. Sordillo. 2011. Immunopathology of Mastitis: Insights into Disease Recognition and Resolution. J Mammary Gland Biol Neoplasia 16:291–304. doi:10.1007/s10911-011-9230-4.

APHA. 2016. Conditions of Use in Relation to the Use of Recycled Manure Solids as Bedding for Dairy Cattle.

APHIS, USDA. 2018. Determining U.S. Milk Quality Using Bulk-Tank Somatic Cell Counts, 2018.

Arcury, T.A., J.M. Estrada, and S.A. Quandt. 2010. Overcoming language and literacy barriers in safety and health training of agricultural workers. J Agromedicine 15:236–248. doi:10.1080/1059924X.2010.486958.

ASTD. 2018. 2018 State of the Industry. ASTD DBA Association for Talent Development (ATD).

Auldist, M.J., and I.B. Hubble. 1998. Effects of mastitis on raw milk and dairy products. Australian Journal of Dairy Technology 53:28–36.

Bannerman, D.D., M.J. Paape, J.-W. Lee, X. Zhao, J.C. Hope, and P. Rainard. 2004. Escherichia coli and Staphylococcus aureus Elicit Differential Innate Immune Responses following Intramammary Infection. Clin Diagn Lab Immunol 11:463–472. doi:10.1128/CDLI.11.3.463-472.2004.

Bar, D., Y.T. Gröhn, G. Bennett, R.N. González, J.A. Hertl, H.F. Schulte, L.W. Tauer, F.L. Welcome, and Y.H. Schukken. 2007. Effect of Repeated Episodes of Generic Clinical Mastitis on Milk Yield in Dairy Cows. Journal of Dairy Science 90:4643–4653. doi:10.3168/jds.2007-0145.

Barbano, D.M., Y. Ma, and M.V. Santos. 2006. Influence of Raw Milk Quality on Fluid Milk Shelf Life1, 2. Journal of Dairy Science 89:E15–E19. doi:10.3168/jds.S0022-

0302(06)72360-8.

Barkema, H.W., M. a. G. von Keyserlingk, J.P. Kastelic, T.J.G.M. Lam, C. Luby, J.-P. Roy, S.J. LeBlanc, G.P. Keefe, and D.F. Kelton. 2015. Invited review: Changes in the dairy industry affecting dairy cattle health and welfare. J. Dairy Sci. 98:7426–7445. doi:10.3168/jds.2015-9377.

Barkema, H.W., Y.H. Schukken, T.J. Lam, M.L. Beiboer, G. Benedictus, and A. Brand. 1999. Management practices associated with the incidence rate of clinical mastitis. J Dairy Sci 82:1643–1654. doi:10.3168/jds.S0022-0302(99)75393-2.

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. Journal of Dairy Science 81:411–419. doi:10.3168/jds.S0022-0302(98)75591-2.

Barker, A.R., F.N. Schrick, M.J. Lewis, H.H. Dowlen, and S.P. Oliver. 1998. Influence of Clinical Mastitis During Early Lactation on Reproductive Performance of Jersey Cows. Journal of Dairy Science 81:1285–1290. doi:10.3168/jds.S0022-0302(98)75690-5.

Bartlett, P.C., G.Y. Miller, S.E. Lance, and L.E. Heider. 1992. Environmental and managerial determinants of somatic cell counts and clinical mastitis incidence in Ohio dairy herds. Preventive Veterinary Medicine 14:195–207. doi:10.1016/0167-5877(92)90016-9.

Belage, E., S.L. Croyle, A. Jones-Bitton, S. Dufour, and D.F. Kelton. 2019. A qualitative study of Ontario dairy farmer attitudes and perceptions toward implementing recommended milking practices. Journal of Dairy Science 102:9548–9557. doi:10.3168/jds.2018-15677.

Bell, B. s. (1), S. i. (2) Tannenbaum, J.(3) Kevin Ford, R. a. (4) Noe, and K.(5) Kraiger. 2017. 100 years of training and development research: What we know and where we should go. Journal of Applied Psychology 102:305–323. doi:10.1037/apl0000142.

Berge, Z.L. 2008. Why it is so hard to evaluate training in the workplace. Industrial and Commercial Training 40:390–395. doi:10.1108/00197850810912270.

Berry, D.P., and W.J. Meaney. 2006. Interdependence and distribution of subclinical mastitis and intramammary infection among udder quarters in dairy cattle. Preventive Veterinary Medicine 75:81–91. doi:10.1016/j.prevetmed.2006.02.001.

Bewley, J.M., L.M. Robertson, and E.A. Eckelkamp. 2017. A 100-Year Review: Lactating dairy cattle housing management. Journal of Dairy Science 100:10418–10431. doi:10.3168/jds.2017-13251.

Bianchi, R.M., C.I. Schwertz, B.S. de Cecco, W. Panziera, C. De Lorenzo, L.C. Heck, G.G.M. Snel, B.C. Lopes, F.S. da Silva, S.P. Pavarini, and D. Driemeier. 2019. Pathological and microbiological characterization of mastitis in dairy cows. Trop Anim Health Prod 51:2057–2066. doi:10.1007/s11250-019-01907-0.

Billikopf, G., and G. González. 2012. Turnover rates are decreasing in California dairies. California Agriculture 66:153–157.

Blum, S.E., D.E. Heller, S. Jacoby, O. Krifuks, U. Merin, N. Silanikove, Y. Lavon, N. Edery, and G. Leitner. 2020. Physiological response of mammary glands to Escherichia coli infection: A conflict between glucose need for milk production and immune response. Scientific Reports 10:9602. doi:10.1038/s41598-020-66612-7.

Blum, S.E., E.D. Heller, and G. Leitner. 2014. Long term effects of Escherichia coli mastitis. The Veterinary Journal 201:72–77. doi:10.1016/j.tvjl.2014.04.008.

Bradley, A.J., K.A. Leach, J.E. Breen, L.E. Green, and M.J. Green. 2007. Survey of the incidence and aetiology of mastitis on dairy farms in England and Wales. Vet Rec 160:253–257. doi:10.1136/vr.160.8.253.

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. International Journal of Food Microbiology 269:36–45. doi:10.1016/j.ijfoodmicro.2017.12.022.

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. doi:10.1017/s0022029900022500.

Bramley, J. 1985. The control of coliform mastitis. Page in Proc. Natl. Mastitis Counc. Ann. Mtg Annual meeting-National Mastitis Council, Inc (USA), Las Vegas, NV.

Breed, R.S., and J.D. Brew. 1917. The control of public milk supplies by the use of the microscopic method. J. Dairy Sci. 1:259–271.

Breen, J.E., M.J. Green, and A.J. Bradley. 2009. Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the United Kingdom. Journal of Dairy Science 92:2551–2561. doi:10.3168/jds.2008-1369.

Brown, J. 2002. Training Needs Assessment: A Must for Developing an Effective Training Program. Public Personnel Management 31:569. doi:10.1177/009102600203100412.

Buelow, K. 2008. Holsum dairy's experience with digested, separated solids. Page in Annual meeting.

Burton, J.L., and R.J. Erskine. 2003. Immunity and mastitis Some new ideas for an old disease. Veterinary Clinics of North America: Food Animal Practice 19:1–45. doi:10.1016/S0749-0720(02)00073-7.

Burvenich, C., V. Van Merris, J. Mehrzad, A. Diez-Fraile, and L. Duchateau. 2003. Severity of E. coli mastitis is mainly determined by cow factors. Vet Res 34:521–564. doi:10.1051/vetres:2003023.

Bushnell, R.B. 1984. Mycoplasma Mastitis. Veterinary Clinics of North America: Large Animal Practice 6:301–312. doi:10.1016/S0196-9846(17)30024-1.

Campos, C.C., F.L. do Prado, J.P.J. dos Reis, L.C. Carneiro, P.R.B. Silva, G.F. de Moraes, and R.M. dos Santos. 2020. Effects of clinical mastitis and puerperal diseases on reproductive efficiency of dairy cows. Trop Anim Health Prod 52:3061–3068. doi:10.1007/s11250-020-02326-2.

Carroll, E.J., and D.E. Jasper. 1980. Coliform populations in bedding materials and coliform mastitis incidence.. Annual Meeting, National Mastitis Council, Inc. February 18-20, 1980. 129–139.

Center of Economic and Policy Research. 2012. How Much Does Employee Turnover Really Cost Your Business? Accessed June 8, 2021. https://www.cepr.net/calculators/turnover_calc.html.

Cha, E., J.A. Hertl, Y.H. Schukken, L.W. Tauer, F.L. Welcome, and Y.T. Gröhn. 2013. The effect of repeated episodes of bacteria-specific clinical mastitis on mortality and culling in Holstein dairy cows. Journal of Dairy Science 96:4993–5007. doi:10.3168/jds.2012-6232.

Chase, L.E., L.O. Ely, and M.F. Hutjens. 2006. Major Advances in Extension Education Programs in Dairy Production. Journal of Dairy Science 89:1147–1154. doi:10.3168/jds.S0022-0302(06)72183-X.

Cheng, B., M. Wang, J. Moormann, B.A. Olaniran, and N.-S. Chen. 2012. The effects of organizational learning environment factors on e-learning acceptance. Computers & Education 58:885–899. doi:10.1016/j.compedu.2011.10.014.

Chiaburu, D.S., and A.G. Tekleab. 2005. Individual and contextual influences on multiple dimensions of training effectiveness. Journal of European Industrial Training 29:604–626. doi:10.1108/03090590510627085.

Condas, L.A.Z., J. De Buck, D.B. Nobrega, D.A. Carson, S. Naushad, S. De Vliegher, R.N. Zadoks, J.R. Middleton, S. Dufour, J.P. Kastelic, and H.W. Barkema. 2017. Prevalence of non-aureus staphylococci species causing intramammary infections in Canadian dairy herds. J Dairy Sci 100:5592–5612. doi:10.3168/jds.2016-12478.

Contreras, G.A., and J.M. Rodríguez. 2011. Mastitis: comparative etiology and epidemiology. J Mammary Gland Biol Neoplasia 16:339–356. doi:10.1007/s10911-011-9234-0.

Costantino, C.A., and C.S. Merchant. 1995. Designing Conflict Management Systems: A Guide to Creating Productive and Healthy Organizations. undefined.

Coulon, J.-B., P. Gasquib, J. Barnouin, A. Ollier, and P. Pradel. 2002. Effect of mastitis and related-germ on milk yield and composition during naturally-occurring udder infections in dairy cows. Animal Research - ANIM RES 51:383–393. doi:10.1051/animres:2002031.

Coulona, J.-B., P. Gasquib, J. Barnouin, A. Ollier, P. Pradel, and D. Pomiès. 2002. Effect of mastitis and related-germ on milk yield and composition during naturally-occurring udder infections in dairy cows. Anim. Res. 51:383–393. doi:10.1051/animres:2002031.

Dalen, G., A. Rachah, H. Nørstebø, Y.H. Schukken, and O. Reksen. 2019. The detection of intramammary infections using online somatic cell counts. J Dairy Sci 102:5419–5429. doi:10.3168/jds.2018-15295.

De Buck, J., V. Ha, S. Naushad, D.B. Nobrega, C. Luby, J.R. Middleton, S. De Vliegher, and H.W. Barkema. 2021. Non-aureus Staphylococci and Bovine Udder Health: Current Understanding and Knowledge Gaps. Front. Vet. Sci. 8. doi:10.3389/fvets.2021.658031.

Dohoo, I.R., and K.E. Leslie. 1991. Evaluation of changes in somatic cell counts as indicators of new intramammary infections. Preventive Veterinary Medicine 10:225–237. doi:10.1016/0167-5877(91)90006-N.

Dohoo, I.R., W. Martin, and H.E. Stryhn. 2003. Veterinary Epidemiologic Research.

Dohoo, I.R., J. Smith, S. Andersen, D.F. Kelton, S. Godden, and Mastitis Research Workers' Conference. 2011. Diagnosing intramammary infections: evaluation of definitions based on a single milk sample. J Dairy Sci 94:250–261. doi:10.3168/jds.2010-3559.

Durst, P.T., S.J. Moore, C. Ritter, and H.W. Barkema. 2018. Evaluation by employees of employee management on large US dairy farms. J. Dairy Sci. 101:7450–7462. doi:10.3168/jds.2018-14592.

Economic Research Service. 2018. Farm Labor. Accessed June 8, 2021. https://www-ers-usda-gov.proxy.library.cornell.edu/topics/farm-economy/farm-labor/#demographic.

Elmoslemany, A.M., G.P. Keefe, I.R. Dohoo, J.J. Wichtel, H. Stryhn, and R.T. Dingwell. 2010. The association between bulk tank milk analysis for raw milk quality and on-farm management practices. Preventive Veterinary Medicine 95:32–40. doi:10.1016/j.prevetmed.2010.03.007.

Erskine, R.J., P.C. Bartlett, J.L. VanLente, and C.R. Phipps. 2002. Efficacy of systemic ceftiofur as a therapy for severe clinical mastitis in dairy cattle. Journal of Dairy Science 85:2571–2575. doi:10.3168/jds.S0022-0302(02)74340-3.

Erskine, R.J., R.O. Martinez, and G.A. Contreras. 2015. Cultural lag: A new challenge for mastitis control on dairy farms in the United States. Journal of Dairy Science 98:8240–8244. doi:10.3168/jds.2015-9386.

Esser, N.M., H. Su, W.K. Coblentz, M.S. Akins, B.A. Kieke, N.P. Martin, M.A. Borchardt, and W.E. Jokela. 2019. Efficacy of recycled sand or organic solids as bedding sources for lactating cows housed in freestalls. J Dairy Sci 102:6682–6698. doi:10.3168/jds.2018-15851.

Farnsworth, R.J. 1993. Microbiologic examination of bulk tank milk. Vet Clin North Am Food

Anim Pract 9:469–474. doi:10.1016/s0749-0720(15)30614-9.

Feiken, M., and W. van Laarhoven. 2012. Verslag van een praktijkonderzoek naar het gebruik van vaste fractie uit gescheiden mest als boxbeddingsmateriaal in ligboxen voor melkvee (Report of a Practical Study on the Use of the Solid Fraction of Separated Manure as Bedding in Cubicles for Dairy Cattle). Valacon Dairy.

Ferraz, P.F.P., G.A. e S. Ferraz, L. Leso, M. Klopčič, M. Barbari, and G. Rossi. 2020. Properties of conventional and alternative bedding materials for dairy cattle. Journal of Dairy Science 103:8661–8674. doi:10.3168/jds.2020-18318.

Fosgate, G.T., I.M. Petzer, and J. Karzis. 2013. Sensitivity and specificity of a hand-held milk electrical conductivity meter compared to the California mastitis test for mastitis in dairy cattle. The Veterinary Journal 196:98–102. doi:10.1016/j.tvjl.2012.07.026.

Fournel, S., S. Godbout, P. Ruel, A. Fortin, K. Duquette-Lozeau, V. Létourneau, M. Généreux, J. Lemieux, D. Potvin, C. Côté, C. Duchaine, and D. Pellerin. 2019a. Production of recycled manure solids for use as bedding in Canadian dairy farms: II. Composting methods. Journal of Dairy Science 102:1847–1865. doi:10.3168/jds.2018-14967.

Fournel, S., S. Godbout, P. Ruel, A. Fortin, M. Généreux, C. Côté, C. Landry, and D. Pellerin. 2019b. Production of recycled manure solids for bedding in Canadian dairy farms: I. Solid–liquid separation. Journal of Dairy Science 102:1832–1846. doi:10.3168/jds.2018-14966.

Fox, L.K. 2012. Mycoplasma Mastitis: Causes, Transmission, and Control. Veterinary Clinics of North America: Food Animal Practice 28:225–237. doi:10.1016/j.cvfa.2012.03.007.

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. Journal of Dairy Science 90:5468–5472. doi:10.3168/jds.2007-0494.

Fuenzalida, M.J., and P.L. Ruegg. 2019a. Negatively controlled, randomized clinical trial to evaluate use of intramammary ceftiofur for treatment of nonsevere culture-negative clinical mastitis. J Dairy Sci 102:3321–3338. doi:10.3168/jds.2018-15497.

Fuenzalida, M.J., and P.L. Ruegg. 2019b. Negatively controlled, randomized clinical trial to evaluate intramammary treatment of nonsevere, gram-negative clinical mastitis. Journal of Dairy Science 102:5438–5457. doi:10.3168/jds.2018-16156.

Gagnon, M., L. Hamelin, A. Fréchette, S. Dufour, and D. Roy. 2020. Effect of recycled manure solids as bedding on bulk tank milk and implications for cheese microbiological quality. Journal of Dairy Science 103:128–140. doi:10.3168/jds.2019-16812.

Ganda, E.K., R.S. Bisinotto, D.H. Decter, and R.C. Bicalho. 2016. Evaluation of an On-Farm Culture System (Accumast) for Fast Identification of Milk Pathogens Associated with Clinical Mastitis in Dairy Cows. PLOS ONE 11:e0155314. doi:10.1371/journal.pone.0155314.

Gao, J., H.W. Barkema, L. Zhang, G. Liu, Z. Deng, L. Cai, R. Shan, S. Zhang, J. Zou, J.P. Kastelic, and B. Han. 2017. Incidence of clinical mastitis and distribution of pathogens on large Chinese dairy farms. Journal of Dairy Science 100:4797–4806. doi:10.3168/jds.2016-12334.

Gáspárdy, A., G. Ismach, Á. Bajcsy, G. Veress, S. Márkus, and I. Komlósi. 2012. Evaluation of the on-line electrical conductivity of milk in mastitic dairy cows. Acta Veterinaria Hungarica 60:145–155. doi:10.1556/avet.2012.012.

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. doi:10.3168/jds.2010-4019.

Geissler, L.R. 1918. A plan for the technical training of Consulting psychologists. Journal of Applied Psychology 2:77–83.

Gieseke, D., C. Lambertz, and M. Gauly. 2020. Effects of cubicle characteristics on animal welfare indicators in dairy cattle. animal 14:1934–1942. doi:10.1017/S1751731120000609.

Gil-Lacruz, M., M.L. Gracia-Pérez, and A.I. Gil-Lacruz. 2019. Learning by Doing and Training Satisfaction: An Evaluation by Health Care Professionals. Int J Environ Res Public Health 16. doi:10.3390/ijerph16081397.

Gioia, G., M.F. Addis, C. Santisteban, B. Gross, D.V. Nydam, A.S. Sipka, P.D. Virkler, R.D. Watters, M. Wieland, M.J. Zurakowski, and P. Moroni. 2021. Mycoplasma species isolated from bovine milk collected from US dairy herds between 2016 and 2019. Journal of Dairy Science 104:4813–4821. doi:10.3168/jds.2020-19171.

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. doi:10.3168/jds.2007-0415.

Griffin, G.M., E.G. Floyd, S.S. Dali, C.M. Dunaway, S.H. Genereaux, and A.L. Olson. 2020. Assessing Mental Health Concerns of Spanish-Speaking Dairy Farm Workers. Journal of Agromedicine 25:115–121. doi:10.1080/1059924X.2019.1656130.

Gröhn, Y.T., R.N. González, D.J. Wilson, J.A. Hertl, G. Bennett, H. Schulte, and Y.H. Schukken. 2005. Effect of pathogen-specific clinical mastitis on herd life in two New York State dairy herds. Preventive Veterinary Medicine 71:105–125. doi:10.1016/j.prevetmed.2005.06.002.

Gröhn, Y.T., D.J. Wilson, R.N. González, J.A. Hertl, H. Schulte, G. Bennett, and Y.H. Schukken. 2004. Effect of Pathogen-Specific Clinical Mastitis on Milk Yield in Dairy Cows. Journal of Dairy Science 87:3358–3374. doi:10.3168/jds.S0022-0302(04)73472-4.

Grusenmeyer, D., and T. Maloney. 2004. In-depth look at Hispanics on dairies. Page Northeast DairyBusiness. Cornell PRO-DAIRY.

Guarín, J.F., C. Baumberger, and P.L. Ruegg. 2017. Anatomical characteristics of teats and premilking bacterial counts of teat skin swabs of primiparous cows exposed to different types of bedding. J. Dairy Sci. 100:1436–1444. doi:10.3168/jds.2016-11514.

Guarín, J.F., and P.L. Ruegg. 2016. Short communication: Pre- and postmilking anatomical characteristics of teats and their associations with risk of clinical mastitis in dairy cows. Journal of Dairy Science 99:8323–8329. doi:10.3168/jds.2015-10093.

Gutierrez-Solano, C., A. Ceballos-Marquez, and Y. Schukken. 2011. Bilingual trainings for milkers in New York State: A success for quality milk.

Hagevoort, G.R., D.I.D.P.M. MBA, and S.J.R.P.C.F. AIHA. 2013. A Review of Health and Safety Leadership and Managerial Practices on Modern Dairy Farms. Journal of Agromedicine 18:265–273. doi:10.1080/1059924X.2013.796905.

Hagnestam, C., U. Emanuelson, and B. Berglund. 2007. Yield losses associated with clinical mastitis occurring in different weeks of lactation. J Dairy Sci 90:2260–2270. doi:10.3168/jds.2006-583.

Hansen, P.J., P. Soto, and R.P. Natzke. 2004. Mastitis and fertility in cattle - possible involvement of inflammation or immune activation in embryonic mortality. Am J Reprod Immunol 51:294–301. doi:10.1111/j.1600-0897.2004.00160.x.

Heikkilä, A.-M., E. Liski, S. Pyörälä, and S. Taponen. 2018. Pathogen-specific production losses in bovine mastitis. Journal of Dairy Science 101:9493–9504. doi:10.3168/jds.2018-14824.

Heikkilä, A.-M., J.I. Nousiainen, and S. Pyörälä. 2012. Costs of clinical mastitis with special reference to premature culling. Journal of Dairy Science 95:139–150. doi:10.3168/jds.2011-4321.

Helmboldt, C.F., E.L. Jungherr, and W.N. Plastridge. 1953. Histopathology of Bovine Mastitis, The.

Hertl, J.A., Y.H. Schukken, D. Bar, G.J. Bennett, R.N. González, B.J. Rauch, F.L. Welcome, L.W. Tauer, and Y.T. Gröhn. 2011. The effect of recurrent episodes of clinical mastitis caused by gram-positive and gram-negative bacteria and other organisms on mortality and culling in Holstein dairy cows. Journal of Dairy Science 94:4863–4877. doi:10.3168/jds.2010-4000.

Hertl, J.A., Y.H. Schukken, F.L. Welcome, L.W. Tauer, and Y.T. Gröhn. 2014. Pathogen-specific effects on milk yield in repeated clinical mastitis episodes in Holstein dairy cows. Journal of Dairy Science 97:1465–1480. doi:10.3168/jds.2013-7266.

Herzberg, F. 1968. One More Time: How Do You Motivate Employees. Harvard Business Review Boston, MA.

Hesse, A., P. Ospina, M. Wieland, F.A.L. Yepes, B. Nguyen, and W. Heuwieser. 2019. Short

communication: Microlearning courses are effective at increasing the feelings of confidence and accuracy in the work of dairy personnel. Journal of Dairy Science 102:9505–9511. doi:10.3168/jds.2018-15927.

Hillerton, J.E. 1998. Mastitis therapy is necessary for animal welfare. Bulletin-International Dairy Federation 4–5.

Hogan, J., and K.L. Smith. 2012. Managing Environmental Mastitis. Veterinary Clinics: Food Animal Practice 28:217–224. doi:10.1016/j.cvfa.2012.03.009.

Hogan, J.S., and K.L. Smith. 1997. Bacteria counts in sawdust bedding. J Dairy Sci 80:1600–1605. doi:10.3168/jds.S0022-0302(97)76090-9.

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, and B.L. Brockett. 1989. Bacterial counts in bedding materials used on nine commercial dairies. J. Dairy Sci. 72:250–258. doi:10.3168/jds.s0022-0302(89)79103-7.

Homerosky, E.F. The Effects of Freezing on Bacterial Counts in Bovine Bedding Materials 1.

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 freestall dairy herds. Journal of Dairy Science 95:2195–2203. doi:10.3168/jds.2011-5105.

Jamali, H., H.W. Barkema, M. Jacques, E.-M. Lavallée-Bourget, F. Malouin, V. Saini, H. Stryhn, and S. Dufour. 2018. Invited review: Incidence, risk factors, and effects of clinical mastitis recurrence in dairy cows. Journal of Dairy Science 101:4729–4746. doi:10.3168/jds.2017-13730.

Jansen, J., C.D.M. Steuten, R.J. Renes, N. Aarts, and T.J.G.M. Lam. 2010. Debunking the myth of the hard-to-reach farmer: Effective communication on udder health. Journal of Dairy Science 93:1296–1306. doi:10.3168/jds.2009-2794.

Jayarao, B.M., and D.R. Wolfgang. 2003. Bulk-tank milk analysis: A useful tool for improving milk quality and herd udder health. Veterinary Clinics: Food Animal Practice 19:75–92. doi:10.1016/S0749-0720(02)00075-0.

Jenkins, P.L., S.G. Stack, J.J. May, and G. Earle-Richardson. 2009. Growth of the Spanish-speaking workforce in the Northeast dairy industry. J Agromedicine 14:58–65. doi:10.1080/10599240802623387.

Jensen, D.B., H. Hogeveen, and A. De Vries. 2016. Bayesian integration of sensor information and a multivariate dynamic linear model for prediction of dairy cow mastitis. Journal of Dairy Science 99:7344–7361. doi:10.3168/jds.2015-10060.

Jensen, M.B., L.J. Pedersen, and L. Munksgaard. 2005. The effect of reward duration on demand functions for rest in dairy heifers and lying requirements as measured by demand functions. Applied Animal Behaviour Science 90:207–217.

doi:10.1016/j.applanim.2004.08.006.

Kandeel, S.A., A.A. Megahed, F.K. Arnaout, and P.D. Constable. 2018. Evaluation and Comparison of 2 On-Farm Tests for Estimating Somatic Cell Count in Quarter Milk Samples from Lactating Dairy Cattle. J Vet Intern Med 32:506–515. doi:10.1111/jvim.14888.

Kayano, M., M. Itoh, N. Kusaba, O. Hayashiguchi, K. Kida, Y. Tanaka, K. Kawamoto, and Y.T. Gröhn. 2018. Associations of the first occurrence of pathogen-specific clinical mastitis with milk yield and milk composition in dairy cows. Journal of Dairy Research 85:309–316. doi:10.1017/S0022029918000456.

Kelly, P., K. O'Sullivan, D. Berry, S. More, W. Meaney, E. O'Callaghan, and B. O'Brien. 2009. Farm management factors associated with bulk tank total bacterial count in Irish dairy herds during 2006/07. Ir Vet J 62:36–42. doi:10.1186/2046-0481-62-1-36.

Kelton, D.F., K.D. Lissemore, and R.E. Martin. 1998. Recommendations for recording and calculating the incidence of selected clinical diseases of dairy cattle. J Dairy Sci 81:2502–2509. doi:10.3168/jds.S0022-0302(98)70142-0.

Keys, 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 Materials1. Journal of Dairy Science 59:1157–1162. doi:https://doi.org/10.3168/jds.S0022-0302(76)84337-8.

Kitchen, B.J. 1981. Review of the progress of dairy science: bovine mastitis: milk compositional changes and related diagnostic tests. J Dairy Res 48:167–188. doi:10.1017/s0022029900021580.

Koivula, M., A. Pitkälä, S. Pyörälä, and E.A. Mäntysaari. 2007. Distribution of bacteria and seasonal and regional effects in a new database for mastitis pathogens in Finland. Acta Agriculturae Scandinavica, Section A — Animal Science 57:89–96. doi:10.1080/09064700701488941.

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. doi:10.3168/jds.S0022-0302(05)73118-0.

Kruze, J., and A.J. Bramley. 1982. Sources of Streptococcus uberis in the dairy herd: II. Evidence of colonization of the bovine intestine by Str. uberis. Journal of Dairy Research 49:375–379. doi:10.1017/S0022029900022512.

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. Journal of Dairy Science 94:4441–4456. doi:10.3168/jds.2010-4046.

Lam, T.J., Y.H. Schukken, F.J. Grommers, J.A. Smit, and A. Brand. 1993. Within-herd and between-herd variation in diagnosis of clinical mastitis in cattle. J Am Vet Med Assoc

202:938–942.

Le Maréchal, C., R. Thiéry, E. Vautor, and Y. Le Loir. 2011. Mastitis impact on technological properties of milk and quality of milk products—a review. Dairy Science & Technol. 91:247–282. doi:10.1007/s13594-011-0009-6.

Leach, K.A., S.C. Archer, J.E. Breen, M.J. Green, I.C. Ohnstad, S. Tuer, and A.J. Bradley. 2015. Recycling manure as cow bedding: Potential benefits and risks for UK dairy farms. Vet. J. 206:123–130. doi:10.1016/j.tvjl.2015.08.013.

Leslie, K., C. Kielland, and S. Millman. 2010. Is mastitis painful and is therapy for pain beneficial? Page in Annual meeting.

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. Journal of Dairy Science 99:1341–1350. doi:10.3168/jds.2015-9809.

Lin, C.-Y., C.-K. Huang, and H. Zhang. 2019. Enhancing Employee Job satisfaction via E-learning: The Mediating Role of an Organizational Learning Culture. International Journal of Human–Computer Interaction 35:584–595. doi:10.1080/10447318.2018.1480694.

Liu, Z., Z.S. Carroll, S.C. Long, A. Roa-Espinosa, and T. Runge. 2017. Centrifuge separation effect on bacterial indicator reduction in dairy manure. Journal of Environmental Management 191:268–274. doi:10.1016/j.jenvman.2017.01.022.

Ma, Y., C. Ryan, D.M. Barbano, D.M. Galton, M.A. Rudan, and K.J. Boor. 2000. Effects of Somatic Cell Count on Quality and Shelf-Life of Pasteurized Fluid Milk. Journal of Dairy Science 83:264–274. doi:10.3168/jds.S0022-0302(00)74873-9.

Makovec, J.A., and P.L. Ruegg. 2003. Results of milk samples submitted for microbiological examination in Wisconsin from 1994 to 2001. J Dairy Sci 86:3466–3472. doi:10.3168/jds.S0022-0302(03)73951-4.

Maloney, T., L. Eiholzer, and B. Ryan. Survey of Hispanic Dairy Workers in New York State 2016 53.

Medrano-Galarza, C., J. Gibbons, S. Wagner, A.M. de Passillé, and J. Rushen. 2012. Behavioral changes in dairy cows with mastitis. J Dairy Sci 95:6994–7002. doi:10.3168/jds.2011-5247.

Miles, A.M., J.A.A. McArt, F.A. Leal Yepes, C.R. Stambuk, P.D. Virkler, and H.J. Huson. 2019. Udder and teat conformational risk factors for elevated somatic cell count and clinical mastitis in New York Holsteins. Preventive Veterinary Medicine 163:7–13. doi:10.1016/j.prevetmed.2018.12.010.

Miller, R.A., D.J. Kent, K.J. Boor, N.H. Martin, and M. Wiedmann. 2015. Different management

practices are associated with mesophilic and thermophilic spore levels in bulk tank raw milk. Journal of Dairy Science 98:4338–4351. doi:10.3168/jds.2015-9406.

Miller, R.H., M.J. Paape, and J.C. Acton. 1986. Comparison of milk somatic cell counts by Coulter and Fossomatic Counters. J Dairy Sci 69:1942–1946. doi:10.3168/jds.S0022-0302(86)80621-X.

Moon, J.S., H.C. Koo, Y.S. Joo, S.H. Jeon, D.S. Hur, C.I. Chung, H.S. Jo, and Y.H. Park. 2007. Application of a new portable microscopic somatic cell counter with disposable plastic chip for milk analysis. J Dairy Sci 90:2253–2259. doi:10.3168/jds.2006-622.

Munksgaard, L., M.B. Jensen, L.J. Pedersen, S.W. Hansen, and L. Matthews. 2005. Quantifying behavioural priorities—effects of time constraints on behaviour of dairy cows, Bos taurus. Applied Animal Behaviour Science 92:3–14. doi:10.1016/j.applanim.2004.11.005.

Murphy, S.I., D. Kent, N.H. Martin, R.L. Evanowski, K. Patel, S.M. Godden, and M. Wiedmann. 2019. Bedding and bedding management practices are associated with mesophilic and thermophilic spore levels in bulk tank raw milk. Journal of Dairy Science 102:6885–6900. doi:10.3168/jds.2018-16022.

Naqvi, S.A., J. De Buck, S. Dufour, and H.W. Barkema. 2018. Udder health in Canadian dairy heifers during early lactation. Journal of Dairy Science 101:3233–3247. doi:10.3168/jds.2017-13579.

Newman, L.E., and J.J. Kowalski. 1973. Fresh sawdust bedding-a possible source of Klebsiella organisms. American Journal of Veterinary Research (EUA) no. 34 p. 979-980.

Nicholas, R.A.J., L.K. Fox, and I. Lysnyansky. 2016. Mycoplasma mastitis in cattle: To cull or not to cull. Vet J 216:142–147. doi:10.1016/j.tvjl.2016.08.001.

Nobrega, D.B., S.A. Naqvi, S. Dufour, R. Deardon, J.P. Kastelic, J. De Buck, and H.W. Barkema. 2020. Critically important antimicrobials are generally not needed to treat nonsevere clinical mastitis in lactating dairy cows: Results from a network meta-analysis. Journal of Dairy Science 103:10585–10603. doi:10.3168/jds.2020-18365.

Noe, R.A. 2017. Employee Training and Development. Seventh edition. McGraw-Hill Education, New York, New York.

Norberg, E., H. Hogeveen, I.R. Korsgaard, N.C. Friggens, K.H.M.N. Sloth, and P. Løvendahl. 2004. Electrical Conductivity of Milk: Ability to Predict Mastitis Status. Journal of Dairy Science 87:1099–1107. doi:10.3168/jds.S0022-0302(04)73256-7.

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. Journal of Dairy Science 91:570–576. doi:10.3168/jds.2007-0452.

Nørstebø, H., G. Dalen, A. Rachah, B. Heringstad, A.C. Whist, A. Nødtvedt, and O. Reksen. 2019. Factors associated with milking-to-milking variability in somatic cell counts from healthy cows in an automatic milking system. Preventive Veterinary Medicine 172:104786. doi:10.1016/j.prevetmed.2019.104786.

Okamoto, E., H. Miyanishi, A. Nakamura, T. Kobayashi, N. Kobayashi, Y. Terawaki, and H. Nagahata. 2018. Bacteriological evaluation of composted manure solids prepared from anaerobic digested slurry for hygienic recycled bedding materials for dairy cows. Animal Science Journal 89:727–732. doi:10.1111/asj.12962.

Olde Riekerink, R.G.M., H.W. Barkema, D.F. Kelton, and D.T. Scholl. 2008. Incidence Rate of Clinical Mastitis on Canadian Dairy Farms. Journal of Dairy Science 91:1366–1377. doi:10.3168/jds.2007-0757.

Olde Riekerink, R.G.M., H.W. Barkema, S. Veenstra, D.E. Poole, R.T. Dingwell, and G.P. Keefe. 2006. Prevalence of contagious mastitis pathogens in bulk tank milk in Prince Edward Island. Can Vet J 47:567–572.

Oliveira, C.S.F., H. Hogeveen, A.M. Botelho, P.V. Maia, S.G. Coelho, and J.P.A. Haddad. 2015. Cow-specific risk factors for clinical mastitis in Brazilian dairy cattle. Preventive Veterinary Medicine 121:297–305. doi:10.1016/j.prevetmed.2015.08.001.

Oliveira, L., C. Hulland, and P.L. Ruegg. 2013. Characterization of clinical mastitis occurring in cows on 50 large dairy herds in Wisconsin. Journal of Dairy Science 96:7538–7549. doi:10.3168/jds.2012-6078.

Oliveira, V.C., F.A. Damasceno, C.E.A. Oliveira, P.F.P. Ferraz, G. a. S. Ferraz, and J. a. O. Saraz. 2019. Compost-bedded pack barns in the state of Minas Gerais: architectural and technological characterization. doi:10.15159/ar.19.179.

Ospina, P., V. Alanis, A. Vasquez, F. Welcome, T. Tomazi, R. Watters, K. Marely, and D. Nydam. 2019. Heifer Mastitis - What About it? Page.

Ostrum, P.G., M.J. Thomas, and R.N. Zadoks,. 2008. Dried manure solids for freestall bedding: Experiences from a Northeast dairy. Pages 149–156 in Proceedings of the NMC annual meeting National Mastitis Council, National Mastitis Counci.

Paduch, J.-H., E. Mohr, and V. Krömker. 2013. The association between bedding material and the bacterial counts of Staphylococcus aureus, Streptococcus uberis and coliform bacteria on teat skin and in teat canals in lactating dairy cattle. J Dairy Res 80:159–164. doi:10.1017/S0022029913000046.

Paixão, M.G., L.R. Abreu, R. Richert, and P.L. Ruegg. 2017. Milk composition and health status from mammary gland quarters adjacent to glands affected with naturally occurring clinical mastitis. Journal of Dairy Science 100:7522–7533. doi:10.3168/jds.2017-12547.

Pantoja, J.C.F., D.J. Reinemann, and P.L. Ruegg. 2009. Associations among milk quality indicators in raw bulk milk. J Dairy Sci 92:4978–4987. doi:10.3168/jds.2009-2329.

Pasteurized Milk Ordinance. 2019. Grade “A” Pasteurized Milk Ordinance.

Patel, K., S.M. Godden, E. Royster, B.A. Crooker, J. Timmerman, and L. Fox. 2019. Relationships among bedding materials, bedding bacteria counts, udder hygiene, milk quality, and udder health in US dairy herds. Journal of Dairy Science. doi:10.3168/jds.2019-16692.

Paudyal, S., P. Melendez, D. Manriquez, A. Velasquez-Munoz, G. Pena, I.N. Roman-Muniz, and P.J. Pinedo. 2020. Use of milk electrical conductivity for the differentiation of mastitis causing pathogens in Holstein cows. animal 14:588–596. doi:10.1017/S1751731119002210.

Petersson-Wolfe, C.S., S. Adams, S.L. Wolf, and J.S. Hogan. 2008. Genomic Typing of Enterococci Isolated from Bovine Mammary Glands and Environmental Sources1. Journal of Dairy Science 91:615–619. doi:10.3168/jds.2007-0253.

Petersson-Wolfe, C.S., K.E. Leslie, and T.H. Swartz. 2018. An Update on the Effect of Clinical Mastitis on the Welfare of Dairy Cows and Potential Therapies. Veterinary Clinics of North America: Food Animal Practice 34:525–535. doi:10.1016/j.cvfa.2018.07.006.

Petrovski, K.R., C. Heuer, T.J. Parkinson, and N.B. Williamson. 2009. The incidence and aetiology of clinical bovine mastitis on 14 farms in Northland, New Zealand. New Zealand Veterinary Journal 57:109–115. doi:10.1080/00480169.2009.36887.

Petzl, W., H. Zerbe, J. Günther, W. Yang, H.-M. Seyfert, G. Nürnberg, and H.-J. Schuberth. 2008. Escherichia coli, but not Staphylococcus aureus triggers an early increased expression of factors contributing to the innate immune defense in the udder of the cow. Vet. Res. 39:1–23. doi:10.1051/vetres:2007057.

Phillips, C.J.C., and S.A. Schofield. 1994. The Effect of Cubicle and Straw Yard Housing on the Behaviour, Production and Hoof Health of Dairy Cows. Animal Welfare 3:37–44.

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. Journal of Dairy Science 94:2933–2944. doi:10.3168/jds.2010-3956.

Pinzón-Sánchez, C., and P.L. Ruegg. 2011. Risk factors associated with short-term post-treatment outcomes of clinical mastitis. Journal of Dairy Science 94:3397–3410. doi:10.3168/jds.2010-3925.

Rainard, P., and C. Riollet. 2006. Innate immunity of the bovine mammary gland. Vet. Res. 37:369–400. doi:10.1051/vetres:2006007.

Rajala-Schultz, P.J., Y.T. Gröhn, C.E. McCulloch, and C.L. Guard. 1999. Effects of Clinical Mastitis on Milk Yield in Dairy Cows. Journal of Dairy Science 82:1213–1220. doi:10.3168/jds.S0022-0302(99)75344-0.

Randall, L.P., F. Lemma, M. Koylass, J. Rogers, R.D. Ayling, D. Worth, M. Klita, A. Steventon, K. Line, P. Wragg, J. Muchowski, M. Kostrzewa, and A.M. Whatmore. 2015. Evaluation of MALDI-ToF as a method for the identification of bacteria in the veterinary diagnostic laboratory. Res. Vet. Sci. 101:42–49. doi:10.1016/j.rvsc.2015.05.018.

Raymond, M.J., R.D. Wohrle, and D.R. Call. 2006. Assessment and Promotion of Judicious Antibiotic Use on Dairy Farms in Washington State. Journal of Dairy Science 89:3228–3240. doi:10.3168/jds.S0022-0302(06)72598-X.

Reksen, O., L. Sølverød, and O. Østerås. 2007. Relationships Between Milk Culture Results and Milk Yield in Norwegian Dairy Cattle. Journal of Dairy Science 90:4670–4678. doi:10.3168/jds.2006-900.

Roberson, J.R. 2003. Establishing treatment protocols for clinical mastitis. Veterinary Clinics of North America: Food Animal Practice 19:223–234. doi:10.1016/S0749-0720(02)00071-3.

Robles, I., D.F. Kelton, H.W. Barkema, G.P. Keefe, J.P. Roy, M. a. G. von Keyserlingk, and T.J. DeVries. 2019. Bacterial concentrations in bedding and their association with dairy cow hygiene and milk quality. Animal 1–15. doi:10.1017/S1751731119002787.

Rodrigues, A.C.O., D.Z. Caraviello, and P.L. Ruegg. 2005. Management of Wisconsin dairy herds enrolled in milk quality teams. J. Dairy Sci. 88:2660–2671. doi:10.3168/jds.S0022-0302(05)72943-X.

Rodriguez, A., G.R. Hagevoort, D. Leal, L. Pompeii, and D.I. Douphrate. 2018. Using mobile technology to increase safety awareness among dairy workers in the United States. Journal of Agromedicine 23:315–326. doi:10.1080/1059924X.2018.1502704.

Rollin, E., K.C. Dhuyvetter, and M.W. Overton. 2015. The cost of clinical mastitis in the first 30 days of lactation: An economic modeling tool. Preventive Veterinary Medicine 122:257–264. doi:10.1016/j.prevetmed.2015.11.006.

Rombach, I., A.M. Gray, C. Jenkinson, D.W. Murray, and O. Rivero-Arias. 2018. Multiple imputation for patient reported outcome measures in randomised controlled trials: advantages and disadvantages of imputing at the item, subscale or composite score level. BMC Medical Research Methodology 18:87. doi:10.1186/s12874-018-0542-6.

Rovai, M., H. Carroll, R. Foos, T. Erickson, and A. Garcia. 2016. Dairy Tool Box Talks: A Comprehensive Worker Training in Dairy Farming. Front. Public Health 4. doi:10.3389/fpubh.2016.00136.

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. Journal of Dairy Science 98:7865–7885. doi:10.3168/jds.2015-9866.

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. Journal of

Dairy Science 99:4707–4717. doi:10.3168/jds.2015-10675.

Rowbotham, R.F., and P.L. Ruegg. 2016b. Bacterial counts on teat skin and in new sand, recycled sand, and recycled manure solids used as bedding in freestalls. Journal of Dairy Science 99:6594–6608. doi:10.3168/jds.2015-10674.

Rowbotham, R.F., and P.L. Ruegg. 2016c. Associations of selected bedding types with incidence rates of subclinical and clinical mastitis in primiparous Holstein dairy cows. J. Dairy Sci. 99:4707–4717. doi:10.3168/jds.2015-10675.

Rowe, S.M., S.M. Godden, E. Royster, J. Timmerman, B.A. Crooker, and M. Boyle. 2019. Cross-sectional study of the relationships among bedding materials, bedding bacteria counts, and intramammary infection in late-lactation dairy cows. Journal of Dairy Science 102:11384–11400. doi:10.3168/jds.2019-17074.

Ruegg, P.L. 2017. A 100-Year Review: Mastitis detection, management, and prevention. Journal of Dairy Science 100:10381–10397. doi:10.3168/jds.2017-13023.

Ruegg, P.L. 2021. What Is Success? A Narrative Review of Research Evaluating Outcomes of Antibiotics Used for Treatment of Clinical Mastitis. Front. Vet. Sci. 8. doi:10.3389/fvets.2021.639641.

Rychert, J. 2019. Benefits and Limitations of MALDI-TOF Mass Spectrometry for the Identification of Microorganisms. Journal of Infectiology 2.

Santman-Berends, I.M.G.A., T.J.G.M. Lam, J. Keurentjes, and G. van Schaik. 2015. An estimation of the clinical mastitis incidence per 100 cows per year based on routinely collected herd data. Journal of Dairy Science 98:6965–6977. doi:10.3168/jds.2015-9642.

Santos, J.E.P., R.L.A. Cerri, M.A. Ballou, G.E. Higginbotham, and J.H. Kirk. 2004. Effect of timing of first clinical mastitis occurrence on lactational and reproductive performance of Holstein dairy cows. Anim Reprod Sci 80:31–45. doi:10.1016/S0378-4320(03)00133-7.

Sargeant, J.M., H.M. Scott, K.E. Leslie, M.J. Ireland, and A. Bashiri. 1998. Clinical mastitis in dairy cattle in Ontario: frequency of occurrence and bacteriological isolates.. Can Vet J 39:33–38.

Sarikaya, H., and R.M. Bruckmaier. 2006. Importance of the Sampled Milk Fraction for the Prediction of Total Quarter Somatic Cell Count. Journal of Dairy Science 89:4246–4250. doi:10.3168/jds.S0022-0302(06)72470-5.

Schalm, O.W., and D.O. Noorlander. 1957. Experiments and observations leading to development of the California mastitis test. J Am Vet Med Assoc 130:199–204.

Schmenger, A., and V. Krömker. 2020. Characterization, Cure Rates and Associated Risks of Clinical Mastitis in Northern Germany. Vet Sci 7. doi:10.3390/vetsci7040170.

Schreiner, D.A., and P.L. Ruegg. 2002. Effects of Tail Docking on Milk Quality and Cow

Cleanliness1. Journal of Dairy Science 85:2503–2511. doi:10.3168/jds.S0022-0302(02)74333-6.

Schreiner, D.A., and P.L. Ruegg. 2003. Relationship Between Udder and Leg Hygiene Scores and Subclinical Mastitis. Journal of Dairy Science 86:3460–3465. doi:10.3168/jds.S0022-0302(03)73950-2.

Schuenemann, G.M., S. Bas, E. Gordon, and J.D. Workman. 2013. Dairy calving management: Description and assessment of a training program for dairy personnel. Journal of Dairy Science 96:2671–2680. doi:10.3168/jds.2012-5976.

Schukken, Y., M. Chuff, P. Moroni, A. Gurjar, C. Santisteban, F. Welcome, and R. Zadoks. 2012. The "other" gram-negative bacteria in mastitis: Klebsiella, serratia, and more. Vet Clin North Am Food Anim Pract 28:239–256. doi:10.1016/j.cvfa.2012.04.001.

Schukken, Y.H., J. Hertl, D. Bar, G.J. Bennett, R.N. González, B.J. Rauch, C. Santisteban, H.F. Schulte, L. Tauer, F.L. Welcome, and Y.T. Gröhn. 2009. Effects of repeated gram-positive and gram-negative clinical mastitis episodes on milk yield loss in Holstein dairy cows. Journal of Dairy Science 92:3091–3105. doi:10.3168/jds.2008-1557.

Sears, P.M., B.S. Smith, P.B. English, P.S. Herer, and R.N. Gonzalez. 1990. Shedding Pattern of Staphylococcus aureus from Bovine Intramammary Infections. Journal of Dairy Science 73:2785–2789. doi:10.3168/jds.S0022-0302(90)78964-3.

Sepúlveda-Varas, P., K.L. Proudfoot, D.M. Weary, and M.A.G. von Keyserlingk. 2016. Changes in behaviour of dairy cows with clinical mastitis. Applied Animal Behaviour Science 175:8–13. doi:10.1016/j.applanim.2014.09.022.

Sharma, N., and D.K. Jeong. 2013. Stem Cell Research: A Novel Boulevard towards Improved Bovine Mastitis Management. Int J Biol Sci 9:818–829. doi:10.7150/ijbs.6901.

Shepley, E., G. Obinu, T. Bruneau, and E. Vasseur. 2019. Housing tiestall dairy cows in deep-bedded pens during an 8-week dry period: Effects on lying time, lying postures, and rising and lying-down behaviors. Journal of Dairy Science 102:6508–6517. doi:10.3168/jds.2018-15859.

Shim, E.H., R.D. Shanks, and D.E. Morin. 2004. Milk Loss and Treatment Costs Associated with Two Treatment Protocols for Clinical Mastitis in Dairy Cows*,†. Journal of Dairy Science 87:2702–2708. doi:10.3168/jds.S0022-0302(04)73397-4.

Siivonen, J., S. Taponen, M. Hovinen, M. Pastell, B.J. Lensink, S. Pyörälä, and L. Hänninen. 2011. Impact of acute clinical mastitis on cow behaviour. Applied Animal Behaviour Science 132:101–106. doi:10.1016/j.applanim.2011.04.005.

Sipka, A., M. Wieland, F. Biscarini, R.M. Rossi, N. Roman, C. Santisteban, P. Moroni, and D.V. Nydam. 2021. Short communication: Comparative performance of 3 on-farm culture systems for detection of mastitis pathogens interpreted by trained and untrained observers. Journal of Dairy Science 104:4936–4941. doi:10.3168/jds.2020-19166.

Sischo, W.M., D.A. Moore, R. Pereira, L. Warnick, D.L. Moore, J. Vanegas, S. Kurtz, K. Heaton, D. Kinder, J. Siler, and M.A. Davis. 2019. Calf care personnel on dairy farms and their educational opportunities. Journal of Dairy Science 102:3501–3511. doi:10.3168/jds.2018-15401.

Sitzmann, T., K. Kraiger, D. Stewart, and R. Wisher. 2006. The comparative effectiveness of web-based and classroom instruction: A meta-analysis. Personnel Psychology 59:623–664. doi:10.1111/j.1744-6570.2006.00049.x.

Smith, K.L. 1999. Suggested interpretation of mastitis terminology. Bulletin-International Dairy Federation 3–26.

Smith, K.L., D.A. Todhunter, and P.S. Schoenberger. 1985. Environmental mastitis: cause, prevalence, prevention. J Dairy Sci 68:1531–1553. doi:10.3168/jds.S0022-0302(85)80993-0.

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 freestalls. J. Dairy Sci. 97:2965–2968. doi:10.3168/jds.2013-7814.

Stangaferro, M.L., R. Wijma, L.S. Caixeta, M.A. Al-Abri, and J.O. Giordano. 2016. Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part II. Mastitis. J Dairy Sci 99:7411–7421. doi:10.3168/jds.2016-10908.

Taponen, S., L. Salmikivi, H. Simojoki, M.T. Koskinen, and S. Pyörälä. 2009. Real-time polymerase chain reaction-based identification of bacteria in milk samples from bovine clinical mastitis with no growth in conventional culturing. Journal of Dairy Science 92:2610–2617. doi:10.3168/jds.2008-1729.

The European Parliament and The Council of the European Union. 2009. Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation).

Tomazi, T., G.C. Ferreira, A.M. Orsi, J.L. Gonçalves, P.A. Ospina, D.V. Nydam, P. Moroni, and M.V. dos Santos. 2018. Association of herd-level risk factors and incidence rate of clinical mastitis in 20 Brazilian dairy herds. Preventive Veterinary Medicine 161:9–18. doi:10.1016/j.prevetmed.2018.10.007.

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. Journal of Dairy Science 98:3071–3078. doi:10.3168/jds.2014-8466.

Tomazi, T., A.C.C.H. Tomazi, J.C.C. Silva, L. Bringhenti, M.L.M.C. Bravo, M.X. Rodrigues, and R.C. Bicalho. 2021. Immunization with a novel recombinant protein (YidR) reduced the risk of clinical mastitis caused by Klebsiella spp. and decreased milk losses and

culling risk after Escherichia coli infections. J Dairy Sci 104:4787–4802. doi:10.3168/jds.2020-19173.

Truitt, D.L. 2011. The Effect of Training and Development on Employee Attitude as it Relates to Training and Work Proficiency. SAGE Open 1:2158244011433338. doi:10.1177/2158244011433338.

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. Journal of Dairy Science 86:521–529. doi:10.3168/jds.S0022-0302(03)73630-3.

Tucker, C.B., G. Zdanowicz, and D.M. Weary. 2006. Brisket Boards Reduce Freestall Use. Journal of Dairy Science 89:2603–2607. doi:10.3168/jds.S0022-0302(06)72337-2.

USDA APHIS | NAHMS Dairy Studies. . Accessed January 20, 2021. https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/monitoring-and-surveillance/nahms/nahms_dairy_studies.

Vasquez, A.K., D.V. Nydam, M.B. Capel, S. Eicker, and P.D. Virkler. 2017. Clinical outcome comparison of immediate blanket treatment versus a delayed pathogen-based treatment protocol for clinical mastitis in a New York dairy herd. J Dairy Sci 100:2992–3003. doi:10.3168/jds.2016-11614.

Verbeke, J., S. Piepers, K. Supré, and S. De Vliegher. 2014. Pathogen-specific incidence rate of clinical mastitis in Flemish dairy herds, severity, and association with herd hygiene. Journal of Dairy Science 97:6926–6934. doi:10.3168/jds.2014-8173.

Villettaz Robichaud, M., J. Rushen, A.M. de Passillé, E. Vasseur, D. Haley, and D. Pellerin. 2019. Associations between on-farm cow welfare indicators and productivity and profitability on Canadian dairies: II. On tiestall farms. Journal of Dairy Science 102:4352–4363. doi:10.3168/jds.2018-14818.

Wald, R., C. Hess, V. Urbantke, T. Wittek, and M. Baumgartner. 2019. Characterization of Staphylococcus Species Isolated from Bovine Quarter Milk Samples. Animals (Basel) 9. doi:10.3390/ani9050200.

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. Veterinary Record 151:199–206. doi:https://doi.org/10.1136/vr.151.7.199.

Watts, J.L. 1988. Etiological agents of bovine mastitis. Vet Microbiol 16:41–66. doi:10.1016/0378-1135(88)90126-5.

Wellnitz, O., and R.M. Bruckmaier. 2012. The innate immune response of the bovine mammary gland to bacterial infection. The Veterinary Journal 192:148–152. doi:10.1016/j.tvjl.2011.09.013.

Wente, N., D. Klocke, J.-H. Paduch, Y. Zhang, M. tho Seeth, V. Zoche-Golob, F. Reinecke, E. Mohr, and V. Krömker. 2019. Associations between Streptococcus uberis strains from the animal environment and clinical bovine mastitis cases. Journal of Dairy Science 102:9360–9369. doi:10.3168/jds.2019-16669.

Whiteside, W.H. 1939. Observations on a new test for the presence of mastitis in milk.. Canadian Public Health Journal 30.

Wilmes, E., and R. Swenson. 2019. Engaging Dairy Farmers in Safety Messages: Values, Moral Norms, Barriers, and Implications for Communication. Journal of Applied Communications 103. doi:10.4148/1051-0834.2204.

Winder, C.B., S.J. LeBlanc, D.B. Haley, K.D. Lissemore, M.A. Godkin, and T.F. Duffield. 2017. Comparison of an online learning module to hands-on training in teaching a cautery disbudding technique for dairy calves including cornual nerve block application. Can Vet J 58:735–740.

Winder, C.B., S.J. LeBlanc, D.B. Haley, K.D. Lissemore, M.A. Godkin, and T.F. Duffield. 2018. Comparison of online, hands-on, and a combined approach for teaching cautery disbudding technique to dairy producers. J Dairy Sci 101:840–849. doi:10.3168/jds.2017-13217.

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. Journal of Dairy Science 101:530–536. doi:10.3168/jds.2016-12358.

Wuytack, A., A. De Visscher, S. Piepers, F. Boyen, F. Haesebrouck, and S. De Vliegher. 2020. Distribution of non-aureus staphylococci from quarter milk, teat apices, and rectal feces of dairy cows, and their virulence potential. Journal of Dairy Science 103:10658–10675. doi:10.3168/jds.2020-18265.

Zadoks, R.N., and M.A. Munoz. 2007. Emergence of Klebsiella as a major mastitis organism. Page in Annual meeting.

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 Sand or Sawdust. Journal of Dairy Science 87:1694–1701. doi:10.3168/jds.S0022-0302(04)73322-6.

Zhao, X., and P. Lacasse. 2008. Mammary tissue damage during bovine mastitis: Causes and control. Journal of animal science 86:57–65. doi:10.2527/jas.2007-0302.

CHAPTER TWO

SHORT COMMUNICATION: COMPARISON AMONG THREE METHODS FOR EVALUATING CLINICAL MASTITIS FREQUENCY IN DAIRY COWS: INCIDENCE RISK AT THE COW LEVEL, INCIDENCE RATE AT THE COW LEVEL, AND INCIDENCE RATE AT THE QUARTER LEVEL

V.M. Alanis[1], T. Tomazi[1], C. Santisteban[1], D. V. Nydam[1], P. A. Ospina[2]

[Manuscript submitted for publication]

[1]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York, USA
[2]Lechear LLC, King Ferry, New York, USA

ABSTRACT

The lack of standardization in reporting clinical mastitis incidence limits the ability to compare results across multiple studies without additional calculations. There is both a biological and statistical rationale for evaluating the at-risk period at the quarter level. This study aimed to: (1) to outline an applied method for calculating clinical mastitis (CM) incidence rate at the quarter level using currently available software; and (2) to present the results of three different measurements: incidence risk at the cow level, incidence rate at the cow level, and incidence rate at the quarter level. In an open population prospective cohort of eight commercial dairy farms monitored from May 15, 2016, to May 31, 2017, all CM cases (n=7,513) were identified by trained on-farm personnel, who collected all milk samples from all quarters with visibly abnormal milk. Microbiological identification was determined by culture and MALDI-TOF. All lactating quarters were at risk for CM. A quarter was at risk for a new CM case if there was at least 14 d between a previously diagnosed case and the current case in the same quarter, or if a different pathogen was isolated in the same quarter within 14 d. A total of 17,513,429 quarters days at risk (QDAR) were estimated. A SAS macro and Structured Query Language (SQL) were used to bring all data together. The monthly incidence rate at the cow level was 16.6 cases per 10,000 cow-days, the monthly incidence rate at the quarter level was 4.4 cases per 10,000 QDAR and the monthly incidence risk at the cow level was 4.8 cases per 100 cows. Although the evaluation of QDAR requires additional computation when compared to other methods, it might allow for a more precise evaluation of the data and a more accurate evaluation of mastitis incidence. Clearly defining the methods used to report mastitis incidence will improve our ability to discuss and learn about the differences and similarities across studies, regions, and countries.

Keywords: clinical mastitis, incidence, calculation

INTRODUCTION

Clinical mastitis (CM) is one of the most complex, frequent, and costly diseases affecting dairy cows. This The complexity arises from many major factors including a variety of infectious pathogens, dynamic host immune response, differing environmental and management factors, and a long risk period. Consequently, mastitis is one of the most investigated dairy cow diseases. However, there is no consensus about how to calculate or report the incidence of CM with some reporting the disease frequency as incidence risk (i.e., a proportion; Persson Waller et al., 2009; Oliveira et al., 2015) and others reporting incidence rates that include time at risk (e.g., cow days, cow years, or quarter days; Barkema et al., 1998; Olde Riekerink et al., 2008; Moosavi et al., 2014).

Although most measures of CM incidence normally use the same numerator after a clear case definition has been established, the denominators can vary (i.e., risk vs. rate or cow vs. quarter), which makes it difficult to compare results between studies. For instance, CM incidence rates were reported as 0.26 cases per 365 cow-days (Barkema et al., 1998), 49 per 10,000 cow-days (Naqvi et al., 2018), or 32.1 cases per 100 cows per year (Santman-Berends et al., 2015); while CM incidence risk as 31% in multiparous cows per year, not including repeated cases (Oliveira et al., 2015).

Even though many studies evaluate mastitis as a rate, the lack of standardization in reporting CM incidence limits the ability to compare results across multiple studies without additional calculations. Several studies have investigated CM rate in dairy herds worldwide; however, some of these studies evaluated the at-risk period at the cow-level, even when they recorded the mastitis occurrence at the quarter level. One recent study performed a quarter-level evaluation considering the quarters days at risk (QDAR) instead of a cow-level estimate (Tomazi et al.,

2018). There is both biological and statistical rationale for evaluating the at-risk period at the quarter level. However, we must be cautious to ensure that all quarters from the study population are truly at risk while calculating the incidence rate.

Evaluation of CM at the quarter level might be challenging because quarters are grouped within udders, and within cows, thus quarter and cow-level factors (e.g., parity, DIM, teat and udder anatomy, immune response, milk production level) can influence the risk of disease. Statistical models that evaluate the incidence of CM should incorporate this hierarchical structure and control for both cow level factors, and perhaps, evaluate the time at risk at the quarter level. In addition, recent some studies suggest that a single quarter with CM can affect the overall immune status of the udder (Barkema et al., 1997; Aitken et al., 2011; Paixão et al., 2017). Thus, the susceptibility to CM of each healthy quarter from a cow with a quarter already infected might differ from those in uninfected cows.

The aim of this study was to calculate the quarter-level CM incidence rate in an open population prospective open cohort of eight commercial dairy farms monitored during one year. The specific objectives were: (1) to outline an applied method for calculating CM incidence rate at the quarter level using currently available software; and (2) to present the results of the three different measurements using the same CM data: incidence risk at the cow level, incidence rate at the cow level, and incidence rate at quarter level.

MATERIALS AND METHODS

A total of 7,513 milk samples from CM cases were collected by trained on-farm personnel from eight commercial dairy herds in Central New York and submitted for microbial identification. Herds were conveniently selected based on a client list of the daily milk sample pick-up and 24-hour result program of Quality Milk Production Services (QMPS) at Cornell

University. The data collection was carried out for 12 months between May 2016 and May 2017. All herds housed cows in free-stall barns and the average herd size was 1,576 cows (ranging from 1,075 to 1,942). The average milk production in these herds was 41 kg/cow/d, ranging from 39 to 43 kg/cow/d. Monthly mean cow SCC was 234,000 cell/ml, ranging from 145,000 to 361,000 cells/ml.

Milk harvest technicians that received bilingual continuous training and education on milk quality basics, Trained farm personnel detected and collected milk samples from all CM cases at every milking. Quarters were forestripped and the identification of the disease was based on changes in the milk appearance (e.g., watery, flakes, clots, serous milk). Milk samples were aseptically collected from affected quarters either before or after milking according to National Mastitis Council guidelines (NMC, 2017). Sample vials were labeled with cow ID, affected quarter, and the date and immediately stored in a refrigerator (≅ 4ºC). If more than one quarter was affected in the same cow, one vial per affected quarter was collected.

After sample collection, farm personnel saved information in Dairy Comp 305 (DC305; Ag Valley Software) as a MAST event, which asks for cow ID, affected quarter, and stamps the date. Every 24 hours (except New Year's Day, Christmas, Easter, 4th of July, and Thanksgiving) a courier from QMPS traveled to the farms to pick up milk samples and downloaded an electronic form containing the information listed above. The samples were transported in coolers with ice packs and the electronic record was uploaded to the QMPS laboratory computers. Upon arriving at the laboratory, samples were processed according to NMC guidelines (NMC, 2017). Colonies from milk cultures were also speciated using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF MS) as described by Rowe (Rowe et al., 2020).

An intramammary infection was defined as the isolation of three or more identical colonies

from cultured milk for all bacteria except non-aureus Staphylococcus, which required ≥6 colonies for confirming the infection. If two different pathogenic colony types were present, the culture was defined as mixed culture. Cultures with ≥3 morphologically distinct colonies were considered contaminated unless a contagious pathogen (e.g., Staphylococcus aureus or Streptococcus agalactiae) was identified based on morphology. A sample was defined as no growth if no colonies were observed on the agar plate after 48 h of incubation.

Preliminary laboratory results were automatically transferred from the QMPS laboratory into the farm computer within 24 h and confirmatory results were sent before 48 h through the internet LOOP (iLOOP). The iLOOP is a transfer method that provides the process for two-way data for DC305 software users. At the farm, affected cows were treated according to individual herd protocols based on culture results.

Every week, the 'save\c' command was used to create a weekly backup file that was stored on the farm computer. These weekly backups were transferred onto a USB flash drive and then stored on the a file storage and synchronization service developed by Google (Google, Mountain View, CA). cloud where every farm had its file according to each week, month and year. These DC305 files were then used to collect additional data and create the monthly data set. The command 'EVENTS\2SI' was used for searching finding the cows that had been recorded as having CM. Additional cow level data such as calving date, parity, date of CM identification, DIM at CM, affected mammary quarter, isolated pathogen, DHIA test dates, linear score, and dry off and/or culling dates (if applicable) were also extracted from the DC305 backups and then transferred to Excel spreadsheets (Microsoft Corp; Redmond, WA).

A main Excel file was used for data analyses including all cows in the selected herds (i.e., those with CM events and those with no CM events). The file scheme used in the analysis was

organized as a long format, meaning that each row is a one-time point (i.e., month) per cow, stating the affected quarters, andin case there was more than one CM event in the same event (Table 1). Therefore, each cow had data in multiple rows and could be present in the final data file up to 12 times (i.e., 12 months tracking).

All lactating quarters were at risk for CM. A quarter was at risk for a new CM case if there was at least 14 d between a previously diagnosed case and the current case in the same quarter, or if a different pathogen was isolated in the same quarter within 14 d. This lag time was chosen due to it can reflect the duration of time to achieve both spontaneous and treatment cure. Specifically, this study started on May 15, 2016, and ended on May 31, 2017. However, the data were collected starting on May 1, 2016, to establish an enrollment baseline.

Descriptive statistical analysis was performed with both Excel and SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The iIncidence risk at the cow level (IRiC), within herds, was calculated monthly as the number of CM cases divided by the number of at-risk cowscows at risk in the population at the beginning of each month and then multiplied by 100. The at-risk period (denominator) for each cow started at the beginning of each month or date of calving within the month and ended at the end of the month, the day of CM, or exit from the study (e.g. culling, death, dry off). Each cow that was present in any interval within these parameters contributed 1 cow at risk to the denominator.

The incidence rate at the cow level (IRaC) was calculated monthly as the number of CM cases divided by the number of cow-days at risk. The at-risk period for each cow started at the beginning of each month or date of calving and ended at the end of the month, the day of CM, or exit from the study (e.g. culling, death, dry off). Each cow that was present in any interval within these parameters contributed with the sum of days that each cow remained without CM and in

the herd during a given month, considering the number of lactating cows at the beginning of the month and then multiplied by 10,000 cow-days.

The incidence rate at the quarter level (IRaQ) was calculated monthly as the number of CM quarters cases divided by the number of QDAR in each month and then multiplied by 10,000 quarters. The number of QDAR was calculated as the sum of days that each quarter remained without CM and in the herd during a given month, considering the number of lactating cows at the beginning of the month and assuming that all cows had four functional quarters. When calculating the incidence rate, an arbitrary multiplier of some power of 10 is generally used (Porta, 2014). The size of this 10,000 multiplier factor was chosen in order to have a final value that would be expressed as a whole number. For example, a monthly incidence rate of 5.0 CM cases per 10,000 quarters-day at risk means that 5 quarters are expected to have CM out of 10,000 healthy quarters daily monitored within the specific month.

The SAS program was used to 1) estimate calculate the time at risk by summarizing the number of days that each cow remained healthy in the herd in each month; 2) summarized estimate the total number of CM quarter cases (numerator) in each month, then transfer this information into Excel; 3) join numerator and denominator files to create a summary file; and 4) estimate the incidence rates (i.e., IRaC and IRaQ). Each month was analyzed separately, therefore SAS macros were used to bring all of the data together. The beginning and the end of each month were specified (i.e., months have 28-31 d). The PROC Structured Query Language (SQL; [Sander and Wauer, 2019]) and the INTNX and INTCK functions were used to create new columns, different data subsets, and sort data within each given month. The code can be found at this link: https://doi.org/10.7298/rts8-mk82. Figure 1 shows the workflow in SAS.

RESULTS AND DISCUSSION

A total of 8,199 CM quarter cases were recorded over the study period. However, 668 (8.1%) were excluded from the analysis because the CM event occurred in the same quarter, with the same culture result, or within 14 days after a previous case and thus not considered new incident cases. For the remaining 7,513 CM cases, a total of 17,513,429 QDAR were estimatedcalculated.

Of these quarter CM cases, 29.6% had a negative culture result, 68.7% had pathogen identification, and 1.7% resulted in contamination. Out of positive samples, Escherichia coli was the most prevalent pathogen (17.9%), followed by Streptococcus uberis (9.9%), Streptococcus dysgalactiae (8.7%), Klebsiella spp. (6.7%), and Staphylococcus aureus (3.1%). Most cows (95%) had CM in only one quarter at a given time.

In this dataset, three methods were evaluated: the IRiC, IRaC, and IRaQ (Table 2). The IRaC was approximately four times the magnitude of the IRaQ. This is because the cow is managed as 1 case yet has 4 times the chances of having CM, assuming 4 quarters, as compared to 1 quarter. The IRiC was similar in magnitude when compared to the IRaQ. Although numerically similar, it is important to note that both calculations (IRiC and IRaQ) represent different outcomes and answers different questions. The IRiC represents how likely a new CM will occur in a lactating cow during a specified time period, such as month in this case. The IRaQ describes how rapidly CM occurs in the population in a given time interval, i.e. the rate at which new quarter cases are occurring per day in any particular month.

The IRiC assumes that the entire population at risk at the beginning of the study period remains at risk for the entire specified time period in terms of CM occurrence without accurately taking into account follow-up losses due to death or culling before the at-risk period is over. That is to say, if a cow is at risk at the beginning of the month she contributes a count of one period to

the time at risk no matter how many days she is at risk during the month. Thus, for example, a cow culled at 10 days into the new month counts the same risk as a cow that is present for the entire month. This method can also result in slight over-estimation of the incidence if cows can contribute to the numerator multiple times (i.e., CM case from different quarters), while only counted in the denominator once. Although CM occurring in multiple quarters at the same time was not common in our study (5% of the cases), it can affect the estimate. This is a common method for estimating CM incidence risk, however, most research restricts CM definitions to only the first CM case or time at risk (e.g., first 30 DIM) to avoid overestimating incidence. To the extent of our knowledge, only one research study clearly states that cows could continue to contribute to the numerator while removed from the denominator (Santman-Berends et al., 2015).

On the other hand, the IRaQ takes into account the sum of the time that each quarter remained under observation and at risk of developing CM. Although the evaluation of QDAR requires additional computation when compared to other methods, it allows for a more precise evaluation of the data and a more accurate evaluation of incidence.

4 Conclusions

In terms of applicability, IRiC could be the easiest to calculate method to measure CM at the farm level. It also likely represents the economic unit of interest when managing mastitis because it is, for example, the entire cow that would be culled due to chronic mastitis, put in a hospital pen after antibiotic treatment, and thus having non-saleable milk. However, for research describing the mastitis dynamics, IRaQ may be a more complete and granular evaluation of CM incidence.

Clear reporting about the methods used to report mastitis incidence can improve our ability to

discuss and learn about the differences and similarities across regions and worldwide. The SAS code and flow chart presented in this report can streamline the process by which researchers can estimate mastitis incidence in a comparable way so that we can continue to make progress on improving milk quality through accurate comparisons between studies, regions, and countries.

Table 2. 1 Example arrangement of the Dairy Comp 305 data of 1 farm extracted and put into the main Excel (Microsoft Corp; Redmond, WA) file used in Clinical Mastitis (CM) incidence analysis (May-June 2016 as an example)

ID	Month	Year	Parity	Dry/cull date	Calving date	First event CM date	DIM	AQ1	Pathogen[2] AQ1	AQ2	Pathogen[2] AQ2	Second event CM date	AQ Second event	Pathogen[2] Second event
6166	May	2016	1	.	8/12/2015	5/10/2016	272	RH	N	.	.	.	.	.
528	May	2016	1	2/21/2016	4/26/2016	5/11/2016	15	LF	E	RF	C	.	.	.
9531	May	2016	1	.	6/10/2015	5/11/2016	336	LH	U	.	.	5/18/20016	LF	U
125	May	2016	4	5/29/2016	6/02/2015	.	.	.	.	.	.	.	.	.
6166	June	2016	1	.	8/12/2015	.	.	.	.	.	.	.	.	.
528	June	2016	1	2/21/2016	4/26/2016	6/29/2016	64	RF	K	.	C	.	.	.
9531	June	2016	1	.	6/10/2015	.	.	.	.		.	.	.	.
125	June	2016	4	5/29/2016	6/02/2015	.	.	.	.	.	.	.	.	.

[1]AQ= affected quarter. AQ1= first affected quarter; first event, AQ2 = second quarter affected; first event (LH =left hind; LF = left front; RH = right hind; RF = right front). Note that a maximum of two affected quarters is listed in this example table due to space limitations.

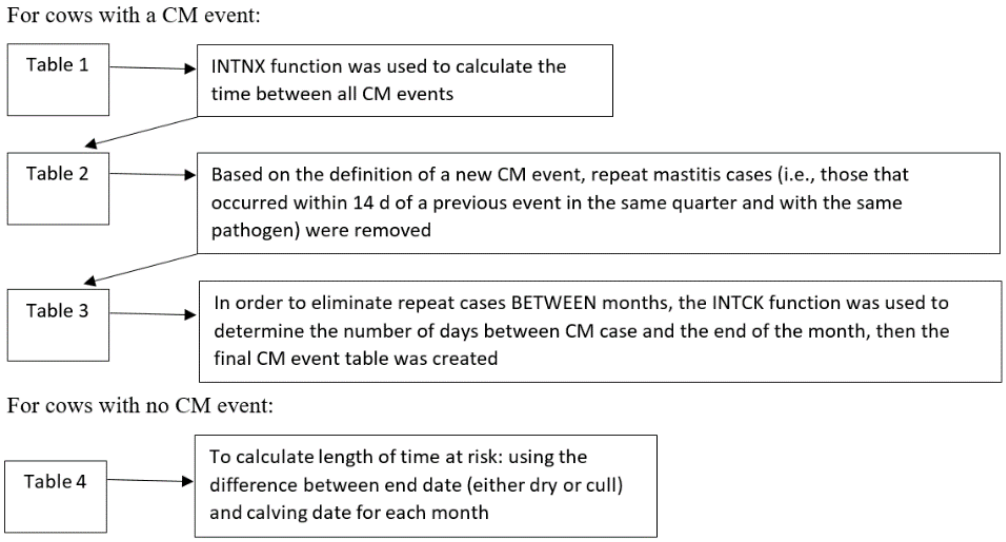
[2] Examples of pathogens isolated in a milk sample if a quarter was affected (E = Escherichia coli; C = Staphylococcus spp; N = no growth; U = *Streptococcus uberis*; K = *Klebsiella* spp)

Table 2. 2 Descriptive characteristics of eight herds from Central New York, United States; evaluated for average monthly clinical mastitis (CM) incidence risk (IRiC) at cow-level, incidence rate at cow-level (IRaC), and incidence rate at quarter-level (IRaQ) from May 2016 to May 2017 (SD in parentheses)

Herd	Lactating cows (monthly average)	Milk yield kg/day	BTSCC[1]	All CM cases	New CM cases	At-risk period QDAR[2]	IRiC[3]	IRaC[4]	IRaQ[5]
A	1,354	43 (0.9)	186	1,442	1,333	1,884,422	7.5	24	7.0
B	1,970	42 (1.3)	145	1,537	1,455	2,719,579	5.7	20	5.3
C	1,075	40 (1.3)	313	900	759	1,488,497	5.4	19	5.1
D	1,593	39 (0.9)	297	1,231	1,075	2,191,178	5.2	18	4.9
E	1,223	41 (0.9)	185	820	790	1,708,832	5.0	17	4.3
F	1,452	39 (1.3)	361	821	770	2,020,090	4.1	15	3.8
G	1,996	43 (0.9)	146	1,134	1,036	2,723,021	4.0	16	3.8
H	1,942	40 (0.9)	198	314	295	2,777,810	1.2	4	1.0
Sum	-	-	-	8,199	7,513	17,513,429	-	-	-
Mean	1,576	41	234	-	-	-	4.8	16.6	4.4

[1]BTSCC – Bulk Tank Somatic Cell Count ($\times 10^3$ cells/mL)
[2]QDAR = Quarters days at risk: sum of days that each quarter remained healthy during a given month, considering the number of lactating cows at the beginning of the month and assuming that all cows had four functional quarters.
[3]IRiC = Incidence risk at cow level: number of CM cases divided by the number of cows in the population at the beginning of each month and then multiplied by 100.
[4]IRaC = Incidence rate at cow level: number of CM cases divided by the number of cow-days at risk (the sum of days that each cow remained healthy during a given month, considering the number of lactating cows at the beginning of the month) and then multiplied by 10,000.
[5]IRaQ = Incidence rate at quarter level: number of CM cases divided by the number of QDAR in each month and then multiplied by 10,000.

Figure 2. 1 Workflow in SAS using Macros and SQL to create tables to evaluate clinical mastitis frequency in dairy cows



CONCLUSIONS

In terms of applicability, IRiC could be the easiest to calculate method to measure CM at the farm level. It also likely represents the economic unit of interest when managing mastitis because it is, for example, the entire cow that would be culled due to chronic mastitis, put in a hospital pen after antibiotic treatment, and thus having non-saleable milk. However, for research describing the mastitis dynamics, IRaQ may be a more complete and granular evaluation of CM incidence.

Clear reporting about the methods used to report mastitis incidence can improve our ability to discuss and learn about the differences and similarities across regions and worldwide. The SAS code and flow chart presented in this report can streamline the process by which researchers can estimate mastitis incidence in a comparable way so that we can continue to make progress on improving milk quality through accurate comparisons between studies, regions, and countries.

REFERENCES

Aitken, S.L., C.M. Corl, and L.M. Sordillo. 2011. Immunopathology of Mastitis: Insights into Disease Recognition and Resolution. J Mammary Gland Biol Neoplasia 16:291–304. doi:10.1007/s10911-011-9230-4.

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. Journal of Dairy Science 81:411–419. doi:10.3168/jds.S0022-0302(98)75591-2.

Barkema, H.W., Y.H. Schukken, T.J.G.M. Lam, D.T. Galligan, M.L. Beiboer, and A. Brand. 1997. Estimation of Interdependence Among Quarters of the Bovine Udder with Subclinical Mastitis and Implications for Analysis. Journal of Dairy Science 80:1592–1599. doi:10.3168/jds.S0022-0302(97)76089-2.

Naqvi, S.A., J. De Buck, S. Dufour, and H.W. Barkema. 2018. Udder health in Canadian dairy heifers during early lactation. Journal of Dairy Science 101:3233–3247. doi:10.3168/jds.2017-13579.

Oliveira, C.S.F., H. Hogeveen, A.M. Botelho, P.V. Maia, S.G. Coelho, and J.P.A. Haddad. 2015. Cow-specific risk factors for clinical mastitis in Brazilian dairy cattle. Preventive Veterinary Medicine 121:297–305. doi:10.1016/j.prevetmed.2015.08.001.

Paixão, M.G., L.R. Abreu, R. Richert, and P.L. Ruegg. 2017. Milk composition and health status from mammary gland quarters adjacent to glands affected with naturally occurring clinical mastitis. Journal of Dairy Science 100:7522–7533. doi:10.3168/jds.2017-12547.

Persson Waller, K., B. Bengtsson, A. Lindberg, A. Nyman, and H. Ericsson Unnerstad. 2009. Incidence of mastitis and bacterial findings at clinical mastitis in Swedish primiparous cows—Influence of breed and stage of lactation. Veterinary Microbiology 134:89–94. doi:10.1016/j.vetmic.2008.09.004.

Porta, M. 2014. A Dictionary of Epidemiology. Oxford University Press.

Rowe, S., S. Godden, D.V. Nydam, P. Gorden, A. Lago, A. Vasquez, E. Royster, J. Timmerman, and M. Thomas. 2020. Evaluation of rapid culture, a predictive algorithm, esterase somatic cell count and lactate dehydrogenase to detect intramammary infection in quarters of dairy cows at dry-off. Preventive Veterinary Medicine 179:104982. doi:10.1016/j.prevetmed.2020.104982.

Sander, A., and R. Wauer. 2019. Integrating terminologies into standard SQL: a new approach for research on routine data. J Biomed Semantics 10. doi:10.1186/s13326-019-0199-z.

Santman-Berends, I.M.G.A., T.J.G.M. Lam, J. Keurentjes, and G. van Schaik. 2015. An estimation of the clinical mastitis incidence per 100 cows per year based on routinely collected herd data. Journal of Dairy Science 98:6965–6977. doi:10.3168/jds.2015-9642.

Tomazi, T., G.C. Ferreira, A.M. Orsi, J.L. Gonçalves, P.A. Ospina, D.V. Nydam, P. Moroni, and M.V. dos Santos. 2018. Association of herd-level risk factors and incidence rate of clinical mastitis in 20 Brazilian dairy herds. Preventive Veterinary Medicine 161:9–18. doi:10.1016/j.prevetmed.2018.10.007.

CHAPTER THREE

CLINICAL MASTITIS AND PATHOGE - SPECIFIC MILK LOSSES IN NEW YORK DAIRY HERDS

V.M. Alanis[1], T. Tomazi[1], P. A. Ospina[2], D. V. Nydam[1],

[Manuscript prepared for publication]

[1]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York, USA
[2]Lechear LLC, King Ferry, New York, USA

ABSTRACT

Considered a multi-etiological disease, bovine mastitis is one of the most common and costly disease in dairy cattle around the world. Studies characterizing the etiology of clinical mastitis (CM) and related milk losses can provide information and better understanding of the impact of this disease on herd profitability. The objectives of this study were to: a) characterize the frequency of CM and pathogen distribution in eight large dairy herds in the State of New York, and b) describe the pathogen-level daily milk losses in primiparous and multiparous cows in dairy herds recording daily milk weights. Eight commercial dairy herds in Central New York State were followed from May 2016 to July 2017. At each milking, trained farm personnel identified CM cases by forestripping all mammary quarters. At CM identification, a milk sample was collected and submitted for microbiological identification to guide the appropriate therapeutic intervention. Daily milk records were available in four out of the eight herds. Mixed-effects models were used to assess the effects of most frequent CM-causing pathogens. Milk losses estimations were based on milk weights recorder 49 days before and after the clinical case. Separate models were fitted for primiparous and multiparous cows. Each pathogen was modeled separately and matched with a control based on herd, DIM and parity; controls were considered as no mastitis cows and used as the reference category in each model. A total of 7,513 clinical cases were recorded. The monthly incidence risk at cow level was 4.8 cases per 100 cows and the monthly incidence rate at quarter level was 4.4 cases per 10,000 quarters days at risk. The overall distribution by pathogen group was 29.6% no growth, 30.8% gram-positive, 26.3% gram-negative, and the remainder cases (13.3%) was made up of mixed culture, non-bacterial pathogens (yeast and *Prototheca* spp.), less-frequent bacteria and contamination. Out of the positive results, *Escherichia coli* was the most prevalent pathogen, cultured in 1,345 (17.9%) of

the cases. There were differences between herds in terms of pathogen-specific distribution. Losses on primiparous cows were greater when caused by *Streptococcus dysgalactie*, *Staphylococcus aureus*, and *E.coli*. Multiparous cows had larger losses when CM was caused by E. coli, Klebsiella spp, and Streptococcus dysgalactie. Long-lasting losses occurred for *E.coli* and *Klebsiella* spp both primiparous and multiparous cows had the greatest milk losses in the first week after CM detection. This study highlights that although these New York herds had similar incidence, the pathogen distribution was different between herds. Moreover, our findings provide accurate estimations of daily milk losses associated with the most prevalent pathogens causing CM in dairy herds, which varies substantially according to the organism identified. Mastitis-control programs to reduce CM caused by environmental mastitis can prevent significant milk losses in dairy herds.

Keywords: clinical mastitis, incidence, pathogens distribution, milk losses

INTRODUCTION

Defined as the inflammation of the mammary gland in response to injury commonly caused by the invasion of pathogenic bacteria, mastitis is one of the most complex, costly, and frequent infectious diseases in dairy cattle (Halasa et al., 2007; Ruegg, 2017). This complexity arises from three major factors: a wide variety of infectious pathogens, a dynamic host-immune response, and a long risk period, which includes the lactation and dry period. Treatment and prevention of clinical mastitis (CM) has been reported as the main reasons for antimicrobial use in dairy herds (Pol and Ruegg, 2007; Gomes and Henriques, 2016; Kuipers et al., 2016; Krömker and Leimbach, 2017). A case of clinical mastitis (CM) in the first 30 DIM can result in a total economic cost of $444 (Rollin et al., 2015). Unsaleable milk and future milk production loss are

among the major costs associated with CM in dairy cows (Seegers et al., 2003; Gröhn et al., 2004; Hogeveen et al., 2011; Kayano et al., 2018). Intramammary infection dynamics at the herd level are determined by the ecology of pathogens and their interactions with the cow's immune-competence, management strategies and treatment interventions. The characterization of etiological and epidemiological profile of CM can be help farmers to control and better estimate the costs associated with this infectious disease in dairy farms.

Several studies have prospectively monitored the frequency and etiology of CM in different countries (Petrovski et al., 2009; Oliveira et al., 2013; Verbeke et al., 2014; Tomazi et al., 2018). In addition, other research groups have evaluated the effect of CM cases on milk production. These effects have been investigated in generic cases (Rajala-Schultz et al., 1999; Bar et al., 2007; Hagnestam et al., 2007), in pathogens-specific (Coulona et al., 2002; Gröhn et al., 2004; Reksen et al., 2007; Heikkilä et al., 2018a; Kayano et al., 2018), as well in repeated cases of CM (Schukken et al., 2009; Hertl et al., 2014). Major milk losses have been associated with infections caused by *Staphylococcus aureus* and gram-negative pathogens, especially coliforms such as *E.coli* and *Klebsiella* spp. The greatest losses have been reported to occur immediately following the CM diagnosis, but often never recovering the initial yield due to the damage to the mammary gland tissue caused by the pathogen and the inflammatory response (Zhao and Lacasse, 2008; Blum et al., 2020). The magnitude of milk yield losses can vary according to the cow's stage of lactation at CM, being greater during early lactation (≤50 DIM) in both primiparous and multiparous cows (Gröhn et al., 2004; Hertl et al., 2014; Heikkilä et al., 2018a). This is particularly important if we use test-day data for the evaluation of short-term losses associated with CM occurring in the early stage of lactation. Despite the fact that cows that develop CM tend to be higher milk producers compared to the non-mastitic cows before

diagnosis (Gröhn et al., 2004; Hertl et al., 2014), different pathogens may have longer or shorter periods of subclinical infection before the appearance of visual clinical signs. For example, cows affected with a CM caused by *S. aureus* had reduction in the milk production starting at the week before CM diagnosis, whereas cows infected with *Trueperella pyogenes* had milk losses starting 4 weeks before CM diagnosis (Gröhn et al., 2004).

The effect estimate on milk production varies across studies, mostly attributable to a different definition and inclusion criteria of CM case, production level, cattle breeds, estimation methods, controls selection, and milk production measurement methods. Besides, the impact on milk production of some pathogens highly isolated from CM are not available in the literature. Excepting Heikkilä et al., 2018a, other studies (Gröhn et al., 2004; Hertl et al., 2014) leave aside the effect of common and important pathogens such as *Streptococcus dysgalactie* and *Streptococcus uberis* (which are generally included in a big general *Streptococcus spp*. category), or even the effect of emergent pathogens such as yeast and *Lactococcus* spp. Another limitation of these studies is the milk production is based on test-day or weekly averages instead of daily milk weights, which may increase the variability between measurements.

Studies characterizing the etiology of CM and pathogen-level milk losses can evolve the understanding of the impact of this disease on herd profitability, in addition to promoting strategies of targeted control of CM. The objectives of this study were to: a) describe the incidence and pathogen distribution of CM in eight large dairy herds located in the State of New York; and b) describe the pathogen-level daily milk losses associated with CM in primiparous and multiparous cows in four dairy farms.

MATERIALS AND METHODS

Population description

Eight commercial dairy herds in Central New York State were followed from May 2016 to July 2017. These herds were conveniently selected based on a client list of the daily milk sample pick-up and 24-hour result program of Quality Milk Production Services (QMPS) at Cornell University. In this program, a courier from QMPS traveled to the herds to pick up milk samples from clinical cases every 24 hours (except New Year's Day, Christmas, Easter, 4th of July, and Thanksgiving). The main breed in these herds was Holstein-Friesian. All herds fed cows with a balanced total mixed ration (TMR) and participated in monthly DHI test (monthly individual SCC and linear score).

These herds use Dairy Comp 305 (DC305; Ag Valley Software, Tulare, California) as management software, and five herds had daily milk production based on milk meter recordings. All herds had a well-established milking routine where every case of CM was identified based on clinical signs (e.g., changes in milk and udder appearance). Every 24 h, a courier from the milk quality laboratory went to the herd and gathered the samples for etiology determination along with an electronic form containing the list of cows identified with CM since the last visit.

Herds with daily milk yield information were A (cows milked 3 times/d; AFI on-herd milk meter [S.A.E. Afikim, Kibbutz Afikim, Israel]), C (cows milked 3 times/d; AFI on-herd milk meter), E (cows milked 3 times/d; Delaval MM27 [DeLaval, Tumba, Sweden]), and H (cows milked 2 times/d; Boumatic on-herd milk meter [Boumatic Dairy Equipment, Madison, WI]).

Clinical mastitis sample collection and processing

Trained farm workers identified all CM cases in the herds at each milking process. Quarters were forestripped and the identification of the disease was based on changes in the milk

appearance (e.g., watery, flakes, clots, serous milk). Once identified in the milking parlor, the cow was sorted and a milk sample was collected from the affected quarter after disinfection of the teat end using cotton pads soaked with 70% ethanol and following National Mastitis Council guidelines (NMC, 2017). Sample collections were performed either before or after milking and the vials were labeled with cow ID, affected quarter, and date of CM. If more than one quarter was affected in the same cow, one vial per affected quarter was collected.

After sample collection, milk samples were immediately on-farm stored in a refrigerator (≅ 4ºC) and the CM-related information (i.e., ID, affected quarter and date) recorded in the DC305 as a MAST event. Every 24 h, a courier from the Quality Milk Production Services Laboratory at Cornell University traveled to the farm to gather the samples along with an electronic form containing the list of cows with CM identified since the previous visit. Milk samples were transported in coolers with ice packs and the electronic record was uploaded to the QMPS laboratory computers.

Laboratory analysis

Upon arriving at the laboratory, approximately 0.03 mL of milk was streaked using a sterile wood handle cotton swab (Puritan Medical Products Company, Guilford, Maine) on trypticase soy agar containing 5% sheep blood and 1% esculin (PML Microbiologicals, Mississauga, ON, Canada), and then incubated aerobically at 37°C for 18 to 24 h.

At 24 h, plates with monoculture or up to two different pathogens, based on colony morphology, were selected for additional analysis. Plates with ≥3 morphologically distinct colonies were considered contaminated unless a contagious pathogen (e.g., *Staphylococcus aureus* or *Streptococcus agalactiae*) was identified based on morphology. This feature was also considered for those results with two different pathogens detected. Colonies were evaluated with

matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF), using a Bruker MALDI-TOF Biotyper (version 3.1.66; Bruker Corp., Billerica, MA) by direct smear method (Bizzini et al., 2010). Results from MALDI-TOF MS with scores of ≥2.000 indicated a species-level identification, a score of 1.700–1.999 indicated a genus-level identification, and a score of ≤1.699 indicated that no reliable identification could be performed.

An intramammary infection was defined as the isolation of three or more identical colonies from cultured milk for all bacteria except non-aureus *Staphylococcus* (NAS), which required ≥6 colonies for confirming the infection. If two different pathogenic colony types were present, the culture was defined as mixed culture. Cultures with ≥3 morphologically distinct colonies were considered contaminated unless a contagious pathogen (e.g., *Staphylococcus aureus* or *Streptococcus agalactiae*) was identified based on morphology. A sample was defined as "no growth" if no colonies were observed on the agar plate after 48 h of incubation.

Preliminary laboratory results were automatically transferred from the QMPS laboratory into the herd computer within 24 h and confirmatory results were sent before 48 h through the internet LOOP (iLOOP). The iLOOP is a transfer method that provides the process for two-way data for DC305 software users. At the herd, affected cows were treated according to individual herd protocols based on culture results.

Data collection

Every month, a weekly backup file that was created and stored on the herd computer was collected using the 'save\c' command. These weekly backups were transferred into a USB flash drive and then stored on the virtual cloud where every herd had its file according to each week, month and year. The DC305 files were used to collect additional cow-level data and to create a monthly dataset including all cows in the herd and then transferred to the main dataset that

included each monthly dataset. The second dataset with daily milk yield for each cow in each herd (regardless of CM occurrence) was created from May 2016 to July 2017. These two datasets were saved to Excel spreadsheets (Microsoft Corp; Redmond, WA).

Daily milk records were available in four (i.e., A, C, E, and H) out of the eight herds in the study. These daily records were available from May 8, 2016, to July 2, 2017.

Selection of mammary-quarter CM cases

All lactating quarters were eligible for inclusion of CM. A quarter was at risk for a new CM case if there was at least 14 d between a previously diagnosed case and the current case, or if a different pathogen was isolated in the same quarter within 14 d. The study started on May 1, 2016, and ended on May 31, 2017; however, the data was collected starting on May 15, 2016, to establish an enrollment baseline (all new cases according to our definition). The at-risk period for a mammary quarter started at the beginning of each month or, at the date of calving and ended at the end of the month, at the day of CM diagnosis, or when cows exited the study (e.g., cull, death, or dry off).

Incidence of Clinical Mastitis

The Incidence risk at the cow level (IRiC), within herds, was calculated monthly as the number of CM cases divided by the number of at-risk cows in the population at the beginning of each month and then multiplied by 100. The incidence rate at the quarter level within a month (IRaQ) was calculated monthly as the number of CM cases divided by the number of quarter-days at risk (QDAR) in each month and then multiplied by 10,000 quarters. The size of this 10,000-multiplier factor was chosen to have a final value that would be expressed as a whole number. The number of QDAR was calculated as the sum of days that each quarter remained without CM and in the herd during a given month, considering the number of lactating cows at

the beginning of the month and assuming that all cows had four functional quarters.

$$\text{IRaQ(monthly)} = \left(\frac{Number\ of\ CM\ cases}{Number\ of\ QDAR}\right) X\ 10{,}000\ \text{quarters}$$

Diseases other than mastitis

We included four diseases in the statistical model as potential confounders: ketosis, displaced abomasum, and retained placenta, which are conditions that may have a drop effect on milk production besides the losses associated with CM cases. The occurrence of these diseases was recorded based on diagnosis performed by the herdsmen and stored into DC305.

Statistical analysis

Data were imported into R version 4.0.4 (RStudio: Integrated Development for R. RStudio, Inc., Boston, MA) to perform statistical analysis and to create the appropriate plots. Descriptive statistics were performed to observe frequency distributions. The table function was used for categorical variables (culture result, affected quarter) using frequency tables. The summary function was used to provide descriptive statistics of continuous variables (e.g., parity, DIM).

The two primary datasets (monthly and daily milk yield) were merged, resulting in the main dataset with cow-specific level information. The dataset included unique herd ID (to avoid repeated numbers between herds), daily milk yield, calving date, parity, DIM at CM detection, affected quarter, isolated pathogen, treatment for CM (yes/no), and other diseases (yes/no)

A mixed-effects model was used to assess the effects of a pathogen from a CM case and other variables over time on milk yield. The lme function from the nlme package (Pinheiro et al., 2017) was used to assess the effects of the mastitis pathogen and their correspondent interaction with the days before and after CM case while controlling other variables (e.g., parity, stage of lactation at CM detection, treatment, and other diseases) on daily milk yield. Using the cow-level

data, the model response for each set of parity (primiparous or multiparous) were daily milk yield measurements for a specific animal. Daily milk yield was modeled from 49 days before to 49 days after the event of mastitis. The explanatory variables were parity, stage of lactation at the time of diagnosis, isolated pathogen, treatment of CM case, and other diseases diagnosed in the same lactation.

The number of lactation was divided into two sets: primiparous (first lactation) and multiparous (≥2 lactations). Within this multiparous set, three levels were included: second, third, and >4 lactations. The stage of lactation at the time of diagnosis with respect to DIM was distributed in 4 categories: early lactation (calving-50 DIM), peak lactation (50-100 DIM), mid-lactation (101-200 DIM), and late lactation (201 DIM to dry-off). For the isolated pathogen, we considered *E. coli*, *Klebsiella* spp., no growth, *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactie*, NAS, *Streptococcus* spp., mixed results, and yeast. We excluded cases in the same cow if occurred within the 14 days evaluation period to avoid the effects of a different pathogen in the estimation in the time variable. Each CM case was matched with a control based on herd, DIM (±7 d), and parity, and those were considered as no mastitis cows and used as the reference category in each model. Cells with zero values in the daily milk yield dataset were evaluated and considered as missing at random (e.g., ID detector malfunction). Multiple imputation using Amelia II package (Honaker et al., 2011) was used to reduce the impact of zero-values on repeated measures.

Because daily milk yield measurements are correlated to each cow and that each cow belongs to a specific herd, we assumed that the milk recording of those cows in the same herd is also correlated within the herd (Heikkilä et al., 2018a). For this reason, we considered a nested model of cows within-herd and milk recordings for each cow within the herd. To incorporate this

attribute into the model, herd and ID were considered random effects and an autoregressive (order 1) correlation structure was used. This structure considers milk yield correlations to be highest for time adjacent times and decreasing with increasing distance between time points; it has also being used by others when analyzing milk losses (Gröhn et al., 2004; Schukken et al., 2009; Heikkilä et al., 2018b).

Three different models were considered: 1) herd as a random effect with no correlation structure; 2) herd and ID as random effects and no correlation structure; and 3) herd and ID as random effects and a first-order autoregressive correlation structure. Models goodness-of-fit was assessed using the method of maximum likelihood to allow comparisons between models using Akaike's information criterion (AIC). The model with the minimum AIC was number 3.
The following model was used for each one of the pathogens preciously stated:

$$Y = \text{mastitis-causing pathogen} + \text{DRTCM} + \text{mastitis-causing pathogen} \times \text{DRTCM} + \text{parity} + \text{stage} + \text{treatment} + \text{other diseases} + \text{herd} + Re,$$

where Y is daily milk yield (kg/d), DRTCM (days relative to CM case: 49 d before and 49 d after) as time variable; parity (primiparous and multiparous according to sub-categories); stage of lactation (4 categories: early-, peak-, mid-, and late-lactation); treatment (yes or no); other diseases (yes or no; retained placenta, ketosis, DA); herd and ID as random effects; and Re a complex error term containing the autoregressive term and random residual.

A fourth model with the time variable being week relative to CM case: 7 weeks before and 7 weeks after was also used to assess the daily milk losses in a given week, where the 7 daily values of each week were summed and divided by 7 to give the average daily milk yield for that particular week relative to the CM event.

RESULTS

Descriptive results and Clinical Mastitis Incidence

Eight large dairy herds located in the State of New York, USA were enrolled in this study (Table 3.1). All lactating cows were housed in free-stalls bedded with four types of materials: manure solids (n=3); paper pulp (n=2); sand (n=2), and recycled sand (n=1). The average herd size was 1,576 lactating cows (ranging from 1,075 to 1,942) with average milk production of 41 kg/cow/d, ranging from 39 to 43 kg/cow/d. Monthly mean SCC was 234,000 cell/mL, ranging from 145,000 to 361,000 cells/mL.

A total of 8,199 CM cases were recorded over the study period (Figure 3.1). However, 668 (8.1%) cases were excluded from the analysis because the CM event occurred in the same quarter within 14 days after a previous case and presented the same culture result. For the remaining 7,513 CM cases, a total of 17,513,429 QDAR were estimated. The monthly IRi was 4.8 cases per 100 cows, ranging from 1.2 to 7.5. The monthly IRaQ mean was 4.4 cases per 10,000 QDAR, ranging from 1.0 to 7.0 (Table 3.1).

Concerning pathogen distribution, no growth was the most common microbiological result with 2,224 (29.6%) samples in this category; followed by 2,316 (30.8%) gram-positive and 1,978 (26.3%) gram-negative. In less proportion, mixed culture was found in 376 (5.1%) samples, 503 (6.6%) were other pathogens isolated less frequently from mastitis, and 128 (1.7%) were contaminated milk samples. Out of the positive results; *Escherichia coli* was the most prevalent pathogen, cultured in 1,345 (17.9%) cases, followed by *Streptococcus uberis* with 744 (9.9%) cases, *Streptococcus dysgalactie* with 654 (8.7%) cases, *Klebsiella* spp. with 503 (6.7%) cases, and *Staphylococcus aureus* with 233 (3.1%) cases (Figure 3.2).

Among all reported cases, 1,450 (19.3%) occurred in early lactation, 1,477 (19.6%) in the

peak, 2,542 (33.8%) during mid-lactation, and 2,044 (27.2%) in late lactation. Most cows (95%) had CM in only one quarter at a given time. In terms of quarter position, 3,734 (49.7%) cases occurred in front quarters and 3,779 (50.3%) on rear quarters.

There were differences between the herds in pathogen-specific distribution. *Staphylococcus aureus* was present in 5 out the 8 herds in different proportions (from 1 to 10% of all cases), while *Escherichia coli* was a common cause of CM in all herds (from 11 to 33% of all cases). As for the minor environmental pathogens, yeast was also present in 5 herds, being isolated in 1 to 6% of all cases. The only result that was constant in all herds was no growth, representing about a third of all culture results in each herd (Figure 3.3).

Milk losses among primiparous (first-lactation) cows by pathogen

A total of 630 primiparous cows and their daily milk weights (with a laboratory result of a pathogen included in the model) were selected. However, 175 were eliminated because there were at least three consecutive missing values. The final number of multiparous cows with CM included in the model was 455, which were compared with their corresponding controls. This final number does not include cows affected by those pathogens in which the number of cases was less than 30. Average daily milk production from those cows corresponding to 49 d before and 49 d after CM diagnosis is presented in the Figure 3.4.

Daily milk loss estimates associated with each pathogen and corresponding confidence intervals are presented in Table 3.2. Figure 3.5 shows the corresponding milk yield prediction based on the mixed model for a control cow (no mastitis) and a cow with CM for each pathogen (A–F). Those primiparous with no growth result had a median of diagnosis at 170 DIM (±125.35), with a milk yield drop of 6.54 kg at CM detection and lasting for 1 week after, losing 4.94 kg/d. The total milk production loss up to 49 d after CM detection was 41.12 kg. (Figure

3.5A).

Primiparous cows affected with *E. coli* had a median of diagnosis at 151 DIM (±97.8) and started the milk production loss 1 week before CM detection (-2.84 kg/d), followed by a severe loss of 16.3 kg that occurred on the day of diagnosis. This loss continued for 4 weeks (until ~36 d after CM detection). The total milk production loss up to 49 d after CM detection was 214.26 kg. Overall, cows identified with *E. coli* CM produced 3.79 kg/d less milk compared to control cows (Figure 3.5B).

Primiparous cows with isolation of *Staphylococcus aureus* had a median diagnosis at 214 DIM (±109.2) and produced 3.26 to 4.38 kg/d less milk compared to their controls from week 7 before CM detection. Those cows showed a severe drop of milk production at CM detection (9.98 kg) and a continued milk loss up to 6 weeks after CM detection. The total milk production loss up to 49 d after CM detection was 259.25 kg (Figure 3.5C).

Cows in their first lactation that were identified with *Streptococcus uberis* had a median diagnosis of 172 DIM (±122.9) and had a drop in milk yield on the day of CM detection of 13.23 kg/d. The reduction of milk production, in comparison to controls, only continued for 1 week after the CM detection, with the cows losing 11.76 kg/d in that week. The total milk production loss up to 49 d after CM detection was 95.55 kg (Figure 3.5D).

Cases with isolation of *Streptococcus dysgalactie* had a median diagnosis at 183 DIM (±88.08). These cows had a drop in milk yield of 13.59 kg on the day of CM detection. Then a slow recovery was observed, which lasted until week 4 after detection. The average milk production was 6.00 kg/d lower than their controls. The total milk production loss up to 49 d after CM detection was 236.05 kg (Figure 3.5E).

Finally, primiparous cows that had CM with isolation of NAS had a median of diagnosis

at 203 DIM (±90). These cows started to show a reduction in milk yield on the day of CM detection, losing 9.39 kg on that day. The total milk production loss up to 49 d after CM detection was 58.67 kg. In addition, cows in this group produced 7.04 kg/d in the first week after detection compared to control cows (Figure 3.5F).

Milk losses among multiparous (>1 lactation) cows by pathogen

A total of 1,950 multiparous cows were selected for the study. Of these, 633 were excluded from data analysis because there were at least three consecutive missing values for daily milk yield. The final number of multiparous cows with CM included in the model were 1,317. All of these cows had a corresponding healthy cow for comparison. The final number did not include cows affected by those pathogens in which the number of cases was less than 30. Figure 3.1 illustrates the average daily milk production from 49 d before to 49 d after CM detection.

Daily milk loss estimates associated with each pathogen and corresponding confidence intervals are presented in Table 3.3. Figure 3.7(A–I) shows the corresponding milk yield for control cows (no mastitis) and for cows with CM caused by one of the major pathogens.

Multiparous cows that had CM with no growth as a result in the milk sample had a median of diagnosis at 138.5 DIM (±100.1). Cows in this group showed a drop in milk yield of 6.77 Kg at the day of CM detection. Losses in milk production (1.95 Kg/d) continued for 2 weeks after CM detection in comparison to non-mastitis cows. The total milk production loss up to 49 d after CM detection was 54.72 kg (Figure 3.5A).

As for multiparous cows infected with *E.coli*, the median DIM at diagnosis was 107 (±88.1). The drop in milk yield started 1 week before the CM detection, with cows losing 2.28 Kg/d. At the day of diagnosis, cows had a severe loss in milk production (22.2 kg), then the milk

losses 2.02 dg/d extended for 6 weeks compared to control cows. The total milk production loss up to 49 d after CM detection was 251.80 kg (Figure 3.5B).

Cows affected with *Klebsiella* spp. CM had a median of diagnosis at 127 DIM (±92.4). Multiparous cows affected by this pathogen showed a severe milk drop (18.66 Kg) at the day of diagnosis compared to control cows. These losses continued until 5 weeks after detection, with cows losing 4.14 kg/d. The total milk production loss up to 49 d after CM detection was 269.96 kg (Figure 3.5C).

Cases with isolation of *Streptococcus uberis* had a median DIM at diagnosis of 108 (±91.5). These cows were higher producers compared to the control cows for 7 weeks after CM detection, then had a remarkable drop in milk yield at CM detection (13.29 Kg). During the first week after diagnosis, CM cows produced 13.89 kg/d less milk than control cows. Significant losses in milk production (3.93 kg/d) continued for 2 weeks after detection in cows with *Streptococcus uberis* CM compared to their controls, with a total milk production loss up to 49 d after CM detection of 138.03 kg (Figure 3.5D).

Multiparous cows with a CM caused by *Streptococcus dysgalactie* had a median time at diagnosis of 138 DIM (±103.6). These cows started to show a drop in milk yield 1 week before CM detection, losing 4.18 kg/d. The greatest loss was observed on the day of CM diagnosis (11.36 Kg), then a slow recovery lasted 4 weeks after detection. During that period, mastitic cows produced 4.35 kg/d less milk compared to their controls and the total loss after CM detection was 205.05 kg (Figure 3.5E).

The median DIM at diagnosis of cows identified with *Streptococcus spp*. CM was 153 (±93.6). At diagnosis, cows affected cows produced 11.18 kg/d less milk than control cows, and these losses continued for the week after detection, when they produced 9.25 kg/d less milk than

their herd controls, losing a total of 75.93 kg after CM detection (Figure 3.5F).

Cows with isolation of NAS had a median of 136 DIM (±101.3) at diagnosis and produced 9.67 kg less milk than control cows at the diagnosis day. Milk losses (6.78 Kg/d) continued only for 1 week after detection. The total milk production loss up to 49 d after CM detection was 57.13 kg (Figure 3.5G).

Clinical mastitis cases with a mixed culture result had a median diagnosis at 132 DIM (±93.1). These cows had a drop of 14.37 kg in milk yield at CM detection. Milk losses continued for at least 5 weeks after detection, during which cows with mastitis produced 4.37 kg/d less milk compared to their controls (Figure 3.5H).

Finally, multiparous cows with isolation of yeast had a median DIM at diagnosis of 124 (±96.9). Unexpectedly, cows that were infected with yeast produced an average 7 Kg/d more milk than their controls before CM diagnosis. However, these same cows produced 15.33 Kg less milk than controls on the day of CM diagnosis. Losses in milk production lasted for 1 week after detection, and the cows never recovered from the initial production yield up to 49 days after CM diagnosis (Figure 3.5I).

Figure 3. 1 Flow-chart showing the enrollment of herds and cows in a cohort study evaluating the CM incidence, pathogen distribution, and daily milk losses related to CM events.

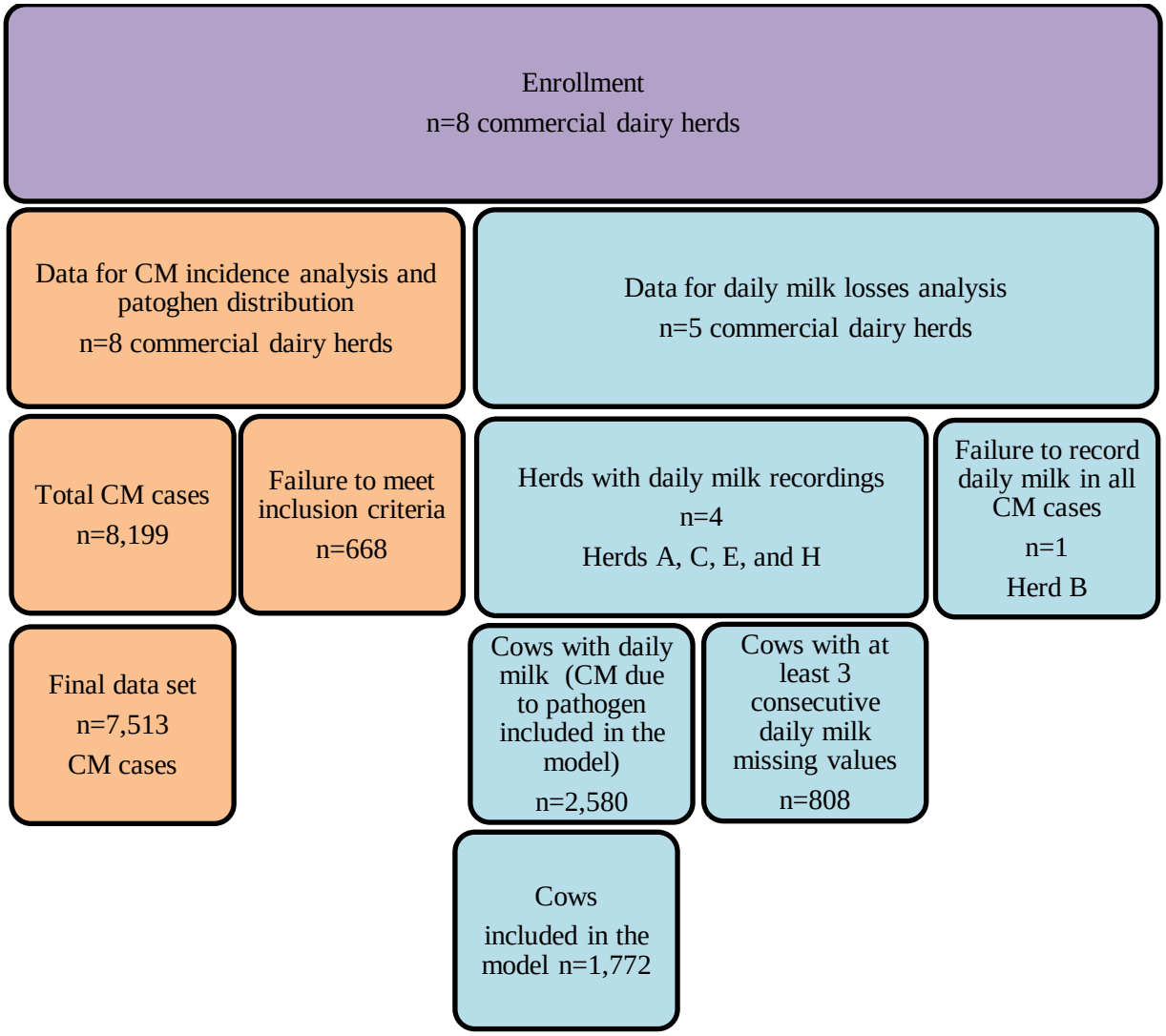


Table 3. 1 Descriptive characteristics of eight herds from Central New York, United States, average monthly clinical mastitis for incidence risk (IRiC) at cow-level and incidence rate at quarter-level (IRaQ) evaluated over 1 year (SD in parentheses).

Herd	Lactating cows (monthly average)	Milk yield kg/day	BTSCC[1]	Bedding material[2]	All CM	IRiC[3]	IRaQ[4]
A	1,354	43 (0.9)	186	Sand	1,333	7.5	7.0
B	1,970	42 (1.3)	145	Manure solids	1,455	5.7	5.3
C	1,075	40 (1.3)	313	Manure solids	759	5.4	5.1
D	1,593	39 (0.9)	297	Manure solids	1,075	5.2	4.9
E	1,223	41 (0.9)	185	Paper pulp	790	5.0	4.3
F	1,452	39 (1.3)	361	Paper pulp	770	4.1	3.8
G	1,996	43 (0.9)	146	Sand	1,036	4.0	3.8
H	1,942	40 (0.9)	198	Recycled sand	295	1.2	1.0
Sum	-	-	-	-	7,513	-	-
Mean	1,576	41	234	-	-	4.8	4.4

[1]BTSCC =Bulk Tank Somatic Cell Count ($\times 10^3$ cells/mL)
[2]Predominant bedding material in lactating pens.
[3]IRiC = Incidence risk at cow level: number of CM cases divided by the number of cows in the population at the beginning of each month and then multiplied by 100.
[4]IRaQ = Incidence rate at the quarter level: number of CM cases divided by the number of QDAR in each month and then multiplied by 10,000.

Figure 3. 2 Overall pathogen-specific distribution of all clinical mastitis cases (n=7,513) occurred in dairy cows from eight commercial herds from Central New York, United States over a year.

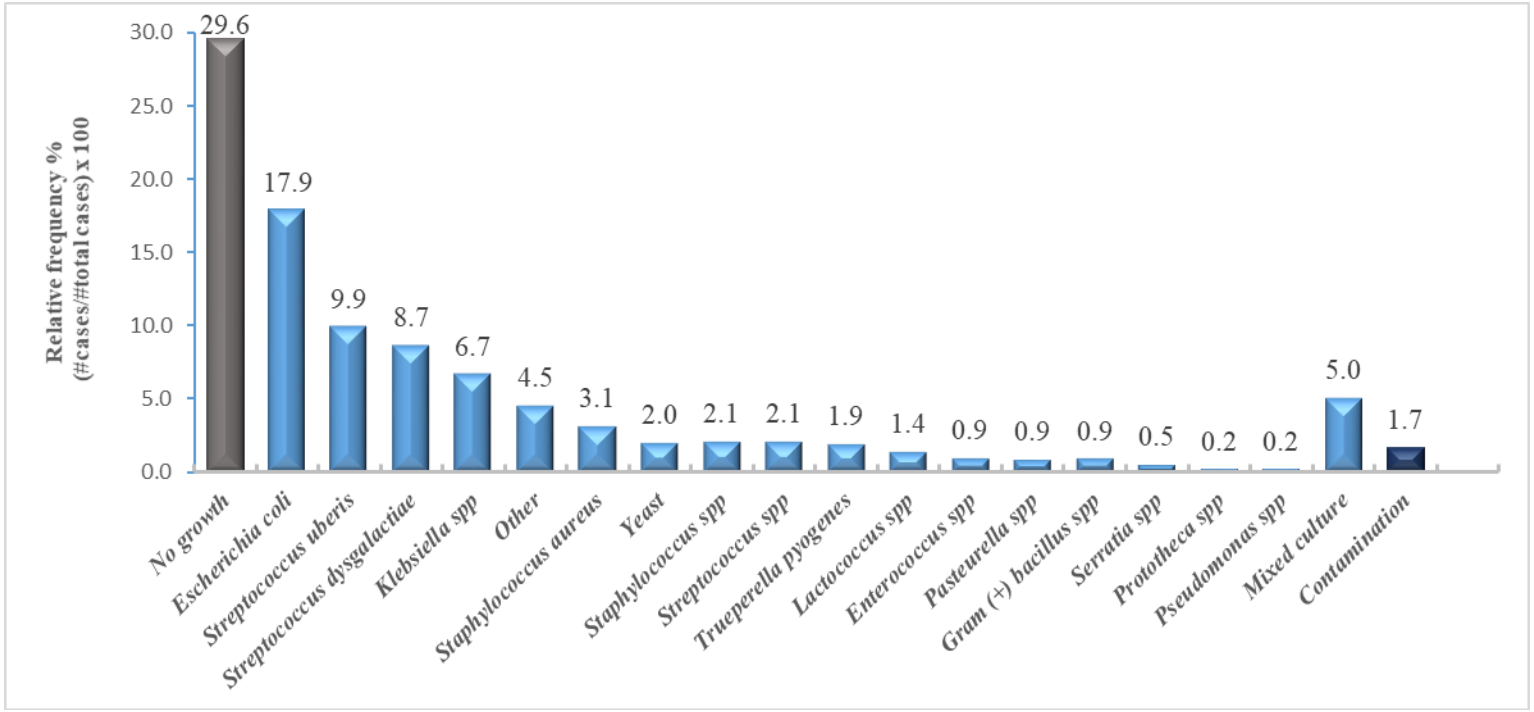


Figure. 3 Pathogen distribution by herd of all clinical mastitis cases (n=7,513) occurred in dairy cows from eight commercial herds from Central New York, United States over a year.

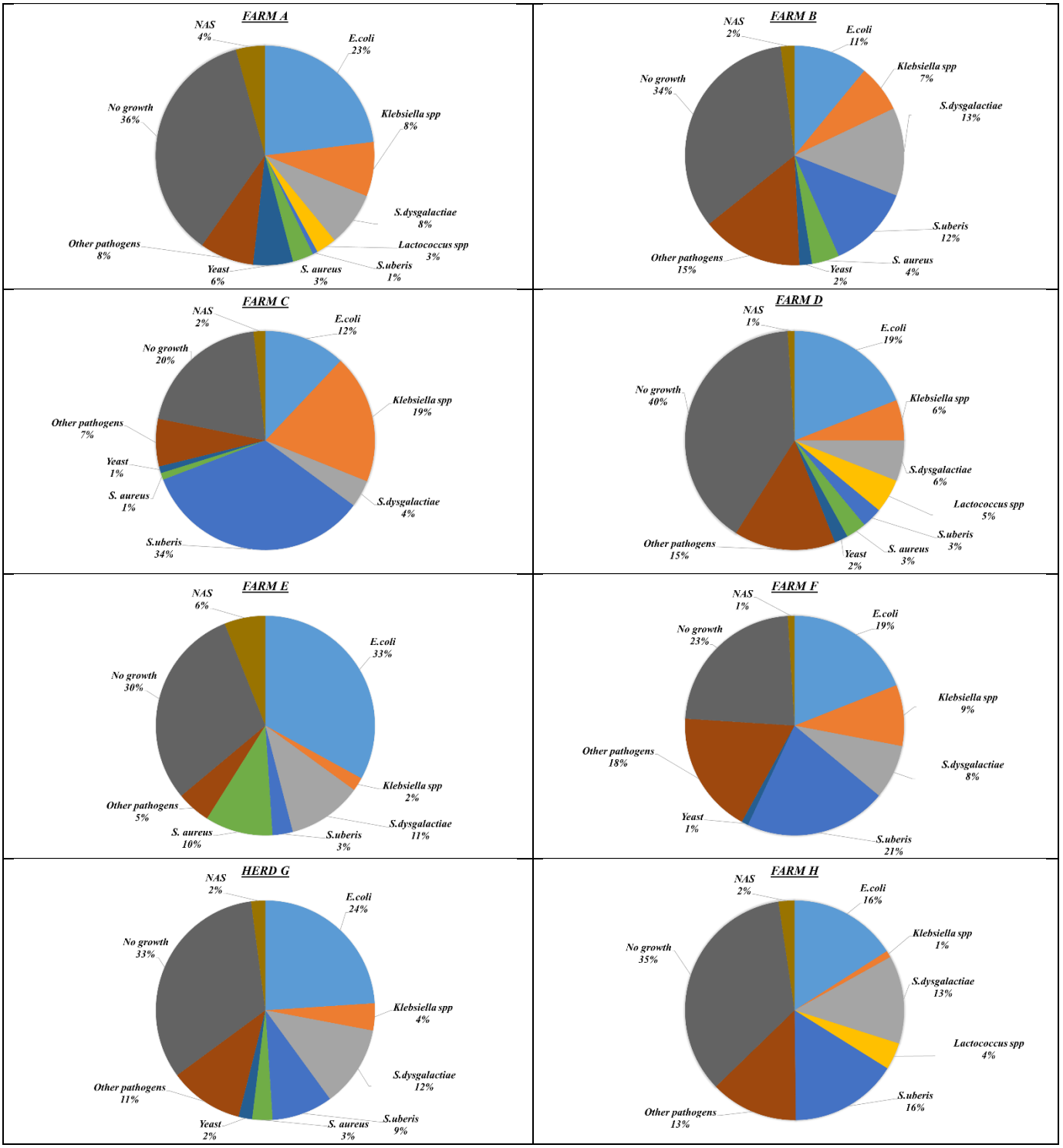
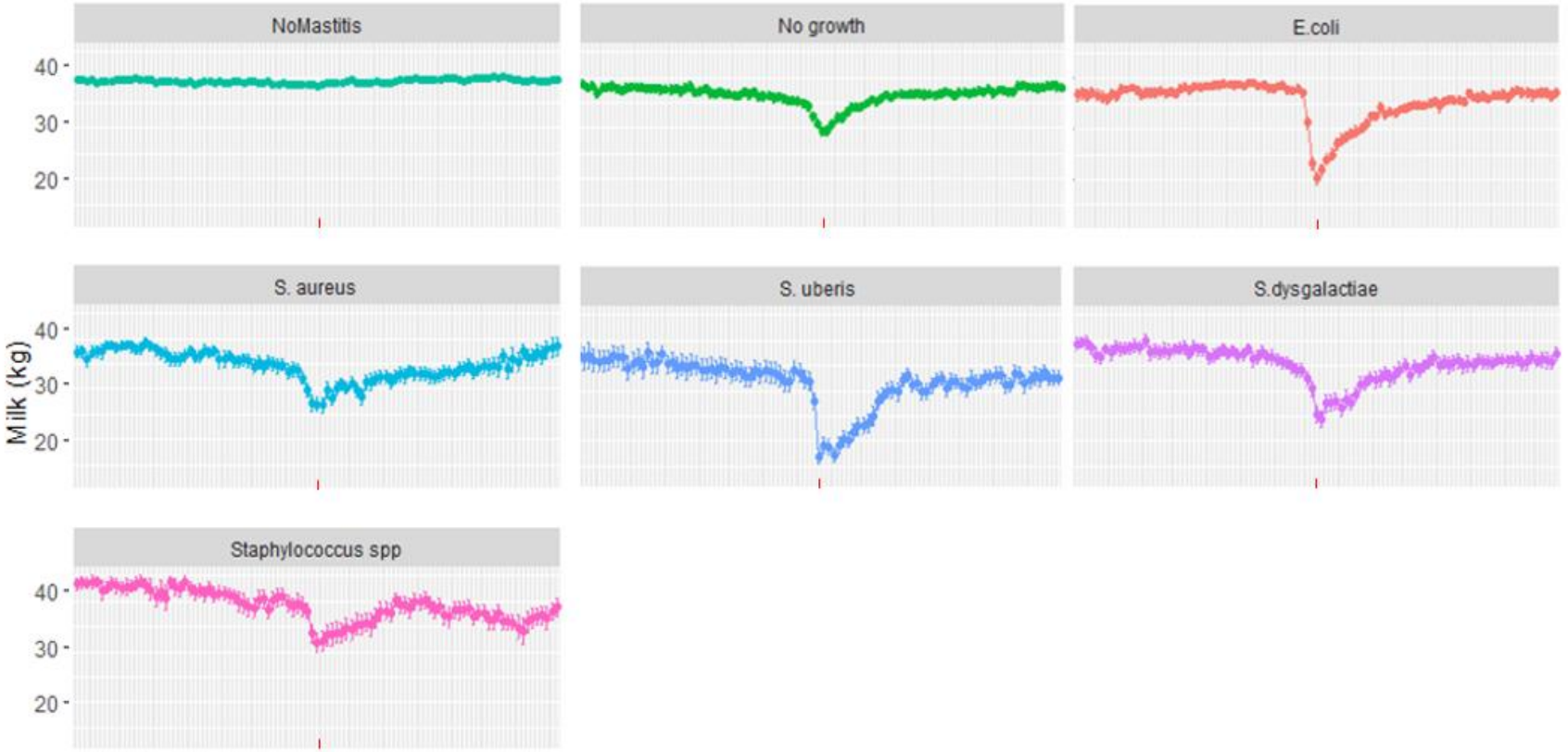


Figure 3. 4 Average daily milk production (kg/d) of primiparous cows (parity =1) in four New York commercial dairy herds[1] followed for one year. Error bars represent the standard deviation (SD). Each graph represents a pathogen-specific and controls (No Mastitis)[2]. Each point represents one day: 49 d before and 49 d after CM detection (Red mark represents Day at CM detection).



[1]Herds with daily weights: A, C, E, and H
[2]Each control (NoMastitis) cow was based on herd and DIM (±7d)

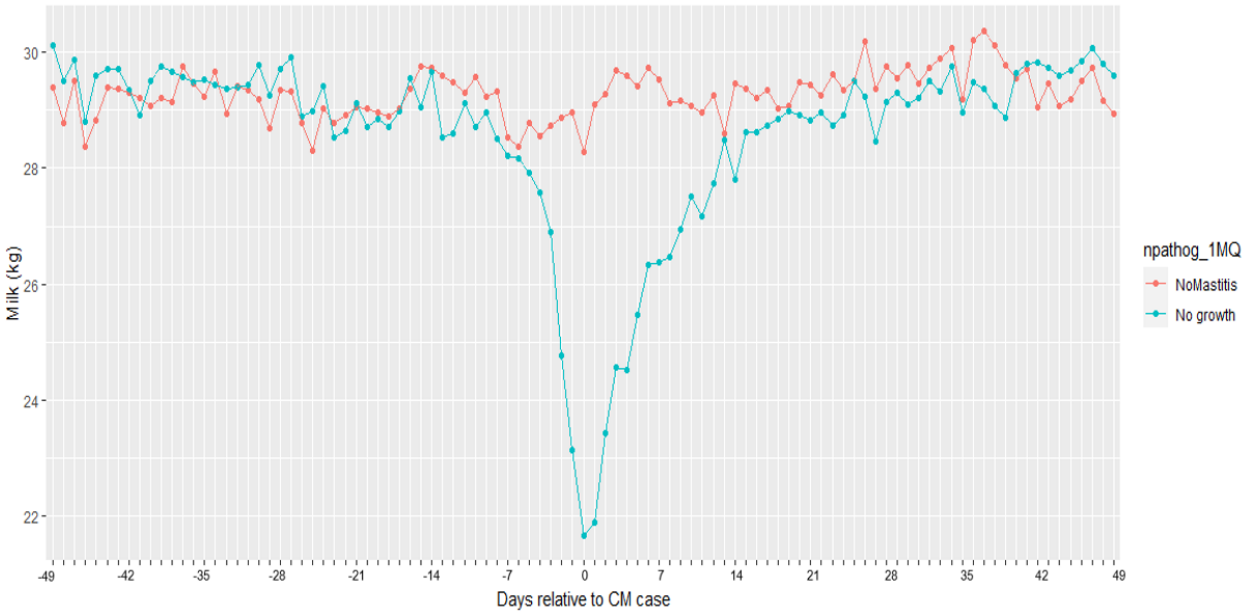
Table 3. 2 Effects of pathogen-specific clinical mastitis on milk yield of primiparous cows in 4 New York State dairy herds. Estimates obtained from the mixed model with autoregressive (AR1) covariance structure. Values in Kg/d, C.I= Confidence interval. Each cow was matched with a control based on herd, DIM (±7d), and parity, and those were considered as no mastitis cows and used as the reference category in each model.

Effect	**No growth**	***E. coli***	***S. aureus***	***S. uberis***	***S. dysgalactie***	***NAS***
	Estimate[1] 95% C.I	Estimate 95% C.I	Estimate 95% C.I	Estimate 95% C.I	Estimate 95% C.I	Estimate 95% C.I
7th week BCM	0.63 -1.72 – 2.99	0.28 -1.81 – 2.37	-3.26* -6.41 – -0.10	4.84 -4.52 – 14.20	-1.16 -6.79 – 4.47	3.27 -1.77 – 8.32
6th week BCM	0.32 -2.03 – 2.67	0.26 -1.82 – 2.36	-2.98 -6.13 – 0.17	5.00 -4.35 – 14.36	-1.52 -7.16 – 4.11	2.66 -2.38 – 7.70
5th week BCM	0.31 -2.03 – 2.66	0.50 -1.59 – 2.58	-4.13* -7.28 – -0.98	4.28 -5.06 – 13.63	-1.75 -7.38 – 3.88	3.09 -1.93 – 8.13
4th week BCM	0.32 .-2.03 – 2.66	0.83 -.125 – 2.92	-3.58* -6.72 – -0.43	3.44 -5.89 – 12.78	-2.97 -8.60 – 2.66	2.42 -2.60 – 7.44
3rd week BCM	-0.06 -2.41 – 2.28	0.77 -1.31 – 2.85	-3.19* -6.33 – -0.05	3.51 -5.81 – 12.84	-3.61 -9.24 – 2.02	1.38 -3.62 – 6.39
2nd week BCM	-0.47 -2.82 – 1.84	0.42 -1.65 – 2.50	-4.38* -7.51 – -1.24	4.00 -5.31 – 13.32	3.90 -9.53 – 1.73	1.17 -3.83 – 6.18
1st week BCM	-1.96 -4.31 – 0.37	-2.84* -4.91 – -0.76	-6.56* -9.69 – -3.43	1.22 -8.09 – 10.54	-5.59 -11.22 – 0.03	1.25 -6.27 – 3.75
CMD	-6.54* -9.20 – -3.89	-16.93* -19.42 – -14.45	-9.98* -13.64 – -6.33	-13.23* -23.09 – -3.38	-13.59* -19.52 – -7.65	-9.39* -15.07 – -3.72
1st week ACM	-4.94* -7.29 – -2.60	-11.20* -13.28 – -9.12	-8.82* -11.96 – -5.68	-11.76* -21.08 – -2.45	-11.55* -17.18 – -5.91	-7.04* -12.05 – -2.03
2nd week ACM	-1.67 -4.03 – 0.67	-5.48* -7.58 – -3.38	-6.79* -9.99 – -3.59	-5.05 -14.84 – 3.82	-7.66* -13.30 – -2.02	-3.51 -8.54 – 1.52
3rd week ACM	-1.61 -4.28 – 1.04	-4.88* -7.41 – -2.35	-4.78* -8.67 – -0.87	-3.26 -13.11 – 6.59	-6.57* -12.52 – -0.61	-1.30 -6.98 – 4.38
4th week ACM	-0.54 -2.89 – 1.80	-3.79* -5.88 – -1.70	-5.92* -9.10 – -2.75	-1.28 -10.61 – 8.04	-6.00* -11.64 – -0.36	-0.43 -5.45 – 4.58
5th week ACM	-0.57 -2.89 – 1.74	-1.86 -3.91 – 0.18	5.82* -8.93 – -2.70	-1.48 -10.76 – 7.80	-4.62 -10.23 – 0.98	-1.83 -6.79 – 3.12
6th week ACM	-0.34 -2.69 – 2.00	-1.11 -3.22 – 0.98	3.48* -6.71 – -0.25	-0.65 -9.99 – 8.69	-4.21 -9.84 – 1.42	-1.57 -6.59 – 3.45
7th week ACM	0.31 -2.04 – 2.66	-0.92 -3.02 – 1.18	-1.08 -4.34 – 2.19	-0.19 -9.54 – 9.15	-3.85 -9.49 – 1.78	0.01 -5.02 – 5.05

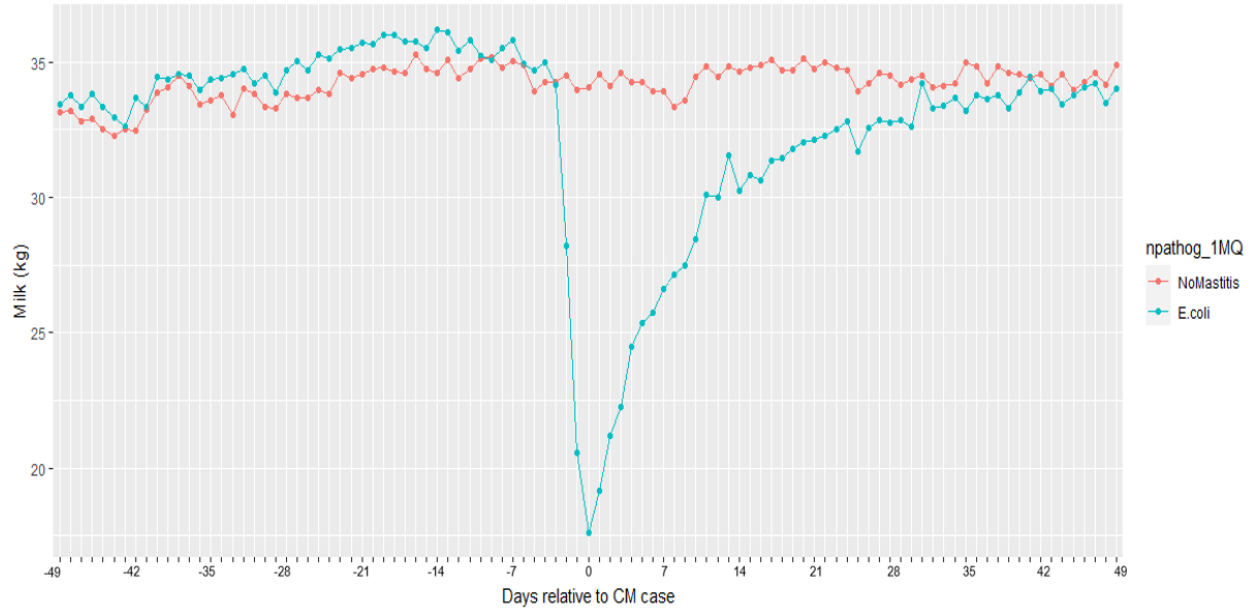
[1]Amount of milk (kg) gained (positive values) or lost (negative values) per day in a given week regarding CM detection
BCM = before clinical case diagnosis; CMD =clinical case diagnosis day; ACM = after clinical case diagnosis
*P < 0.05

Figure 3. 5 Average daily milk production prediction based on the model for primiparous cows (parity =1) infected with pathogens versus non-clinical controls [1], in 4 dairy commercial herds[2].

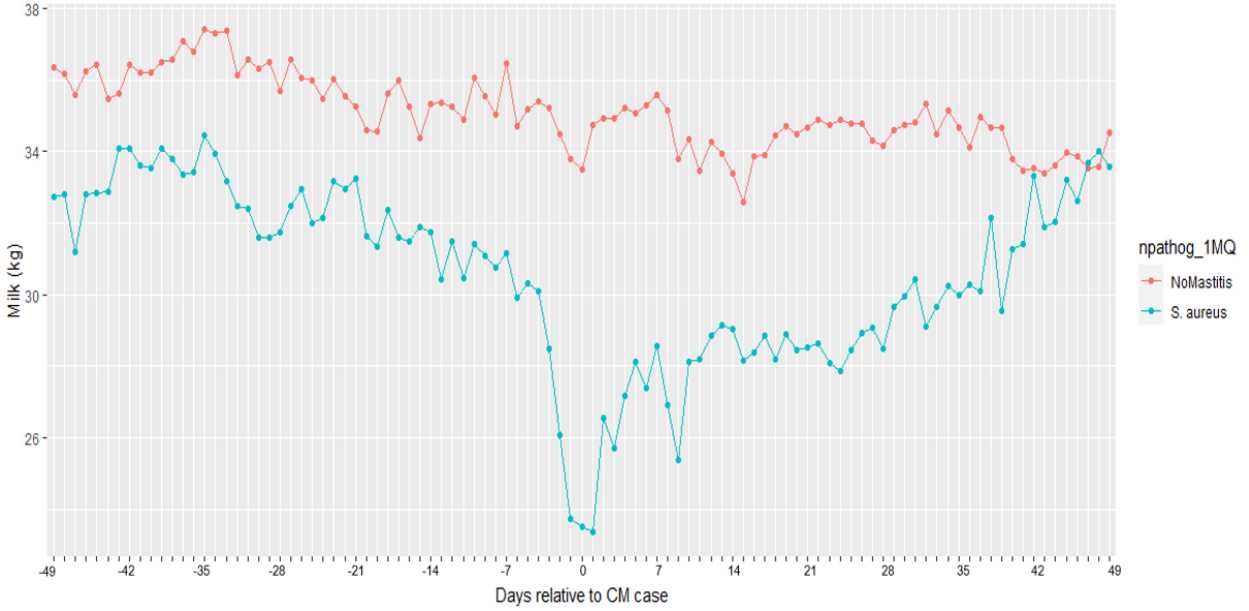
A) No growth (n=150)



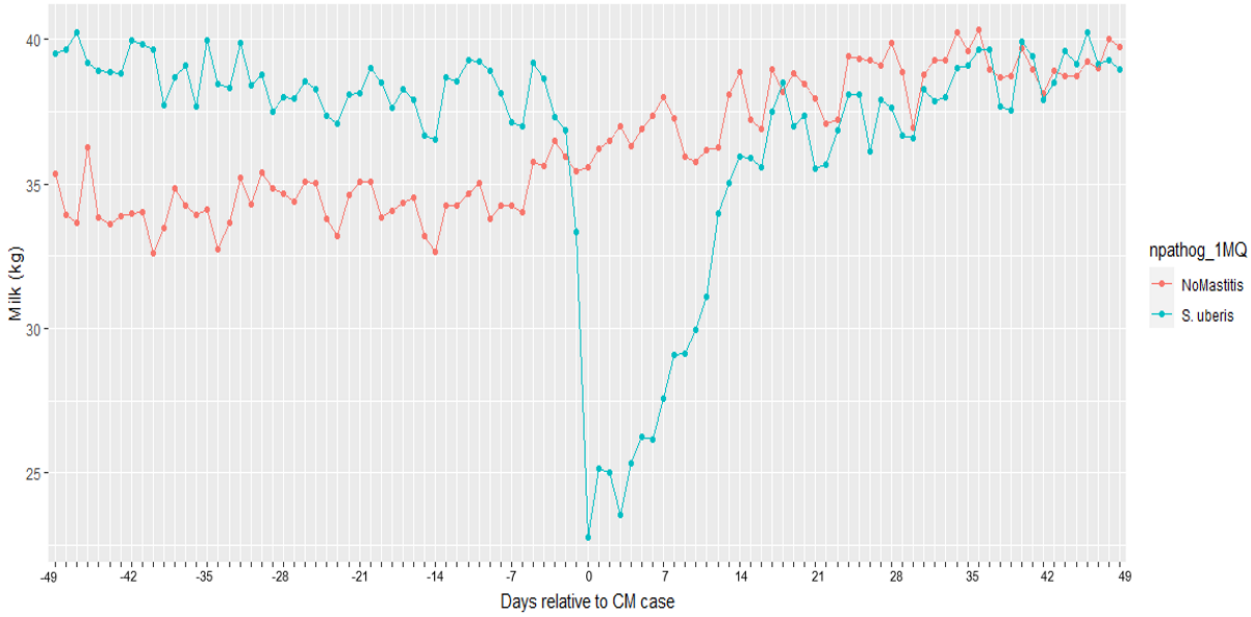
B) *E. coli* (n=124)



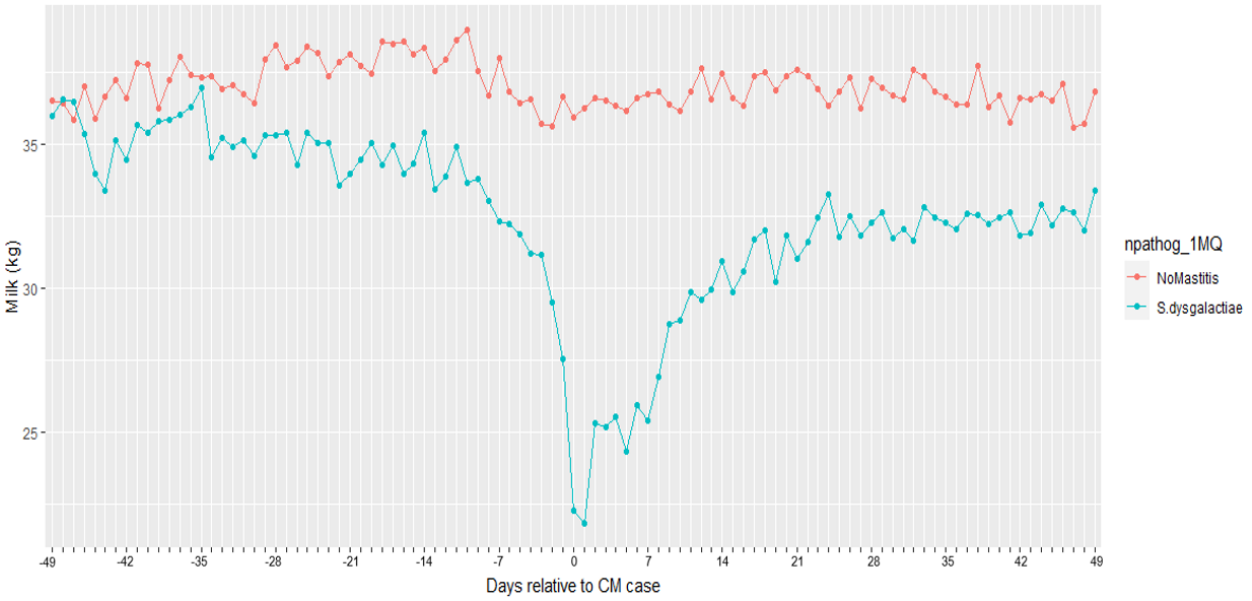
C) *Staphylococcus aureus (n=48)*



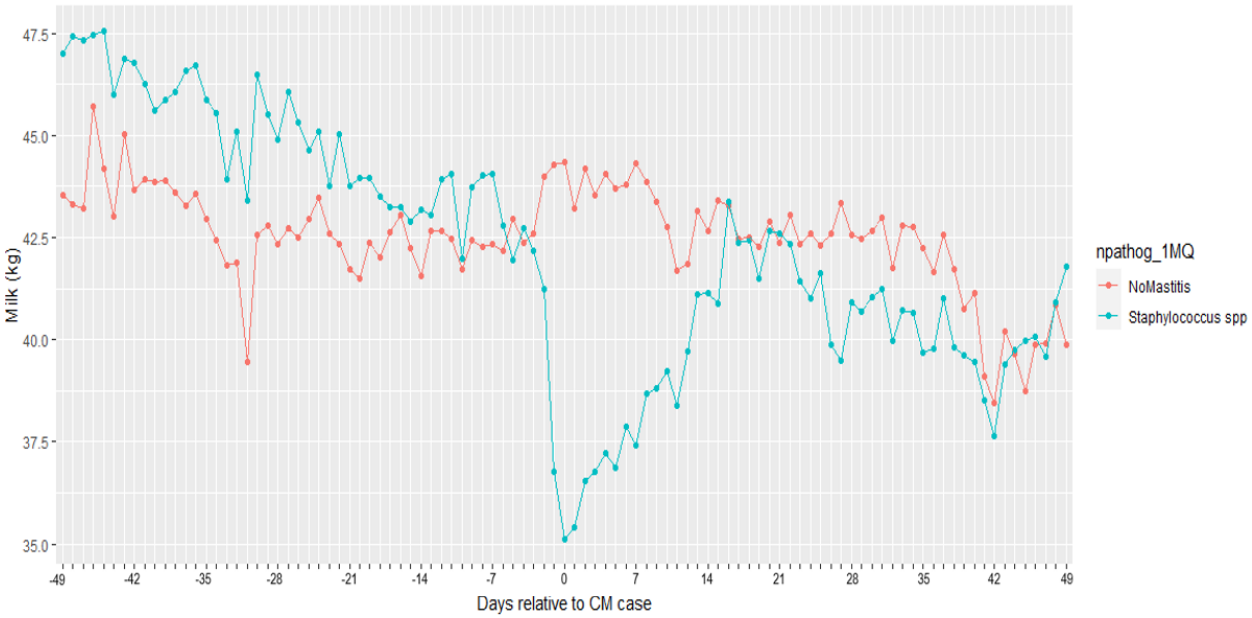
D) *Streptococcus uberis (n=53)*



E) *Streptococcus dysgalactie* (n=46)

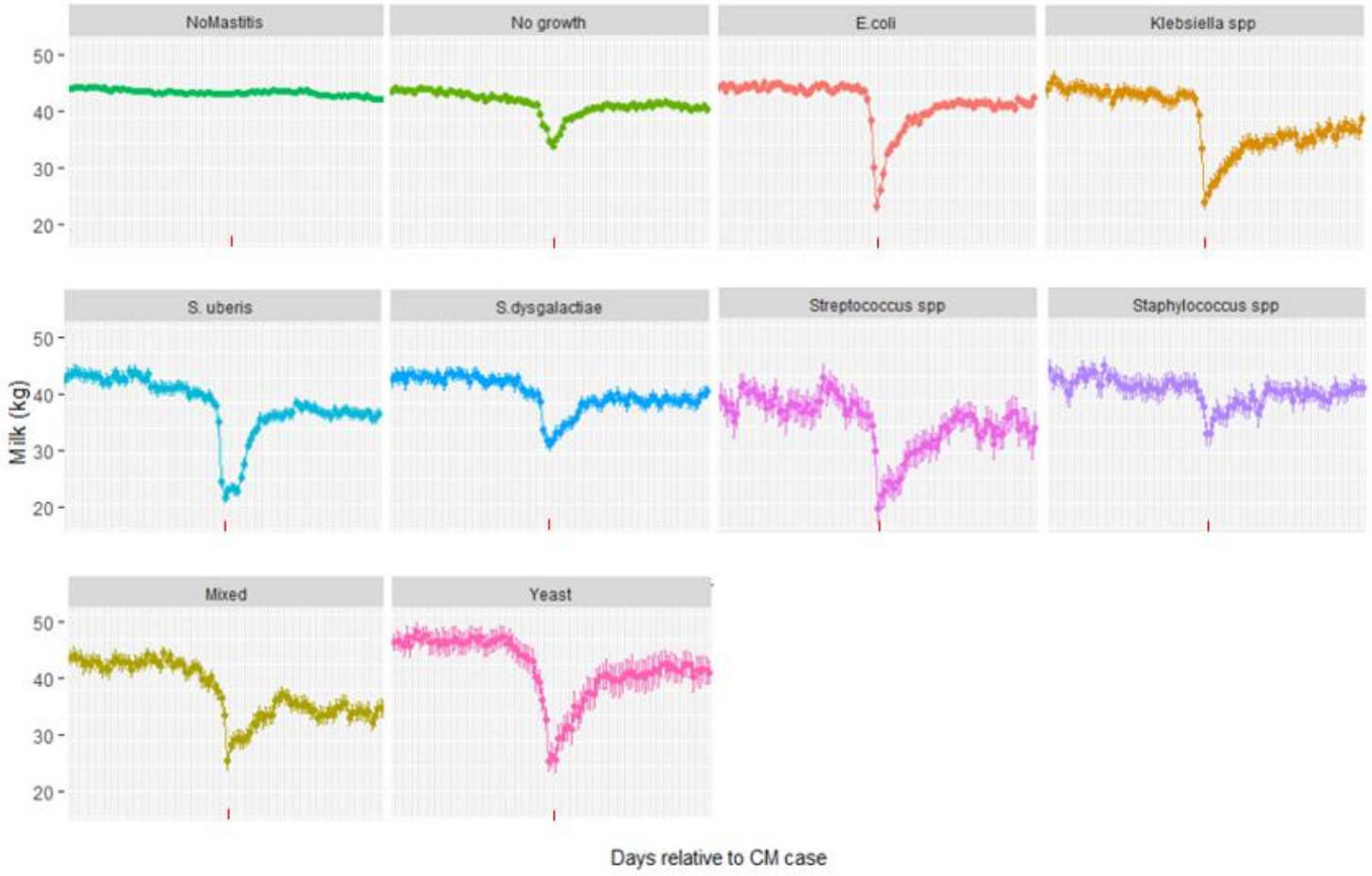


F) Non-aureus *Staphylococcus* spp (n=34)



[1]Each control (NoMastitis) cow was matched based on herd and DIM (±7d)
[2]Herds with daily weights: A, C, E, and H.

Figure 3. 6 Average daily milk production (kg/d) of multiparous cows (parity ≥2), in four New York commercial dairy herds[1] followed for a year. Error bars represent SD. Each graph represents a pathogen-specific and controls (No Mastitis)[2]. Each point represents one day: 49 d before and 49 d after CM detection (Red mark represents Day at CM detection).



[1]Herds with daily weights: A, C, E, and H
[2]Each control (NoMastitis) cow was based on herd, DIM (±7d), and parity

Table 3. 3 Effects of pathogen-specific clinical mastitis on milk yield of multiparous cows in 4 New York State dairy herds. Estimates obtained from the mixed model with autoregressive (AR1) covariance structure. Values in Kg/d, C.I= Confidence interval. Each cow was matched with a control based on herd, DIM (±7d), and parity, and those were considered as no mastitis cows and used as the reference category in each model.

Effect	No growth	*E. coli*	*Klebsiella* spp	*S. uberis*	*S. dysgalactie*	*Streptococcus spp*	*NAS*	*Mixed*	*Yeast*
	Estimate[1] 95% C.I	Estimate 95% C.I	Estimate 95% C.I	Estimate 95% C.I	Estimate 95% C.I	Estimate 95% C.I	Estimate 95% C.I	Estimate 95% C.I	Estimate 95% C.I
7th week BCM	1.28 0.40 –2.95	0.87 0.74 – 2.78	2.15 0.99 – 5.30	4.87* 2.82 – 8.29	0.21 -3.74 – 4.22	4.83 -3.06 – 12.71	-4.33 -9.91 – 1.25	3.22 -0.33 – 6.77	7.43* 1.04 – 13.82
6th week BCM	1.09 0.59– 2.78	0.73 0.88 – 2.34	1.88 1.25– 5.02	4.72* 1.34 – 8.10	0.24 -3.77 – 4.19	7.98 0.10 – 15.86	-3.14 -8.71 – 2.43	3.07 -0.47 – 6.61	7.15* 0.76 – 13.53
5th week BCM	1.28 0.40– 1.48	1.15 0.45 – 2.75	1.83 1.30 – 4.97	4.60* 1.23 – 7.98	0.86 -3.12 – 4.84	5.06 -2.80 – 12.93	-1.60 -7.16 – 3.95	2.37 -1.16 – 5.91	7.37* 2.34 – 13.76
4th week BCM	2.52 0.85 – 2.55	1.16 0.43 – 2.76	2.44 0.68 – 5.58	5.64* 2.27 – 9.02	0.11 -3.86 – 4.09	7.41 -0.44 – 15.26	-0.68 -6.23 – 4.87	3.14 -0.38 – 6.67	7.08* 0.70 – 13.46
3rd week BCM	0.81 0.87 – 2.49	1.67 0.07 – 3.26	1.89 1.23 – 5.01	4.72* 1.36 – 8.09	-0.72 -4.71 – 3.24	8.59 0.73 – 16.44	-0.50 -6.06 – 5.04	3.32 -0.19 – 6.84	7.16* 0.78 – 13.55
2nd week BCM	0.72 0.96 – 2.40	1.75 0.16 – 3.35	1.96 1.16 – 5.07	5.32* 1.97 – 8.68	-0.54 -4.53 – 3.42	6.58 -1.23 – 14.42	-1.04 -6.59 – 4.49	2.18 -1.33 – 5.69	5.71 -0.66 – 12.09
1st week BCM	-1.17 -0.50 –2.85	-2.28* -3.87 – -0.72	0.89 2.22 – 4.01	1.54 -1.80 – 4.90	-4.18* -8.17 – -0.20	5.15 -2.98 – 12.98	-1.55 -7.08 – 3.98	0.09 -3.41 – 3.60	-2.53 -8.91 – 3.85
CMD	-6.77* -8.72 – -4.81	-22.2* -24.26 – -20.14	-18.66 * -22.44 – -14.28	-13.29* -17.23 – -10.55	-11.36* -15.74 – -6.98	-11.18* -20.32 – -2.05	-9.67* -16.13 – -3.22	-14.37* -18.71 – -10.02	-15.33* -22.31 – -8.37
1st week ACM	-4.90* -6.59 – -3.22	-12.49* -14.09 – -10.93	-12.93* -16.06– -6.80	-13.89* -17.25 – -10.55	-9.00* -12.98 – -5.01	-9.25* -17.08 – -1.42	-6.78* -12.33 – -1.23	-9.43* -12.94 – -5.92	-10.00* -16.39 – -3.62
2nd week ACM	-1.95* -3.65 – 0.26	-5.88* -7.51 – -4.25	-7.64* -10.81 – -4.73	-3.93* -7.31 – -0.57	-5.57* -9.57 – -1.57	-2.07 -9.94 – 5.81	-3.59 -9.18 – 1.99	-6.00* -9.54 – -2.46	-3.93 -10.93 – 2.52
3rd week ACM	-1.28 -3.27 – 0.69	-4.82* -70.00 – -2.64	5.35* -9.28 – -2.68	-1.59 -5.52 – 2.34	-4.57* -8.98 – -0.16	-2.03 -11.17 – 7.11	-2.20 -8.71 – 4.31	-5.52* -9.93 – -1.11	-0.75 -7.95 – 6.44
4th week ACM	-1.21 -2.90 – 0.47	-3.24* -4.85 – -1.63	-5.84* -8.99 – -2.69	-1.03 -4.39 – 2.32	-4.35* -8.34 – -0.36	2.88 0.73 – 10.71	-2.17 -7.73 – 3.38	-3.10 -6.62 – 0.42	-0.86 -7.32 – 5.57
5th week ACM	-0.16 -1.82 – 0.19	-2.07* -3.63 – -0.52	-4.14* -7.22 – -1.07	0.10 -3.20 – 3.40	-3.66 -7.62 – 0.28	3.12 -4.59 – 10.84	-2.19 -7.66 – 3.28	-4.37* -7.81 – -0.92	0.76 -5.60 – 7.13
6th week ACM	-0.06 -1.75 –1.63	-2.02* -3.64 – -0.40	-2.10 -5.27 – 1.30	0.07 -3.28 – 3.43	-2.91 -6.91 – 1.07	3.39 -4.49 – 11.27	-1.47 -7.04 – 4.09	-3.38 -6.92 – 0.15	1.13 -5.29 – 7.58
7th week ACM	-0.14 -1.55 –1.88	-0.82 -2.44 – 0.88	-0.62 -3.80 – 2.55	-0.40 -3.76 – 2.96	-2.43 -6.42 – 1.56	3.28 -4.63 – 11.19	0.01 -5.56 – 5.59	3.00 -6.55 – 0.53	0.16 -6.24 – 6.60

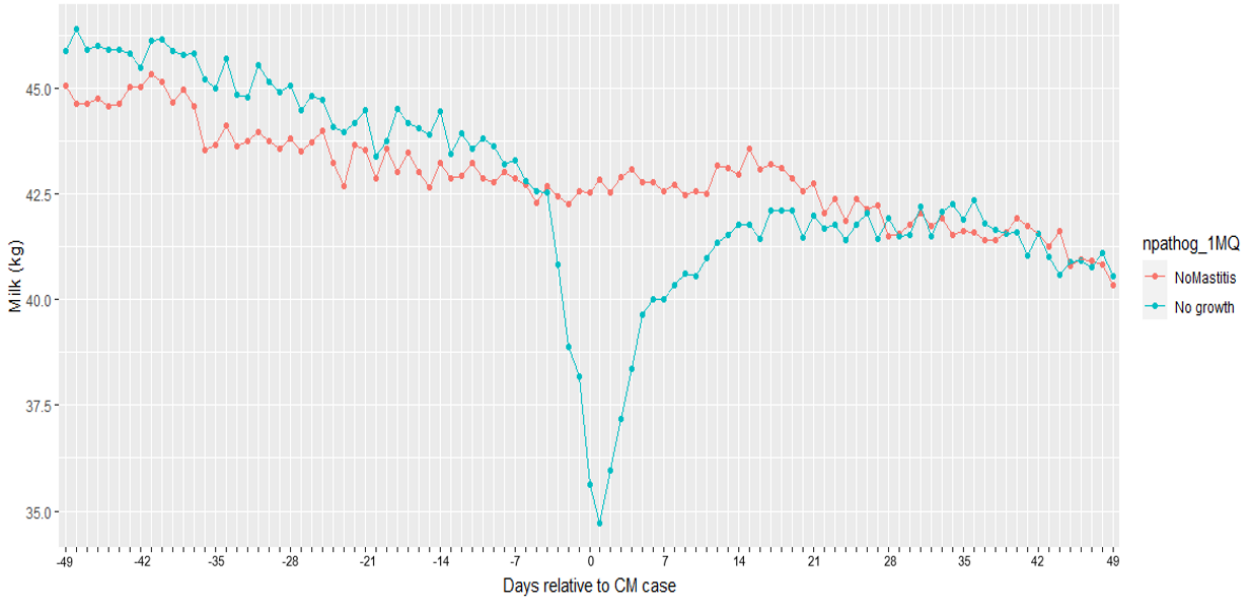
[1]Amount of milk (kg) gained (positive values) or lost (negative values) per day in a given week regarding CM detection

BCM = before clinical case diagnosis; CMD =clinical case diagnosis day; ACM = after clinical case diagnosis

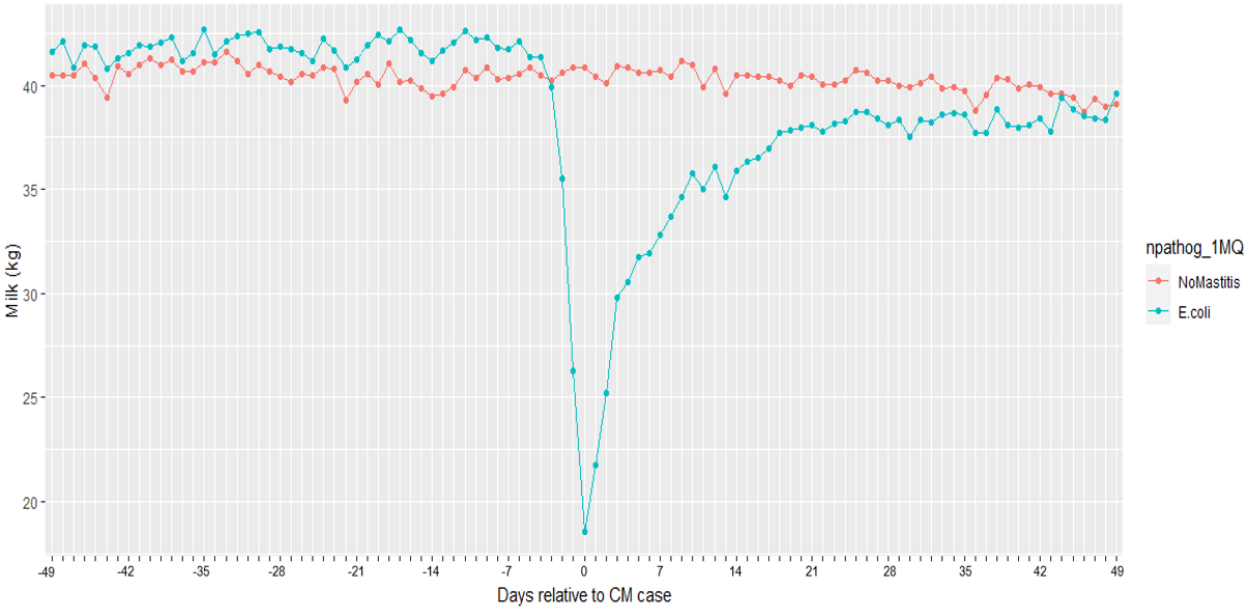
*P < 0.05

Figure 3. 7 Average daily milk production prediction based on the mixed model for multiparous cows (parity >1) infected with pathogens versus non-clinical controls[1], in 4 commercial dairy herds[2].

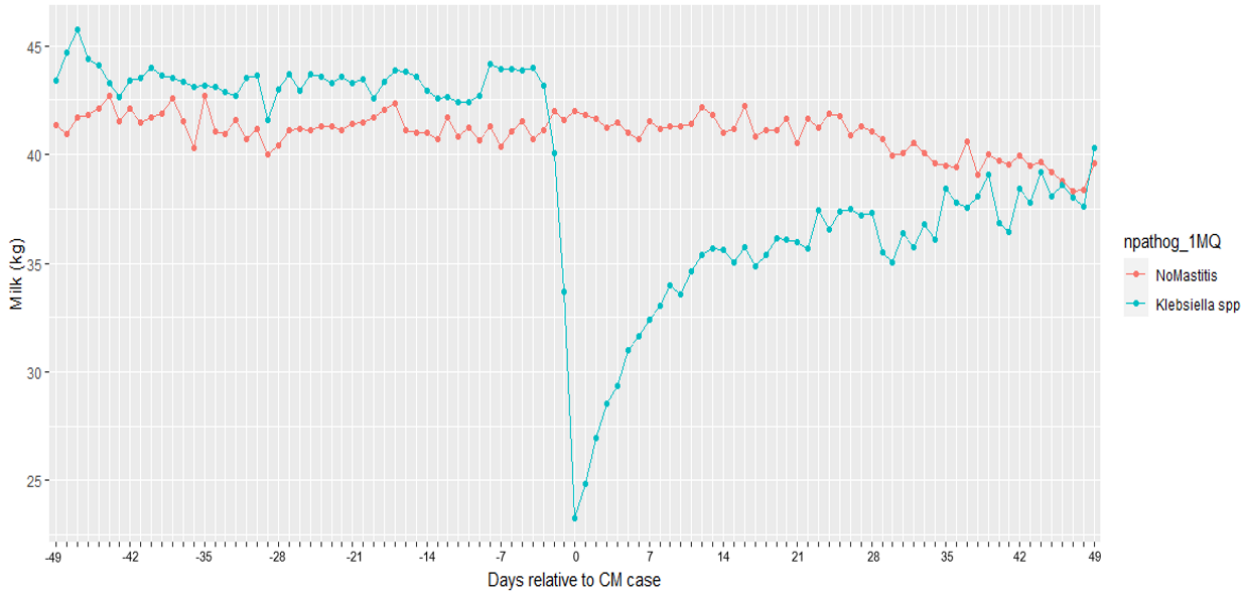
A) No growth (n=426)



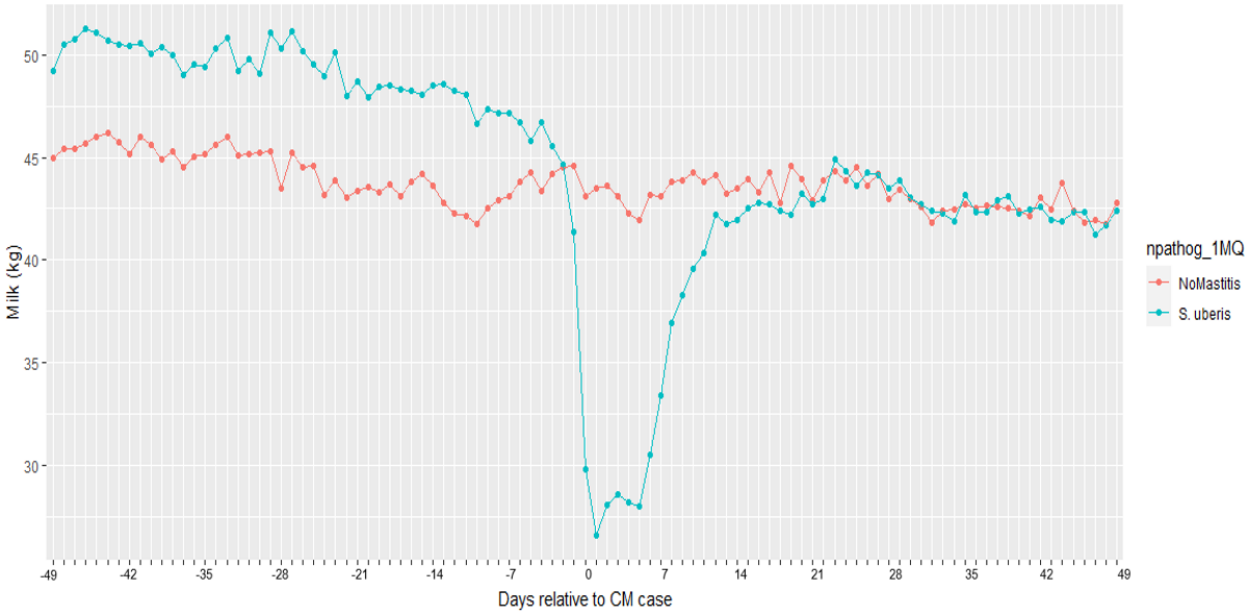
B) *E. coli* (n=262)



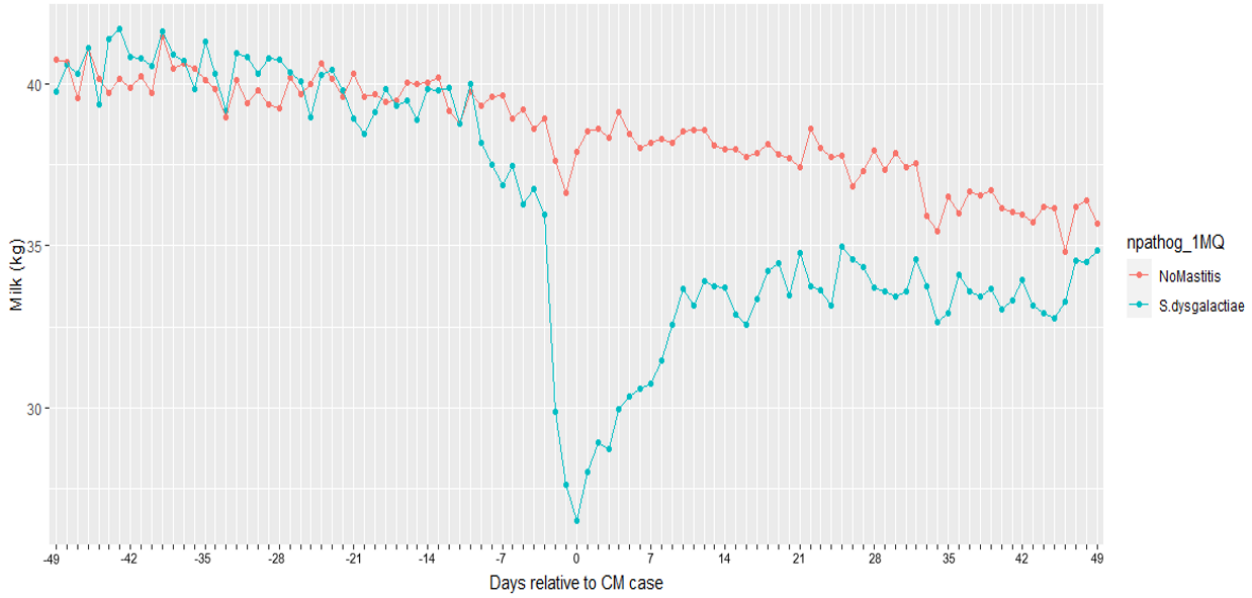
C) *Klebsiella* spp (n=135)



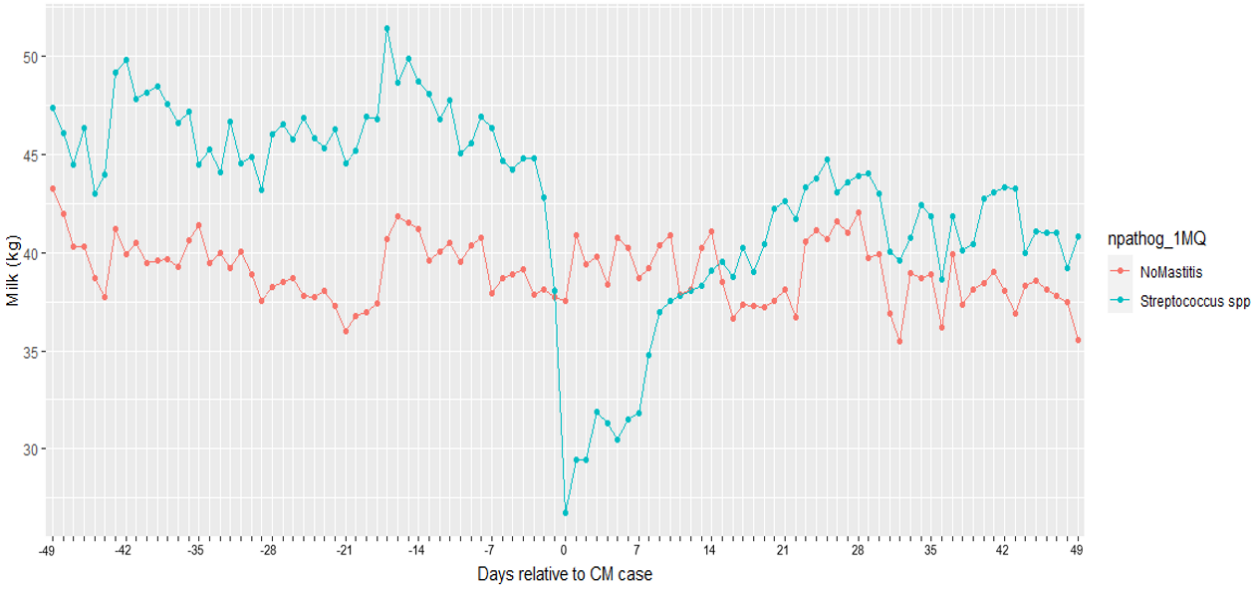
D) *Streptococcus uberis (n=168)*



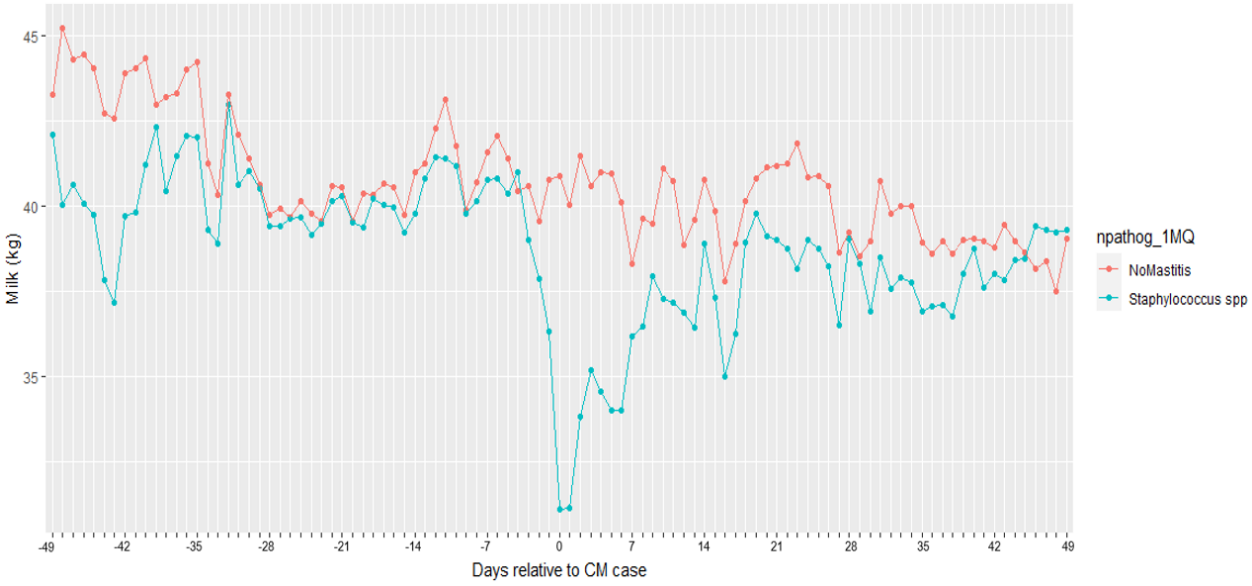
E) *Streptococcus dysgalactie* (n=115)



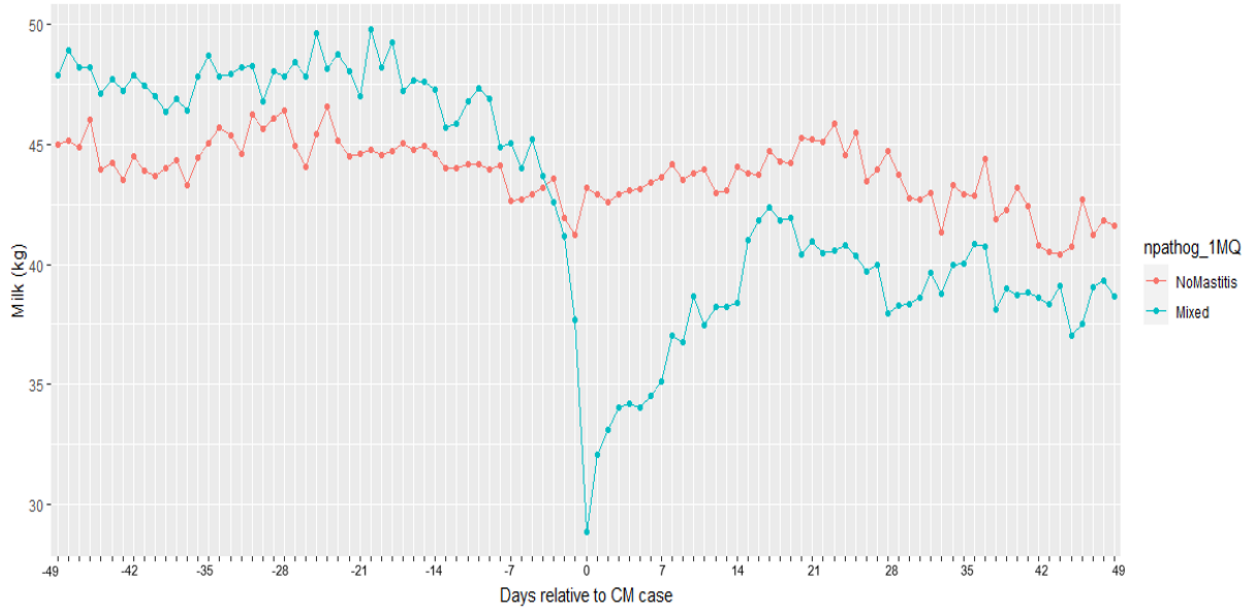
F) *Streptococcus* spp (n=33)



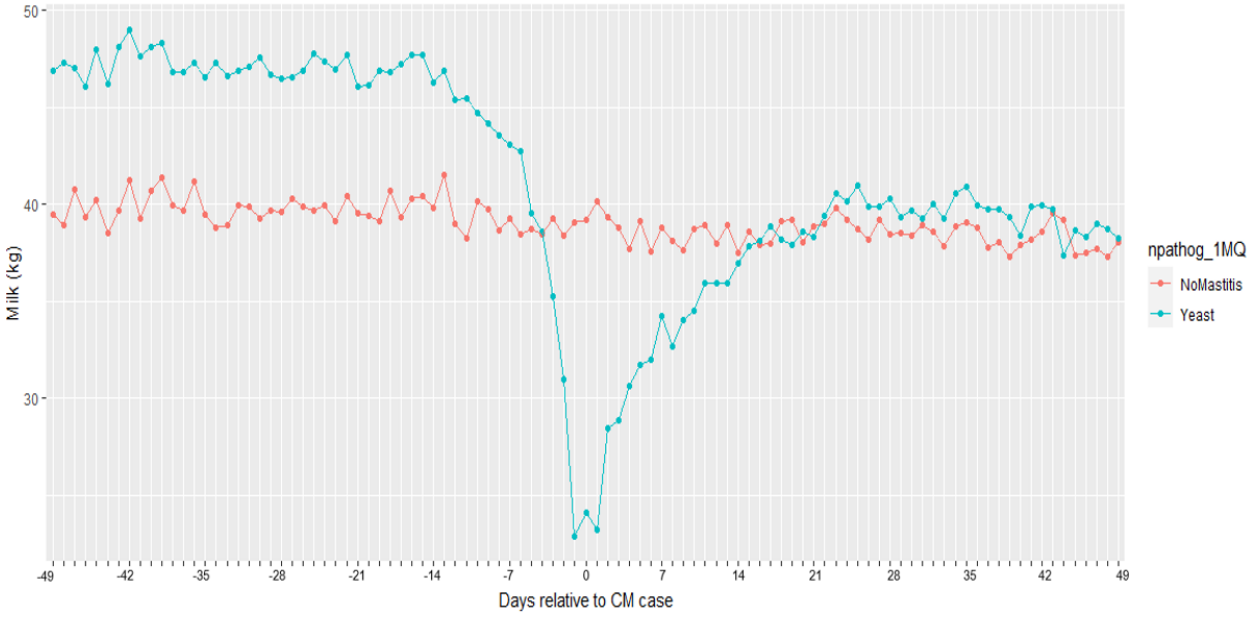
G) Non-aureus *Staphylococcus* spp (n=48)



H) Mixed infection (n=95)



I) Yeast (n=35)



[1]Each control (NoMastitis) cow was matched based on herd, DIM (±7d), and parity
[2]Herds with daily weights: A, C, E, and H.

DISCUSSION

Research that follows for an extended period all clinical cases in herds with accurate pathogens-species identification, and with daily milk weights to evaluate the effect pathogen-specific patterns are uncommon. This study aimed to describe pathogen distribution in dairy herds where every case of mastitis was recorded and to describe daily milk losses by pathogen. Evaluate milk losses using daily milk weights by pathogen will facilitate understanding specific differences during a CM case.

Although milkers identified all CM cases in this study, they were trained and accustomed to detect changes in milk during forestrip, which helps to standardize the criteria in the definition of a mastitis case. Besides, these herds follow this 24 hour-result program as routine management, which ensures that all cases no matter severity to be sampled and recorded. Another study showed that herder's diagnostic capability to detect clinical mastitis was not a source of bias and did not have a negative influence on validity (Lam et al., 1993). While it is true that the number of herds in this cohort study does not allow us to make inferences; the characteristics of these herds are highly representative of commercial dairy herds in Upstate New York.

In this study, we used MALDI-TOF as the method for organism identification, a more reliable method to discriminate one species from another compared to more traditional o molecular methods (Rychert, 2019) that could be time-consuming or technically challenging. Additionally, to addresses the milk losses, we used daily milk weights and multiple imputation to reduce the impact of missing values on repeated measures; bringing the option to use more and balance data and may provide more precise estimates (Rombach et al., 2018). A mixed model with autoregressive covariance structure similar to reported by other studies (Gröhn et al., 2004; Hertl et al., 2011; Heikkilä et al., 2018) was used to evaluate the effects of pathogen-specific on

milk yield, including both herd and cow as random terms (Heikkilä et al., 2018).

Over the study period, the monthly incidence rate at the quarter level was 4.4 cases per 10,000 QDAR. This means that per 10,000 quarters-day at risk, 4.4 quarters are expected to have CM out of 10,000 healthy quarters daily monitored within the specific month. The incidence risk over the study period was 4.8%, meaning that out of 100 cows at risk for mastitis during each month, about five of them will experience a new CM case.

No growth was the most common laboratory result in 29.6% of the CM cases, similar to the 28.1% reported in 50 herds in Wisconsin (Oliveira et al., 2013), as to the 27% reported in 114 herds in Minnesota, Wisconsin, and Ontario, Canada (Lago et al., 2011); which is within of the usual 20-40% stated that not require antibiotic therapy (Roberson, 2003). In research done in herds outside the US, no growth is also reported in a range of 15-40% of all milk samples from cows with clinical signs (Bradley et al., 2007; Koivula et al., 2007; Olde Riekerink et al., 2008; Gao et al., 2017; Tomazi et al., 2018; Schmenger and Krömker, 2020). Possible explanations for these results include pathogen's load below to detection levels in the laboratory due to either cow's immune system has already cleared the infection or that the pathogen not shedding at the time that the sample was taken. (Sears et al., 1990; Taponen et al., 2009). Special growth and media conditions for specific pathogens such as *Mycoplasma* spp could be also another reason, even though the global incidence of this pathogen is not as high in all these different countries, which makes it a low option to explain the similarities in the number reported. A study evaluating the use of real-time PCR assay on milk samples with no growth results reported that bacterial DNA was not detected in 58% of the samples. Besides, these cows also showed significantly milder clinical signs compared with those with a positive finding, which could also restate bacterial has been eliminated earlier by the immune system (Taponen et al., 2009).

The distribution of mastitis-causing pathogens observed in this cohort is comparable to other studies of commercial dairy herds in the United States (Lago et al., 2011; Pinzón-Sánchez and Ruegg, 2011; Oliveira et al., 2013; Stangaferro et al., 2016). Environmental pathogens were predominant in the CM cases in these eight herds, with *E. coli* as the most common pathogen cultured in 17.9% of the samples, followed by *Streptococcus uberis* in 9.9%, *Streptococcus dysgalactie* 8.7%, and *Klebsiella* spp in 6.7%.

E. coli remains the number one coliform to cause mastitis in dairy herds. As for *Klebsiella* spp, a previously stated emerging pathogen in North America (Zadoks and Munoz, 2007), now is the second coliform causing mastitis in several studies; accounting for up to 30.2%% of cases in a large study in Wisconsin, United States (Oliveira et al., 2013). The importance is such that nowadays is one of the main targets in the development of vaccines (Tomazi et al., 2021). *Streptococcus uberis* and *Streptococcus dysgalactiae*, the two most frequently environmental streptococci found in milk samples from CM, cause acute and recurrent episodes of this disease, leading to a variety of forms ranging from mild to severe. A recent study in 15 German dairy herds showed a limited variety of *Streptococcus uberis* strains in mastitis samples within a herd but similar strains were found in different environmental spots, indicating modes of transmission include environmental sources in a contagious pattern and might be herd-specific (Wente et al., 2019). Some of the called minor pathogens (i.e. *Streptococcus* spp and non-aureus *Staphylococcus*) represented each one 2% of the cases, lower to the reported in other studies (Lago et al., 2011; Pinzón-Sánchez and Ruegg, 2011; Oliveira et al., 2013; Rowbotham and Ruegg, 2016a). These variations in distribution could be explained to the different species in this group, so it may be species more likely to be isolated from different milk samples: healthy quarters, quarters with subclinical, or with clinical signs (Condas et al., 2017; Wuytack et al.,

2020). Non-aureus *Staphylococcus* has been more frequently isolated in milk samples of subclinical mastitis (Tomazi et al., 2015; Wald et al., 2019). Current research categorized Non-aureus *Staphylococcus* species to identify which ones should be considered more important on control programs. This categorization was based on different discriminating factors: nature of the interaction with the udder (commensal interaction to a pathogenic interaction), strength and specialization behind this interaction (environmental organism to obligate symbiont), and impact on the milk microbiome and major mastitis pathogens (De Buck et al., 2021)

As for contagious pathogens, the only cultured from clinical cases was *Staphylococcus aureus*, accounting for 3.1% of the milk samples, similar to the 3.3% reported by Oliveira et al and below to the 7% reported by Lago et al.; and the 6.3% by Rowbotham and Ruegg. This value was lower to other countries where the incidence of this pathogen is higher, ranging from 9.6 to 21.7% (Olde Riekerink et al., 2008; Levison et al., 2016; Gao et al., 2017; Tomazi et al., 2018), showing that commercial dairy herds in the US had an improvement in the management of contagious pathogens.

Mastitis treatment without knowledge of etiology results in unnecessary antimicrobial use (i.e., no growth and gram negatives pathogens, with some cases exceptions) and makes it more difficult to evaluate treatment success (Ruegg, 2021). Strategic treatment protocols based on severity and/or pathogen have the potential to efficiently reduce antimicrobial use (Lago et al., 2011; Vasquez et al., 2017; Fuenzalida and Ruegg, 2019a; Nobrega et al., 2020). Our results reaffirm the need to have reliable and fast identification methods as an important step to reduce the use of antibiotics in dairy herds, and that current udder health programs should have an emphasis on the control of environmental pathogens.

Cows affected with CM suffered a sharp drop in production even before clinical signs

detection. The sharper drops in milk yield occurred during the CM detection and the first 7 days in most of the pathogens, and depending on the pathogen continued for several weeks, including yields that never return to the one before the CM occurrence. These differences could be associated with the mammary tissue damage and immune response level produced by each pathogen.

Full recovery of the mammary gland from cows affected by *E. coli* may take a longer time than clinical recovery. Our results show that milk losses in multiparous lasted up to 6 weeks after CM detection and for up to 4 weeks in primiparous. Frequently associated with acute clinical cases resolution depend on the health status of the cow, as well as the infecting strain. Previous studies have shown how cows failed to gain a complete recovery, showing a long loss pattern (Coulona et al., 2002; Gröhn et al., 2004; Hertl et al., 2014; Heikkilä et al., 2018) or even a complete ceased of milk production (Blum et al., 2014). Even if *E.coli* cases are considered of short duration, this study confirms what was stated by other authors, long-term effects are present in milk yield.

Our study shows that cows affected by *Klebsiella* spp are also losing large amounts of milk after a CM case and that these losses remain for at least 6 weeks after detection. Our findings are similar to the ones reported in previous New York dairy herds (Gröhn et al., 2004; Hertl et al., 2014). Unfortunately, the number of *Klebsiella* spp cases with complete daily data in primiparous were lower than 30, so the milk losses due to this pathogen in this group of animals in this population cannot be described. Compared to *E.coli* cases, cows affected by *Klebsiella* spp are more likely to have greater milk losses and less survival on herd after a CM case (Erskine et al., 2002; Gröhn et al., 2005). This pathogen is also more likely to have an increased incidence in well-managed herds that keep the SCC under <150,000 cells/mL (Barkema et al., 1998),

which makes it a current challenge in commercial dairy herds. In addition *Klebsiella* spp has a more severe innate response compared to other coliforms; with a higher concentration of TNF-α and cytokine IL-10 (Schukken et al., 2012), low spontaneous cure rate, and low rates of response to antibiotic therapy (Fuenzalida and Ruegg, 2019b; Nobrega et al., 2020).

Milk losses produced by environmental streptococcus have been established in previous research (Coulona et al., 2002; Gröhn et al., 2005; Hertl et al., 2014; Heikkilä et al., 2018), although losses evaluated separately by species was done only by Heikkilä et al., 2018. They described that the milk yield between cows without a CM and cows with *Streptococcus dysgalactie* and *Streptococcus uberis* seems to be similar with a moderate loss of 1.2 and 1.3 kg/d, respectively. In our study, multiparous cows affected by *Streptococcus uberis* were higher producers than then controls, producing 7 kg/d more, and never recovering this initial yield. As for *Streptococcus dysgalactie* showed similar losses in primiparous and multiparous cows, with losses that continued for up to 4 weeks after CM detection. As for multiparous cows affected by *Streptococcus* spp, these showed a milk loss that lasted for 1 week after CM detection, and a rapid recovering after that.

Primiparous cows affected by *S. aureus* showed a lower initial yield compared to their controls, and could be related to subclinical infections and/or chronically infected animals.

Cows that had a CM event with a no-growth laboratory result, both primiparous and multiparous had lower milk losses and recovered the initial yield faster compared to other pathogens. These findings are different from the ones reported by (Gröhn et al., 2004), where they found similar loss patterns to gram negatives. This could indicate that severity was lower and the tissue damage was less, so this type of mastitis infection did not appear to be of concern in terms of milk losses.

As for mixed infections (meaning 2 pathogens detected in the same milk sample), the milk losses showed to be an important type of mastitis, due to the milk losses that lasted for up to 5 weeks after CM detection. To the best of our knowledge, this is the first study reporting milk losses due to mixed infection and showed to have important detrimental effects that need to be considered when having a case in this category.

Finally, cows infected with Yeast were higher producers before the CM detection and never return to the initial yield. The most common species of yeast include *Cryptococcus neoformans* and *Candida albicans* involved in bovine mastitis. This is considered a minor opportunistic pathogen that can be found in moist places that are rich in organic matter and frequently associated with repetitive intramammary treatment (Moretti et al., 1998; Dworecka-Kaszak et al., 2012).

CONCLUSIONS

This study shows some insights regarding the incidence of CM and associated pathogens in large dairy herds in Central New York. As one of the major production limiting and most common infectious affecting dairy cattle, there is a need to know the distribution of mastitis-causing pathogens, allowing identifying and prioritizing management areas to improve control programs, treatment options, and prevention strategies. Although herds in this study may have similar mastitis incidence, the pathogen distribution was different between herds, thus practices to control these mastitis-causing pathogens and treatment protocols need to be different between herds. With this information, we can conclude that environmental pathogens are the current challenge of mastitis control.

Regarding milk yield losses, the most important pathogen causing losses both in primiparous and multiparous is *E. coli*, with losses that lasted longer than any other pathogen in

the analysis. Cases with a no-growth result caused fewer milk losses and more rapid recovery compared to other more commonly isolated in this population. The environmental streptococci such as *Streptococcus uberis* and *Streptococcus dysgalactie* had important milk losses, especially in multiparous cows, where the milk yield was higher compared to the control cows several weeks before CM detection. NAS has subtle milk losses. As for milk losses due to mixed infections and yeast should be considered of importance, and take into consideration when this occurs in the herd.

In conclusion, our findings showed that milk loss varies according to the pathogens causing the event, nonetheless a program and control of environmental mastitis would bring a significant increase in milk production to the herds.

REFERENCES

Bar, D., Y.T. Gröhn, G. Bennett, R.N. González, J.A. Hertl, H.F. Schulte, L.W. Tauer, F.L. Welcome, and Y.H. Schukken. 2007. Effect of Repeated Episodes of Generic Clinical Mastitis on Milk Yield in Dairy Cows. Journal of Dairy Science 90:4643–4653. doi:10.3168/jds.2007-0145.

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. Journal of Dairy Science 81:411–419. doi:10.3168/jds.S0022-0302(98)75591-2.

Bizzini, A., C. Durussel, J. Bille, G. Greub, and G. Prod'hom. 2010. Performance of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry for Identification of Bacterial Strains Routinely Isolated in a Clinical Microbiology Laboratory. J Clin Microbiol 48:1549–1554. doi:10.1128/JCM.01794-09.

Blum, S.E., D.E. Heller, S. Jacoby, O. Krifuks, U. Merin, N. Silanikove, Y. Lavon, N. Edery, and G. Leitner. 2020. Physiological response of mammary glands to Escherichia coli infection: A conflict between glucose need for milk production and immune response. Scientific Reports 10:9602. doi:10.1038/s41598-020-66612-7.

Blum, S.E., E.D. Heller, and G. Leitner. 2014. Long term effects of Escherichia coli mastitis. The Veterinary Journal 201:72–77. doi:10.1016/j.tvjl.2014.04.008.

Bradley, A.J., K.A. Leach, J.E. Breen, L.E. Green, and M.J. Green. 2007. Survey of the incidence and aetiology of mastitis on dairy farms in England and Wales. Vet Rec 160:253–257. doi:10.1136/vr.160.8.253.

Condas, L.A.Z., J. De Buck, D.B. Nobrega, D.A. Carson, S. Naushad, S. De Vliegher, R.N. Zadoks, J.R. Middleton, S. Dufour, J.P. Kastelic, and H.W. Barkema. 2017. Prevalence of non-aureus staphylococci species causing intramammary infections in Canadian dairy herds. J Dairy Sci 100:5592–5612. doi:10.3168/jds.2016-12478.

Coulona, J.-B., P. Gasquib, J. Barnouin, A. Ollier, P. Pradel, and D. Pomiès. 2002. Effect of mastitis and related-germ on milk yield and composition during naturally-occurring udder infections in dairy cows. Anim. Res. 51:383–393. doi:10.1051/animres:2002031.

De Buck, J., V. Ha, S. Naushad, D.B. Nobrega, C. Luby, J.R. Middleton, S. De Vliegher, and H.W. Barkema. 2021. Non-aureus Staphylococci and Bovine Udder Health: Current Understanding and Knowledge Gaps. Front. Vet. Sci. 8. doi:10.3389/fvets.2021.658031.

Erskine, R.J., P.C. Bartlett, J.L. VanLente, and C.R. Phipps. 2002. Efficacy of systemic ceftiofur as a therapy for severe clinical mastitis in dairy cattle. Journal of Dairy Science 85:2571–2575. doi:10.3168/jds.S0022-0302(02)74340-3.

Fuenzalida, M.J., and P.L. Ruegg. 2019a. Negatively controlled, randomized clinical trial to

evaluate use of intramammary ceftiofur for treatment of nonsevere culture-negative clinical mastitis. J Dairy Sci 102:3321–3338. doi:10.3168/jds.2018-15497.

Fuenzalida, M.J., and P.L. Ruegg. 2019b. Negatively controlled, randomized clinical trial to evaluate intramammary treatment of nonsevere, gram-negative clinical mastitis. Journal of Dairy Science 102:5438–5457. doi:10.3168/jds.2018-16156.

Gao, J., H.W. Barkema, L. Zhang, G. Liu, Z. Deng, L. Cai, R. Shan, S. Zhang, J. Zou, J.P. Kastelic, and B. Han. 2017. Incidence of clinical mastitis and distribution of pathogens on large Chinese dairy farms. Journal of Dairy Science 100:4797–4806. doi:10.3168/jds.2016-12334.

Gomes, F., and M. Henriques. 2016. Control of Bovine Mastitis: Old and Recent Therapeutic Approaches. Curr Microbiol 72:377–382. doi:10.1007/s00284-015-0958-8.

Gröhn, Y.T., R.N. González, D.J. Wilson, J.A. Hertl, G. Bennett, H. Schulte, and Y.H. Schukken. 2005. Effect of pathogen-specific clinical mastitis on herd life in two New York State dairy herds. Preventive Veterinary Medicine 71:105–125. doi:10.1016/j.prevetmed.2005.06.002.

Gröhn, Y.T., D.J. Wilson, R.N. González, J.A. Hertl, H. Schulte, G. Bennett, and Y.H. Schukken. 2004. Effect of Pathogen-Specific Clinical Mastitis on Milk Yield in Dairy Cows. Journal of Dairy Science 87:3358–3374. doi:10.3168/jds.S0022-0302(04)73472-4.

Hagnestam, C., U. Emanuelson, and B. Berglund. 2007. Yield losses associated with clinical mastitis occurring in different weeks of lactation. J Dairy Sci 90:2260–2270. doi:10.3168/jds.2006-583.

Halasa, T., K. Huijps, O. Østerås, and H. Hogeveen. 2007. Economic effects of bovine mastitis and mastitis management: a review. Vet Q 29:18–31. doi:10.1080/01652176.2007.9695224.

Heikkilä, A.-M., E. Liski, S. Pyörälä, and S. Taponen. 2018a. Pathogen-specific production losses in bovine mastitis. J Dairy Sci 101:9493–9504. doi:10.3168/jds.2018-14824.

Heikkilä, A.-M., E. Liski, S. Pyörälä, and S. Taponen. 2018b. Pathogen-specific production losses in bovine mastitis. Journal of Dairy Science 101:9493–9504. doi:10.3168/jds.2018-14824.

Hertl, J.A., Y.H. Schukken, D. Bar, G.J. Bennett, R.N. González, B.J. Rauch, F.L. Welcome, L.W. Tauer, and Y.T. Gröhn. 2011. The effect of recurrent episodes of clinical mastitis caused by gram-positive and gram-negative bacteria and other organisms on mortality and culling in Holstein dairy cows. Journal of Dairy Science 94:4863–4877. doi:10.3168/jds.2010-4000.

Hertl, J.A., Y.H. Schukken, F.L. Welcome, L.W. Tauer, and Y.T. Gröhn. 2014. Pathogen-specific effects on milk yield in repeated clinical mastitis episodes in Holstein dairy cows. Journal of Dairy Science 97:1465–1480. doi:10.3168/jds.2013-7266.

Hogeveen, H., K. Huijps, and T.J.G.M. Lam. 2011. Economic aspects of mastitis: new developments. N Z Vet J 59:16–23. doi:10.1080/00480169.2011.547165.

Honaker, J., G. King, and M. Blackwell. 2011. **Amelia** II: A Program for Missing Data. J. Stat. Soft. 45. doi:10.18637/jss.v045.i07.

Kayano, M., M. Itoh, N. Kusaba, O. Hayashiguchi, K. Kida, Y. Tanaka, K. Kawamoto, and Y.T. Gröhn. 2018. Associations of the first occurrence of pathogen-specific clinical mastitis with milk yield and milk composition in dairy cows. Journal of Dairy Research 85:309–316. doi:10.1017/S0022029918000456.

Koivula, M., A. Pitkälä, S. Pyörälä, and E.A. Mäntysaari. 2007. Distribution of bacteria and seasonal and regional effects in a new database for mastitis pathogens in Finland. Acta Agriculturae Scandinavica, Section A — Animal Science 57:89–96. doi:10.1080/09064700701488941.

Krömker, V., and S. Leimbach. 2017. Mastitis treatment—Reduction in antibiotic usage in dairy cows. Reproduction in Domestic Animals 52:21–29. doi:10.1111/rda.13032.

Kuipers, A., W.J. Koops, and H. Wemmenhove. 2016. Antibiotic use in dairy herds in the Netherlands from 2005 to 2012. Journal of Dairy Science 99:1632–1648. doi:10.3168/jds.2014-8428.

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. Journal of Dairy Science 94:4441–4456. doi:10.3168/jds.2010-4046.

Lam, T.J., Y.H. Schukken, F.J. Grommers, J.A. Smit, and A. Brand. 1993. Within-herd and between-herd variation in diagnosis of clinical mastitis in cattle. J Am Vet Med Assoc 202:938–942.

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. Journal of Dairy Science 99:1341–1350. doi:10.3168/jds.2015-9809.

Nobrega, D.B., S.A. Naqvi, S. Dufour, R. Deardon, J.P. Kastelic, J. De Buck, and H.W. Barkema. 2020. Critically important antimicrobials are generally not needed to treat nonsevere clinical mastitis in lactating dairy cows: Results from a network meta-analysis. Journal of Dairy Science 103:10585–10603. doi:10.3168/jds.2020-18365.

Olde Riekerink, R.G.M., H.W. Barkema, D.F. Kelton, and D.T. Scholl. 2008. Incidence Rate of Clinical Mastitis on Canadian Dairy Farms. Journal of Dairy Science 91:1366–1377. doi:10.3168/jds.2007-0757.

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

doi:10.3168/jds.2012-6078.

Petrovski, K.R., C. Heuer, T.J. Parkinson, and N.B. Williamson. 2009. The incidence and aetiology of clinical bovine mastitis on 14 farms in Northland, New Zealand. New Zealand Veterinary Journal 57:109–115. doi:10.1080/00480169.2009.36887.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, S. Heisterkamp, B. Van Willigen, and R. Maintainer. 2017. Package 'nlme'. Linear and nonlinear mixed effects models, version 3.

Pinzón-Sánchez, C., and P.L. Ruegg. 2011. Risk factors associated with short-term post-treatment outcomes of clinical mastitis. Journal of Dairy Science 94:3397–3410. doi:10.3168/jds.2010-3925.

Pol, M., and P.L. Ruegg. 2007. Treatment Practices and Quantification of Antimicrobial Drug Usage in Conventional and Organic Dairy Farms in Wisconsin. Journal of Dairy Science 90:249–261. doi:10.3168/jds.S0022-0302(07)72626-7.

Rajala-Schultz, P.J., Y.T. Gröhn, C.E. McCulloch, and C.L. Guard. 1999. Effects of Clinical Mastitis on Milk Yield in Dairy Cows. Journal of Dairy Science 82:1213–1220. doi:10.3168/jds.S0022-0302(99)75344-0.

Reksen, O., L. Sølverød, and O. Østerås. 2007. Relationships Between Milk Culture Results and Milk Yield in Norwegian Dairy Cattle. Journal of Dairy Science 90:4670–4678. doi:10.3168/jds.2006-900.

Roberson, J.R. 2003. Establishing treatment protocols for clinical mastitis. Veterinary Clinics of North America: Food Animal Practice 19:223–234. doi:10.1016/S0749-0720(02)00071-3.

Rollin, E., K.C. Dhuyvetter, and M.W. Overton. 2015. The cost of clinical mastitis in the first 30 days of lactation: An economic modeling tool. Preventive Veterinary Medicine 122:257–264. doi:10.1016/j.prevetmed.2015.11.006.

Rombach, I., A.M. Gray, C. Jenkinson, D.W. Murray, and O. Rivero-Arias. 2018. Multiple imputation for patient reported outcome measures in randomised controlled trials: advantages and disadvantages of imputing at the item, subscale or composite score level. BMC Medical Research Methodology 18:87. doi:10.1186/s12874-018-0542-6.

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. Journal of Dairy Science 99:4707–4717. doi:10.3168/jds.2015-10675.

Rowbotham, R.F., and P.L. Ruegg. 2016b. Associations of selected bedding types with incidence rates of subclinical and clinical mastitis in primiparous Holstein dairy cows. J. Dairy Sci. 99:4707–4717. doi:10.3168/jds.2015-10675.

Ruegg, P.L. 2017. A 100-Year Review: Mastitis detection, management, and prevention. J. Dairy Sci. 100:10381–10397. doi:10.3168/jds.2017-13023.

Ruegg, P.L. 2021. What Is Success? A Narrative Review of Research Evaluating Outcomes of Antibiotics Used for Treatment of Clinical Mastitis. Front. Vet. Sci. 8. doi:10.3389/fvets.2021.639641.

Rychert, J. 2019. Benefits and Limitations of MALDI-TOF Mass Spectrometry for the Identification of Microorganisms. Journal of Infectiology 2.

Schmenger, A., and V. Krömker. 2020. Characterization, Cure Rates and Associated Risks of Clinical Mastitis in Northern Germany. Vet Sci 7. doi:10.3390/vetsci7040170.

Schukken, Y., M. Chuff, P. Moroni, A. Gurjar, C. Santisteban, F. Welcome, and R. Zadoks. 2012. The "other" gram-negative bacteria in mastitis: Klebsiella, serratia, and more. Vet Clin North Am Food Anim Pract 28:239–256. doi:10.1016/j.cvfa.2012.04.001.

Schukken, Y.H., J. Hertl, D. Bar, G.J. Bennett, R.N. González, B.J. Rauch, C. Santisteban, H.F. Schulte, L. Tauer, F.L. Welcome, and Y.T. Gröhn. 2009. Effects of repeated gram-positive and gram-negative clinical mastitis episodes on milk yield loss in Holstein dairy cows. Journal of Dairy Science 92:3091–3105. doi:10.3168/jds.2008-1557.

Sears, P.M., B.S. Smith, P.B. English, P.S. Herer, and R.N. Gonzalez. 1990. Shedding Pattern of Staphylococcus aureus from Bovine Intramammary Infections. Journal of Dairy Science 73:2785–2789. doi:10.3168/jds.S0022-0302(90)78964-3.

Seegers, H., C. Fourichon, and F. Beaudeau. 2003. Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet. Res. 34:475–491. doi:10.1051/vetres:2003027.

Stangaferro, M.L., R. Wijma, L.S. Caixeta, M.A. Al-Abri, and J.O. Giordano. 2016. Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part II. Mastitis. J Dairy Sci 99:7411–7421. doi:10.3168/jds.2016-10908.

Taponen, S., L. Salmikivi, H. Simojoki, M.T. Koskinen, and S. Pyörälä. 2009. Real-time polymerase chain reaction-based identification of bacteria in milk samples from bovine clinical mastitis with no growth in conventional culturing. Journal of Dairy Science 92:2610–2617. doi:10.3168/jds.2008-1729.

Tomazi, T., G.C. Ferreira, A.M. Orsi, J.L. Gonçalves, P.A. Ospina, D.V. Nydam, P. Moroni, and M.V. dos Santos. 2018. Association of herd-level risk factors and incidence rate of clinical mastitis in 20 Brazilian dairy herds. Preventive Veterinary Medicine 161:9–18. doi:10.1016/j.prevetmed.2018.10.007.

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. Journal of Dairy Science 98:3071–3078. doi:10.3168/jds.2014-8466.

Tomazi, T., A.C.C.H. Tomazi, J.C.C. Silva, L. Bringhenti, M.L.M.C. Bravo, M.X. Rodrigues, and R.C. Bicalho. 2021. Immunization with a novel recombinant protein (YidR) reduced

the risk of clinical mastitis caused by Klebsiella spp. and decreased milk losses and culling risk after Escherichia coli infections. J Dairy Sci 104:4787–4802. doi:10.3168/jds.2020-19173.

Vasquez, A.K., D.V. Nydam, M.B. Capel, S. Eicker, and P.D. Virkler. 2017. Clinical outcome comparison of immediate blanket treatment versus a delayed pathogen-based treatment protocol for clinical mastitis in a New York dairy herd. J Dairy Sci 100:2992–3003. doi:10.3168/jds.2016-11614.

Verbeke, J., S. Piepers, K. Supré, and S. De Vliegher. 2014. Pathogen-specific incidence rate of clinical mastitis in Flemish dairy herds, severity, and association with herd hygiene. Journal of Dairy Science 97:6926–6934. doi:10.3168/jds.2014-8173.

Wald, R., C. Hess, V. Urbantke, T. Wittek, and M. Baumgartner. 2019. Characterization of Staphylococcus Species Isolated from Bovine Quarter Milk Samples. Animals (Basel) 9. doi:10.3390/ani9050200.

Wente, N., D. Klocke, J.-H. Paduch, Y. Zhang, M. tho Seeth, V. Zoche-Golob, F. Reinecke, E. Mohr, and V. Krömker. 2019. Associations between Streptococcus uberis strains from the animal environment and clinical bovine mastitis cases. Journal of Dairy Science 102:9360–9369. doi:10.3168/jds.2019-16669.

Wuytack, A., A. De Visscher, S. Piepers, F. Boyen, F. Haesebrouck, and S. De Vliegher. 2020. Distribution of non-aureus staphylococci from quarter milk, teat apices, and rectal feces of dairy cows, and their virulence potential. Journal of Dairy Science 103:10658–10675. doi:10.3168/jds.2020-18265.

Zadoks, R.N., and M.A. Munoz. 2007. Emergence of Klebsiella as a major mastitis organism. Page in Annual meeting.

Zhao, X., and P. Lacasse. 2008. Mammary tissue damage during bovine mastitis: Causes and control. Journal of animal science 86:57–65. doi:10.2527/jas.2007-0302.

CHAPTER FOUR

DESCRIPTION OF THE CHARACTERISTICS OF 5 BEDDING MATERIALS AND ASSOCIATION WITH BULK TANK MILK QUALITY ON 5 NEW YORK DAIRY HERDS

V. M. Alanis[1], M. Zurakowski[2], D. Pawloski[2], T. Tomazi[1], D. V. Nydam[1], P. A. Ospina[3]

Frontiers in Veterinary Science
https://doi.org/10.3389/fvets.2021.636833

[1]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York, USA
[2] Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Cobleskill, NY 12043.
[3]Lechear LLC, King Ferry, New York, USA

ABSTRACT

Environmental mastitis represents a major challenge on dairy farms where contagious pathogens are controlled by improved milking procedures. Therefore, research focused on the environment is important to improve udder health programs. The objectives of this prospective and descriptive study were to: 1) describe bedding bacterial counts, pH, and dry matter (DM) of 5 different bedding types (organic: manure solids, straw, paper fiber; inorganic: sand and recycled sand), and 2) explore the association between bedding bacterial counts with bulk tank milk quality. This study took place on 5 conveniently selected commercial dairy herds, each with a predominant bedding material in lactating pens. Bedding samples (used n=237; fresh n=53) were collected monthly from July 2018 to July 2019 following a Standard Operating Procedure (SOP) to minimize sampling variability. Additionally, a bulk tank (BT) milk sample (n=40) was collected on the same day unless milk had been picked up prior to arrival. Both BT and bedding samples were submitted to the laboratory for culture and bacterial identification and quantification of Streptococcus spp, Coliforms, and Non-coliforms, as well as detection of several pathogens of mastitis importance. Somatic cell count was evaluated in BT samples. Within bedding type, the correlation between bedding characteristics and bacterial counts in bedding was evaluated using Pearson correlation. Within bedding type, the correlation between bacterial counts in bedding samples and bacterial counts in BT were determined. The Kruskal-Wallis test was used to evaluate the bacterial count by bedding type, and to evaluate BT somatic cell count differences based on bedding type. In fresh bedding, bacterial counts were generally higher for manure solids for all bacterial groups compared to other materials. In used samples, organic materials had the highest levels of all bacterial groups. The proportion of samples with detectable organisms of mastitis importance varied within and among herds, both in bedding and

BT samples throughout the study period. In bedding samples, a higher DM content had the lowest levels of bacterial growth compared to those with lower DM content. Most bedding samples were on the alkaline side within a pH range of 8-11. No relationship between bacterial counts and pH was observed. No associations between BT bacteria counts and bedding bacterial counts were observed. No association between bulk tank somatic cell counts based on bedding type were observed. Despite using an SOP for bedding sampling in an effort to consistently collect samples, we still observed a large amount of variability, both within and among bedding samples. This variability may have obscured any potential association between BT milk quality and bedding type.

Keywords: bedding, bacteria count, milk quality, environmental mastitis

INTRODUCTION

As a multifactorial disease, bovine mastitis is one of the most complex, frequent, and costly diseases of dairy herds associated with decreased milk yield and quality (Gröhn et al., 2004; Barbano et al., 2006; Schukken et al., 2009; Heikkilä et al., 2018). Research has shown that coliform and *Streptococcus* spp pathogens cause impactful milk losses (Schukken et al., 2009; Hertl et al., 2014; Heikkilä et al., 2018) and that these losses varied between primiparous and multiparous cows. Raw milk with high somatic cell count (**SCC**) often has higher lipolysis and proteolysis than in low SCC milk, and also has effects in pasteurized milk such as decreasing shelf life and sensory defects, including rancidity, bitterness, and astringency (Ma et al., 2000). In the last years, there have been some changes in the distribution and patterns of mastitis in dairy herds in developed countries, with an important decrease of cows with contagious forms of mastitis, but persistent environmental forms (Lago et al., 2011; Pinzón-Sánchez and Ruegg,

2011; Oliveira et al., 2013; Ruegg, 2017).

Coliforms (including *Escherichia spp*, *Klebsiella* spp, and other gram-negative bacteria), Streptococcus species (including *Streptococcus uberis* and *Streptococcus dysgalactiae*), and Non-aureus *Staphylococcus* are among the most common environmental bacteria causing mastitis in US dairy herds (USDA, 2014). This distribution of mastitis pathogens was also identified in a recent study from 8 commercial herds in New York (Ospina et al., 2019). Additionally, cows with at least 1 clinical mastitis case due to environmental pathogens such as *E. coli*, *Klebsiella* spp, and *T. pyogenes* have greater risks of culling (Cha et al., 2013) compared with non-mastitic cows. Further, gram-negative cases increased the risk of mortality, as stated in a study from 30,233 lactations in cows of 7 dairy farms in New York State (Hertl et al., 2011).

These environmental mastitis pathogens have been isolated from bedding materials, soil, rumen, feces, vulva, lips, nares, and feed samples (Bramley, 1982; Kruze and Bramley, 1982; Petersson-Wolfe et al., 2008; Paduch et al., 2013) which demonstrates their nearly ubiquitous risk to environmental and teat end contamination. Like any other types of bacteria, they require appropriate moisture, temperature, and nutrients to live. Appropriate conditions are often present on dairy farms to allow bacterial numbers to increase. Therefore, the number of these bacteria on teat skin is a reflection of the cow's exposure to the contaminating environment (Hogan and Smith, 2012). Bedding material itself has physical and biochemical properties that support bacterial growth along with external factors that influence it (Godden et al., 2008).

Extensive research has demonstrated that both heifers and cows need 12-14 hours of lying daily and that they prioritize it over other activities (Jensen et al., 2005; Munksgaard et al., 2005). Considering this strong behavioral need to rest, a fundamental issue to consider is bedding materials that provide adequate cushion, but also one that can reduce udder and teat exposure to

environmental pathogens. Exposure to these pathogens when the cow lies down could result in intramammary infections with a possible mastitis outcome (Hogan and Smith, 2012). Several studies have shown that bacteria can be transferred between the lying surface and the teats (Zdanowicz et al., 2004; Rowbotham and Ruegg, 2016a; Guarín et al., 2017; Wolfe et al., 2018). Because environmental pathogens are highly influenced by management practices, like housing system, cow comfort, manure collection method, proper bedding, and pen cleanliness (Bartlett et al., 1992; Barkema et al., 1999), one of the most difficult dairy farm challenges is to minimize the level of exposure to environmental mastitis pathogens at the teat level between milkings to maintain good udder hygiene.

Few studies have focused on the association between bedding material and bulk tank (**BT**) milk quality (i.e. bacterial load and somatic cell counts). Among these few studies, there has been few consistent results. One prospective study using data from BT test results from 325 dairy herds in Wisconsin using the same bedding in all pens during the 2-yr study period (Rowbotham and Ruegg, 2015), showed that total bacterial counts in the BT were not associated with bedding type, but bulk milk somatic cell score (**BTSLS**) was lower for farms using inorganic materials.

A cross-sectional study using data from 125 herds in the United Kingdom (Bradley et al., 2018), showed no significant differences between bedding material in bacterial counts in milk for any of the organisms studied, and no significant correlations between bacterial load in used bedding and milk. More recently another cross-sectional study using data from 167 herds from 17 states in the United States (Patel et al., 2019), showed a wide variation of pathogen load in bedding among farms, with organic material bedding showing the highest coliform levels compared to inorganic materials, and manure solids showing the highest counts for streptococci-

like organisms. They established a guide for monitoring bedding hygiene in commonly used organic and inorganic bedding. Looking at another aspect of milk quality, research focused on food safety showed that bedding management practices (e.g., re-bedding frequency, raking frequency) were associated with mesophilic and thermophilic spore levels, and used organic bedding spore levels were positively related to those in bulk tank milk (Murphy et al., 2019).

The objectives of this prospective and descriptive study with repeated measures were to 1) describe the variability in bedding bacterial counts, pH, and dry matter (DM) of 5 different bedding types (manure solids, sand, straw, paper fiber, and recycled sand) and 2) explore the association between bedding bacterial counts with bulk tank milk quality in 5 conveniently selected New York dairy farms using 1 of 5 bedding materials in lactating pens.

MATERIALS AND METHODS

Herd selection and sample collection

Five commercial dairy herds in central New York State with an average herd size of approximately 1,400 cows (ranging from 838 to 2,050) were conveniently selected based on the willingness of the producers to participate and the proximity of the herds to the Quality Milk Production Services laboratory (**QMPS**), at the Animal Health Diagnostic Center, Cornell University (Ithaca, New York). Each herd used a predominant bedding material for lactating pens: manure solids (**MS**), paper fiber (**PF**), straw (**ST**), recycled sand (**RS**), or sand (**SD**).

All herds used Dairy Comp 305 (**DC305**; Ag Valley Software) as the management software. Participating herds used a well-established milking routine and every case of mastitis was identified by trained on-farm personnel, who collected all milk samples from all quarters with visibly abnormal milk, stored in a refrigerator (≅ 4ºC), and saved information in DC305. These milk samples were submitted to the QMPS laboratory for culture and matrix-assisted laser

desorption/ionization time-of-flight (MALDI-TOF) identification. These herds also had a regular DHIA testing program (monthly individual SCC and linear score) and were fed a balanced total mixed ration (TMR).

Farms were visited once monthly for a period of 6 to12 months from July 2018 to July 2019 by the same observer. The sample collection period among the herds varied in one herd because they changed bedding type mid-study. At each visit, used and fresh bedding samples were collected, as well as a BT sample and a DC305 backup.

Herd bedding practices

The herd using RS used a modified plug-flow aerobic digestion system with recirculation and mixing and a multi-stage sand separation system. The herd using MS used a screw press as a manure separation system. Herds using PF, SD, and ST purchased the materials and stored it in clean and dry storage are inside the herd.

They were also asked to notify investigators of any changes to these management practices during the study.

Bedding samples

The samples were collected once a month from lactation pens. A Standard Operating Procedure (**SOP**) was followed to minimize sampling variability. The day that the fresh bedding was due to be applied and after the routine cleaning, used bedding from 3-5 stalls from each pen was collected. Wearing clean disposable gloves, samples were collected from a 60 cm x 60 cm area, avoiding any manure spots, where the udder would touch the stall after scraping 3 cm off the top of the bedding material. Samples were transferred to a new 1-quart storage freezer bag. Using new and clean disposable gloves, fresh bedding samples were collected after asking the worker to dump extra bedding material in 5 stalls distributed throughout the pen. Fresh bedding

was collected from the top of this pile to form a combined sample. The sample was transferred to a new 1-quart storage freezer bag. Each used and fresh sample bag was labeled with the herd name, pen number, and date. Samples were placed in ice coolers, transported the same day within 2 hours after sampling, and frozen at -18°C for up to 4 weeks for analysis at QMPS.

Bulk tank milk samples

Unless milk had been picked up prior to arrival, the same day bedding was sampled, 1 BT sample was collected directly from the bulk tank using a clean and sanitized dipper into a 10 ml vial. Sampling was performed following the Dairy Practices Council (DPC) guidelines (i.e., mechanically agitate the milk for at least 5 min until sufficient homogeneity is obtained and 10 minutes for tanks more than 1,500 gallons). Each vial was labeled with the herd name and date. Samples were placed in ice coolers at 1°C, transported the same day within 2 hours after sampling, and frozen at -18°C for up to 4 weeks for analysis at QMPS.

Laboratory analysis and bacteria quantification

Frozen bedding and BT samples were submitted for bacterial identification and quantification for *Streptococcus* spp, Coliforms, and Non-coliforms at QMPS, as well as detection of other pathogens associated with mastitis.

Bedding samples

Frozen samples were allowed to thaw at refrigeration temperature (2-8°C) for 1 to 4 hours, depending on the bedding material to be analyzed. The sample was placed into a large, clean zip-type bag that allowed thorough mixing and breaking up of any clumps. For straw samples, pieces were cut into approximately 2.5 cm in length using sterile scissors. Using a weight verified scale, bedding material was weighed 10±1% (9.90-10.10) grams into a stomacher bag (manure solids, sand, and paper fiber) or sterile vial (recycled sand, straw) by taking small

sub-samples from at least three random locations within the mixed sample. Then 90 ml of sterile PBS was added to the 10-gram test sample and mixed two minutes using a stomacher set at blending speed 2 (2 strokes/second) or vortex for 40 seconds at setting 7 (1,800 rpm) for vials. Approximately 10 ml of this suspension was decanted into an empty sterile dilution tube. This was the 10^{-1} dilution. The 10^{-2} dilution was made by vortexing the 10^{-1} dilution for a minimum of 5 seconds and removing 1 ml using a micropipette and adding it to 9 ml of PBS. This dilution process continued until the 10^{-5} dilution.

BT samples

Frozen samples were allowed to thaw at refrigeration temperature 2-8°C and mixed thoroughly by shaking. The 10^{-1} dilution was made by removing 1 ml and adding it to 9 ml of PBS and vortex for a minimum of 5 seconds after a vortex has been achieved. This dilution process continued until the 10^{-2} dilution.

Plate Inoculation and Incubation Parameters (bedding and BT samples)

For each bedding and BT sample, 50 µl of each dilution was inoculated on different selective media. Edwards media was inoculated to test for *Streptococcus* spp and "streptococci-like" organisms. MacConkey media was inoculated to test for Coliforms and Non-coliforms. Hayflick media was inoculated with 50 µl of used bedding material from dilutions 10^{-2}, $10^{-3,}$ and 10^{-4} to test for Mycoplasma and placed in a CO_2 incubator. For BT samples, Trypticase Soy Agar with 5.0% blood and 0.1% esculin media was inoculated to test for Total count of all organisms (TBC).

In addition to the organisms that were quantified, the following organisms of mastitis significance were identified and counted as detected or not detected: *Staphylococcus aureus*, *Streptococcus agalactiae*, *E. coli*, *Klebsiella* spp, *Serratia* spp, *Pasteurella* spp, *Pseudomonas*

spp, *Prototheca* spp, *Trueperella pyogenes*, Yeast, mold, other fungi, and other microorganisms (*Listeria* spp, *Nocardia* spp, and *Salmonella* spp). Experienced technicians in microbiology used visual cues and biochemical tests (NMC, 2017) along with colony morphology of the plate to identify these pathogens. The presence of even one colony would be considered as detected.

Plates were incubated at 35°C to 38°C. After18-24 hours of incubation plates were observed using standard microbiology procedures. At 18-24 hours, the lactose-positive gram negatives colonies were counted, *E. coli* and *Klebsiella* were observed and recorded. Plates were placed back in the incubator at 35°C to 38°C for an additional 18-24 hours.

Bacteria Counts calculation

Plates were removed from the incubator and the number of colony-forming units (**CFU**; CFU/g for bedding samples and CFU/ml for BT samples) counted by an experienced laboratory technician from the dilution plate (up to 10^{-5} for bedding samples and up to 10^{-2} for BT samples) that presented 25-250 colonies whenever possible. All counts and the dilution plate were recorded into an internal form. The formulas used are as follows:

Bedding
((A(1000/B)*9)/C)/(D/E)

$$\left(\frac{\left(\frac{A\ CFU}{50\ \mu L}\right)*\left(\frac{1000\ \mu L}{1\ mL}\right)}{\left(\frac{10\ g}{90\ mL}\right)}\right)*C\ *\left(\frac{E}{D}\right)$$

$$=\ \left(\left(\left(\left(\frac{A\ CFU}{50\ \mu L}\right)*\left(\frac{1000\ \mu L}{1\ mL}\right)\right)*\ \left(\frac{90\ mL}{10\ g}\right)\right)*(10^{n})\right)*\left(\frac{E}{D}\right)=X\ CF\frac{U}{g}$$

Where:

A = number of colonies (CFU)

B = inoculation volume = 50 µl

C = dilution factor, n (10^{-n})

D = dry weight (g)

E = wet weight (g)

% moisture

((A+B)-C)*100/10

Where:

A = empty dish

B = bedding weight (added to the dish to go into the oven)

C = after drying (dish + bedding)

Bulk tank
A(1000/B)/C

Where:

A = number of colonies (CFU)

B = inoculation volume (µl)

C = dilution value of the plate counted or dilution factor, n (10^{-n})

Moisture Content (Dry matter content) estimation

The drying dish was weighed. The scale was tared and 10 ±1% (9.90-10.10) grams of bedding material was added, and evenly spread. The dish containing the 10 grams of bedding was placed into the oven and dried for at least 4 hours at 100±10°C. After drying, the sample was weighed and the total weight to two decimals was recorded.

pH estimation

A flip-top vial was placed on the scale and tared and 10.00 grams of bedding material was added by taking small sub-samples from at least three random locations within the mixed sample. Next 90 ml of deionized water was added using a 100 ml graduated cylinder and mixed

well. The pH probe from a pH meter was verified with appropriate buffers (7 and 10 buffers for calibration as most bedding material fit that range). If a bedding material ended up with a lower pH after calibration with the 7 and 10 buffer, the pH meter was recalibrated using 4 and 7 buffer. This probe was placed into the mixture and pH was recorded to two decimals.

Somatic cell count

Bulk tank milk Somatic Cell Counts (**BTSCC**) were analyzed using a DeLaval cell counter (DCC). The DCC analyses were performed using samples at 10-40^{o}C following the manufacturer's instructions. To transform BTSCC into bulk tank somatic cell scores (BTSLS) the following equation was applied: BTSLS = log2 (BTSCC/100) + 3.

Statistical analysis

Data collected and laboratory results were transferred to Excel spreadsheets (Microsoft Corp; Redmond, WA). Data were imported into R version 4.0.3 (RStudio: Integrated Development for R. RStudio, Inc., Boston, MA) to perform statistical analysis and to create the appropriate plots. All graphical representations were made using the ggplot2 package. The normality of continuous variables (i.e. bacteria counts) was visually assessed with density plots and quantile-quantile plots. These were not normally distributed; therefore, bacteria count values greater than zero were log10 transformed. When no bacteria were identified, a value of log10+1 CFU/g for bedding and log10+1 CFU/ml for BT was used, assuming that at least 10 CFU were present in a given sample. The decision to use this arbitrary value was due to the potential losses on each dilution before having the final count. An additional outcome was created where the counts of each bacterial group isolated (*Streptococcus* spp, Coliforms, and Non-Coliforms) in bedding samples were summed. This new outcome was named Sum Bacterial Count (**SBC**).

Within bedding type, the correlation between bedding characteristics and bacterial counts

in bedding were evaluated using Pearson correlation. For bacterial count analysis in bedding samples, the Kruskal-Wallis test was used to evaluate the differences based on bedding type running the kruskal.test function. When appropriate (meaning following a Kruskal-Wallis test at $P<0.05$.), Dunn's multiple comparison test among the 5 bedding material and Bonferroni correction were used as a post-hoc non-parametric test running dunn.test function. Correlations between bedding characteristics (pH and DM) and bacterial counts were determined using the Pearson correlation coefficient running the cor.test function. Within bedding type, the correlation between bacterial counts in bedding samples and bacterial counts in BT were determined. The Kruskal-Wallis test was used to evaluate the bacterial count by bedding type, and to evaluate BT somatic cell count differences based on bedding type.

For bacterial count analysis in BT samples and to evaluate differences between BTSLS based on bedding type; the Kruskal-Wallis test was used to evaluate the differences based on bedding type running the kruskal.test function. When appropriate (meaning following a Kruskal-Wallis test at $P<0.05$.), Dunn's multiple comparison test among the 5 bedding material and Bonferroni correction were used as a post-hoc non-parametric test running dunn.test function. The proportion of bedding and BT samples with detectable organisms from the list of pathogens of mastitis importance was also described. Correlation between bedding bacterial counts and bacterial counts was determined using the Pearson correlation coefficient running the cor.test function, with the average value of used bedding samples per time point.

RESULTS

Study herds

The mean number of lactating cows was 1,400 and the daily mean milk production of 37 kg. The mean BTSCC was 130,000 cells/ml. All farms used a consistent milking routine with pre-dipping, foremilk stripping, and wiping teats with either cloth (MS, PF, RS, and SD) or paper

towels (ST). All farms used iodine-based disinfectant solutions as pre-dipping and post-dipping. Basic farm descriptors, design, and management of bed descriptors are displayed in Table 4.1. Additionally, the results of cow positioning, bedding quantity, and quality can also be found in Table 4.1. Generally, most cows had adequate positioning (>70%, except MS herd with 25%).

Bacterial counts in bedding samples

All collected samples were evaluated in the laboratory. Although the goal was to collect 12 fresh samples (1 representative stall per month from each herd bedding type) and 60 used samples (5 representative stalls per month from each herd bedding type) from 12 monthly visits (n= 360 total samples), only a total of 290 bedding samples (used n=237; fresh n=53) were collected for final analysis. The difference in the number of samples was due to lack of bedding available to sample (18 visits among herds) or equipment malfunction (12 visits among herds) on the follow-up visit. Due to cold storage space and laboratory time limitations, the number of used bedding samples collected from each farm visit was changed to 3 in the second half of the study. Lastly, we started sampling in the ST herd later compared to the other herds, which affected the final number of samples, besides this herd changed bedding mid-way through the study which severely limited the number of used samples of this bedding type. Thus inferences from ST should be interpreted in light of the small number of observations. Comparatively the fresh samples were not as strongly impacted. The final analysis consisted of: MS=54 (used n=44; fresh n =10), PF=86 (used n=70; fresh n=16), ST=24 (used n=18; fresh n=6), RS=74 (used n=60; fresh n=14) and SD=52 (used n=45; fresh n=7).

Bacterial counts (log_{10} CFU/g) from fresh and used samples during the entire study period are summarized in Figure 4.1. The ST samples showed a wider variation on all bacterial counts compared to the other bedding types. The SD bedding type had 4 fresh samples with no

detectable levels of *Streptococcus* spp and no detectable levels of coliforms.

There was a clear increase in bacterial counts in used bedding samples compared to fresh samples for all bedding materials. *Streptococcus* spp, Coliforms, and Non-coliforms counts in inorganic materials (RS and SD) were generally lower than organic materials (MS, PF, and ST). For example Coliforms counts were different between all bedding types, being the highest on ST, then equally highest on MS and ST, and equally lowest on RS and SD (MS vs SD $P<0.0001$, MS vs RS $P<0.0001$, ST vs SD $P<0.0001$, ST vs RS $P<0.0001$). All pairwise comparisons are shown in Figure 4.1. A similar relationship was seen with SBC counts, in which inorganic materials were approximately 1 $\log_{10}$ less than the organic materials. The variability between used samples collected on the same day is illustrated in Figure 4.2.

Detection of specific bacteria in bedding

A summary of the proportion of bedding samples where bacteria were positively identified is shown in Figure 4.3 (i.e., if bacteria were not detected in fresh or used bedding these bacteria are not included in the Figure).

Dry matter content and pH

The % DM content and pH values for fresh and used bedding samples during the entire study period are shown in Figure 4.4. Generally, inorganic bedding samples were dryer than organic. Regarding pH values, fresh samples were on the alkaline side within a range of 8-11 except for ST, with acidic values (5.8 ±1.4). For used bedding samples, all materials were in the alkaline range of 8-9. Relationships between DM content and bacterial count in fresh and used samples are shown in Figures 4.5 and 4.6, respectively. For example, correlation analysis showed a negative linear relationship between DM content and bacterial count in used samples: SBC ($r = -0.61$, $P<0.001$), *Streptococcus* spp ($r = -0.60$, $P<0.001$), Coliforms ($r = -0.56$, $P<0.001$) and for

Non-Coliforms (r = -0.53, $P<0.001$), suggesting drier bedding material had lower bacterial counts.

Bulk tank bacterial counts

On several visits (n=15), the BT had recently been picked up and a BT sample was not available. A total of 40 BT samples were collected for the final analysis: MS (n=11); PF (n=7); RS (n= 8); SD (n=8); and ST (n=6).

The bacterial groups evaluated in BT samples are summarized in Figure 4.7. Dunn's test with Bonferroni correction for multiple comparisons indicated that Coliforms counts on ST herd (0.19) were observed to be different from those on RS (2.24) (P=0.04), although it is important to notice that only 1 BT sample from this herd had detected levels of this bacterial group. In the other bacterial groups among herds based on the Kruskal-Wallis test the p-values were: *Streptococcus* spp (P=0.19), *Staphylococcus* spp (P=0.08), TBC (P=0.57).

A correlation analysis of the average of Coliforms and *Streptococcus* spp counts in used bedding samples and those counts in BT was performed, the results showed a limited association, with values of -0.09 (P=0.5), and 0.06 (P=0.6), respectively.

Detection of specific bacteria in BT

A summary of the proportion of BT samples with detectable pathogens of mastitis importance are illustrated in Figure 4.8 (i.e., those without detectable organisms are not displayed).

Bulk tank Somatic Cell Linear Score

The overall BTSLS among herds was 3.54, ranging from 2.80 to 5.35 (Figure 4.9). The p-value for the Kruskal-Wallis test for the differences observed among bedding materials and BTSLS was 0.13.

Table 4. 1 Herd characteristics from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens

Herd	Bedding type	Milking cows (n)[1]	DIM[2]	Average milk (kg)[3]	Average SCC[4]	Type of stalls	Re-bedding frequency[5]	Rake/groom bedding surface frequency	Milking frequency per day	Milking parlor type/Number of stalls	Towel material	Dry-off routine
A	Manure solids	2,050	189	41.7	143	Deep beds	Daily	3x daily	3x	Rotatory/80	Cloth	Blanket
B	Paper fiber	1,214	184	39.4	166	Mattress	Twice a week	2x daily	2x	Parallel/Double 18	Cloth	Blanket
C	Straw	838	161	32.2	211	Concrete	Daily	2x daily	2x	Parallel/Double 12	Paper	Blanket
D	Recycled sand	1,750	172	40.3	187	Deep beds	Weekly	3x daily	3x	Parallel/Double 17	Cloth	Selective
E	Sand	1,170	169	39.9	148	Deep beds	Weekly	3x daily	3x	Parallel/Double 18	Cloth	Blanket

[1]Lactating cows monthly average
[2]Average days in milk
[3]Average of total daily milk produced (kg)
[4]Test day average somatic cell count (SCC; ×1,000 cells/mL) over the year of study
[5]Frequency of adding new bedding material to resting area and stalls

Figure 4. 1 Average bacterial counts (log10 CFU/g) for fresh and used bedding samples collected from July 2018 to July 2019 from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens. One fresh bedding sample and 3-5 used bedding samples were collected monthly following a Standard Operating Procedure at each visit (unless there was no bedding available due to lack of supply or equipment malfunction on the follow-up visit). Error bars represent SD. The same letters are not different at P≤0.01 (P-values adjusted for multiple contrasts). SBC (Sum Bacterial Count) = Streptococcus spp, Coliforms and Non-coliforms summed.

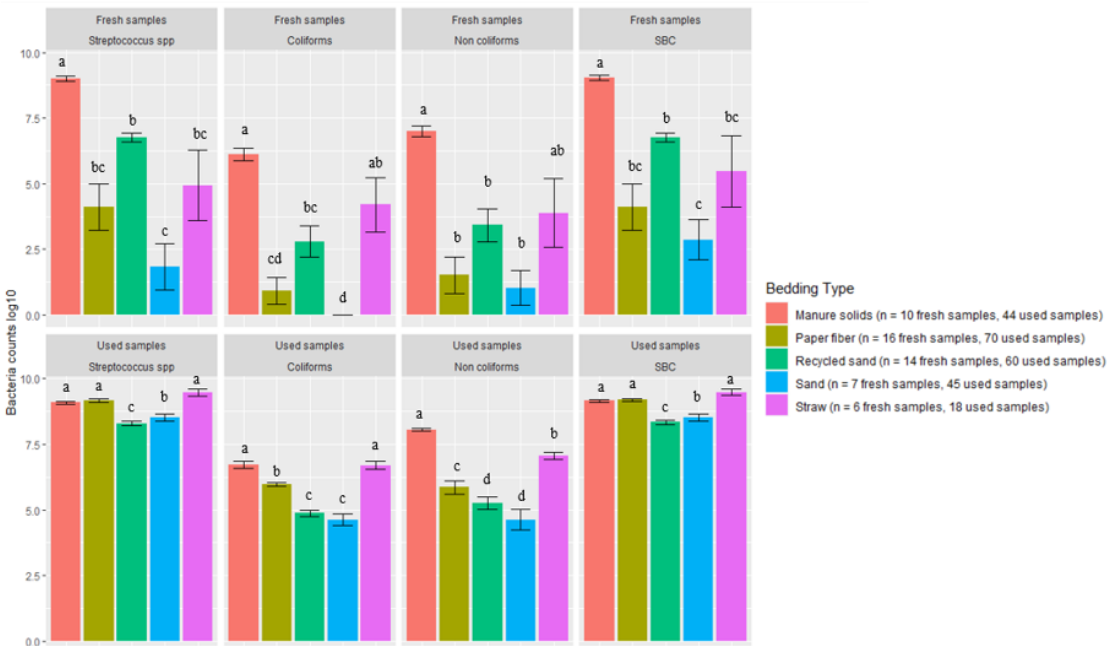


Figure 4. 2 Boxplots showing 25th, 50th (median), and 75th percentiles of the distribution of bacteria counts (log10 CFU/g) for used bedding samples collected from July 2018 to July 2019 from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens. Used bedding samples (3-5) were collected monthly following a Standard Operating Procedure at each visit (unless there was no bedding available due to lack of supply or equipment malfunction on the follow-up visit).

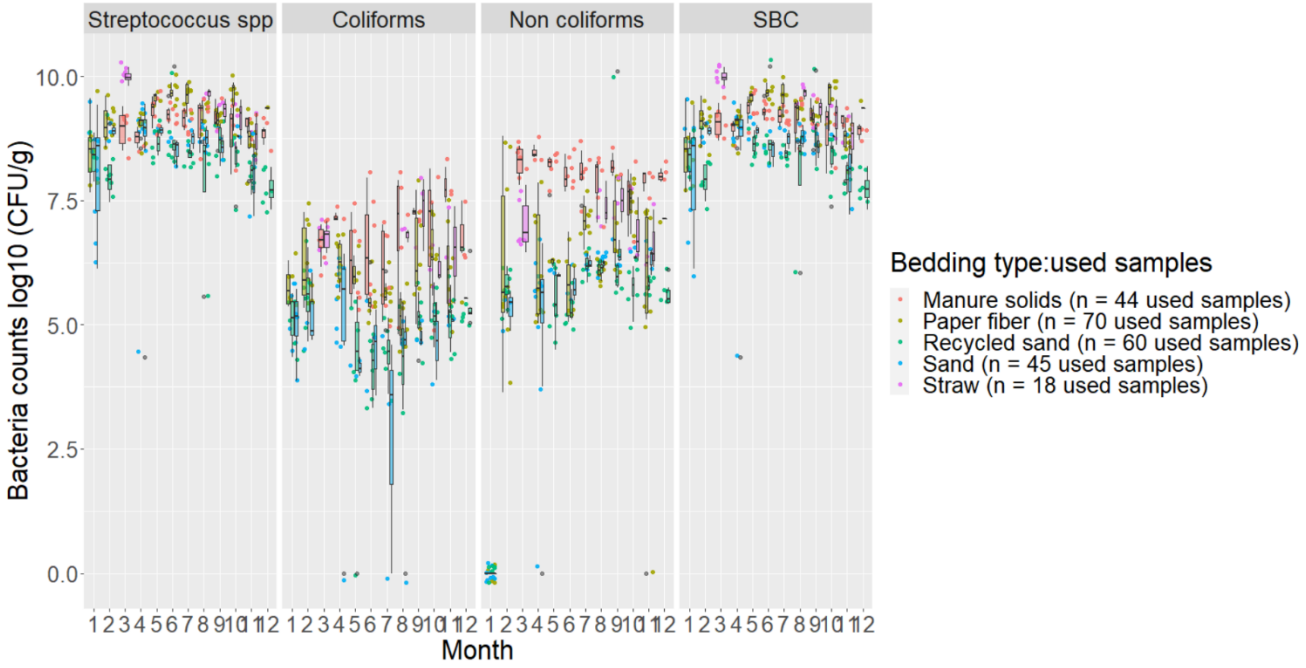


Figure 4. 3 Proportion of used or fresh bedding samples with detectable organisms of mastitis importance. Fresh and used bedding samples collected from July 2018 to July 2019 from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens. One fresh bedding sample and 3-5 used bedding samples were collected monthly following a Standard Operating Procedure at each visit (unless there was no bedding available due to lack of supply or equipment malfunction on the follow-up visit).

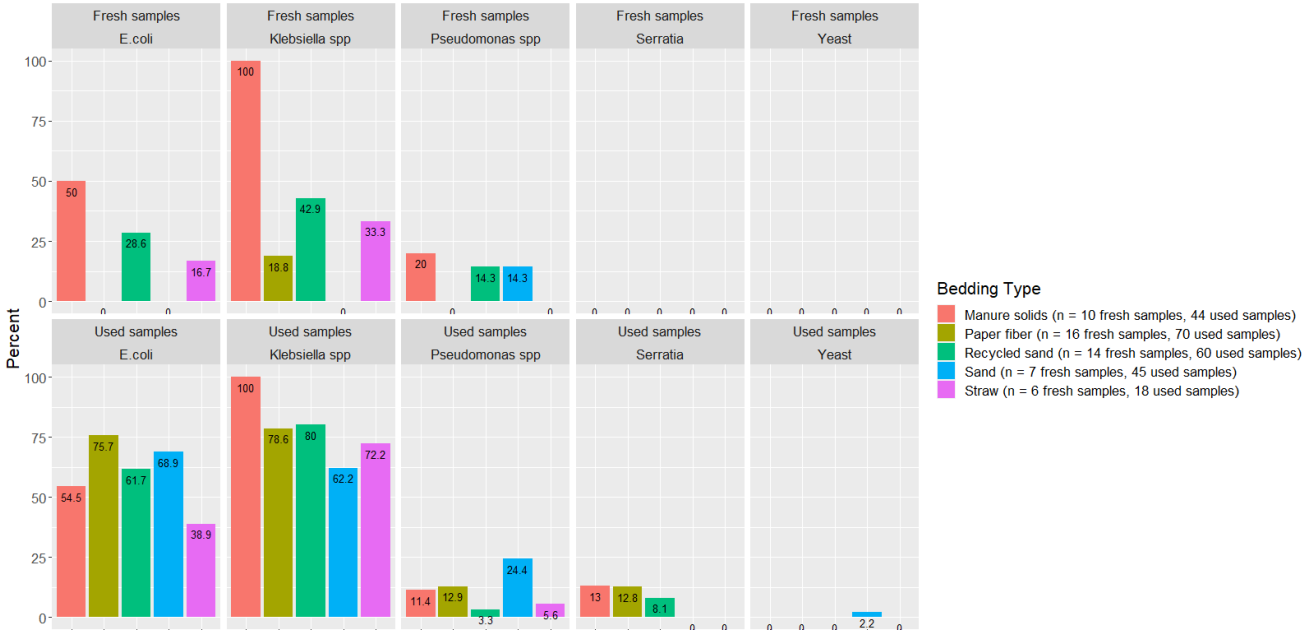


Figure 4. 4 Average Dry Matter content (% DM) and pH values (Error bars represent SD) for fresh and used bedding samples collected from July 2018 to July 2019 from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens. One fresh bedding sample and 3-5 used bedding samples were collected monthly following a Standard Operating Procedure at each visit (unless there was no bedding available due to lack of supply or equipment malfunction on the follow-up visit).

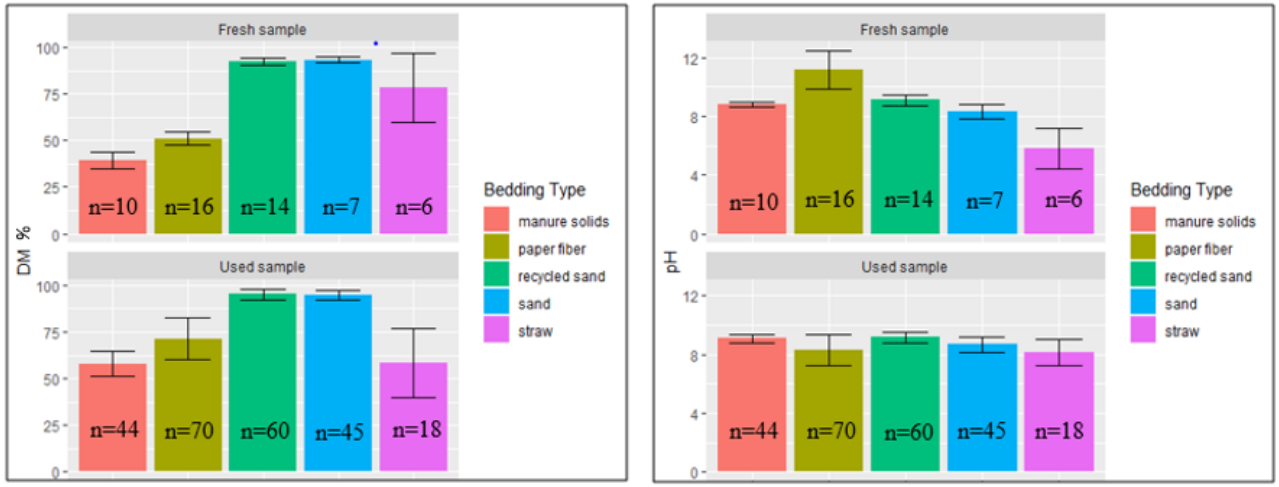


Figure 4. 5 Scatter plot of dry matter content (% DM) vs bacteria counts (log10 CFU/g) by bacteria group from fresh samples collected from July 2018 to July 2019 from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens. One fresh bedding sample was collected monthly following a Standard Operating Procedure at each visit (unless there was no bedding available due to lack of supply or equipment malfunction on the follow-up visit). When no bacteria were identified, a value of $\log_{10}$ + 1 CFU/g was given, assuming that at least 10 CFU were present. SBC (Sum Bacterial Count) = *Streptococcus* spp, Coliforms and Non-coliforms summed.

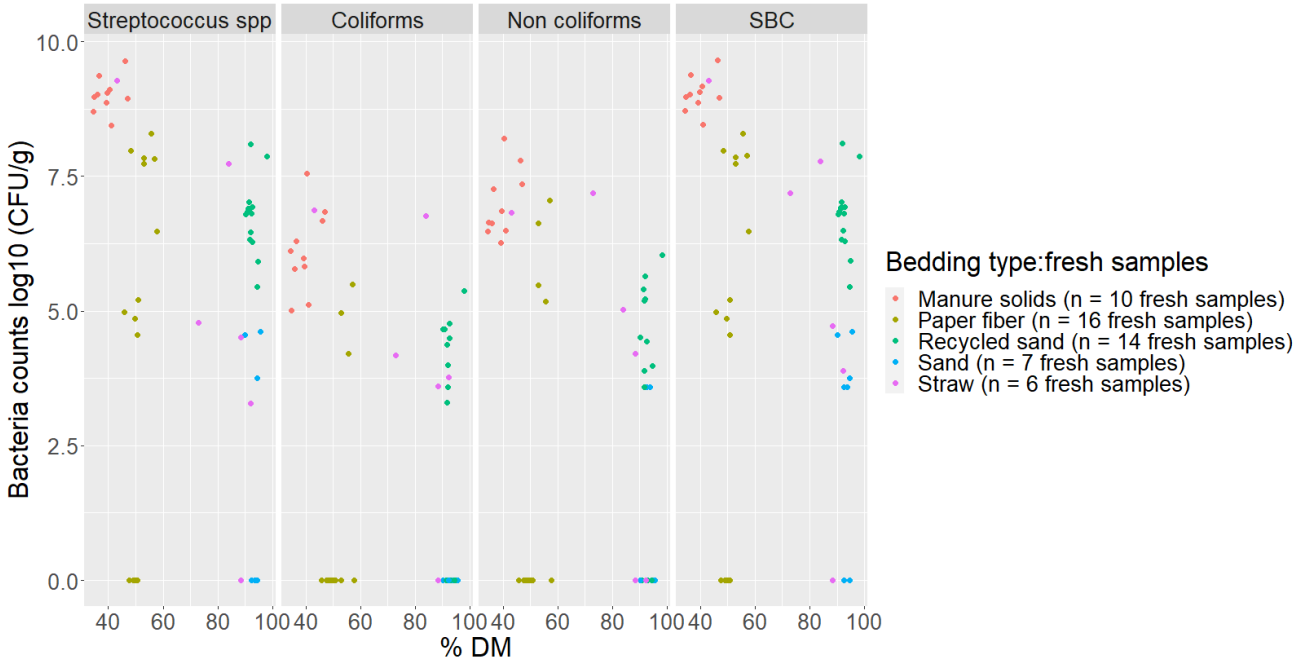


Figure 4. 6 Scatter plot of dry matter content (% DM) vs bacteria counts (log10 CFU/g) by bacteria group and bedding type in used bedding samples collected from July 2018 to July 2019 from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens. Three to five used bedding samples were collected monthly following a Standard Operating Procedure at each visit (unless there was no bedding available due to lack of supply or equipment malfunction on the follow-up visit). When no bacteria were identified, a value of $\log_{10}$ + 1 CFU/g was given, assuming that at least 10 CFU were present. SBC (Sum Bacterial Count) = *Streptococcus* spp, Coliforms and Non-coliforms summed.

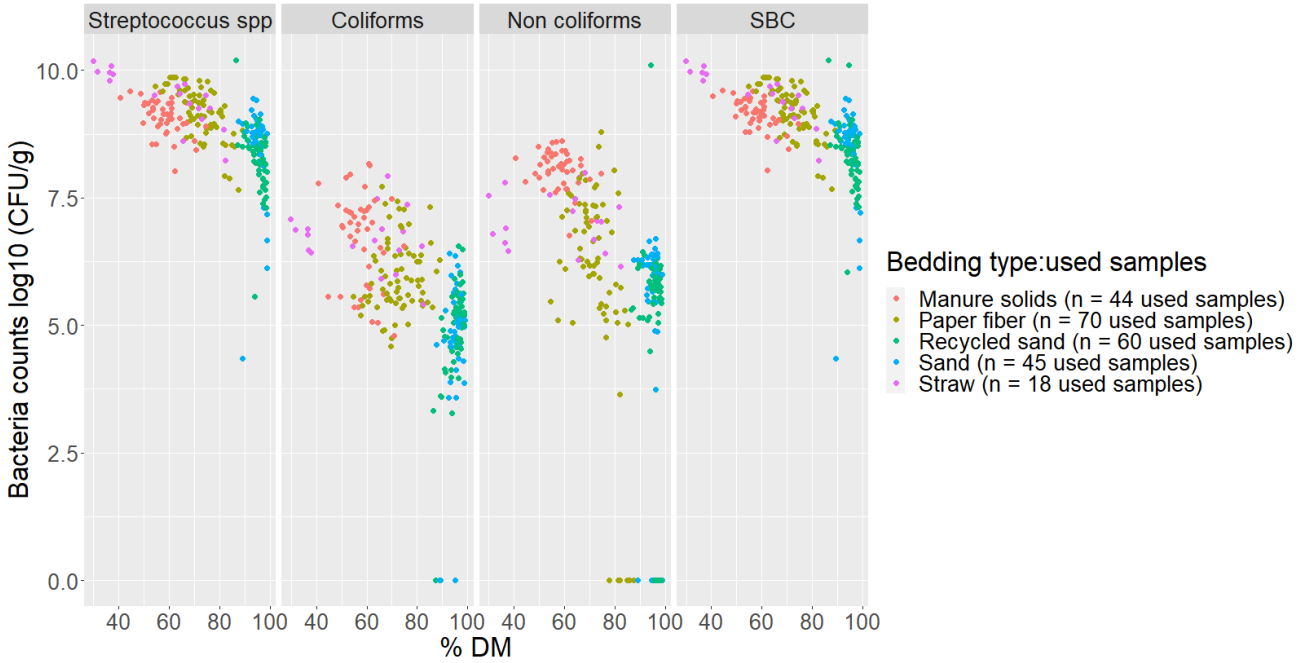


Figure 4. 7 Average bacteria counts (log10 CFU/ml) in milk samples monthly collected (unless milk had been picked up prior to arrival for follow up visit) from the bulk tank after mechanically agitating the milk for at least 5 min until sufficient homogeneity is obtained, from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens. Error bars represent SD. TBC = Total Bacteria count. When no bacteria was identified, a value of $\log_{10} + 1$ CFU/ml was given, assuming that at least 10 CFU were present.

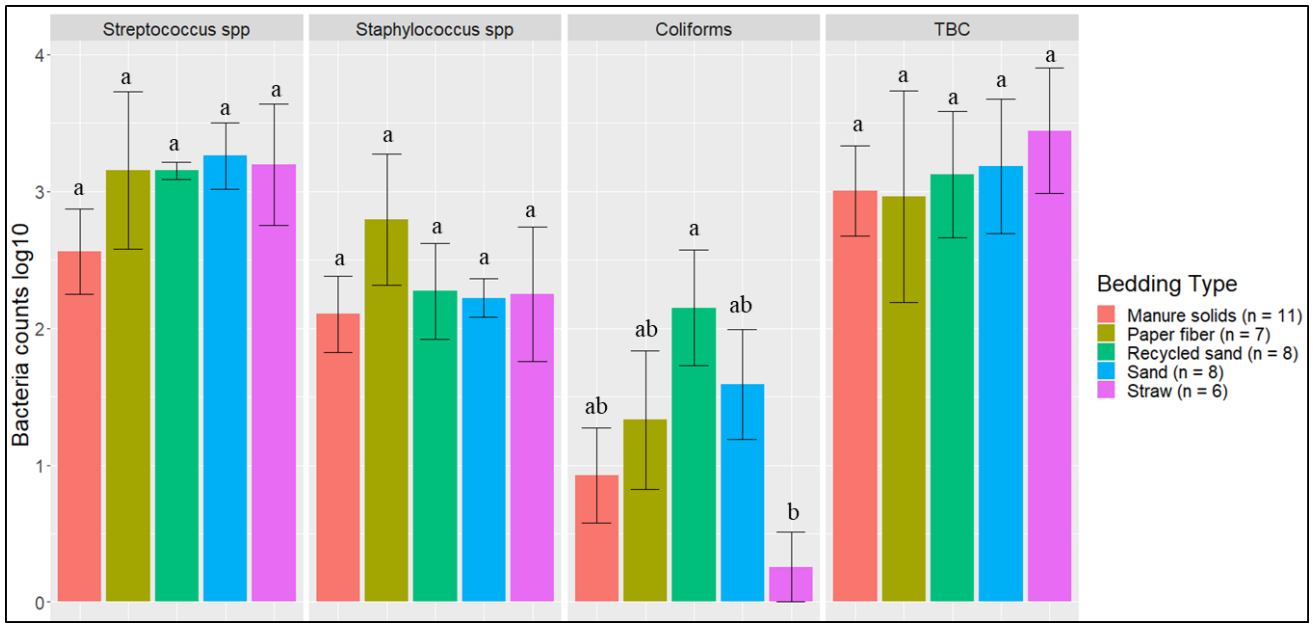


Figure 4. 8 Displayed only the proportion from BT milk samples with detectable organisms of mastitis importance. Milk samples monthly collected (unless milk had been picked up prior to arrival for follow up visit) from the bulk tank after mechanically agitating the milk for at least 5 min until sufficient homogeneity is obtained, from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens.

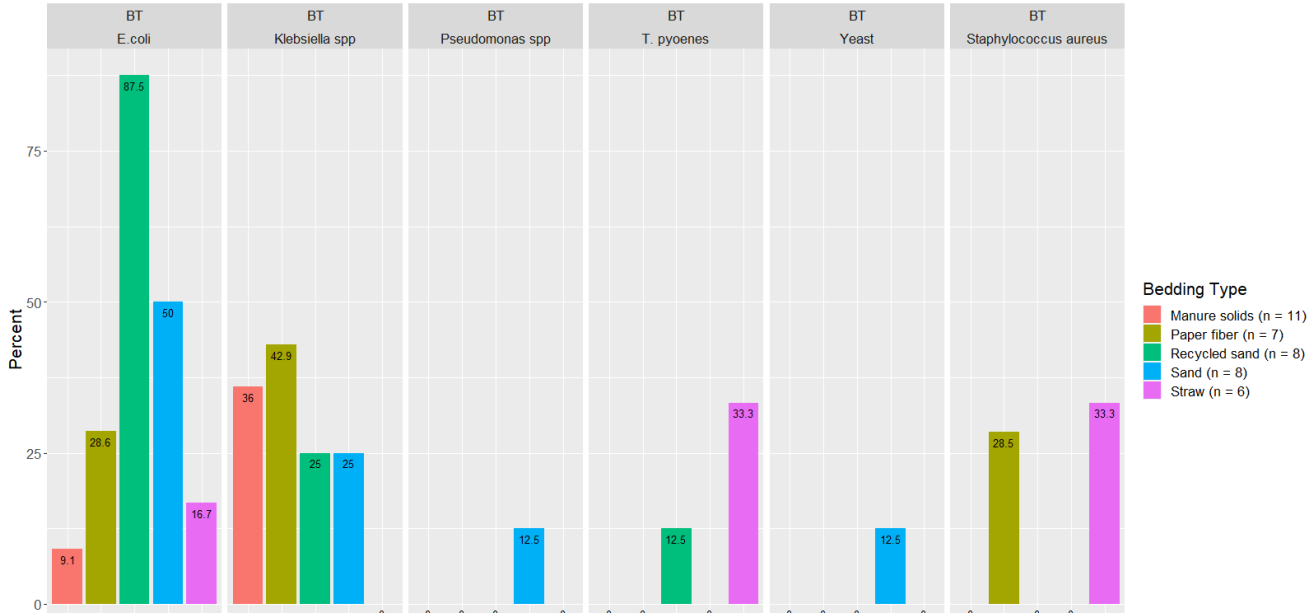
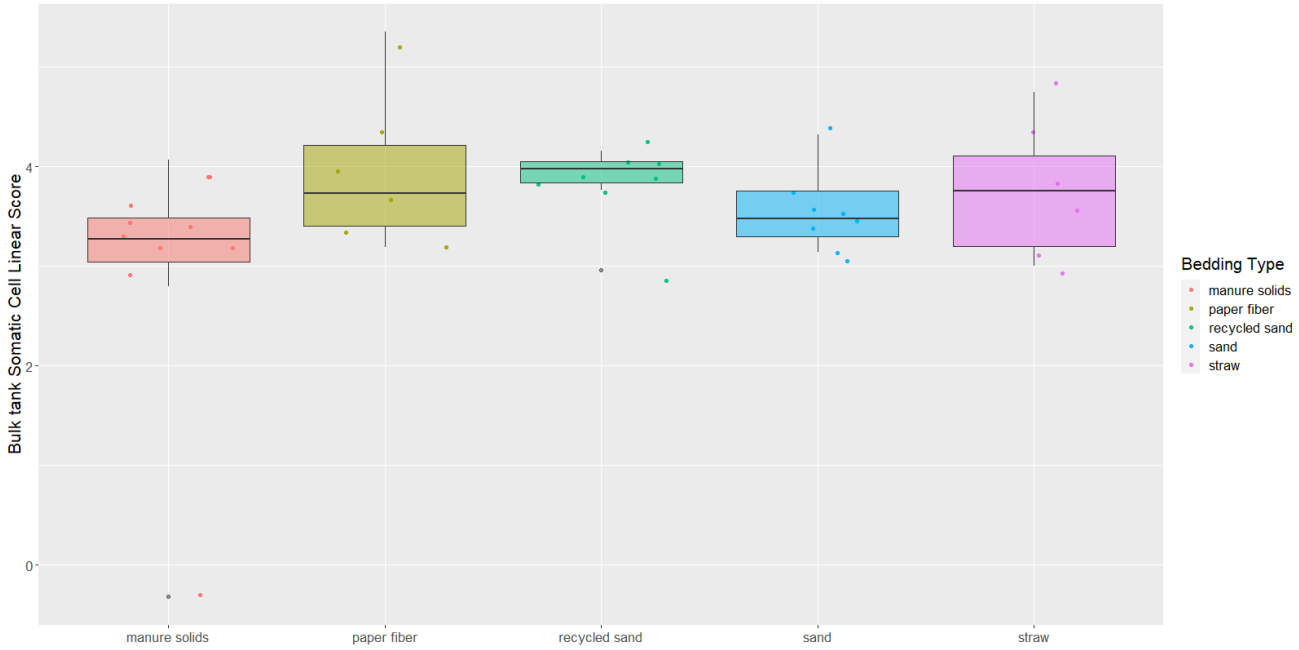


Figure 4. 9 Boxplots showing 25th, 50th (median), and 75th percentiles of somatic cell score in milk samples monthly collected (unless milk had been picked up prior to arrival for follow up visit) from the bulk tank after mechanically agitating the milk for at least 5 min until sufficient homogeneity is obtained, from 5 conveniently selected New York dairy herds using 1 of 5 bedding materials in lactating pens. Milk samples analyzed using DeLaval cell counter (DCC) to get Somatic Cell Counts and transformed into somatic cell scores (BTSLS) by applying the following equation: BTSLS = log2 (BTSCC/100) + 3.



DISCUSSION

This study describes characteristics (i.e., bacteria counts, pH, and DM) for fresh and used bedding samples, as well as bacterial counts and somatic cell counts from BT samples. These samples were collected, following a strict sampling SOP, monthly over 1 year from 5 conveniently selected New York dairy farms. Each farm used 1 of 5 bedding materials in lactating pens. In addition to describing these characteristics, this sampling scheme allowed us to demonstrate the variability within bedding samples in the same farm.

The first objective of this study was to describe bedding bacterial counts, pH, and dry matter. It is known that bedding material (especially organic material) can support bacterial growth due to contained nutrients, and even inorganic bedding once soiled with feces, urine, or any other cow secretion can grow bacteria. Our results confirm this with bacterial counts higher in used samples compared to fresh samples, which agrees with what has been stated by other research groups (Hogan et al., 1989; Bradley et al., 2018; Patel et al., 2019; Rowe et al., 2019). Evaluating these bedding characteristics is important because organic bedding material has been associated with higher bacterial load (Hogan et al., 1989; Patel et al., 2019), and with higher bacterial counts on teat skin (Zdanowicz et al., 2004; Rowbotham and Ruegg, 2016a). Our results on bacterial counts were generally highest for MS on fresh bedding samples for all bacterial groups, which is similar to what is described by other researchers (Rowbotham and Ruegg, 2016a; Patel et al., 2019). Particularly, coliforms counts were not different between RS, ST, and PF. As for used samples, we observed that organic materials supported the highest levels of all bacterial groups (Figure 4.1). In herds bedding with inorganic bedding material, *Streptococcus* spp levels were lowest in RS compared to SD, but similar to previous research (Kristula et al., 2005) there was no difference in the number of coliforms and Non-coliforms.

Several organisms of mastitis importance were not quantified, but rather their presence evaluated because we focused on the pathogens that we can manage in bedding. In other words, we manage all *Streptococcus* species as a whole, but we can't specifically manage *Streptococcus uberis* or *Streptococcus dysgalactiae*. *E. coli* was detected in only 50 and 54% of MS fresh and used bedding samples, respectively, which was surprising given that *E. coli* is known to exist in high quantities in feces. Apparently, in the herd studied here that bedded with MS, the manure and bedding processing procedures reduced *E. coli* to levels below detection. However, another fecally shed organism, *Klebsiella* spp, was found in 100% of fresh and used MS samples (Figure 4.3) suggesting that at least on this farm the manure processing and bedding procedures did not eliminate *Klebsiella* spp leaving it as a risk factor for intramammary infections.

In our study, DM content was higher for RS and SD (~92%) in fresh samples, similar to Canadian farms (Robles et al., 2019) and within the ranges reported for used samples (~95%) by Zdanowicz, Patel, and Kristula (Zdanowicz et al., 2004; Kristula et al., 2005; Patel et al., 2019). These values seem to have low variability across studies. Fresh MS had a DM average content of 39.5% (34.2 - 47.0%), similar to the values reported by Robles (Robles et al., 2019). However, a different study (Patel et al., 2019), reported a much wider range (21.4 – 96.3%) in samples collected from 17 states across the US. That variability might be explained by different MS processing techniques (i.e., digested, compost, or fresh), and possibly due to the sampling variability (e.g., time in relation to when were applied). In our study, used MS samples had a higher DM content (57.8%, range of 40.6 – 74.6%) compared to MS fresh samples. This observation has been reported by others (Husfeldt et al., 2012; Sorter et al., 2014; Robles et al., 2019).

The relationship of DM content and bacterial growth suggest that drier bedding material

impedes bacterial growth for all bacterial groups in all bedding types. The correlations between these variables are similar to the ones reported by Zdanowicz (Zdanowicz et al., 2004). As a result, high % DM (e.g., as for RS or SD) supported the lowest levels of growth of *Streptococcus* spp, Coliforms, and Non-coliforms compared to those bedding materials with lower % DM (Figures 4.5 and 4.6).

Regarding pH values, most of the bedding materials samples were on the alkaline side within a range of 8-11 except for ST, with acidic values (5.8 ±1.4) in fresh samples (Figure 4.4). This is similar to those reported in other research (Patel et al., 2019). This can be of importance when controlling some bacteria species that do not multiply well in low-pH environments (Godden et al., 2008).

Our results show that even following a standardized sampling protocol, the bacterial count distribution in used samples within the same day of sampling had a noticeable variation, especially in PF, RS, and ST materials. The MS and SD appeared to have counts that were more constant within the same day of sampling, although differed throughout the study period (Figure 4.2). This suggests that using summarized data such as averages might not be a good way to analyze bedding bacteria because one might lose a lot of important information about the variability. This is important to consider when evaluating bedding samples and a specific outcome and when using only a few samples from a specific point in time in an attempt to describe bedding data.

The second objective was to evaluate the association between bedding type with milk quality. When evaluating the association between BT bacteria and bedding bacteria counts, our results show the greatest difference was in Coliforms in the RS and ST bedding (Figure 4.7). However, ST is also the bedding where the farm was only present for 6 months of the study so

these findings should be interpreted with caution. Other studies have shown a similar lack or marginal association between bulk tank total bacterial count and bedding type ((27, 28), respectively). Bradley reported a marginal difference, where it was higher for farms using recycled manure solids bedding, followed by wood products, straw, and sand. The detected organisms of mastitis importance varied across BT samples; surprisingly *E. coli* was detected in only 9.1% of samples from MS herd, whereas 87.5% in RS farm (Figure 4.8). We did not find an association between bedding type and BTSLS (Figure 4.9).

It is important to note that other cross-sectional bedding studies used only 2 points in time during different seasons (winter and summer) and did not take into account the variability during an extended period. Even though these researchers showed the variability among farms, they did not take into account the variability in bacterial counts within the same farm, on the same day of sampling, or even how the sampling method can affect these parameters.

STRENGTHS AND LIMITATIONS

This was a descriptive study that prospectively evaluated bedding bacterial counts over time. The two main strengths of this study are the consistent sampling SOP and serial sampling of bedding through time. These features can reduce the variability in sample procurement and improve the understanding of bedding bacteria count variability among sampling times.

However, missing bedding and BT samples decreased the number of complete evaluations. Another possible limitation is the use of frozen samples which can result in possible measurement error in bacterial counts. Although Homerosky (Homerosky) reported a decrease in gram-negative and coliform bacteria counts after freezing, the QMPS laboratory did not find any difference in bacteria counts. In the aforementioned data from QMPS, bacteria counts were evaluated weekly from 20 bedding samples and did not show a significant difference between

each day for up to 21 days (M. Zurakowski, unpublished data).

Finally, this study only involved 5 herds, each with 1 bedding type. Thus, only 1 experimental unit per bedding type was included in this analysis, and this limits the ability to generalize the findings to other farms using these types of bedding material. Nonetheless, our results showed that even conducting repeated sampling within farm, there was a significant variation in the bacterial count within the sampling day and throughout the study period (monthly samples). These findings indicate that results from studies evaluating the association between bedding material and bulk tank bacterial load should be interpreted with caution, especially if a single or few samples collections were carried out over time. That may be a concern even in studies enrolling several herds per bedding material.

The herds enrolled in our study were well managed and conveniently selected; therefore, our findings should not be generalized to herds with different management practices and different bedding processing. Differences in management practices in each herd may likely influence the bedding bacterial counts and the association between bedding type and BT parameters. However, it is important to mention that the main objective of this study was to report the variability in bacterial counts within the farms over time and its association with the bacterial load present in the BT milk. The association assessment between bedding bacterial counts and particular herd management practices was not in the scope of the study.

CONCLUSIONS

Bedding sample results can be difficult to interpret because bacteria counts in bedding are not easily linked to bacteria counts in BT or milk quality. Results from this study show that there is a lot of variability in bedding samples, even when collected under strict SOP guidelines. In bedding samples, a higher DM content had the lowest levels of bacterial growth compared to

those with lower DM content. No associations between BT bacteria counts and bedding bacterial counts were observed. No association between bulk tank somatic cell counts based on bedding type were observed. Despite using an SOP for bedding sampling in an effort to consistently collect samples, we still observed a large amount of variability, both within and among bedding samples. This variability may have obscured any potential association between BT milk quality and bedding type.

REFERENCES

Barbano, D.M., Y. Ma, and M.V. Santos. 2006. Influence of Raw Milk Quality on Fluid Milk Shelf Life1, 2. Journal of Dairy Science 89:E15–E19. doi:10.3168/jds.S0022-0302(06)72360-8.

Barkema, H.W., Y.H. Schukken, T.J. Lam, M.L. Beiboer, G. Benedictus, and A. Brand. 1999. Management practices associated with the incidence rate of clinical mastitis. J Dairy Sci 82:1643–1654. doi:10.3168/jds.S0022-0302(99)75393-2.

Bartlett, P.C., G.Y. Miller, S.E. Lance, and L.E. Heider. 1992. Environmental and managerial determinants of somatic cell counts and clinical mastitis incidence in Ohio dairy herds. Preventive Veterinary Medicine 14:195–207. doi:10.1016/0167-5877(92)90016-9.

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. International Journal of Food Microbiology 269:36–45. doi:10.1016/j.ijfoodmicro.2017.12.022.

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. doi:10.1017/s0022029900022500.

Cha, E., J.A. Hertl, Y.H. Schukken, L.W. Tauer, F.L. Welcome, and Y.T. Gröhn. 2013. The effect of repeated episodes of bacteria-specific clinical mastitis on mortality and culling in Holstein dairy cows. Journal of Dairy Science 96:4993–5007. doi:10.3168/jds.2012-6232.

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. doi:10.3168/jds.2007-0415.

Gröhn, Y.T., D.J. Wilson, R.N. González, J.A. Hertl, H. Schulte, G. Bennett, and Y.H. Schukken. 2004. Effect of Pathogen-Specific Clinical Mastitis on Milk Yield in Dairy

Cows. Journal of Dairy Science 87:3358–3374. doi:10.3168/jds.S0022-0302(04)73472-4.

Guarín, J.F., C. Baumberger, and P.L. Ruegg. 2017. Anatomical characteristics of teats and premilking bacterial counts of teat skin swabs of primiparous cows exposed to different types of bedding. J. Dairy Sci. 100:1436–1444. doi:10.3168/jds.2016-11514.

Heikkilä, A.-M., E. Liski, S. Pyörälä, and S. Taponen. 2018. Pathogen-specific production losses in bovine mastitis. Journal of Dairy Science 101:9493–9504. doi:10.3168/jds.2018-14824.

Hertl, J.A., Y.H. Schukken, D. Bar, G.J. Bennett, R.N. González, B.J. Rauch, F.L. Welcome, L.W. Tauer, and Y.T. Gröhn. 2011. The effect of recurrent episodes of clinical mastitis caused by gram-positive and gram-negative bacteria and other organisms on mortality and culling in Holstein dairy cows. Journal of Dairy Science 94:4863–4877. doi:10.3168/jds.2010-4000.

Hertl, J.A., Y.H. Schukken, F.L. Welcome, L.W. Tauer, and Y.T. Gröhn. 2014. Pathogen-specific effects on milk yield in repeated clinical mastitis episodes in Holstein dairy cows. Journal of Dairy Science 97:1465–1480. doi:10.3168/jds.2013-7266.

Hogan, J., and K.L. Smith. 2012. Managing Environmental Mastitis. Veterinary Clinics: Food Animal Practice 28:217–224. doi:10.1016/j.cvfa.2012.03.009.

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, and B.L. Brockett. 1989. Bacterial counts in bedding materials used on nine commercial dairies. J. Dairy Sci. 72:250–258. doi:10.3168/jds.s0022-0302(89)79103-7.

Homerosky, E.F. The Effects of Freezing on Bacterial Counts in Bovine Bedding Materials 1.

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 freestall dairy herds. Journal of Dairy Science 95:2195–2203. doi:10.3168/jds.2011-5105.

Jensen, M.B., L.J. Pedersen, and L. Munksgaard. 2005. The effect of reward duration on demand functions for rest in dairy heifers and lying requirements as measured by demand functions. Applied Animal Behaviour Science 90:207–217. doi:10.1016/j.applanim.2004.08.006.

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. doi:10.3168/jds.S0022-0302(05)73118-0.

Kruze, J., and A.J. Bramley. 1982. Sources of Streptococcus uberis in the dairy herd: II. Evidence of colonization of the bovine intestine by Str. uberis. Journal of Dairy Research 49:375–379. doi:10.1017/S0022029900022512.

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. Journal of Dairy Science 94:4441–4456. doi:10.3168/jds.2010-4046.

Ma, Y., C. Ryan, D.M. Barbano, D.M. Galton, M.A. Rudan, and K.J. Boor. 2000. Effects of Somatic Cell Count on Quality and Shelf-Life of Pasteurized Fluid Milk. Journal of Dairy Science 83:264–274. doi:10.3168/jds.S0022-0302(00)74873-9.

Munksgaard, L., M.B. Jensen, L.J. Pedersen, S.W. Hansen, and L. Matthews. 2005. Quantifying behavioural priorities—effects of time constraints on behaviour of dairy cows, Bos taurus. Applied Animal Behaviour Science 92:3–14. doi:10.1016/j.applanim.2004.11.005.

Murphy, S.I., D. Kent, N.H. Martin, R.L. Evanowski, K. Patel, S.M. Godden, and M. Wiedmann. 2019. Bedding and bedding management practices are associated with mesophilic and thermophilic spore levels in bulk tank raw milk. Journal of Dairy Science 102:6885–6900. doi:10.3168/jds.2018-16022.

Oliveira, L., C. Hulland, and P.L. Ruegg. 2013. Characterization of clinical mastitis occurring in cows on 50 large dairy herds in Wisconsin. Journal of Dairy Science 96:7538–7549. doi:10.3168/jds.2012-6078.

Ospina, P., V. Alanis, A. Vasquez, F. Welcome, T. Tomazi, R. Watters, K. Marely, and D. Nydam. 2019. Heifer Mastitis - What About it? Page.

Paduch, J.-H., E. Mohr, and V. Krömker. 2013. The association between bedding material and the bacterial counts of Staphylococcus aureus, Streptococcus uberis and coliform bacteria on teat skin and in teat canals in lactating dairy cattle. J Dairy Res 80:159–164. doi:10.1017/S0022029913000046.

Patel, K., S.M. Godden, E. Royster, B.A. Crooker, J. Timmerman, and L. Fox. 2019. Relationships among bedding materials, bedding bacteria counts, udder hygiene, milk quality, and udder health in US dairy herds. Journal of Dairy Science. doi:10.3168/jds.2019-16692.

Petersson-Wolfe, C.S., S. Adams, S.L. Wolf, and J.S. Hogan. 2008. Genomic Typing of Enterococci Isolated from Bovine Mammary Glands and Environmental Sources1. Journal of Dairy Science 91:615–619. doi:10.3168/jds.2007-0253.

Pinzón-Sánchez, C., and P.L. Ruegg. 2011. Risk factors associated with short-term post-treatment outcomes of clinical mastitis. Journal of Dairy Science 94:3397–3410. doi:10.3168/jds.2010-3925.

Robles, I., D.F. Kelton, H.W. Barkema, G.P. Keefe, J.P. Roy, M. a. G. von Keyserlingk, and T.J. DeVries. 2019. Bacterial concentrations in bedding and their association with dairy cow hygiene and milk quality. Animal 1–15. doi:10.1017/S1751731119002787.

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. Journal of Dairy Science 98:7865–7885. doi:10.3168/jds.2015-9866.

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. Journal of Dairy Science 99:4707–4717. doi:10.3168/jds.2015-10675.

Rowe, S.M., S.M. Godden, E. Royster, J. Timmerman, B.A. Crooker, and M. Boyle. 2019. Cross-sectional study of the relationships among bedding materials, bedding bacteria counts, and intramammary infection in late-lactation dairy cows. Journal of Dairy Science 102:11384–11400. doi:10.3168/jds.2019-17074.

Ruegg, P.L. 2017. A 100-Year Review: Mastitis detection, management, and prevention. Journal of Dairy Science 100:10381–10397. doi:10.3168/jds.2017-13023.

Schukken, Y.H., J. Hertl, D. Bar, G.J. Bennett, R.N. González, B.J. Rauch, C. Santisteban, H.F. Schulte, L. Tauer, F.L. Welcome, and Y.T. Gröhn. 2009. Effects of repeated gram-positive and gram-negative clinical mastitis episodes on milk yield loss in Holstein dairy cows. Journal of Dairy Science 92:3091–3105. doi:10.3168/jds.2008-1557.

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 freestalls. J. Dairy Sci. 97:2965–2968. doi:10.3168/jds.2013-7814.

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. Journal of Dairy Science 101:530–536. doi:10.3168/jds.2016-12358.

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 Sand or Sawdust. Journal of Dairy Science 87:1694–1701. doi:10.3168/jds.S0022-0302(04)73322-6.

CHAPTER FIVE

SHORT COMMUNICATION: DAIRY FARM WORKER MILKING EQUIPMENT TRAINING WITH AN E-LEARNING SYSTEM

Valeria M. Alanis[1], Paula A. Ospina[2], W. Heuwieser[3], Paul D. Virkler[1]

[Manuscript prepared for submission]

[1]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853
[2]Lechear LLC, King Ferry, New York, USA
[3]Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, 14163 Berlin, Germany

ABSTRACT

In many farms, the logistics of on-farm training are a limiting factor, due to communication, cultural challenges, and limited access to industry professionals. The use of E-learning systems may help bridge the communication gap and can be sensitive to cultural challenges. The objectives of this study were to 1) identify how many of the high priority problems in the milking parlor relate to milker training in the areas of milking equipment and milking routine, 2) design and test an E-learning training system for dairy farm milkers related to milking equipment, , and 3) gain feedback targeted to improve subsequent E-learning training modules. An interactive online training course on basic checks of the milking equipment was developed with a cloud-based authoring software. A total of 15 commercial dairy farms resulting in training of 95 dairy farm workers in Northern New York State participated in this study. Milk quality experts performed an initial evaluation of three main areas: equipment analysis, milker assessment, and cow assessment. Based on this, risk factors for mastitis were summarized for each farm and the top three risk factors were identified and placed into priority categories. A one-hour training event was scheduled with milkers guided by a bilingual professional in milk quality. Over 50% of the farms (8/15) had one or more of the top three priorities with milking equipment, which milkers could have detected and reported to management. All participants completed the module and 95% stated that they felt capable of checking the equipment before milking, 86% felt more confident in reporting equipment problems to the manager after having taken the course. There were also discrepancies between managers and milkers on whether milking equipment training was offered. This can be explained due to the lack of or secondary to poor communication between managers and employees on training objectives and goals on the farm. Our results also show that milking equipment issues which milkers could detect and report

are common on dairy farms and reinforces the need for additional milker training in this area.

Keywords: training, E-learning, milking equipment

INTRODUCTION

Learning is defined as the absorption of information aimed at increasing knowledge, skills, and behaviors by employees to apply it in real-life situations, while training is aimed to facilitate this process and depends on the quality of the transfer of the desired information (Noe, 2017). As in any competitive business, a dairy farm should have both learning and training as key components in its management plan. There are generally three options for training: in-person training by on-farm personnel or industry professionals, access to E-learning systems, which is an educational and training platform that takes place over the internet, and finally a combination of both.

In the last years, US dairy farms have seen an increase in the number of Latino employees (Jenkins et al., 2009; Menger et al., 2016), which can impact the business through communication and cultural challenges, and increase safety and occupational health risk (Arcury et al., 2010; Hurley and Lebbon, 2012). This complicates management-employee relationships and results in more rapid employee turnover (Barkema et al., 2015; Erskine et al., 2015). The primary native language of twenty-five percent of these Latino employees is an indigenous language, and sometimes this is not in written form (Arcury et al., 2010). In addition, the education distribution in this group is not homogenous, ranging from zero years of education to individuals with professional degrees (Rodriguez et al., 2018; Sischo et al., 2019; Maloney et al.). This deficient formal education and the challenge to effectively communicate creates additional barriers such as lack of confidence in their ability to learn, limited access to training

tools, and a reduced likelihood to perform managerial functions compared to their English-speaking counterparts (Stack et al., 2006). Recently published studies, however, showed that employers oftentimes underestimate the employee's interest in learning and commitment to the success of the farm (Durst et al., 2018) and that the absence of training or training materials negatively impacts employee recruitment and retention (Moore et al., 2020). For that reason, training should include bilingual content, as it has been demonstrated that this benefits the employees (Chase et al., 2006; Raymond et al., 2006; Rovai et al., 2016).

Achieving or maintaining high standards of milk quality still relies heavily on dairy employees. Therefore, milkers should be well trained and conscientious about milking routines and milking equipment. Lack of training has been claimed to be one of the main reasons for lower detection of animal health problems, poor animal handling and management of calving events, and poor milking routines (Gutierrez-Solano et al., 2011; Schuenemann et al., 2013). Farms with frequent training of milking personnel achieve faster milking speeds and lower rates of clinical mastitis (Rodrigues et al., 2005).

Although most dairy producers and industry professionals would agree that both initial hire and ongoing employee training are essential to assuring proper adherence to protocols (Jansen et al., 2010; Erskine et al., 2015; Belage et al., 2019), the practical logistics of on-farm training are a limiting factor. This has been especially true during the COVID-19 pandemic, which has severely limited in-person training and revealed opportunities for new ways to deliver training. The use of online information has become a part of normal life worldwide and it seems reasonable to incorporate it as a training tool in dairy farms. Besides, online training has been shown to be effective at creating a feeling of confidence and accuracy in work performance

(Hesse et al., 2019) and is an effective way to deliver safety awareness training to dairy employees (Rodriguez et al., 2018). The objectives of this study were to 1) identify how many of the high priority problems in the milking parlor relate to milker training in the areas of milking equipment and milking routine, 2) design and test an E-learning training system for dairy farm milkers related to milking equipment, , and 3) gain feedback targeted to improve subsequent E-learning training modules.

MATERIALS AND METHODS

An interactive online training course on basic checks of the milking equipment was developed with a cloud-based authoring software (GomoLearning, Brighton, UK). The course consisted of 5 modules (i.e., liner alignment, checking vents, checking pulsators, assessing vacuum levels, and preparing the milkhouse) and was available both in English and Spanish. Each module took the user 6 to 8 minutes to complete with the entire course lasting approximately 30 to 40 minutes. The modules were designed to be user-friendly and straightforward to ensure an engaging and non-intimidating learning experience. The content was displayed in a step-by-step fashion based on images and videos. Textual information was reduced to a minimum and aimed at how to perform each step of the equipment check, why each check is important to udder health, and the quality of the milk produced. In two of the modules, there was an option for the participant to choose between reading the texts and listening to audio. Four sets of three questions each were embedded into the modules to collect data on the background information of the participants, the farm, and perceptions of the modules. At the end of each module, there were quiz questions to gauge how well the participant understood the main concept. An introductory module was provided to familiarize oneself with the major components of the milking system and explained the function of each component. This section was designed

for employees with limited milking experience. Participants could choose to explore each component on their own or have it taught to them through a narrated video. A glossary of relevant key terms in the modules was available throughout including images and brief definitions.

After completion and beta-testing in one farm with 10 milkers and guided in both Spanish and English, the modules were used in a field study conducted on 15 commercial dairy farms across four counties in Northern New York State between September 2020 and January 2021. These farms were a convenience sample based on long-term working relationships and the willingness of the farm to participate in this research. The main breed of these farms was Holstein-Friesian, with an average farm size of 800 cows (ranging from 250 to 2400) and an average milk production of 38.6 kg/cow/d (ranging from 32.7 to 49.1). Monthly mean somatic cell count (SCC) was 178,000 cell/ml, (ranging from 100,000 to 310,000 cells/ml). The average number of milkers per farm was 6 (ranging from 3 to 14).

For each of the farms, an initial extension survey was performed to assess the risk factors for mastitis. An extension survey is a service provided by Quality Milk Production Services (QMPS) from the Animal Health Diagnostic Center at Cornell University; often used by dairy producers who have large herds, whereby milking procedures, management, housing, equipment, and mastitis control are evaluated. This service is performed by QMPS professionals, including veterinarians with parlor experience and skilled technicians. The information is then used to make recommendations for improved management and mastitis control.

Each extension survey consisted of an assessment of 3 main areas: 1) equipment analysis involving average claw vacuum, milk line vacuum during milking, and graphing all pulsators, 2)

milker assessment involving milking routine, milk flow rate analysis, unit alignment scoring, teat end cleanliness scoring, and dip coverage, and 3) cow assessment involving teat scoring, strip yields, udder hygiene scoring, and an assessment of the environment.

During equipment analysis, the average claw vacuum at peak flow and the pulsation parameters were measured with the unit on the cow and milk flowing through the claw per the NMC guidelines for dynamic testing (NMC Procedures, 2012). Milkline vacuum stability was assessed per NMC guidelines over an approximately 30 minute period at the milk inlet closest to the receiver jar during normal milking operations. All pulsators were also tested statically per NMC guidelines.

For milker assessment, milking routine timing focused on initial stimulation time, pre-dip contact time, and the lag time from stimulation to unit attachment. The milk flow rates of individual cows were measured using an electronic milk flow meter (Lactocorder, WMB, Balgach, Switzerland. Unit alignment was measured using a two-category scoring system (proper or improper unit alignment) and was assessed within the first two minutes after unit attachment, with any three-quarter cow not scored. Teat end cleanliness was performed after teat preparation but before unit attachment. A 10x10 cm gauze soaked in alcohol was used to swab the teat end. The scores were recorded using a one to a four-category system (Cook and Reinemann, 2007). Dip coverage was assessed by visually observing all surfaces of the teat including the teat end and all sides of the barrel, and evaluating whether or not dip was present.

For cow assessment, teat scoring was performed within one minute of unit detachment using the Teat Club International scoring system (Mein et al., 2001). At least 20% of the farm was scored in the categories measuring the short and long-term effects on the teats. Strip yields

were performed immediately after unit detachment. Each teat was stripped for a maximum of 15 seconds and the total volume of milk from all four teats recorded. Udder hygiene was scored using a one to a four-category system (Schreiner and Ruegg, 2002). The environment was assessed by walking the lactating cow stalls and scoring in the categories of cleanliness, bedding levels, and cow positioning.

Based on the data from the extension survey, the risk factors for mastitis were summarized and ranked by importance for each farm. The three most important risk factors for each farm were then placed into the following categories: milking equipment malfunction, equipment malfunctions that could be detected by milkers, inadequate milking routine, and other.

After the extension survey, a one-hour training event was scheduled with milkers in each farm to apply and test the online course in a real-life situation. Milkers, and farm managers or owners, completed an initial written questionnaire at the beginning of each session regarding background and training on farm (Table 5.2).

As the online course can be run on any web-enabled device, milkers were asked either to use their cellphones or a tablet that we provided. Each participant received a unique login ID based on a random number and linked to the farm to assure anonymity and create a safe working environment for the milkers. A description of the terms and conditions of use and the privacy and data protection policies was provided on the first page of the course. This training project was performed following the oral consent guidance from the Cornell University Institutional Review Board for Human Participants.

Milkers were asked to complete the module during the training session that lasted about 50 minutes. Any person with reading disabilities was guided by a bilingual project collaborator

and any question regarding the content was clarified when necessary. All milkers completing the modules received a printed certificate with their name on it. Oral feedback was collected about how they felt about the module, and if anyone had suggestions for improvements.

RESULTS AND DISCUSSION

Based on the extension survey results, fourteen of 15 farms had a milking equipment problem as at least one of the top three risk factors for mastitis. Eight farms had one or more of the top three risk factors that involved milking equipment malfunction, which milkers could have detected and reported to management. Inadequate milking routine also accounted for a large portion of the risk factors with 13 of the farms having this as one or more of the top three risk factors. For the category of equipment malfunctions that milkers could have detected or corrected, pulsators and the use of the manual button (canceling the automatic cluster remover) accounted for 36% (4 out of 11) and 27% (3 out of 11) respectively. A short lag time from stimulation to attachment (47%, 7 out of 15) and teat end cleanliness (40%, 6 out of 15), were the most common problems found in the inadequate milking routine category (Table 5.1). Within the category of milking equipment malfunction the higher proportion was related to automatic take-off (ATO) adjustments (40%, 6 out of 15), and pulsator function or adjustments (33%, 5 out of 15).

A total of 95 milkers participated in this study, with 90 and 5 of these milkers having Spanish and English as their native language, respectively.

From the initial oral questions, almost half (46%) of the milkers had not milked cows before and 40% had worked less than six months on the current farm. For milkers who have been at the farm more than 6 months, they stated that the last time they had received training on the

farm was more than 6 months ago, and 83% had received some type of training when they started the position.

From the written questions. 67% of milkers stated that they had received milking equipment training when they started the position. In 59% of the cases, this training was conducted by another milker. Seven farms indicated that they provided milking equipment training to their milkers. But there were some discrepancies with milkers on four of those farms, where at least one of the milkers contradicted this statement. On the other hand, in three out of the eight farms that indicated they did not provide such training at least 80% of the milkers in each farm contradicted this. This can be explained as the lack of communication between managers and employees on what training is in respect to milking equipment. About 69% of milkers reported that they were expected to fix milking equipment problems on their farms, but 45% were not trained at all or were not satisfied with their training in milking equipment (Table 5.3). As for hours per pay period, 77 milkers reported less than 80 h, 7 milkers 80-130 h, and 10 more than 130 h. One milker did not mark any option.

All milkers completed the online course and nine relaunched it after completion. This completion rate was much higher compared to a previous study (only 6%) in which milkers worked at their discretion (P. Virkler, unpublished data). This shows mere availability online is not sufficient. Instead, it seems important to provide the employees with dedicated time to complete the training. Interestingly, 70% of the milkers stated to prefer the text compared to the audio recordings. However, 73% of the milkers reported that they wanted audio recordings in future training. One explanation for this discrepancy is that the participants were reluctant to admit a limited reading proficiency for the online course and therefore opted for audio recordings

in future materials.

We did not conduct a before and after training comparison, but the questions after each module were answered correctly in more than 80% of the cases in three of the five modules, and 75% in the milk house module. At the end of the training, 95% of the milkers stated they were able to test the equipment before milking, and 87% that they were confident to communicate milking equipment-related issues to the manager. We hypothesize that the basic concepts learned will motivate the milkers to perform the skills covered and improve the reporting of milking equipment problems (Table 5.4).

Our results show that milking equipment issues are common on dairy farms and reinforce the need for additional milker training in this area. Even though there was a certain percentage of milkers reporting some training, the percentage of milkers that were not trained at all, were not satisfied with the training, or were unsure if they were trained or satisfied was considerable (45%). This finding shows an opportunity for efficient training materials that employers could use for new employees to train them on how to detect milking equipment problems. This could be particularly valuable as many of the incoming employees are lacking at least basic knowledge or skills from growing up on a farm or from previous working experience on a farm (Hagevoort et al., 2013; Erskine et al., 2015).

All herd managers agreed on the need for training tools to better educate their employees on milking equipment. Nonetheless, only one of the herd managers launched the module and they failed to complete it. This is interesting due to supervisor support is crucial to training effectiveness, playing an important role in motivation (Chiaburu and Tekleab, 2005). Farm owners were willing to pay employees to be trained in a dedicated session; however, just giving

milkers time to complete training at their own initiative was not successful (P. Virkler, unpublished data). This observation may also demonstrate the importance of providing owners with a systematic approach to training and training materials to establish a training culture with systematic and continuous training for milkers.

This technology may help to simplify training by providing access to more remote areas through online learning approaches. Further research is warranted to demonstrate that online training is effective in improving measurable outcomes related to milking equipment issues. This could be achieved by adding a follow-up assessment to test certain manual skills. This would allow a herd manager to determine whether a milker has retained the knowledge and adequately learned the new skills.

This study provides more evidence that there is a lack of a learning culture on some farms and a lack of a structured training program. More work is needed to help farms realize the importance of developing a learning culture where training and feedback are provided to milkers regularly to promote continuous improvement and job satisfaction.

Table 5. 1 Priorities based on extension surveys1 used to identify mastitis risk factors in 4 main areas: milking equipment malfunction, equipment malfunctions that could be detected by milker, milking routine errors, and other2 in 15 commercial dairy farms in Northern New York State.

Farm	Number of milkers	Milking equipment malfunction	Equipment malfunctions that could be detected/corrected by milker	Inadequate milking routine	Other issues
A	6	Pulsator adjustment ATO adjustment	Not Detected	Lag time too short	Not Detected
B	5	Pulsator adjustment	Abnormal ATOs	Lag time too short	Not Detected
C	6	Pulsator function	Units on manual	Poor use of unit alignment device	Not Detected
D	5	Units on manual	Units on manual	Teat end cleanliness	Not Detected
E	5	Not Detected	Shut-offs not working Plugged vents	Lag time too short	Not Detected
F	14	ATO adjustment	Pulsators not working	Lag time too long	Not Detected
G	7	ATO adjustment	Pulsators not working	Lag time too short	Not Detected
H	3	Pulsator adjustment	Not Detected	Teat end cleanliness	Bedding levels
I	7	System vacuum incorrect	Not Detected	Lag time too short Teat end cleanliness	Not Detected
J	6	Not Detected	Units on manual Pulsators not working	Lag time too short	Not Detected
K	8	Not Detected	Not Detected	Teat end cleanliness Pre-dip coverage	Bedding levels
L	10	System vacuum incorrect ATO adjustment	Not Detected	Not Detected	Bedding levels
M	7	Not Detected	Units not retracting correctly	Teat end cleanliness	Teat skin condition/ post-dip
N	3	ATO adjustment Unit alignment devices	Pulsators not working	Not Detected	Not Detected
O	3	System vacuum incorrect ATO adjustment	Not Detected	Teat end cleanliness	Not Detected

[1]Service provided by Quality Milk Production Services (QMPS) from the Animal Health Diagnostic Center at Cornell University. QMPS professionals, including veterinarians with parlor experience and skilled technicians, that evaluate management, milking routine, housing, equipment, and mastitis control.
[2]Issues not related to milking equipment or milkers performance that could have an impact on mastitis risk.
Not Detected= No related deviations/malfunctions detected during extension survey

Table 5. 2 Initial questions to 95 milkers and 15 herd managers in 15 commercial dairy farms in Northern New York State prior training and milking equipment

Category	Questions
Milker	
Background (oral questions)	Have you milked cows before you worked on this farm? How long have you milked dairy cows on this farm? When was the last time you received training on this farm? Have you ever had any kind of training
Training on farm (multiple choice)	Have you ever had training on the milking equipment? Are you satisfied with that training? Who trained you on the equipment on this farm?
Other	How many hours do you work per pay period?
Herd manager	
Training on farm (multiple choice)	Do you provide training on the milking equipment? How is milking equipment training done on the farm?
Communication between milkers and herd managers (multiple choice)	How often do milkers bring up milking equipment problems to you? How do milkers communicate milking equipment problems with you?
Equipment function (5-point Likert scale)	The milking equipment works well on our farm most of the time How quickly do you fix milking equipment issues?
Other	How many hours does your average milker work per pay period?

Table 5. 3 Anonymous responses (no.; % in parentheses[1]) from 95 milkers in 15 commercial dairy farms in Northern New York State concerning training and milking equipment

Question	Milkers
Have you ever had training on the milking equipment? (How it works? What to do if it breaks?)	
Yes	64 (67.4)
No	28 (29.5)
Not sure	3 (3.1)
Training provided in milking equipment	
In agreement w/manager	64 (67.4)
In disagreement w/manager	31 (32.6)
Are you satisfied with that training?	
Yes	68 (71)
No	18 (19)
Not sure	9 (10)
Who trained you on the equipment on this farm?	
Another milker	56 (59)
Manager	12 (13)
External professional	12 (13)
Nobody	10 (11)
Other	5 (5)
How many hours do you work per pay period?	
Less than 80 h	77
More than 80 but less than 130 h	7
More than 130 h	10
No answer	1

[1]Due to rounding, percentages do not always add up to exactly 100%

Table 5. 4 Responses (no.; % in parentheses[1]) from 57 participants who completed a survey embedded in an E-learning module on milking equipment in 15 commercial dairy farms in Northern New York State

Question	Milkers
Do you feel confident now to tell the management that there is an equipment problem?	
Yes	49 (86)
No	3 (5.3)
Not sure	5 (8.8)
After this training, are you able to check the equipment before milking?	
Yes	55 (95.6)
No	0 (0)
Not sure	2 (4.4)
Would you like to have audio recording in future training modules?	
Yes	41 (70.7)
No	5 (8.6)
Not sure	11 (19.0)

[1]Due to rounding, percentages do not always add up to exactly 100%

REFERENCES

Arcury, T.A., J.M. Estrada, and S.A. Quandt. 2010. Overcoming language and literacy barriers in safety and health training of agricultural workers. J Agromedicine 15:236–248. doi:10.1080/1059924X.2010.486958.

Barkema, H.W., M. a. G. von Keyserlingk, J.P. Kastelic, T.J.G.M. Lam, C. Luby, J.-P. Roy, S.J. LeBlanc, G.P. Keefe, and D.F. Kelton. 2015. Invited review: Changes in the dairy industry affecting dairy cattle health and welfare. J. Dairy Sci. 98:7426–7445. doi:10.3168/jds.2015-9377.

Belage, E., S.L. Croyle, A. Jones-Bitton, S. Dufour, and D.F. Kelton. 2019. A qualitative study of Ontario dairy farmer attitudes and perceptions toward implementing recommended milking practices. Journal of Dairy Science 102:9548–9557. doi:10.3168/jds.2018-15677.

Chase, L.E., L.O. Ely, and M.F. Hutjens. 2006. Major Advances in Extension Education Programs in Dairy Production. Journal of Dairy Science 89:1147–1154. doi:10.3168/jds.S0022-0302(06)72183-X.

Chiaburu, D.S., and A.G. Tekleab. 2005. Individual and contextual influences on multiple dimensions of training effectiveness. Journal of European Industrial Training 29:604–626. doi:10.1108/03090590510627085.

Cook, N., and D. Reinemann. 2007. A Tool Box for Assessing Cow , Udder and Teat Hygiene. Pages 31–43 in 46th Annual NMC meeting 46th Annual Meeting of the National Mastitis Council, San Antonio, TX.

Durst, P.T., S.J. Moore, C. Ritter, and H.W. Barkema. 2018. Evaluation by employees of employee management on large US dairy farms. J. Dairy Sci. 101:7450–7462. doi:10.3168/jds.2018-14592.

Erskine, R.J., R.O. Martinez, and G.A. Contreras. 2015. Cultural lag: A new challenge for mastitis control on dairy farms in the United States. Journal of Dairy Science 98:8240–8244. doi:10.3168/jds.2015-9386.

Gutierrez-Solano, C., A. Ceballos-Marquez, and Y. Schukken. 2011. Bilingual trainings for milkers in New York State: A success for quality milk.

Hagevoort, G.R., D.I.D.P.M. MBA, and S.J.R.P.C.F. AIHA. 2013. A Review of Health and Safety Leadership and Managerial Practices on Modern Dairy Farms. Journal of Agromedicine 18:265–273. doi:10.1080/1059924X.2013.796905.

Hesse, A., P. Ospina, M. Wieland, F.A.L. Yepes, B. Nguyen, and W. Heuwieser. 2019. Short communication: Microlearning courses are effective at increasing the feelings of confidence and accuracy in the work of dairy personnel. Journal of Dairy Science 102:9505–9511. doi:10.3168/jds.2018-15927.

Hurley, D.T., and A.R. Lebbon. 2012. A Comparison of Nonfatal Occupational Injuries and Illnesses Among Hispanic Versus Non-Hispanic Workers in the United States. Hispanic Journal

of Behavioral Sciences 34:474–490. doi:10.1177/0739986312448316.

Jansen, J., C.D.M. Steuten, R.J. Renes, N. Aarts, and T.J.G.M. Lam. 2010. Debunking the myth of the hard-to-reach farmer: effective communication on udder health. J Dairy Sci 93:1296–1306. doi:10.3168/jds.2009-2794.

Jenkins, P.L., S.G. Stack, J.J. May, and G. Earle-Richardson. 2009. Growth of the Spanish-speaking workforce in the Northeast dairy industry. J Agromedicine 14:58–65. doi:10.1080/10599240802623387.

Maloney, T., L. Eiholzer, and B. Ryan. Survey of Hispanic Dairy Workers in New York State 2016 53.

Mein, G.A., F. Neijenhuis, W.F. Morgan, D.J. Reinemann, J.E. Hillerton, J.R. Baines, I. Ohnstad, M.D. Rasmussen, L. Timms, and J.S. Britt. 2001. Evaluation of bovine teat condition in commercial dairy herds: 1. Non-infectious factors. Pages 347–351 in Proceedings of the 2nd International symposium on mastitis and milk quality. Citeseer.

Menger, L.M., J. Rosecrance, L. Stallones, and I.N. Roman-Muniz. 2016. A Guide to the Design of Occupational Safety and Health Training for Immigrant, Latino/a Dairy Workers. Front. Public Health 4. doi:10.3389/fpubh.2016.00282.

Moore, S.J., P.T. Durst, C. Ritter, D. Nobrega, and H.W. Barkema. 2020. Effects of employer management on employee recruitment, satisfaction, engagement, and retention on large US dairy farms. J Dairy Sci 103:8482–8493. doi:10.3168/jds.2019-18025.

NMC Procedures. 2012. NMC Procedures for Evaluating Vacuum Levels and Air Flow in Milking Systems. 2012. Green Book. Published by the NMC Organization.

Noe, R.A. 2017. Employee Training and Development. Seventh edition. McGraw-Hill Education, New York, New York.

Raymond, M.J., R.D. Wohrle, and D.R. Call. 2006. Assessment and Promotion of Judicious Antibiotic Use on Dairy Farms in Washington State. Journal of Dairy Science 89:3228–3240. doi:10.3168/jds.S0022-0302(06)72598-X.

Rodrigues, A.C.O., D.Z. Caraviello, and P.L. Ruegg. 2005. Management of Wisconsin dairy herds enrolled in milk quality teams. J. Dairy Sci. 88:2660–2671. doi:10.3168/jds.S0022-0302(05)72943-X.

Rodriguez, A., G.R. Hagevoort, D. Leal, L. Pompeii, and D.I. Douphrate. 2018. Using mobile technology to increase safety awareness among dairy workers in the United States. Journal of Agromedicine 23:315–326. doi:10.1080/1059924X.2018.1502704.

Rovai, M., H. Carroll, R. Foos, T. Erickson, and A. Garcia. 2016. Dairy Tool Box Talks: A Comprehensive Worker Training in Dairy Farming. Front. Public Health 4. doi:10.3389/fpubh.2016.00136.

Schreiner, D.A., and P.L. Ruegg. 2002. Effects of Tail Docking on Milk Quality and Cow Cleanliness1. Journal of Dairy Science 85:2503–2511. doi:10.3168/jds.S0022-0302(02)74333-6.

Schuenemann, G.M., S. Bas, E. Gordon, and J.D. Workman. 2013. Dairy calving management: Description and assessment of a training program for dairy personnel. Journal of Dairy Science 96:2671–2680. doi:10.3168/jds.2012-5976.

Sischo, W.M., D.A. Moore, R. Pereira, L. Warnick, D.L. Moore, J. Vanegas, S. Kurtz, K. Heaton, D. Kinder, J. Siler, and M.A. Davis. 2019. Calf care personnel on dairy farms and their educational opportunities. Journal of Dairy Science 102:3501–3511. doi:10.3168/jds.2018-15401.

Stack, S.G., P.L. Jenkins, G. Earle-Richardson, S. Ackerman, and J.J. May. 2006. Spanish-speaking dairy workers in New York, Pennsylvania, and Vermont: results from a survey of farm owners. J Agromedicine 11:37–44. doi:10.1300/J096v11n02_07.

CHAPTER SIX

OVERALL CONCLUSIONS AND FUTURE DIRECTIONS

This research aimed to facilitate our understanding of bovine clinical mastitis and to have effective management in a dairy farm. Methods used to report mastitis incidence must clearly be described in research studies to improve our ability to discuss similarities and make appropriate comparisons across countries and regions. The use of incidence rate at the quarter level may be a more complete and granular evaluation of clinical mastitis.

The distribution of pathogens observed in this research was similar to other studies in US farms, where environmental pathogens, being the most common *E.coli*, *Klebsiella* spp, and environmental streptococcus plus those with no growth results, caused around 75% of the cases. The presence of *Staphylococcus aureus* represented 3.1% of all clinical cases. Based on the milk losses due to clinical mastitis analysis, we can confirm that these depend on the pathogen causing the event and those caused by coliforms such as *E.coli* and *Klebsiella* spp have the most negative and lasting effects. On the other hand, in cases in which pathogens were not recovered in the laboratory (no growth); negative effects on milk production were less severe and shorter in duration. Based on these conclusions, practitioners should consider not treat cows with no growth, but targeted strategies to control environmental pathogens need to be considered when designing udder health programs. One of the strengths of this study is that we included all clinical cases no matter the severity hence no selection bias was present. Our approach on using multiple imputations allows us to create complete datasets, and the opportunity to use all daily available data in comparison to other studies assessing milk losses.

The primary source of the most common pathogens cultured from clinical samples is considered the cow's environment, which includes bedding materials. Based on our study results, organic bedding materials had the highest levels of all bacterial groups studied. On the other hand, bedding materials with higher dry matter content had the lowest levels of bacterial growth

compared to those with lower dry matter content. However, no associations between bulk tank bacteria counts and bedding bacterial counts were observed, nor between bulk tank somatic cell counts. Our approach was to consistently collect bedding samples following a standardized operational procedure to reduce variability and serial sampling. Based on our results, the observed significant variation in the bacterial count within the sampling day and throughout the study period indicate that results from studies evaluating the association between bedding material and bulk tank bacterial load should be interpreted with caution, especially if a single or few samples collections were carried out over time.

Herd managers and owners are responsible for providing adequate training to their employees, but this should be prepared based on-farm needs. Our results show that milking equipment malfunctions and inadequate milking routines are common on dairy farms and reinforce the need for additional milker training in this area. In addition, our results reaffirm the lack of communication between managers and employees, which restate the necessity to state objectives and goals on every training. Furthermore, this study showed that the use of online training is a reasonable alternative for dairy farms, increasing employee's confidence by providing a more detailed training content.

To better understand the implications of these results, future studies could address some research needs. In regards to milk losses there a few cohort studies looking for the clinical mastitis repeated events. This may help farmers to make better management decisions for cows with recurrent cases that can be used for economic analysis. As for bedding material, it would be interesting to describe the pathogen distribution in quarters of cows through lactation by comparing those housed in different bedding material types, and identify possible associations

between bedding type and milk microbiome and differentiate milk profiles, including different production systems.

Further research is needed to evaluate the effectiveness of the e-learning model, as this research completed the first two levels of the Kirkpatrick model. Remaining changes in the last two levels: behavior and results along with a return of investment analysis can measure the cost of this training against the benefits to both employees and farmers.