# MICROBIAL AND COST BENEFIT ANALYSES OF ON-FARM INTERVENTIONS THAT REDUCE SPOREFORMING BACTERIA LEVELS IN RAW MILK

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by
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# ABSTRACT

Bacterial spores are a major challenge for the dairy industry as they can survive pasteurization, as well as other processing hurdles such as drying, and deteriorate the quality of many dairy products including fluid milk, cheese, and dairy powders. Research has shown that sporeforming bacteria can enter the raw milk supply at the farm level through udders and teat ends contaminated with feed, manure, and bedding. However, little has been done to give farmers evidence-based approaches to reduce the levels of sporeforming bacteria in their raw milk. This research aims to i) provide farmers with easy to implement management practices that can reduce bacterial spore levels in raw milk, and ii) provide a cost benefit analysis of these management practices for both dairy processors looking to increase the quality of their products and for dairy farmers. Our data suggest applying a combination of interventions including i) washing towels with bleach; ii) drying towels completely; and iii) a milking employee training is a low-cost strategy to reducing the mesophilic and thermophilic spore counts in bulk tank raw milk. Future studies should analyze different training programs that can be used for milking parlor employees.

# BIOGRAPHICAL SKETCH

Rachel Lynn Evanowski was raised in Riegelsville, Pennsylvania and attended Easton Area High School. Rachel earned a Bachelor of Science Degree in Biological Sciences with a minor in Animal Science at Cornell University. As an undergraduate student, she gained an interest in the dairy industry through coursework, and she became interested in research after she researched a candidate gene for horse size in an equine genetics laboratory. After graduating, she started working in the Milk Quality Improvement Program where her passion for dairy research and dairy extension grew. Her job as a technician allowed her to participate in applied research projects aimed at improving the quality of raw milk on dairy farms. Rachel hopes to continue working with dairy farmers through research and extension roles. Outside of academia, Rachel enjoys raising puppies for Guiding Eyes for the Blind, milking cows, and playing on the Wiedmann Boorior soccer team.

For Salsa- thank you for reminding me that

there is always time for happy circles and snuggles

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# CHAPTER 1

# INTRODUCTION

Bacterial spores pose a threat to the dairy industry due to their ability to survive processing hurdles such as heating (e.g. pasteurization of fluid milk) and drying (e.g. dairy powder processing). In vegetative form, they can grow at a wide range of temperatures, pH, and water activity which makes them challenging for the food industry as they can grow in a large variety of finished products and will often cause quality deterioration (Ranieri and Boor, 2009; Carlin, 2011).

Bacterial spores are costing the dairy industry money through both loss due to spoiled products that are not consumed and through additional investment in steps to reduce the chances of sporeforming bacteria spoilage of fluid milk. We define spoilage as reaching the Pasteurized Milk Ordinance (PMO) limit of 20,000 CFU/mL during shelf life (FDA, 2015). A survey conducted by the United States Department of Agriculture (USDA) found that dairy products represent 17% of all food loss in the United States, a 27 billion dollar value, at retailer and consumer levels (Buzby et al., 2014). One cause of this food loss is psychrotolerant sporeforming bacteria which are responsible for 50% of fluid milk spoilage (Ranieri and Boor, 2009; Alles et al., 2018; Reichler et al., 2018). A recent study investigated the ability of sporeforming bacteria to produce proteolytic and lipiolytic enzymes and revealed that sporeforming bacteria are capable of producing enzymes that are capable of degrading fluid milk quality (Trmčić et al., 2015). For example, *Bacillus weihenstephanensis* is a psychrotolerant sporeforming bacteria that can produce proteolytic enzymes in fluid milk and cause a defect known as "sweet curdling" (Berkeley, 2008).

In addition to fluid milk, sporeforming bacteria are a concern for both cheesemakers and dairy powder processors. *Clostridium tyrobutyricum* and other anaerobic butyric acid bacteria (BAB) cause a "late blowing" defect in semi-hard cheeses through butyric acid fermentation (Klijn et al., 1995). Late blowing in cheese is not included in the estimated cost of dairy food loss predicted above, as that prediction does not include loses before the product gets to the retailer. However, because it often ruins entire batches of cheese at once, the late blowing defect has severe economic impacts for cheesemakers, who go to great lengths to reduce the risk of the late blowing defect. Some of these prediction methods include the addition of nitrate or lysozyme, bactofugation, membrane filtration, and the addition of cultures that can inhibit *Clostridium* sp. growth such as reuterin- producing *Lactobacillus reuteri* and glycerol (Lodi and Stadhouders, 1990; van den Berg et al., 2004; Gómez-Torres et al., 2014). However, prevention methods can be expensive and time consuming for cheesemakers. Furthermore, dairy powder processors, who often export their products, are becoming increasingly concerned with mesophilic and thermophilic sporeforming bacteria levels and are placing more stringent requirements on spore counts of the raw milk entering their plants to prevent spoilage in reconstituted products (Watterson et al., 2014).

Due to this potential of economic losses, reducing the number of bacterial spores that get into the bulk tank raw milk on dairy farms is desirable mitigation step for the dairy industry. Bacterial spores are ubiquitous in the dairy farm environment and can be found in soil, manure, bedding material, and silage (Carlin, 2011; Gleeson et al., 2013). Multiple studies have shown that spores exist in high levels in the dairy farm environment. For example, a study in the Netherlands sampled soil, unused bedding, teat end swabs, and manure on dairy farms and found levels of 5.52 log CFU/g, 4.08 log CFU/g, 2.82 log CFU/g, and 5.20 log CFU/g in each, respectively (Slaghuis et al., 1997). Another study surveyed 190 farms in 18 states across the United States and found mesophilic and thermophilic spore levels in unused organic bedding (e.g. manure solids and straw) that ranged from 3.21 to 6.03 log CFU/mL for mesophilic spores and 2.79 to 5.41 log CFU/mL for thermophilic spores (Murphy et al., in press). Additionally, Huck and colleagues (2007) collected bedding, feed, soil, manure, and water samples on a farm in New York and found spore levels that ranged from 2.57 to 6.08 log CFU/mL (Huck et al., 2007).

Multiple studies have sought to link farm management practices to spore levels. For example, Masiello and colleagues (2014) sampled 99 dairy farms across NY and found that farms with >25% of cows with dirty udders are 3.15 times more likely to have higher amounts of psychrotolerant sporeforming bacteria in their raw milk when compared to cows with clean udders (Masiello et al., 2014). Another study that sampled bulk tank raw milk and surveyed farm management practices on ten NY farms monthly for a year found that using a skid steer to clean the housing area, cleaning the bulk tank area more frequently, and segregating problem cows during milking were all management practices associated with low spore milk (Masiello et al., 2017). Additionally, drying teats prior to unit attachment has been associated with lower bulk tank raw milk spore counts while feeding silage has been associated with an increase in bacterial spores in raw milk (O'Connell et al., 2013).

While many studies have looked at management practices associated with low spore milk, we are not aware of any studies that identify specific methods for farmers to use to reduce the number of spores in their bulk tank raw milk. Chapter 2 identifies an easy to apply combination of on-farm interventions that includes i) using chlorine bleach and detergent when washing towels, ii) drying towels completely, and iii) training milking parlor employees on the importance of teat end cleaning before milking unit attachment. These methods were applied concurrently on farms and reduced mesophilic and thermophilic spore counts in milk by 37% and 40%, respectively. Chapter 3 investigates the economic implications of these interventions for both producers and processors using a Monte Carlo simulation that was designed based on data from a previous study (Buehler et al., 2018). The model predicts that a reduced percentage of simulated half gallon containers of milk will reach spoilage levels, defined as 20,000 CFU/mL as outlined by the Pasteurized Milk Ordinance (PMO), on days 14 and 21 post-pasteurization if the combination of interventions from Chapter 2 is applied versus if it was not applied (FDA, 2015).

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# CHAPTER 2

# MILKING TIME HYGIENE INTERVENTIONS ON DAIRY FARMS REDUCE SPORE COUNTS IN RAW MILK*

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# ABSTRACT

Sporeforming bacteria, such as *Paenibacillus* sp. and *Clostridium* sp., can survive pasteurization in spore form and affect the quality of dairy products (e.g. spoilage in fluid milk and late blowing in certain cheeses). With the demand for higher quality finished products that have longer shelf-lives and that can be distributed further and to new markets, dairy processors are becoming more interested in obtaining low spore raw milk supplies. One tool for reducing spores in the dairy system will require disrupting the transmission of spores from environmental locations, where they are often found at high concentrations (e.g., manure, bedding, etc.), into bulk tank raw milk. Previous research has suggested that cow hygiene factors (e.g., udder hygiene, level of spores in milk from individual cows, etc.) are important for the transmission of spores into bulk tank raw milk, suggesting that intervention strategies to reduce spores in bulk tank milk should target cow hygiene in the parlor. To that end, we conducted a study on five New York dairy farms over a 15-month period to evaluate the impact of a combination of intervention strategies, which were applied together, on the levels of aerobic spores in bulk tank raw milk. The combination of interventions included i) training milking staff to focus on teat end cleaning during milking preparation and ii) implementing changes in laundered towel preparation (i.e., use of detergent, chlorine bleach, and drying). Study design involved collecting bulk tank raw milk samples for a week before and a week after initiating the combination of interventions (i.e., training on the importance of teat end cleaning and towel treatment). Observations on teat end condition, udder hygiene scores, and number of kickoffs during milking were also collected for 24h prior to and after the implementation of the interventions. Mean bulk tank raw milk mesophilic and thermophilic spore counts were 2.1 CFU/mL and 2.4 CFU/mL respectively prior to the combination of interventions being applied and 1.6 and 1.5 CFU/mL respectively after the combination of interventions was applied. These reductions represent a 37% and 40% decrease in bulk tank raw milk mesophilic spores and thermophilic spores, respectively. Importantly, spore reductions were seen during each of the three visits once the interventions were applied, and the largest reduction in spores was recorded for the first sampling after the milking staff training. Further, when a higher proportion of very rough teat ends were observed, bulk tank milk thermophilic spore counts were significantly higher. The intervention strategies tested here represent easy to execute cleaning strategies (e.g., focusing on teat end hygiene and towel washing procedures) that can reduce bulk tank raw milk spore levels. Future studies should validate the impact of on-farm interventions for reduced spore raw milk on corresponding processed product quality.

## INTRODUCTION

Sporeforming bacteria, such as *Bacillus* sp., *Paenibacillus* sp., and *Clostridium* sp., are important spoilage organisms in the dairy industry (De Jonghe et al., 2010; Ivy et al., 2012).  Sporeforming bacteria are responsible for roughly 50% of the cases where HTST pasteurized fluid milk exceedes the Pasteurized Milk Ordinance (PMO) bacterial limit of 20,000 CFU/mL at refrigeration temperatures and are therefore, a limiting factor of fluid milk shelf life (Ranieri and Boor, 2009; Alles et al., 2018; Reichler et al., 2018). Sporeforming bacteria have been shown to produce a number of enzymes capable of degrading the quality of processed dairy products (Trmčić et al., 2015). For example, the psychrotolerant sporeforming bacteria *Bacillus weihenstephanenesis* produces proteolytic enzymes in fluid milk that cause a defect known as “sweet curdling” (Berkeley, 2008). Beyond fluid milk, spores are also a concern for dairy powders, since processors need to meet export specifications due to the potential of spoilage in reconstituted products, and certain styles of cheese. Specifications for mesophilic and thermophilic sporeforming bacteria have become more stringent in dairy powders, and processors are increasingly concerned with the levels of these microorganisms in their products (Watterson et al., 2014). *Clostridium tyrobutyricum* are capable of metabolizing lactate into hydrogen gas and causing a “late blowing” defect in semi-hard cheeses (Klijn et al., 1995).

Sporeforming bacteria are particularly difficult for dairy processors to control because in spore form, they are capable of surviving pasteurization, drying, exposure to sanitizers, and low pH conditions (Logan and De Vos, 2009). Because of their ability to survive common dairy processing techniques, eliminating or reducing the entry of sporeforming bacteria into raw milk is important. However, this has proven to be a challenge because sporeforming bacteria are found ubiquitously in the dairy farm environment including in soil, silage and other feed sources, bedding, and manure (Carlin, 2011; Postollec et al., 2012).

Numerous studies have identified farm level management practices that are associated with spores in raw milk (Murphy et al., in press; Magnusson et al., 2006; Masiello et al., 2017). For example, Masiello and colleagues (2017) found that cleaning the bulk tank area frequently, using a skid steer to clean the housing area, and separating sick cows during milking were all associated with lower psychrotolerant spores in raw milk (Masiello et al., 2017). Magnusson and colleagues tested a variety of pre-milking teat cleaning methods and found that using a moist washable towel followed by drying with a dry paper towel was most effective in removing *Clostridium tyrobutyricum* spores from teats that were inoculated before cleaning methods were tested (Magnusson et al., 2006). Several studies found that high spore counts in used bedding are associated with higher spore counts in bulk tank raw milk (Murphy et al., in press; Magnusson et al., 2007). Murphy and colleagues (in press) also identified removing udder hair as a factor associated with lower thermophilic spores in raw milk, housing cows in bedded packs as a factor associated with higher mesophilic spores in raw milk, and having a higher proportion of cows with dirty udders as a factor associated with higher mesophilic and thermophilic spores in raw milk (Murphy et al., in press). While it is clear that certain practices are associated with lower spore raw milk, we are not aware of published studies that have examined how changing farm practices may affect the levels of spores in bulk tank raw milk. To that end our study was designed to assess easy to implement management intervention strategies for their ability to reduce the level of spores in bulk tank raw milk.

## MATERIALS AND METHODS

A 15-month intervention study on New York State dairy farms was conducted. Interventions included washing towels with bleach, drying towels completely, and a milking employee training focused on cleaning teat ends.

***Farm selection and characteristics.*** Farms in Upstate New York were recruited to participate in this study based on participation in a previous study of spores in dairy farm environments (Martin et al., accepted). Five dairy farms who participated in the previous study were selected to participate based on willingness and location (within 70 miles of Ithaca, NY). All farms enrolled used conventional management practices (i.e., “non-organic”), milked cows in a parlor, and used laundered towels to clean teat ends during milking. However, the farms had varying towel washing and milking parlor employee training procedures (Table 2.1). Three of the farms washed towels in detergent but did not use chlorine bleach, and these three farms all dried their towels after laundering (Table 2.1). One farm washed towels in chlorine bleach and detergent but did not dry their towels, and one farm washed with chlorine bleach and detergent and dried their towels (Table 2.1). All the farms trained their employees on the job upon hire but did not offer formal training sessions. The farms ranged in size from 500 to 1,500 lactating cows with a mean lactating herd size of 930.

Table 2.1. Pre-intervention towel management practices and observations collected by farm for each implementation of the combination of interventions

| | | Pre-intervention towel management practices[1] | | Observations collected[2] | | | | |
|---|---|---|---|---|---|---|---|---|
| Farm ID | Implementation[3] | Bleach use | Drying | Udder hygiene scores 1 and 2 (%) | Udder hygiene scores 3 and 4 (%) | Teat condition scores 1 and 2 (%) | Teat condition scores 3 and 4 (%) | Kick-offs (%) |
| A | 1 | no | no | 77 | 23 | 69 | 31 | 1.5 |
| A | 2 | no | yes | 83 | 17 | 83 | 17 | 1.0 |
| A | 3 | no | yes | 85 | 15 | 68 | 32 | 0.4 |
| B | 1 | no | yes | 82 | 18 | 89 | 11 | 0.5 |
| B | 2 | no | yes | 75 | 25 | 91 | 9 | 0.4 |
| B | 3 | no | yes | 81 | 19 | 80 | 20 | 0.2 |
| C | 1 | yes | no | 66 | 34 | 82 | 18 | 1.8 |
| C | 2 | yes | no | 86 | 14 | 73 | 27 | 1.0 |
| C | 3 | yes | no | 90 | 10 | 81 | 19 | 1.1 |
| D | 1 | no | yes | 84 | 16 | 89 | 11 | 1.8 |
| D | 2 | no | yes | 90 | 10 | 81 | 19 | 1.8 |
| D | 3 | no | yes | 87 | 13 | 89 | 11 | 1.1 |
| E | 1 | yes | yes | 86 | 14 | 92 | 8 | 1.4 |
| E | 2 | yes | yes | 90 | 10 | 83 | 17 | 0.9 |
| E | 3 | yes | yes | 88 | 12 | 84 | 16 | 1.3 |

[1] towel management practices the farms had in place prior to each implementation of interventions

[2] observations were conducted for 24 h before and 24 h after each implementation of interventions

[3] each farm implemented the combination of interventions once every 5 months for 15 months; the combination of interventions was the same for all farms and for all implementations

***Experimental design and sample collection***. A total of three intervention trials, in which the same combination of interventions was repeated for each implementation on every farm, were conducted on each of the five study farms between April 2017 to June 2018. Over the 15-month period, one farm per month was sampled on a rotating basis so that each farm had four months between implementations. Each trial period lasted two weeks and consisted of one week of baseline sample collection of bulk tank raw milk and towels followed by one week of sample collection of bulk tank raw milk and towels with the combination of interventions applied.

Sample collection occurred on alternating days (days 1, 3, 5, and 7) of each week (Table 2.2) and included the collection of one clean laundered towel in a 1625 mL Whirl-Pak® bag each day and one sample of bulk tank raw milk (~295 mL) collected in a sterile vial at the end of each milking shift after 5 min. of bulk tank agitation and using a dipper sanitized in 200 ppm chlorine prior to raw milk collection.

Table 2.2. Type of interventions and observations that occurred each sampling day

| Sampling Day[1] | Observation occurred?[2] | Intervention Type[3] |
|---|---|---|
| -7 | no | NA |
| -5 | no | NA |
| -3 | no | NA |
| -1 | yes | NA |
| 1 | yes | Combination: training and towel |
| 3 | no | Combination: training and towel |
| 5 | no | Combination: training and towel |
| 7 | no | Combination: training and towel |

[1] Time 0 = the day the interventions were applied. One bulk tank milk sample was collected each milking shift on each sampling day, and one clean towel sample was collected each sampling day. The combination of interventions was applied at time = 0 and continued through sampling day 7. Training was only given once, at time =0.

[2] Observations occurred in the milking parlor during milking and included hygiene and teat end scores on 20% of the herd during each milking shift

[3] The training intervention included a classroom style training on the importance of teat end hygiene during milking and the towel intervention included washing towels with 200 ppm bleach and detergent as well as drying them completely. NA (not applied) = no intervention applied

The raw milk samples were collected from the top of the bulk tank. If the farm switched bulk tanks during milking, raw milk samples were taken from both tanks, stored in separate vials, and ultimately processed as two different samples. Samples were stored at -20°C at each farm until the end of the intervention trial when researchers collected the frozen samples in coolers packed with ice and transported them to the Milk Quality Improvement Program (MQIP) laboratory (Ithaca, NY).

On day 7 of the first week of the sampling period and on day 1 of the second week of the sampling period (Table 2.2), udder hygiene and teat condition scores were observed for 20% of the milking cows in the parlor during each milking shift. A random number generator was used to select cows for teat end condition and udder hygiene scoring as cows came into the parlor. Udder hygiene was scored using the University of Wisconsin Milk Quality “Udder Hygiene Scoring Chart” by research staff as the cows entered the milking parlor (Ruegg and Schreiner, 2002). Teat ends were scored using the University of Wisconsin Milk Quality “Teat Condition Scoring Chart” as soon as the milking unit detached (Ruegg and Reinemann, 2005). Both udders and teat ends were scored on a 1 to 4 scale with 1 being the best possible score and 4 being the worst. Teat ends with a score of 1 had no ring around the teat canal, teat ends with a score of 2 had a smooth or slight ring with no keratin, teat ends with a score of 3 had a rough ring with occasional keratin that extended no more than 1-3 mm, and teat ends with a score of 4 had a very rough ring of keratin that extended more than 3 mm and had a flowered appearance. Cows with an udder hygiene score of a 1 had minimal dirt on them (< 2% of the surface area), udders that scored a 2 had 2-10% of surface area covered in dirt, udders that scored a 3 had 11-30% of surface area covered in dirt, and cows with an udder hygiene score of 4 had more than 30% of their surface area covered with dirt and manure.

***Interventions.*** The combination of interventions was implemented on day 0, one week after baseline sample collection began (Table 2.2), and included i) a short, classroom style training focusing on teat end cleanliness during milking; this 15-30 minute training was conducted by MQIP personnel and attended by workers in the milking parlor; ii) using chlorine bleach in a 200 ppm concentration when washing the towels in addition to the detergent they were currently using for towel washing; and iii) drying towels completely. The training occurred between shift changes so that both nighttime and daytime milking parlor employees could attend, and the towel washing protocol started before the training so that all towels used after the training session were bleached during the wash cycle and dried afterwards. Chlorine bleach amount was calculated based on the amount of water used by the washer during the wash cycle and was 320 mL for most of the farms in the study. The farms were asked to apply the combination of interventions for one week after implementation and the same combination of interventions, including the same training, was applied for every implementation.

***Microbiological analysis.*** Frozen samples were thawed in a 6°C incubator for ~24 h prior to microbiological analyses. Raw milk samples were shaken in accordance with Standard Methods for the Examination of Dairy Products (Laird et al., 2004) before ~50 mL of each sample was transferred into individual sterile screw capped glass tubes and stored at 6°C until heat treatment. Towel samples were placed in a 1625 mL Whirl-Pak® filter bag with 270 mL of Butterfield's buffer and manually agitated for 1 minute. Approximately 50 mL of the liquid portion of the sample was transferred into individual sterile screw capped glass tubes and stored at 6°C until heat treatment.

Samples were heat treated at 80°C for 12 minutes in accordance with Standard Methods for the Examination of Dairy Products to eliminate vegetative cells and select for bacterial spores (Frank and Yousef, 2004). Samples were cooled on ice until they reached 6$^o$C. All samples were pour plated in brain heart infusion (**BHI**) agar using 1 mL of sample per plate in replicates of ten plates for a total of 10 mL plated per sample per incubation temperature. Plates were incubated at three different temperatures to select for aerobic psychrotolerant, mesophilic, and thermophilic sporeforming bacteria, including 6°C for 10 days, 32°C for 48 h, or 55°C for 48 h for aerobic psychrotolerant spore count (**PSC**), aerobic mesophilic spore count (**MSC**) and aerobic thermophilic spore count (**TSC**) respectively prior to enumeration. Enumeration was performed on an automated colony counter (Q-count, Advanced Instruments, Norwood, MA) according to the manufacturer's instructions.

***Data management and statistical analysis***. Data was stored in a Microsoft Access database (Microsoft Access, Redmond, WA). All statistical analyses were performed in R (version 3.4.3; R project, Vienna, Austria). The PSC test results were not included in the analysis due to a high proportion of samples with below detection limit outcomes in raw milk (352/355 BTM samples) and towels (94/117 towel samples). For each test type (MSC and TSC), a linear mixed-effects model was fit using the lmerTest package to the log-transformed spore count of the raw milk (Kuznetsova A. et al., 2017). Five variables were included as fixed effects, including: i) visit; ii) an indicator for intervention (i.e., whether the sample collected before or after the combination of interventions was applied); iii) proportion of cows with a hygiene score of 2, score of 3, or score of 4; iv) proportion of cows with smooth, rough, or very rough teats; and v) number of kickoffs per hundred cows. Random effects included; i) farm and ii) visit nested within farm. Farm was selected as a random effect since the combination of interventions was designed to be applicable to any dairy farm. Whether or not the farms used bleach to wash their towels prior to each implementation and whether or not the farms dried their towels prior to each implementation was originally included in the model, but both were removed after an Akaike information criterion analysis (AIC) was performed. Both the model with and the model without these variables had the same AIC values and the simpler model (i.e. the model with fewer variables) was chosen. All data and code used for this study can be found at github.com/mqip/fis.

## RESULTS

***Psychrotolerant spores showed lowest frequency and levels***. A total of 355 bulk tank milk samples and 117 towel samples were collected. Mesophilic spore counts in the bulk tank raw milk (BTM) ranged from -1.0 log CFU/mL to 1.7 log CFU/mL with a mean of 0.3 log CFU/mL and thermophilic spore counts in raw milk ranged from < -1.0 log CFU/mL to 1.4 log CFU/mL with a mean of 0.3 log CFU/mL. All mesophilic spore counts were within detection limits and only one thermophilic spore count for a bulk tank milk sample was below the detection limit. To calculate arithmetic means, the BTM TSC below the detection limit (< -1.0 log CFU/mL) was substituted with a value equal to half the detection limit. Psychrotolerant spore counts (PSC) ranged from < -1.0 log CFU/mL to -1.0 log CFU/mL and were removed from further analysis since 352/355 of the BTM samples were below detection.

The towel MSCs ranged from 0.0 log CFU/mL to 3.0 log CFU/mL with a mean of 2.0 log CFU/mL, the towel TSCs ranged from < -0.3 log CFU/mL to 2.6 log CFU/mL with a mean of 1.5 log CFU/mL, and the PSCs ranged from < -0.3 log

CFU/mL to 1.8 log CFU/mL and were removed from further analysis since 94/117 towel samples were below detection.

***Post-intervention spore counts in bulk tank raw milk and towels were lower than pre-intervention spore counts.*** Overall, BTM MSC was reduced from an average 0.3 log CFU/mL (2.1 CFU/mL) before the interventions to an average of 0.2 log CFU/mL (1.6 CFU/mL) once the interventions were applied (Supplemental Table 2.1), a 37% (0.2 log CFU/mL) reduction of spores ($p < 0.001$; Figure 2.1).

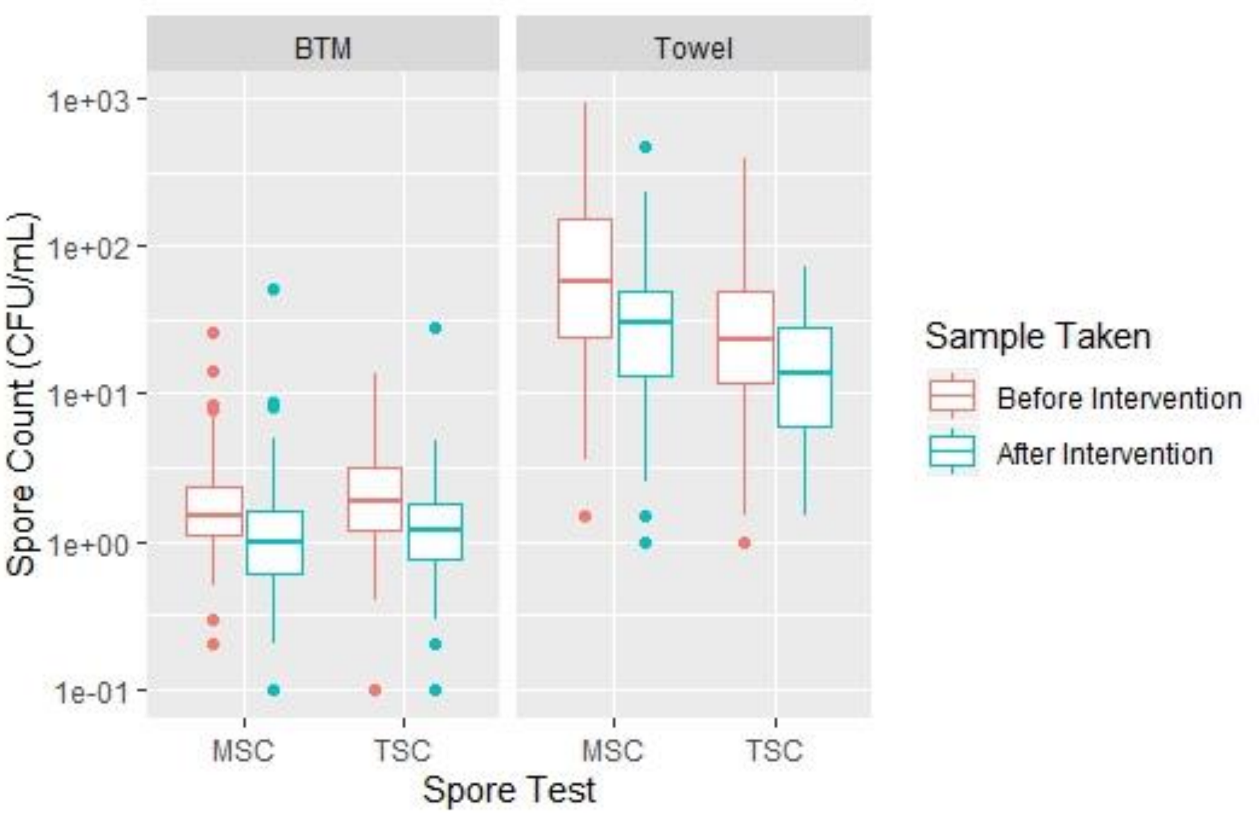


Figure 2.1. Boxplots representing the distribution of mesophilic spore counts (MSC) and thermophilic spore counts (TSC) for bulk tank milk samples (n=355) and towels (n=117) collected before the combination of interventions was applied (orange) and

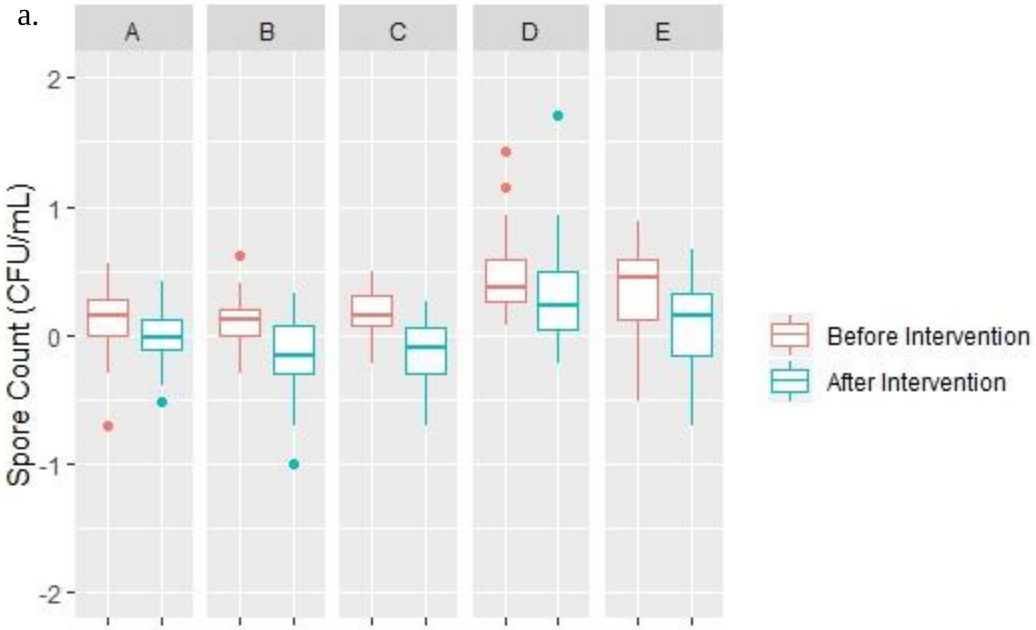
after the combination of interventions was applied (blue) throughout the whole study (all implementations). The combination of interventions includes a parlor employee training that focused on teat end cleanliness as well as towel washing procedures that included washing towels with bleach and detergent before drying them completely. Bold, horizontal lines within the boxplots represent median spore count values; ends of each box represent the first and third quartiles; whiskers represent minimum and maximum values (excluding outliers), and dots represent outliers.

The BTM TSC was reduced from 0.4 log CFU/mL (2.4 CFU/mL) to 0.2 log CFU/mL (1.5 CFU/mL), a 40% (0.2 log CFU/mL) reduction of spores ($p < 0.001$; Supplemental Table 2.2). Towel MSC was reduced from 2.2 log CFU/mL (156.2 CFU/mL) to 1.7 log CFU/mL (45.8 CFU/mL), a 51% (0.31 log CFU/mL) reduction of spores ($p < 0.001$; Supplemental Table 2.3). Towel TSC was reduced from 1.7 log CFU/mL (45.8 CFU/mL) to 1.3 log CFU/mL (18.6 CFU/mL), a 14.9% (0.07 log CFU/mL) reduction of spores ($p > 0.1$; Supplemental Table 2.4).

Mesophilic and thermophilic spore levels in raw milk were lower after the interventions were applied for every farm. However, some farms saw bigger decreases than others (Figure 2.2). For example, farm B showed the smallest reduction in MSC with a mean of 0.09 log CFU/mL (1.23 CFU/mL) before the interventions were applied and -0.14 log CFU/mL (0.72 CFU/mL) while the interventions were applied. Farm D saw the largest reductions with a mean of 0.47 log CFU/mL (2.95 CFU/mL) MSC and 0.45 log CFU/mL (2.82 CFU/mL) TSC before interventions and 0.32 log

CFU/mL (2.09 CFU/mL) MSC and 0.24 log CFU/mL (1.74 CFU/mL) TSC after the interventions were applied.

a.



b.

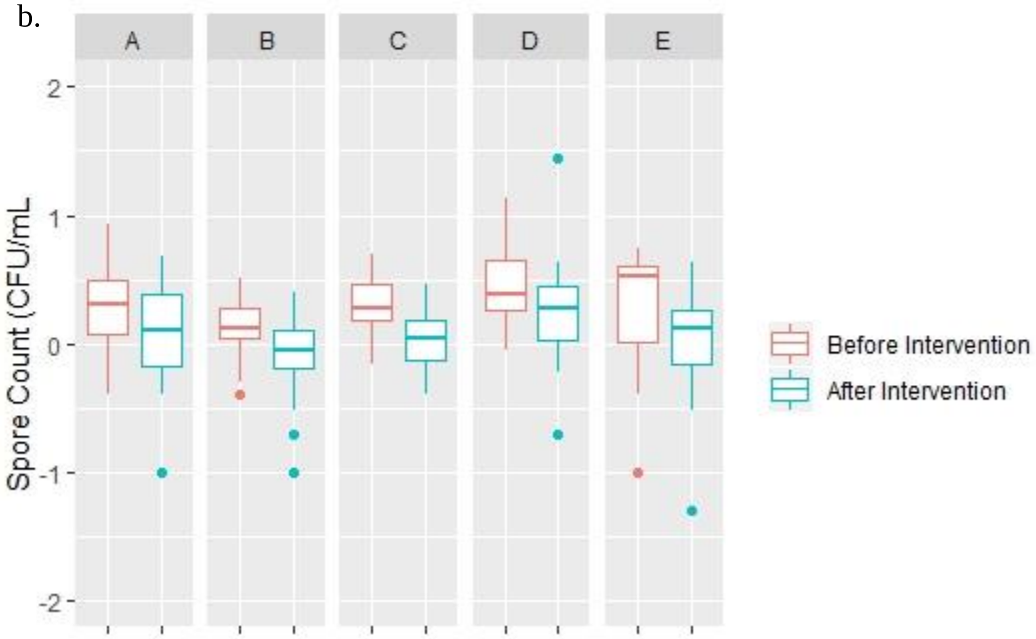


Figure 2.2. Boxplots representing the distribution of (a) mesophilic spore counts (MSC) and (b) thermophilic spore counts (TSC) before the combination of interventions was applied (orange) and after the combination of interventions was applied (blue) for the five farms in the study. Each panel, labeled A-E, represents each study farm. Bold, horizontal lines within the boxplots represent median spore count values; ends of each box represent the first and third quartiles; whiskers represent minimum and maximum values (excluding outliers), and dots represent outliers.

***Spore levels decreased each time the interventions were applied; however, the lowest mean spore counts were observed after the first intervention.*** Mean bulk tank MSC prior to and after implementation of the interventions was 0.08 log CFU/mL (1.21 CFU/mL) and -0.15 log CFU/mL (0.71 CFU/mL), 0.30 log CFU/mL (2.00 CFU/mL) and 0.08 log CFU/mL (1.21 CFU/mL), and 0.25 log CFU/mL (1.78 CFU/mL) and 0.11 log CFU/mL (1.29 CFU/mL) during the first, second, and third implementations of the combination of interventions, respectively (Table 2.3).

Table 2.3. Mesophilic spore counts (MSC) and thermophilic spore counts (TSC) for bulk tank milk samples before and after interventions were applied for each implementation of the combination of interventions[1]

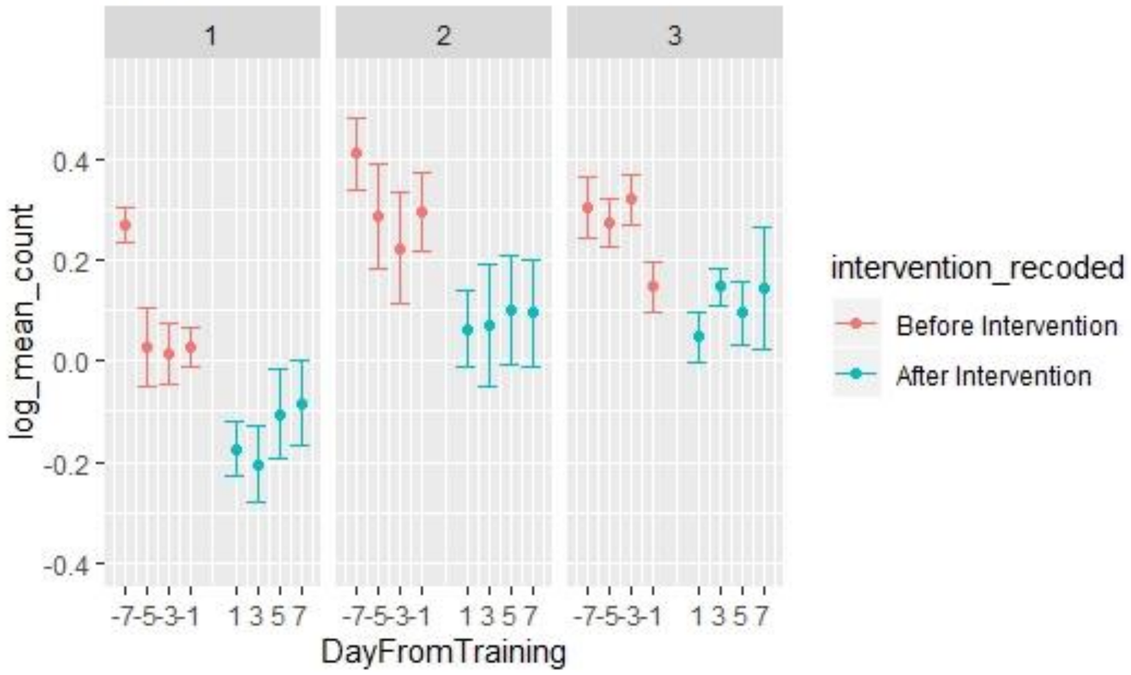
| | | Mean MSC (CFU/mL) | ΔMSC (CFU/mL) | Mean TSC (CFU/mL) | ΔTSC (CFU/mL) |
|---|---|---|---|---|---|
| Implementation 1 | Pre-Intervention | 1.21 | 0.51 | 1.32 | 0.59 |
| | Post-Intervention | 0.71 | | 0.72 | |
| Implementation 2 | Pre-Intervention | 2.00 | 0.78 | 2.14 | 0.85 |
| | Post-Intervention | 1.21 | | 1.29 | |
| Implementation 3 | Pre-Intervention | 1.78 | 0.49 | 2.40 | 0.81 |
| | Post-Intervention | 1.29 | | 1.58 | |

[1] Each farm implemented the combination of interventions, which included a milking parlor employee training on the importance of teat end cleaning as well as washing towels with bleach and drying towels, three times in a 15-month period

While the largest change in MSC was observed during the second implementation of the combination of interventions (0.78 CFU/mL decrease), the lowest mean MSC after applying the intervention combination was observed during the first implementation (Table 2.3). Similarly, mean bulk tank TSC prior to and after applying the combination of interventions was 1.32 and 0.72 CFU/mL, 2.14 and 1.29 CFU/mL, and 2.40 and 1.58 CFU/mL during the first, second, and third implementations respectively (Table 2.3). Similar to the bulk tank MSC, the largest change in bulk tank TSC was observed during the second implementation (-0.07 log CFU/mL or 0.85 CFU/mL decrease) and the lowest mean TSC was observed during the first implementation after the combination interventions was applied. The first time that farms were visited, the mean MSCs and TSC were 0.08 log CFU/mL and 0.12 log

CFU/mL respectively before the combination of interventions was applied (Figure 2.3). These baseline spore counts were lower than the baseline spore counts that were observed for the second and third implementations for TSC which had a 0.49 log CFU/mL increase from Implementation 1 to Implementation 2 (p= 0.051) and a 0.47 increase from Implementation 1 to Implementation 3 (p= 0.061) and for MSC which had a 0.42 log CFU/mL increase from Implementation 1 to Implementation 2 (p=0.094). The baseline spore counts were 0.30 log CFU/mL and 0.33 log CFU/mL for MSC and TSC respectively for the second implementation and 0.25 log CFU/mL and 0.38 log CFU/mL for MSC and TSC respectively for the third implementation. While the first implementation had lower spore counts throughout, the second implementation had the largest spore reduction for both mesophilic and thermophilic spore counts (Table 2.3). However, no statistical difference was seen between intervention implementations (Supplemental Tables 2.1 and 2.2).

a.


1
2
3
0.4
0.2
0.0
-0.2
-0.4
log_mean_count
-7-5-3-1
1 3 5 7
DayFromTraining
intervention_recoded
Before Intervention
After Intervention

b.

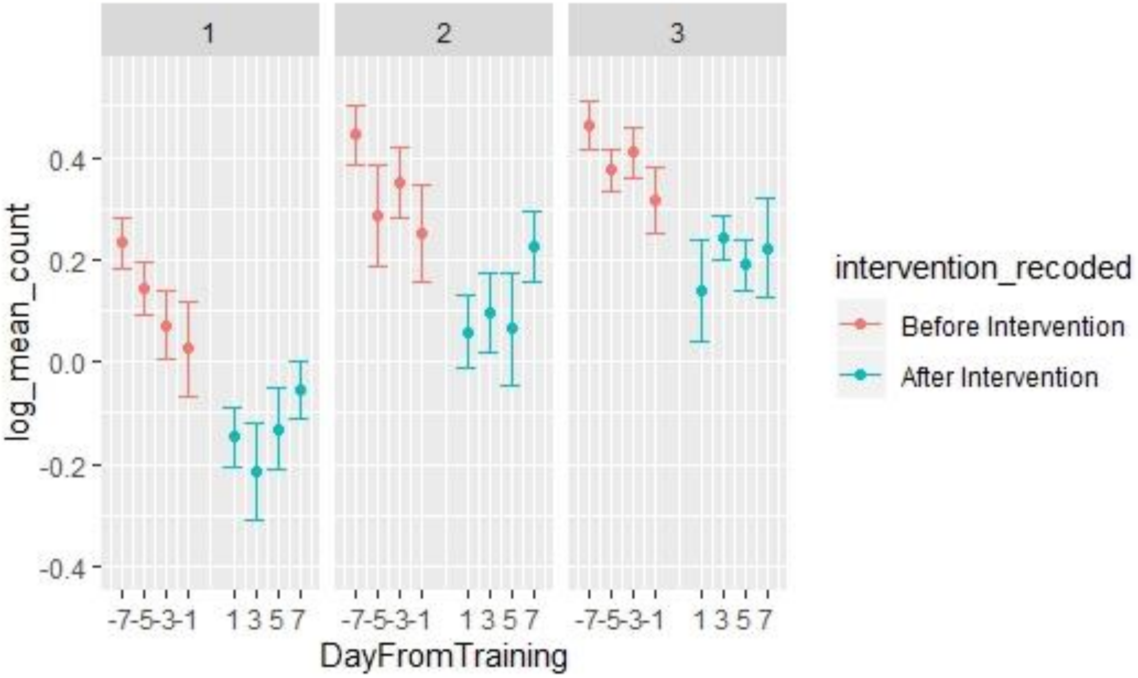

1
2
3
0.4
0.2
0.0
-0.2
-0.4
log_mean_count
-7-5-3-1
1 3 5 7
DayFromTraining
intervention_recoded
Before Intervention
After Intervention

Figure 2.3. Error bars representing the distribution of (a) mesophilic spore counts (MSC) and (b) thermophilic spore counts (TSC) by each day samples were collected before the combination of interventions was applied (orange) and after the combination of interventions was applied (blue). The dots represent median values and bars span from the highest spore count observed during the sampling day to the lowest spore count observed during the sampling day. Each panel labeled 1-3 represents each implementation, or trial, of the combination of interventions and each bar represents a sampling day. Samples were collected on 7, 5, 3, and 1 day before the interventions were applied and 1, 3, 5, and 7 days after the combination of interventions were applied.

***Proportion of cows with very rough teat ends was significantly associated with higher bulk tank thermophilic spores.*** For each of the three intervention trials, or implementations, on a given farm, we observed the number of kickoffs and scored teat end condition on each farm on days -1 and 1 of sampling (Table 2.2). The number of kickoffs per 100 cows ranged from 0.22% on farm B during visit 3 prior to the interventions being applied to 1.84% on farm C during the first visit after the start of interventions (Table 2.1). Farms had a range of 23 to 50% of observed cows with very smooth teat ends, 29 to 59% with smooth teat tends, 7.2 to 25% with rough teat ends and 0.8 to 10% with very rough teat ends (Table 2.1). Mixed effects linear regression analysis indicated that the proportion of teat ends with a score of 4, or “very rough,” was associated with higher BTM TSC (p= 0.04) with an effect size of 0.14 log CFU/mL (Supplemental Table 2.2). No significant association was observed between teat end condition and BTM MSC. These analyses also revealed that both MSC and TSC were reduced by 0.06 log CFU/mL (p=0.02) and 0.09 log CFU/mL (p=0.001), respectively in bulk tank milk during the milking shifts when teat end and kickoff observations were occurring by research staff 24 h prior to and 24 h after training occurred.

## DISCUSSION

***Spores in bulk tank milk and towels can be reduced with interventions.*** Our study showed that applying a combination of on-farm interventions focused on teat end cleanliness (e.g., training and towel preparation interventions) resulted in significant reduction in bulk tank raw milk MSC and TSC as well as towel MSC and TSC. Other studies have found that the materials that udders and teats come into contact with, namely manure and bedding, often have high levels of spores (Slaghuis et al., 1997; Huck et al., 2008). For example, Slaghuis et al. (1997) found aerobic mesophilic spore levels of 5.20 log CFU/g in manure and 4.08 log CFU/g in unused bedding (Slaghuis et al., 1997). Similarly, Huck et al. evaluated sporeforming bacteria levels in manure and bedding at a farm in NY and found that spore levels to be 2.82 log CFU/g in fresh sawdust bedding and 5.88 to 6.00 CFU/g in manure (Huck et al., 2008). We hypothesized that the spores in these materials are transferred to the bulk tank raw milk if the teat end is insufficiently cleaned, or if it is cleaned with towels that have high levels of bacterial spores, during milking preparation. This hypothesis is consistent with the findings in our current study that instructing parlor employees to focus on teat end cleaning during milking preparation and washing towels with bleach and drying them completely prior to use results in lower spore bulk tank milk. Our findings are also consistent with previous work that has found that udder hygiene, as well as housing area hygiene, is associated with the presence and levels of spores in bulk tank milk (Martin et al., in review; Vissers et al., 2007; O'Connell et al., 2013). For example, a study conducted by Vissers and colleagues (2007) assessed the amount of dirt transferred from the teat end to the bulk tank milk by using aerobic mesophilic spores as markers on 11 randomly selected Dutch farms and found that the amount transmitted varied from ~3 to 300 mg/L with a mean of 59 mg/L (Vissers et al., 2007). Vissers et al. (2007) consequently proposed that limiting dirt on teat ends could be used to limit the number of spores in bulk tank milk (Vissers et al., 2007). A study by Martin and colleagues (accepted) reports that udder cleanliness is an important factor in spore presence and levels in bulk tank raw milk (Martin et al., accepted). Additionally, O'Connell et al. observed that farms that cleaned teat ends before milking had lower *Bacillus cereus* counts in their raw milk (O'Connell et al., 2013). Overall, we found that applying the combination of interventions on farms resulted in lower spore counts in towels and in bulk tank raw milk which supports our original hypothesis.

***Worker training is likely critical to spore reduction.*** Our data show that milk samples collected during milking shifts where research staff were present had significantly lower spore counts than milk samples collected during shifts where research staff were not present. However, there was no significant difference between the spore count of the towels that were collected while research staff were present and the spore count of the towels that were collected while research staff were not present. We hypothesize that our presence affected the actions of the parlor employees and how thoroughly they performed teat end cleaning during udder preparation. Observer effects have been described numerous times and are thought to have an impact on people's behaviors, especially in research settings (Agar, 1980; Monahan and Fisher, 2010). We further hypothesize that teat end cleaning, which can be influenced by workers' behavior, is driving the decrease in spore levels in bulk tank raw milk compared with washing towels with chlorine bleach since workers' habits can be influenced by research staff observing milking shifts. This is not unexpected as previous research has shown that training workers in the food industry reduces the number of food safety violations, and it has also been shown that more frequent inspections leads to even fewer violations which is consistent with our results and indicates that it might be useful for farmers to observe their parlor employees during milking more frequently (Mathias et al., 1995; Coffill et al., 1998).

During the current study, average baseline spore counts were lower for the first implementation than the second or third implementations which further supports our hypothesis that spore reductions are driven by parlor employee behaviors. The first data point collected during our first implementation had higher levels of spore counts than the rest of the baseline samples collected during that implementation. We hypothesize that this is because collection of that sample occurred before the milking parlor staff at the farms knew we were collecting milk samples and was therefore more representative of a normal level of spores for that farm. After the initial sample collection, workers were aware of the study even though they did not know the details and responded by carefully performing milking preparation. We further hypothesize that workers were desensitized to our presence after the first implementation, which is why we saw overall higher spore counts during the second and third implementations. A follow-up study will be needed to observe the two interventions separately and to determine how to drive behavior change at the farm level.

***Spore reduction strategies benefit producers beyond low spore milk.*** Our study showed that teat ends with a condition score of 4 (very rough) are associated with higher TSCs in bulk tank milk (p=0.04). Teat ends are commonly damaged by over-milking caused by milking units left on for too long during milking (Shearn and Hillerton, 1996; Edwards et al., 2013) leading to hyperkeratosis. Hyperkeratosis can lead to reduced ability of the teat sphincter to close following milking which allows both spores and mastitis organisms to enter the teat canal from environmental sources, including bedding and manure (Zecconi et al., 2002). Further, once teat ends have hyperkeratosis, they are more difficult to clean leading to, we hypothesize, higher levels of spores that transfer into the bulk tank raw milk. Finally, poor teat end condition is more uncomfortable for the cows which could lead to more kicking, and therefore manure splashing and more unit kickoffs, in the parlor (Rousing et al., 2004). Having a herd with good teat end condition scores is not only associated with having lower TSCs, it also helps with numerous other cow health factors as well. Applying the combination of interventions used in this study not only reduces sporeforming bacteria in raw milk, but the combination of interventions is also consistent with recommendations for reducing the risk of mastitis (Ruegg, 2017).

## CONCLUSIONS

The current study shows that milking parlor employee training on teat end hygiene combined with bleaching and drying towels can lead to a reduction in sporeforming bacteria in the bulk tank raw milk. Based on these results, farms should focus on employee training and cow hygiene before milking unit attachment. Future studies should focus on testing different styles of employee training as well as testing these practices from interventions on the farm through pasteurized ready-to-eat products to determine spore reduction and possible shelf life extension in the finished product. These management practices, especially when combined with good bedding and housing area management practices, will provide farmers with easy to implement changes that can lower sporeforming bacteria counts in milk and create a high-quality product.

## ACKNOWLEDGEMENTS

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# SUPPLEMENTAL MATERIAL

Supplemental Table 2.1. Model parameters for all fixed effects in the mesophilic spore count model for bulk tank milk samples. Farm and visit nested within farm were included in the model as random effects.

| Response Variable | Fixed Effects | Levels | Estimate | Standard Error | *P*-value | Significance |
|---|---|---|---|---|---|---|
| BTM mean MSC (log cfu/ml) | Implementation | 1 | Ref[1] | | | |
| | | 2 | 0.421 | 0.156 | 0.094 | . |
| | | 3 | 0.395 | 0.164 | 0.117 | |
| | Observed | N | Ref | | | |
| | | Y | -0.063 | 0.028 | 0.027 | * |
| | Udder Hygiene Score | 1 | Ref | | | |
| | | 2 | -0.010 | 0.028 | 0.730 | |
| | | 3 | -0.008 | 0.016 | 0.644 | |
| | | 4 | 0.092 | 0.101 | 0.406 | |
| | Teat Condition Score | 1 | Ref | | | |
| | | 2 | 0.016 | 0.015 | 0.578 | |
| | | 3 | -0.013 | 0.021 | 0.338 | |
| | | 4 | 0.074 | 0.043 | 0.186 | |
| | Proportion of kick-offs | | 0.292 | 0.164 | 0.1400 | |
| | Intervention | Before | Ref | | | |
| | | After | -0.201 | 0.02 | $2.25 \times 10^{-15}$ | *** |

[1] Ref indicates reference; therefore, estimates, standard error, and *P*-values are not calculated

****P* < 0.001; ***P* < 0.01; **P* < 0.05; • *P*<0.1

Supplemental Table 2.2. Model parameters for all fixed effects in the thermophilic spore count model for bulk tank milk samples. Farm and visit nested within farm were included in the model as random effects.

| Response Variable | Fixed Effects | Levels | Estimate | Standard Error | *P*-value | Significance |
|---|---|---|---|---|---|---|
| BTM mean TSC (log cfu/ml) | Implementation | 1 | Ref[1] | | | |
| | | 2 | 0.491 | 0.131 | 0.052 | . |
| | | 3 | 0.471 | 0.138 | 0.061 | . |
| | Observed | N | Ref | | | |
| | | Y | -0.092 | 0.028 | 0.001 | ** |
| | Udder Hygiene Score | 1 | Ref | | | |
| | | 2 | -0.022 | 0.024 | 0.413 | |
| | | 3 | -0.011 | 0.014 | 0.472 | |
| | | 4 | 0.125 | 0.088 | 0.231 | |
| | Teat Condition Score | 1 | Ref | | | |
| | | 2 | 0.031 | 0.012 | 0.095 | . |
| | | 3 | -0.037 | 0.018 | 0.127 | |
| | | 4 | 0.137 | 0.036 | 0.040 | * |
| | Proportion of kick-offs | | 0.169 | 0.144 | 0.305 | |
| | Intervention | Before | Ref | | | |
| | | After | -0.226 | 0.024 | < $2x10^{-16}$ | *** |

[1] Ref indicates reference; therefore, estimates, standard error, and *P*-values are not calculated

****P* < 0.001; ***P* < 0.01; **P* < 0.05; • *P*<0.1

Supplemental Table 2.3. Model parameters for all fixed effects in the mesophilic spore count model for towel samples. Farm and visit nested within farm were included in the model as random effects.

| Response Variable | Fixed Effects | Levels | Estimate | Standard Error | *P*-value | Significance |
|---|---|---|---|---|---|---|
| Towel mean MSC (log cfu/ml) | Implementation | 1 | Ref[1] | | | |
| | | 2 | 0.141 | 0.493 | 0.796 | |
| | | 3 | 0.322 | 0.439 | 0.522 | |
| | Observed | N | Ref | | | |
| | | Y | 0.097 | 0.074 | 0.192 | |
| | Udder Hygiene Score | 1 | Ref | | | |
| | | 2 | -0.008 | 0.082 | 0.935 | |
| | | 3 | -0.021 | 0.048 | 0.707 | |
| | | 4 | 0.093 | 0.624 | 0.895 | |
| | Teat Condition Score | 1 | Ref | | | |
| | | 2 | 0.010 | 0.054 | 0.866 | |
| | | 3 | -0.005 | 0.055 | 0.933 | |
| | | 4 | -0.006 | 0.087 | 0.952 | |
| | Proportion of kick-offs | | 0.033 | 0.164 | 0.851 | |
| | Intervention | Before | Ref | | | |
| | | After | -0.310 | 0.062 | $3.7x10^{-6}$ | *** |

[1] Ref indicates reference; therefore, estimates, standard error, and *P*-values are not calculated

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; • $P<0.1$

Supplemental Table 2.4. Model parameters for all fixed effects in the thermophilic spore count model for towel samples. Farm and visit nested within farm were included in the model as random effects.

| Response Variable | Fixed Effects | Levels | Estimate | Standard Error | *P*-value | Significance |
|---|---|---|---|---|---|---|
| Towel mean TSC (log cfu/ml) | Implementation | 1 | Ref[1] | | | |
| | | 2 | -0.529 | 0.716 | 0.599 | |
| | | 3 | -1.197 | 0.664 | 0.398 | |
| | Observed | N | Ref | | | |
| | | Y | 0.014 | 0.137 | 0.918 | |
| | Udder Hygiene Score | 1 | Ref | | | |
| | | 2 | -0.039 | 0.097 | 0.744 | |
| | | 3 | -0.034 | 0.059 | 0.629 | |
| | | 4 | 0.107 | 0.750 | 0.902 | |
| | Teat Condition Score | 1 | Ref | | | |
| | | 2 | -0.002 | 0.066 | 0.978 | |
| | | 3 | -0.041 | 0.070 | 0.618 | |
| | | 4 | 0.078 | 0.109 | 0.548 | |
| | Proportion of kick-offs | | -0.254 | 0.291 | 0.690 | |
| | Intervention | Before | Ref | | | |
| | | After | 0.071 | 0.116 | 0.542 | |

[1] Ref indicates reference; therefore, estimates, standard error, and *P*-values are not calculated

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; • $P<0.1$

# CHAPTER 3

# COST OF ON-FARM INTERVENTIONS TO REDUCE SPORES IN BULK TANK RAW MILK AND ASSESSMENT OF BENEFITS*

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# ABSTRACT

Bacterial spores can survive many harsh processing conditions, including pasteurization and drying. Spores are present in raw milk and can be reduced through multiple farm management practices. Low spore count raw milk is desirable for processors, especially those who ship their product long distances or make semi-hard or hard cheeses that are susceptible to late blowing, a defect caused by anaerobic sporeforming bacteria. A recent study examined the impact of two low-cost interventions, including washing towels with chlorine bleach and drying them completely as well as training milking parlor employees to focus on teat end cleaning, on spore levels in bulk tank raw milk. Costs associated with these interventions were calculated based on farm size and ranged from $9.48 and $13.10 per cow per year. A Monte Carlo model was used to predict the shelf-life of fluid milk processed from raw milk before and after these low-cost interventions were applied, based on experimental data collected from a previous study. The model predicted that the percentage of simulated half gallon containers of milk that reach 20,000 CFU/mL, the Pasteurized Milk Ordinance limit for total bacterial levels in pasteurized Grade “A” fluid milk at 14 and 21 days after pasteurization would be significantly lower in milk processed from raw milk after the interventions were applied as compared with milk processed from raw milk before the interventions were applied. Finally, a survey of consumer milk use was conducted and revealed that over 50% of fluid milk consumers surveyed continue to consume fluid milk after the date indicated on the package (e.g., code date), indicating that consumers are exposed to fluid milk that is likely to have high levels of growth by bacterial sporeformers. This further highlights the importance of

reducing spore levels in raw milk to extend pasteurized fluid milk shelf-life, and thereby reducing the risk of adverse consumer experience. Processors who are interested in extending fluid milk shelf-life by controlling the levels of spores in the raw milk supply should consider incentivizing low-spore raw milk.

## INTRODUCTION

Bacterial spores are found ubiquitously in the dairy farm environment and can survive processing hurdles that are commonly used in the dairy industry including pasteurization and drying (Carlin, 2011; Postollec et al., 2012). The primary factor that limits High Temperature Short Time (HTST) pasteurized fluid milk shelf-life are sporeforming bacteria capable of growing at refrigeration temperatures, otherwise known as psychrotolerant sporeforming bacteria (Ranieri and Boor, 2009). Psychrotolerant sporeforming bacteria are responsible for 50% of fluid milk reaching the Pasteurized Milk Ordinance (PMO) bacterial limit of 20,000 CFU/mL (FDA, 2015). These bacteria can produce proteolytic enzymes that cause spoilage (Trmčić et al., 2015; Alles et al., 2018). For example, the psychrotolerant sporeforming bacteria, *Bacillus weihenstephanenesis*, is responsible for “sweet curdling,” a defect in fluid milk (Berkeley, 2008). Sporeforming bacteria are a biological limit to fluid milk shelf-life extension which limits the ability of milk to be distributed farther distances.

Additionally, anaerobic sporeforming bacteria can grow during the aging process for certain types of cheeses and cause butyric acid fermentation which causes a defect known as “late blowing” (Klijn et al., 1995). This defect, predominantly caused by *Clostridium tyrobutyricum* and other butyric acid bacteria (BAB), is responsible for severe economic losses for hard and semi-hard cheese processors since it often affects the entire batch of cheese the product is not saleable (Klijn et al., 1995). Cheesemakers go to great lengths to minimize the risk of *C. tyrobutyricum* in their raw milk supply. Some of these methods focus on reducing sporeforming bacteria on the farm side while others mechanically remove sporeforming bacteria prior to processing. For example, there are penalties used to regulate the number BAB in raw milk that is used for cheesemaking in the Netherlands (Berg et al., 1989). Centrifugation and microfiltration are both mechanical methods used in the dairy industry to remove spores from milk prior to pasteurization (Guerra et al., 1997; Gésan-Guiziou, 2010). Additional methods for reducing bacterial spore levels in cheese include adding lysozyme to cheese milk to prevent the growth of BAB, and therefore prevent the late blowing defect (Zucali et al., 2015). However, these methods are expensive and time consuming. Ultimately, bacterial spores are costing the dairy industry money both through product spoilage and through the steps processors take to reduce the chances or effects of contamination. Reducing the number of bacterial spores in raw milk at the farm level using low-cost interventions is an alternative strategy to remove spores.

Several studies have analyzed farm management practices that are associated with low spore raw milk. For example, Zucali et al. (2015) found that if a herd has a large proportion of dirty udders, they are more likely to have higher anaerobic sporeforming bacteria counts in their raw milk (Zucali et al., 2015). Additionally, forestripping has also been associated with lower anaerobic sporeforming bacteria counts (Zucali et al., 2015). Other studies have found that removing udder hair and using inorganic bedding (e.g. sand) are also associated with lower spore counts in raw milk (Murphy et al., in press). In Chapter 2, a combination of interventions that included washing towels with bleach, drying towels, and giving a one-time training to milking parlor employees on the importance of teat end cleanliness, was found to lower the spore count in raw milk. Using the combination of interventions, a 37% and 40% decrease in bulk tank raw milk mesophilic spores and thermophilic spores was observed, respectively, compared to spore levels in raw milk before the combination of interventions was applied. In this chapter, a cost-benefit analysis is performed on the same interventions from both the perspective of the producer (i.e. dairy farmers) as well as the processors selling dairy products.

## MATERIALS AND METHODS

***On-farm interventions***. Details of intervention strategies and spore count analyses can be found in Chapter 2. Briefly, interventions applied to the farms included i) adding chlorine bleach to the wash cycle for the towels used during udder preparation prior to milking unit attachment, ii) drying the towels completely, and iii) training milking parlor employee on the importance of teat end cleaning during milking. All interventions were applied concurrently on each farm, and farms were sampled three times over 15 months. Farms implemented the combination of interventions on a rotating basis once each month. For each implementation, samples were collected every other day for one week prior to the combination of interventions being applied and every other day for a week after the combination of interventions was applied.

***Model design***. A predictive model of fluid milk spoilage caused by sporeforming bacteria was based off the model developed by Buehler et al. (2018), which was designed to predict how the shelf-life of fluid milk was affected by varying levels of contamination with psychrotolerant sporeforming bacteria (Buehler et al., 2018). Buehler's model, or version 1 of the model, is designed to predict the effect the interventions have on bacterial counts in milk and the rate of spoilage. Our baseline, pre-intervention model used the same data and values as reported in Buehler et al. (2018). However, version 2 of the model, which was developed here, incorporates a temperature distribution instead of assuming the milk was stored at a static temperature as was done in version 1 of the model. Specifically, the temperature for version 2 of the model is drawn from a normal distribution with a mean of 4.096$^{o}$C and a standard deviation of 2.381. As with version 1 of the model, the initial log most probable number (MPN) counts were sampled from a normal distribution with a mean of -0.723 log CFU/mL for version 2. To simulate the results of the interventions, a 0.22 log reduction was applied to this distribution and sample using a mean of -0.943 log CFU/mL. This reduction and distribution were based on the reduction and distribution of mesophilic and thermophilic spores observed in Chapter 2. We assumed that the reduction of psychrotolerant spores would be similar to the reductions observed for mesophilic and thermophilic spores. Each model was run for 10,000 iterations. All model design and statistical analyses were performed in R (version 3.4.3; R project, Vienna, Austria).

***Cost of interventions by farm size***. The cost-benefit analysis assumes farms will apply a combination of interventions (e.g., washing towels with bleach, drying towels, and giving a one-time training to milking parlor employees on the importance of teat end cleanliness) at the same time. Assumptions were made when calculating the cost of the interventions, including; i) one dryer can do 15 one hour loads of 100 towels per day, ii) the cost of a dryer is $1000, iii) the average lifespan for a dryer is 3 years, iv) the cost of electricity is $0.12/kWh, v) the average dryer uses 3.3 kW of energy per hour, vi) a 3 pack of concentrated bleach (Clorox) cost $14.15, vii) milking employee training costs $100 per year, viii) cows are milked three times per day, and ix) farms averaged milk yields of 12700 kg/cow/year (28,000 lbs/cow/year). Yearly costs were calculated based on herd size. Since four of the five farms in the study conducted in Chapter 2 dried their towels before the start of the study, an additional analysis was done to calculate the cost of the interventions if the farm did not need to purchase a dryer or pay for additional electricity.

***Survey design and distribution***. A survey to evaluate consumer milk usage was designed on Qualtrics (Qualtrics, Provo, UT) and was distributed electronically through the Cornell University Food Science Sensory Evaluation Center as well as through social media. The survey was developed to determine consumer fluid milk consumption patterns and consisted of 25 questions with 531 respondents (Supplemental Table 3.1).

## RESULTS AND DISCUSSION

***Significant reductions in bulk tank raw milk spore levels can be achieved with low-cost on-farm interventions.*** Farm-level interventions, including washing towels with bleach, drying towels, and providing a one-time training to milking parlor employees, were found in a previous study to significantly reduce bulk tank spore levels (Chapter 2). Our analysis determined that the cost to implement these interventions ranged between $9.48 and $13.10 per cow per year based on herd size (Table 3.1), or between $0.03 and $0.05 per hundred weight (CWT) depending on herd size assuming a herd average of 12,700 kg (28,000 lbs) produced per cow per year (Table 3.1).

Table 3.1. Cost of the combination of interventions per year by cow and by hundred weight (cwt) of milk based on herd size

| | 100 Lactating Cows | 500 Lactating Cows | 1000 Lactating Cows | 1500 Lactating Cows |
|---|---|---|---|---|
| Number of dryers[1] | 1 | 1 | 2 | 3 |
| Cost of dryer[2] | $333.33/year | $333.33/year | $666.67/year | $1000/year |
| Number of dryer loads/day[3] | 3 loads/day | 15 loads/day | 30 loads/day | 45 loads/day |
| Electricity cost[4] | $438/year | $2,190/year | $4,380/year | $6,570/year |
| Bleaching towels[5] | 93 gal/year | 463 gal/year | 926 gal/year | 1387 gal/year |
| Cost of bleach/year[6] | $438.96/year | $2,185.36/year | $4,370.72/year | $6,546.64/year |
| Milker training[7] | $100 | $100 | $100 | $100 |
| Cost of interventions/cow/year | $13.10 | $9.62 | $9.52 | $9.48 |
| Cost of interventions per cwt[8] | $0.05 | $0.03 | $0.03 | $0.03 |
| Cost of interventions/cow/year without dryer[9] | $5.39 | $4.57 | $4.47 | $4.43 |
| Cost of interventions/cwt without dryer[9] | $0.02 | $0.02 | $0.02 | $0.02 |

[1] assumed 1 dryer can do 15 loads per day

[2] assumed 1 dryer lasts 3 years; $1000 dryer cost averaged over 3 years

[3] assumed 100 towels/load; 1 load takes 1 hour in the dryer

[4] assumed $0.12/kWh; 1 hour loads; 3.3 kilowatts of energy = average dryer; $0.40/load

[5] based on 320mL bleach/load (10mL/1 galon = 200 ppm); 3785mL/gal

[6] 3 pack of concentrated bleach = $14.15 ($4.72/gal)

[7] 1 training given per year

[8] based on 28,000 lbs/cow/year or 280 cwt/cow/year

[9] assumed farm was previously drying towels and would not have additional electric or dryer costs

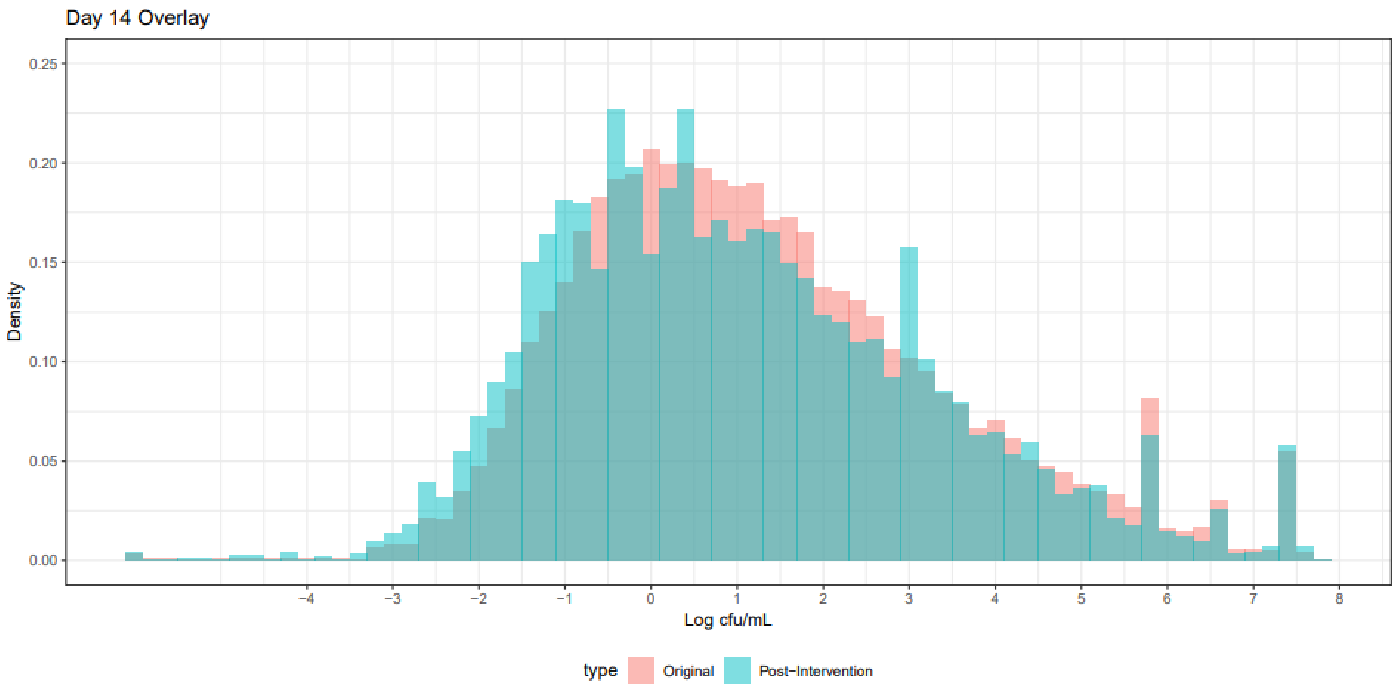
According to the 2017 New York State Dairy Business Summary, which evaluated 156 dairy farms across the state, the cost of applying these interventions is low when compared to the costs associated with a dairy cow each year (Karszes et al., 2018). It was calculated that the average cost of a dairy cow in New York State in 2017 was $5,052 per year and the cost of a dairy cow per CWT of milk produced was $19.49. Furthermore, many dairy farms dry their towels and would have no additional dryer or electric costs. Of the farms who participated in the study in Chapter 2, four of the five farms already dried towels before using them. If a farm does not need to add drying towels to their towel washing protocol, the intervention cost will range from $4.43 to $5.39 per cow per year depending on the size of the farm or will cost about $0.02 per CWT assuming a herd average of 12,700 kg (28,000 lbs) produced per cow per year (Table 3.1).

Importantly, our analysis indicates that the cost per cow for applying intervention strategies drops as the herd size increases. Others have described how economies of scale, or the cost advantages that are seen as a business grows, can save dairy farmers money including Mosheim et al. (2009) who concluded that economies of scale have even more of an effect than efficiency on dairy farms (Mosheim and Lovell, 2009).

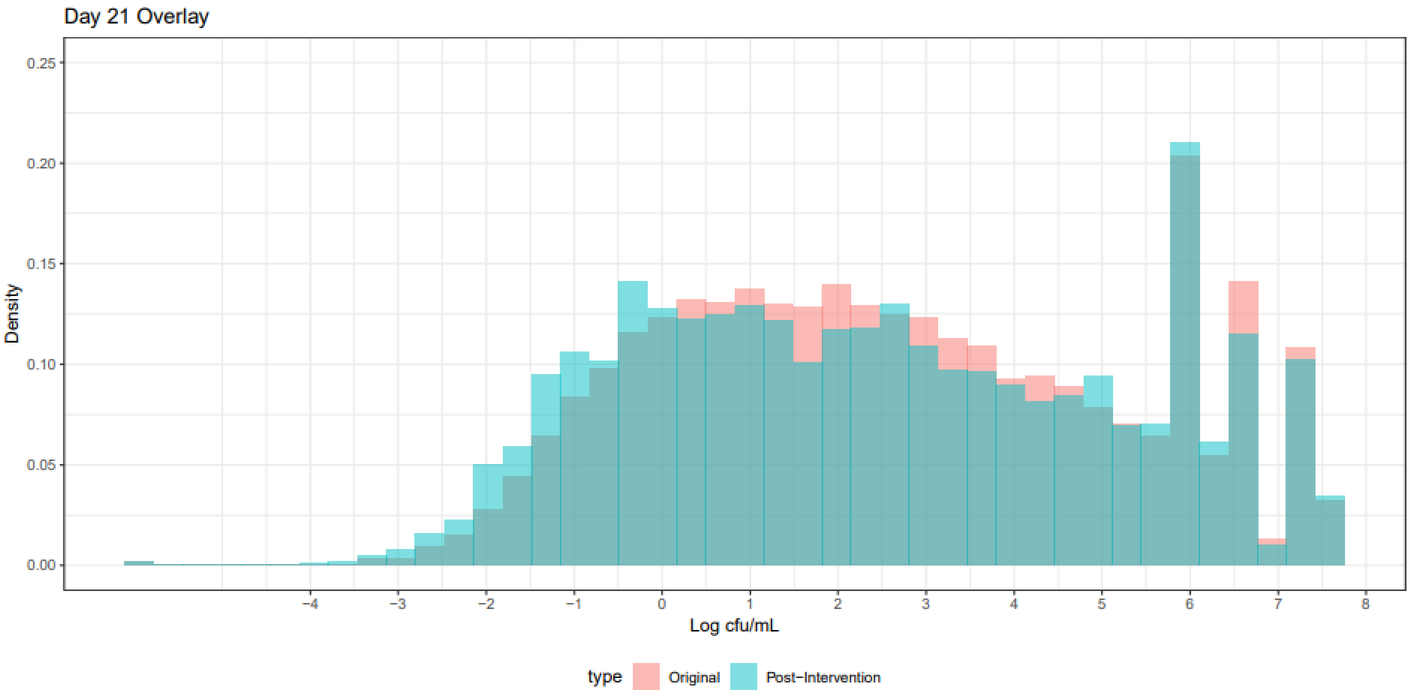
Furthermore, we hypothesize that this combination of on-farm interventions will also reduce the risk of mastitis and high somatic cell counts as the interventions focus on cleaner teat ends and good milking parlor practices. Mastitis is one of the most common and costly diseases in the dairy industry and reducing the chances cows will get it with low a low-cost combination of interventions would be worthwhile for producers (Seegers et al., 2003). One part of the combination of interventions applied on the farms was a milking parlor employee training that emphasized the importance of teat end cleaning before unit attachment. Several studies have found that cleaner teat ends are associated with fewer cases of mastitis (Schreiner and Ruegg, 2003; Breen et al., 2009; de Pinho Manzi et al., 2012). For example, de Pinho Mazi and colleagues conducted a study to evaluate the relationship between teat end condition and mastitis and found that cows with poor teat end condition scores were 30% more likely to develop mastitis and cows with dirtier udders were 47% more likely to develop mastitis (de Pinho Manzi et al., 2012). While mastitis can be reduced by having cleaner udders and teats, it is one of the most expensive costs to a dairy farm since they not only have to pay to treat the cow and withhold the milk for either high somatic cell count or for antibiotics if she is treated, but the cow's lifetime milk yield potential is reduced (Raubertas and Shook, 1982; Fleischer et al., 2001). It is estimated that production losses due to mastitis cost $110 per cow annually for the average dairy farm (Ruegg, 2005). Since our low-cost combination of on-farm spore interventions involves a milking parlor employee training on the importance of teat end cleanliness during milking, it seems likely that it could reduce the incidence of mastitis on a farm, but more research will need to be done to determine the impact.

***Reduced spore raw milk significantly reduces bacterial counts over pasteurized fluid milk shelf-life.*** We hypothesized that a reduction in bulk tank raw milk spore levels, such as those seen as a result of the on-farm interventions applied in Chapter 2 will reduce spoilage in dairy products, including fluid milk. Fluid milk was used as a model system to evaluate the influence of reduced spore raw milk on pasteurized product shelf-life, because sporeforming bacteria are responsible for approximately

50% of fluid milk reaching the PMO limit (Alles et al., 2018; Reichler et al., 2018). A previously developed fluid milk Monte Carlo model (Buehler et al., 2018) was used to estimate the effect the on-farm interventions, and the resultant reduction in bulk tank spore levels, have on the shelf-life of fluid milk, indicating that on days 14 and 21 after pasteurization, total bacteria counts were significantly lower in post-intervention simulations ($p < 0.001$; Figure 3.1). Based on model simulations, half gallons predicted to be spoiled by day 14 were reduced by 11% and by 0.7% by day 21. These simulated reductions assume that we would observe the same decrease in psychrotolerant spore counts that we saw for the mesophilic and thermophilic spore counts in Chapter 2. Most psycrotolerant spore counts in Chapter 2 were below the detection limit and could not be used in analysis.



Day 14 Overlay



Day 21 Overlay

Figure 3.1. Histograms representing the simulated distribution of bacterial spore counts in fluid milk 14 days and 21 days after pasteurization for original milk samples without the combination of interventions applied (red bars) and for the post-intervention samples with the interventions applied (blue bars). Interventions included washing towels with bleach, drying towels, and giving a one-time training to milking parlor employees on the importance of teat end cleanliness.

Having a lower percentage of milk spoiling at 14 and 21 days post-pasteurization is important as many code dates for milk are 17 days post-pasteurization. In a survey distributed electronically to consumers, 92% (490/531) of respondents either consume milk or live with someone who consumes milk. Of those 92%, 55% (257/471) of survey respondents drink milk past the code date printed on the container. Therefore, a reduction in the amount of fluid milk spoilage caused by sporeforming bacteria, even at the end of shelf-life, would ensure that over half of the milk-drinking population is exposed to less instances of reduced quality fluid milk. Additionally, a study by Quelch and Ash (1980) investigated consumer complaints and how consumers react after experiencing a product that did not meet their expectations (Quelch and Ash, 1980). While they found that only 2% of consumers complain to the manufacturer, they report that 14.7% will complain to the store which could influence whether or not a store continues to carry that product (Quelch and Ash, 1980). Importantly, 19.6% of consumers decided to not buy that brand again and 11.6% of consumers stated that they warn their friends and family about the product which is likely an underestimated percentage with the advent of social media (Quelch and Ash, 1980). Therefore, significantly reducing the number of half gallon containers that experience spoilage is desirable for fluid milk processors. Extended fluid milk shelf-life also provides opportunities for processors to ship further distances and to new markets.

Additionally, fluid milk that has a longer shelf-life will reduce food loss and waste which will assist in sustainably feeding a growing human population. A survey conducted by the United States Department of Agriculture (USDA) found that dairy represents 17% of food loss at retail and consumer levels which makes it one of the food groups with the highest amount of food loss (Buzby et al., 2014). This represents a 27 billion dollar value that could be reduced with better quality milk (Buzby et al., 2014).

## CONCLUSIONS

Applying an easy to implement combination of interventions on dairy farms will not only reduce sporeforming bacteria in the bulk tank raw milk, but it is a low-cost strategy for dairy farmers to implement. Low-spore fluid milk is an important step to extending the shelf-life of milk which will help to feed the growing human population worldwide since it will allow fluid milk to travel further and to new markets and reduce consumer waste. Furthermore, the low spore milk provides a lower risk of late blowing in cheese, a defect that has severe economic effects on cheesemakers. Our data provides farms ways to assess the economic benefits of spore reductions and can help processors set premiums for low spore milk. Since many consumers consume milk past the code date printed on the container and since many processors are affected by the negative impacts of bacterial spores in milk, it would be worthwhile for dairy processors to incentivize the implementation of this combination of interventions through premiums to producers. Future studies should focus on the effectiveness of this combination of interventions and its impact on the incidence of mastitis as well as developing models that can predict the affect these interventions would have on cheeses and dairy powders.

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## SUPPLEMENTAL MATERIAL

Supplemental Table 3.1. Milk consumption survey questions and responses for 531 consumers[1]

| Survey question | Responses[2] |
|---|---|
| Do you or any member of your household consume milk? | Yes (490), No (41) |
| Who in your household consumes whole milk? | Me (257), My partner/spouse (131), My kid(s) (111), My roommate(s) (43), Other (25), No one (162) |
| Who in your household consumes 2% milk? | Me (227), My partner/spouse (117), My kid(s) (72), My roommate(s) (36), Other (29), No one (190) |
| Who in your household consumes 1% milk? | Me (133), My partner/spouse (69), My kid(s) (33), My roommate(s) (17), Other (14), No one (313) |
| Who in your household consumes skim milk? | Me (118), My partner/spouse (45), My kid(s) (18), My roommate(s) (19), Other (11), No one (329) |
| Who in your household consumes flavored milk? | Me (146), My partner/spouse (90), My kid(s) (107), My roommate(s) (26), Other (20), No one (208) |
| How frequently do you consume milk? | Daily (154), 4-6 times/week (82), 2-3 times/week (83), Once/week (37), <once/week but > once/month (55), <once/month (40), Never (26) |
| How do you most frequently consume milk? | In a glass (143), With cereal (152), For cooking/baking (67), In coffee/tea (113) |
| Who in your household purchases milk? | Me only (180), Another household member only (47), Equally shared between me and another member of household (228), My roommates (11), Other (6) |
| How frequently do you buy milk? | > once/week (77), once/week (165), once every 10 days (71), once every 14 days (70), Once a month (37), < once /month (35), never (16) |
| What size container do you or the people in your household typically buy? | Gallon (263), Half gallon (146), Quart (48), 16oz (7), Half pint (7) |
| When purchasing milk, do you typically notice the best by, use by, or code date? | Yes (424), No (47) |
| Why not? | I've never noticed it (6), I'm in too much of a hurry (12), I can't always find it (2), It's not something that is important to me (18), Other (9) |
| Do you ever drink milk past the date printed on the container? | Yes (257), No (214) |
| Why not? | I always finish my milk before the date printed on the container (115), I discard my milk when it reaches the date printed on the container (90), Other (9) |
| How much of the milk is consumed after the date printed on the container? | 100% of the container (16), 75% of the container (5), 50% of the container (8), 25% of the container (53), <25% of the container (175) |
| Do you have milk in your refrigerator right now? | Yes (401), No (70) |
| How much milk is left in the container in your refrigerator right now? | 100% (35), 75% (95), 50% (142), 25% (71), <25% (45) |
| What is your age? | Under 18 (0), 18-22 (94), 23-34 (195), 35-45 (117), 46-60 (62), 61 or older (31) |
| You are… | Male (102), Female (395), Nonconforming (2), Prefer not to say (0) |
| Are you… | Single-living alone (76), Single-living with roommates (101), Single- living with kids (18), Single-living with a partner (39), Married- living with a partner (76), Married-living with a partner and kids (146), Other (43) |
| Do you have any children living with you? | Yes (179), No (320) |
| How old are the children that live with you? | Under 1 year (18), 1-4 years (71), 5-10 years (79), 11-13 years (34), 14-18 years (49), over 18 years (32) |
| How many people are in your household including you? | 1 (76), 2 (151), 3 (107), 4 (98), 5 (18), 6 or more (18) |
| How many people in your household, including you, consume cow's milk? | 0 (35), 1 (88), 2 (158), 3 (110), 4 (68), 5 (29), 6 or more (11) |

[1] survey was developed through Qualtrics (Qualtrics, Provo, UT) and was distributed electronically through the Cornell University Food Science Sensory Evaluation Center as well as through social media. The survey was developed to determine consumer fluid milk consumption patterns and consisted of 25 questions that received 531 responses

[2] number of responses shown in parentheses

# CHAPTER 4

# CONCLUSIONS

As the global population continues to expand, it is imperative that we find ways to optimize our food supply, which involves reducing food loss and food waste. In the dairy industry, bacterial spores are a biological limitation to the shelf life of fluid milk, as they can survive pasteurization in spore form and can grow at refrigeration temperatures and cause quality deterioration in vegetative form. Additionally, bacterial spores are responsible for multiple dairy product defects including late blowing in cheese which affects cheese during the aging process and ultimately leads to severe economic impacts for cheesemakers as it creates a product that is not saleable.

One way to extend the shelf life of milk, and therefore allow it to be shipped further and to new markets, as well as reduce the likelihood of late blowing in cheese, is to reduce the number of bacterial spores in the milk supply. While this can be done mechanically through centrifugation and microfiltration, low-cost interventions that can be applied at the farm level are a cost-effective alternative. A combination of low-cost interventions including washing towels with bleach, drying towels, and giving a one-time milking parlor employee training were analyzed in Chapter 2. While we found that applying the combination of interventions at one time significantly reduced the bacterial spore load in the raw milk, more research will need to be performed to assess washing towels with bleach, drying towels, and providing training to milking parlor employees on the importance of teat end cleanliness individually to see if any were more effective or less effective than the others. Additional future studies should focus on applying this combination of interventions to farms of varying sizes and in various locations as well as for extended periods of time.

Chapter 3 analyzed the cost of performing these interventions for a year on dairy farms of varying sized and concluded that the cost of the interventions is low compared to the yearly costs of a lactating dairy cow. Dairy producers benefit from receiving low spore raw milk from farms due to its ability to have a longer shelf life and its decreased risk of causing costly defects such as sweet curdling and late blowing and should therefore consider incentivizing low spore milk by providing premiums to farms who achieve this goal. Future studies should focus on the effectiveness of these interventions at reducing cases of mastitis on farms to allow for a more holistic assessment of the financial benefits of the interventions found here to be effective to reduce raw milk spore counts.

Overall, continuing to strive for low spore milk will provide the dairy industry with higher quality milk with fewer spoilage problems while allowing processors to ship milk farther and to new markets. The work provided in this thesis provides dairy farmers with low-cost interventions that can be used as a first step to producing cost-effective low spore count milk.