

AN ASSESSMENT OF ORGANIC AND CONVENTIONAL DAIRY PRODUCTION IN THE
UNITED STATES: MILK QUALITY AND MANAGEMENT

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The work included in this thesis is focused on research regarding the organic dairy community and milk quality. Our research is dedicated to assessing differences and best management practices on organic and conventional dairy farms throughout New York, Wisconsin and Oregon. We concentrated on modeling management associations with regularly used milk quality indicators somatic cell count, standard plate count, coliform count, as well as the presence of *Staphylococcus aureus* (*S. aureus*) in bulk tank milk. We also assessed general differences among conventional non-grazing, conventional grazing and organic dairy farms in the study population. Finally, we assessed the presence of methicillin-resistant *S. aureus* in bulk tank milk of the study population. As a whole, our results indicated that while the organic dairy community faces many unique management challenges and in achieving optimal milk quality, the factors affecting the organic community are much the same as those affecting the conventional community. An interesting point from our research is that farms that tend to use more outside resources and external inputs tend to have better quality. In addition to this finding, conventional farms are more likely to use external resources than organic farms. Finally, we determined that there is a low prevalence of methicillin-resistant coagulase negative *Staphylococcus* spp. and *S. aureus* in the bulk tank milk of both organic and conventional dairies in our study population. Overall, we have found that while both organic and conventional dairy farms, while facing their own individual challenges, are not all that different in the US.

BIOGRAPHICAL SKETCH

Kellie Marie Cicconi Hogan is seeking a Ph.D. in the field Comparative Biomedical Sciences, with minors in Epidemiology and Microbiology, from the Department of Population Medicine and Diagnostic Sciences at the Cornell College of Veterinary Medicine. She received a Bachelor of Science *cum laude* with Biology departmental honors from Lycoming College in Williamsport, PA in 2007. Her Ph.D. work was supervised by Dr. Ynte Schukken, Dr. Linda Tikofsky, Dr. Craig Altier, Dr. Yrjo Gröhn and Dr. Martin Wiedmann. Kellie is studying organic dairy production and management on US dairy farms. Her projects involved assessing management associations with milk quality and the presence of antimicrobial resistance in bulk tank milk.

DEDICATION

To my husband, Josh – I would not have finished this without you.

To my parents, Pam and Lou – you guys are pretty amazing parents. Thank you for everything

To the dairy community of New York

And last – but certainly not least:

To my babies, Cami and Hunter – For never letting me get as much work done as I should have and loving me unconditionally (as long as I filled the food bowls).

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LIST OF ABBREVIATIONS

AI = artificial insemination
BLAST = Basic Local Alignment Search Tool
BTM = bulk tank milk
BVD = bovine viral diarrhea
CC = coliform count
cfu/mL = colony forming units per milliliter
CMT = California Mastitis Test
CON = conventional
CON-GR = conventional grazing
CON-NG = conventional nongrazing
DHIA = Dairy Herd Improvement Association
DMI = dry matter intake
DNA = deoxyribonucleic acid
EIS = external input score
LPC (LP) = laboratory pasteurized count
LSCC = \log_{10} transformation of somatic cell count
LSPC = \log_{10} transformation of standard plate count
mL = milliliter
MRSA = methicillin-resistant *Staphylococcus aureus*
MR-CNS = methicillin-resistant coagulase negative *Staphylococcus species*
NAHMS = National Animal Health Monitoring System
NOP = National Organic Program
ORG = organic
OSS = outside support score
PCR = polymerase chain reaction
PI = preliminary incubation
RAPD = random amplification of polymorphic DNA
rRNA = ribosomal ribonucleic acid
SCC = somatic cell count
SCC_{mec} = staphylococcal chromosome cassette
SPC = standard plate count
TMR = total mixed ration
USDA = United States Department of Agriculture
 μ L = microliter

CHAPTER ONE

Introduction

Organic Agriculture

In the past decade, the demand for organic agricultural products has increased rapidly in the United States and worldwide. Sales of organic foods have reached \$25 billion in the United States, accounting for approximately 3.7% of total U.S. food sales. From 2000 to 2008, the number of certified organic cows in the U.S. increased from 38,196 to 249,766 (Economic Research Service, 2008). With the current rate of growth in organic food consumption, it is likely that the demand for organically produced dairy products will continue to increase (Batte et al., 2007). Consumers are becoming increasingly concerned with animal welfare and the environmental impact of the dairy industry as a whole (Sundrum, 2001); however, due to the recent surge in organic consumption, organic dairy production is getting much attention. As it stands, scientific data on milk quality, herd management methods and animal welfare on organic farms in the US has been lacking prior to the research from this study. Our goal was to close the informational gap by studying the management and milk quality of a large number of both organic and conventional herds in the US.

The National Organic Program defines the standards and regulations for the transition to organic production in the US (National Organic Program, 2007). At the starting point of a completely conventional dairy system, the land must be transitioned to organic status over three years, without the use of any substances prohibited by the National Organic Program on the animals or the land. Over the course of the third year the dairy animals must be managed 100% organically and be fed a completely certified organic diet. Organic standards require that all

dairy animals have access to pasture for the full length of the grazing season. It is also required that a minimum of 30% of DMI is provided during the grazing season from pasture. Most organic farmers feed a ration comprised primarily of hay crops, including dry hay, in addition to the pasture (NOFA, 2009). This combination provides high forage content in rations for organic dairy cattle, relative to conventionally fed dairy cattle. Many organic farms have eliminated corn silage from the ration, as it is difficult to grow organically, and instead feed organic molasses as an important source of energy (NOFA, 2009). All animals that comprise the organic herd must be on the farm at the start of the third year of transition, with no additions from conventional farms during that final transition year. Calves entering an organic farm must be raised organically from the last third of gestation (NOFA, 2009). Any animal that is certified organic may not receive prohibited substances, including, but not limited to, antimicrobials or synthetic reproductive drugs.

There are some key differences between US and European organic standards. Antimicrobials are permitted as a treatment on organic farms in the European Union (EU), with the diagnosis and administration by a veterinarian. Dairy production animals that are given an antimicrobial must have their milk segregated from the bulk tank or their meat kept from sale for double the traditional withholding time as their conventional counterparts. However, these animals can return to the herd and do not lose their organic status (CEC, 1999), unlike animals on organic dairy farms in the US, which must be removed from the herd and cannot be sold as organic. In the case of a sick or injured animal, producers are prohibited from withholding life saving treatments in order to preserve organic status (National Organic Program, 2007). Organic management in the US therefore, in principle, heavily promotes preventative measures to handle disease through measures such as vaccination (Ruegg, 2009).

As of 2008, most organic dairies in the US were former conventional farms that had transitioned to organic management within the past 10 years. Organic dairy farms are generally smaller, have lower milk production per cow and tend to be family run operations (Ruegg et al. 2008). Organic farms receive a higher price for their milk, which has allowed many smaller farms to switch to organic production and still remain economically viable. Due to the recent surge in the number of organic farms, the need for established best management practices and overall herd health information on organic dairy farms is growing, but the industry lacks sufficient scientific information based on contemporary organic practices. This lack of scientific information on best management practices is particularly true in the US, as organic management has been studied in more detail on European organic farms (Bennedsgaard et al., 2010; Ellis et al., 2007; Padel et al., 2009; Nauta et al., 2006). There have been a few comparisons of antimicrobial use (and lack thereof) and mastitis management on organic and conventional dairy operations in the US (Zwald et al., 2004; Pol and Ruegg, 2007; Ruegg, 2009), but few studies have been conducted that investigate the well-being and milk quality of dairy animals on similarly sized and location matched organic and conventionally managed herds.

Milk Quality

Milk quality is of major interest to both consumers and dairy farmers alike. The most common and cost effective way to assess milk quality is through regular bulk tank milk (**BTM**) testing. There are many studies that report associations between various management practices measures of animal health and milk quality (Schukken et al., 2003; Elmoslemany et al., 2009; Elmoslemany et al., 2010), but few that focus specifically on organic dairy management associated risk factors.

The most commonly used and validated milk quality measurement is the somatic cell count (**SCC**) of the bulk tank. The SCC is an excellent way to assess the presence of mammary infection in the herd, which is a major indicator of udder health (Dohoo and Leslie, 1991). It is used as an international standard for milk quality and has major influence on the price and marketability of the milk sold by farmers (Dufour et al., 2011). Premiums are often determined based on BTM results showing high quality milk and low incidence of mastitis (Jayarao et al., 2004). Many management practices, such as a lack of pre- and post-dip use, use of fewer towels during a pre-milking routine, and not keeping disease records, have been associated with an increased disease risk and consequently, a higher bulk milk SCC (Dohoo and Meek, 1982; Schukken et al., 2003; Barkema et al., 1998). Other commonly tested parameters for milk quality include **SPC**, which determines the total number of bacteria in a milliliter of milk; **LPC**, which detects the amount of pasteurization-resistant bacteria in a milliliter of milk; coliform count (**CC**); and **PI** count, which is used to detect the amount of bacteria that continue to grow at refrigeration (55°F) temperatures.

In a recent study (Dufour et al., 2011), several variables were found to be associated with a lower bulk tank SCC, such as wearing gloves during milking, post-dipping, milking problem cows last, use of a freestall as primary housing for lactating cows, regular use of dry-cow therapy, udder hair removal and the regular use of a California Mastitis Test (**CMT**). Most of the studies reviewed by Dufour et al. (2011) were studies on conventional farms and it is expected that risk factors on organic farms would not necessarily be the same as on conventional farms. There has been a significant amount of research in Europe on organic dairy farms and milk quality. Ellis et al. (2007) focused on cleanliness on organic and conventional dairy farms in the United Kingdom, and found that organic cows were cleaner in the winter months than the conventional

cows, although there was no difference in cleanliness in the summer months. This study also reported that cleaner cows were associated with a lower SCC. Haskell et al. (2009) found that there was no influence of organic dairy production on SCC, and that there were not any major differences between the organic and conventional dairy farms surveyed. Langford et al. (2009) reported that organic farms tend to produce less milk, feed a less concentrated ration, cull less than the conventional farms studied. While there is some research on organic dairy production in the US, many have conflicting information regarding bulk tank milk quality (Wilhelm et al., 2009) or do not have adequately size matched controls. Some studies have found no significant difference between SCC of organic and conventional farms, while others have found organic farms to have a higher or lower SCC than conventional farms (Wilhelm et al., 2009).

The presence of antimicrobial-resistance organisms in agriculture has been a major source of scientific interest and consumer concern in recent years, due to the potential public health risk of antimicrobial-resistant organisms in the food supply (McEwen and Fedorka-Cray, 2002; Silbergeld et al., 2008). One such organism that has gotten much attention recently is methicillin-resistant *Staphylococcus aureus* (**MRSA**). Because *S. aureus* is a major mastitis pathogen, and MRSA is a public health risk, there has been some question of how frequently MRSA is isolated from milk. There have been reports of MRSA isolated from bulk tank milk (Kreusikon et al., 2012) as well as the discovery of a *mecA* homologue from milk samples in Europe (García-Álvarez et al., 2011; Paterson et al., 2012). In the United States, recent studies have found a low prevalence of MRSA and methicillin-resistance coagulase negative *Staphylococcus* (**MR-CNS**) in bulk tank milk (Virgin et al., 2009; Haran et al., 2012); however, these studies have not specifically investigated the prevalence of MRSA in organic bulk tank milk in the US. The lack of information regarding MRSA in US organic bulk tank milk, in

combination with the recent *mecA* homologues in Europe led us to explore the prevalence of methicillin-resistance in the bulk tank samples from our project.

As mentioned previously, the lack of scientific data on organic management in the US in combination with the growing organic market has created a need for more precise data regarding risk factors for milk quality in the organic population. The goal of our study was to use both survey and sampling methods to properly assess the bulk tank milk and associated risk factors, as a way to create a set of best management practices for not only organic dairy farms, but the dairy industry as a whole.

Project C.O.W.

Project C.O.W. is a collaboration of researchers at Cornell University, Oregon State University and The University of Wisconsin-Madison. The project was funded through the USDA Integrated Organic Program from August 2008 to August 2012. The project was designed to provide a complete picture of organic and conventional dairy production in New York, Oregon and Wisconsin. The goals were as follows, as defined from the project narrative (Ruegg, 2008): 1) Identify the unique methods that organic dairy farmers use to detect and treat disease. 2) Compare prevalence of disease and identify management factors that affect animal well-being and farm profitability. 3) Use the information gathered to provide dairy producers with benchmarks to help recognize and diagnose disease. 4) Provide farmers with milk quality information. 5) Use data gathered to develop extension suggestions for preventative health management of dairy cattle. For this thesis, we focused primarily on management variables, both in how they differed between grazing systems and also how they affected bulk tank milk quality.

A total of 192 organic (**ORG**) and 100 conventional (**CON**) herds were visited between March 2009 and May 2011 from dairy herds located in New York, Oregon, and Wisconsin. One ORG farm from Vermont and one CON farm from Pennsylvania (frequency matched to a similarly sized NY organic farm within a 50 mile radius) were included in the NY, as they were in close proximity to the NY border. Herds were matched by dairy production system (ORG vs. CON) with differing ratios by state, due to various herd demographics in the 3 locations. The ratios were as follows: 3 ORG to 1 CON in New York, 1 ORG to 1 CON in Oregon, and 2 ORG to 1 CON in Wisconsin.

Organic herds were initially identified by listings from organic certifying agencies, county extension agents, and other various personal contacts. A list of potential CON herds was assembled by acquiring a list of licensed dairy farms from each state's department of agriculture. Recruitment letters were sent to all ORG and randomly selected CON, size matched farms in the same county, or within a 50-mile radius of the matched ORG farms. All letters included a participation reply postcard requesting contact information and basic demographic material. Positive responders were contacted by phone or mail to assess eligibility. In New York, 400 ORG farms were contacted with approximately 80 positive responses; in Oregon, 32 ORG farms were contacted with 24 positive responses; and in Wisconsin, 600 ORG farms were contacted with 120 positive responses.

Herd Inclusion and Exclusion Criteria. To fulfill study entrance conditions, ORG farms had to be certified organic and shipping organic milk for at least 2 full years prior to their participation in the study, and have a minimum of 20 lactating cows. Conventional herds included in the study were required to have a minimum of 20 cows and must have been shipping milk for at least 2 years. Farms with fewer than 20 adult cows were excluded from the study, as

the objective of the study was to assess management strategies on established commercial dairy operations, and including farms with less than 20 cows would open the study up to farms that served primarily as a hobby or a side project instead of a primary source of income and employment. Conventional farms were matched based on location and herd size. Farms were characterized into three groups: 20 – 99 adult cows, 100 – 199 adult cows, and ≥ 200 adult cows.

Questionnaire. The study questionnaire (available at <http://milkquality.wisc.edu/organic-dairies/project-cow/>) was adapted from previously published surveys (Zwald et al., 2004; Pol and Ruegg, 2007). It was reviewed by questionnaire developers, and tested prior to use in the study with volunteer ORG and CON dairy farmers in each state. The Institutional Review Board at Oregon State University approved the use of human subjects for the questionnaire.

Farmers were asked to refer to all obtainable records to ensure accuracy of the data collected. Recall was often limited to the 12 months prior to the herd visit or less. Questionnaire information addressed questions under each of the following themes: animal health, personnel, herd inventory and expansion, milk production, reproductive information, housing, feed and water systems, milking procedures, other routine procedures, disease definitions and treatment, mastitis, management of Johne's disease, veterinarian involvement, calf and heifer management, and Dairy Herd Improvement Association (DHIA) information (if available).

Data Collection. A single member of the study team conducted all interviews within each state. Before herd visits began, all study personnel were trained together on administration of the questionnaire and scoring systems used in the study. In most herds, the individual directly responsible for animal health and farm management was interviewed. Retrospective treatment data including calf and adult cattle disease, mastitis, culling, vaccinations and veterinarian use was collected for the 60-day period before the visit using on-farm records or farmer recall. The farmer was asked to record all herd health events that occurred in the 60-day period after the herd visit on predefined recording forms. Farmers were also asked to take milk samples from cases of subclinical or clinical mastitis during the 60-day prospective period. Prepaid mailers were left with the farmer to return recording forms and milk samples. If no prospective data was received after the 60-day period had lapsed, the farmer was called and reminded to send in the information.

Samples of bulk milk collected by study personnel were sent to Quality Milk Production Services at Cornell University. Samples were tested by real-time PCR for foodborne pathogens *Salmonella species* (detecting the presence of the *invA* gene) (Nam et al., 2005), *Listeria monocytogenes* (detecting the gene *hly*) (Jothikumar et al., 2003), and Shiga toxin producing *E. coli* (detecting for the presence of the *stx1* or *stx2* gene) (Reischl et al., 2002; Manning et al., 2008). Samples were also tested for *Mycoplasma bovis* (Hogan et al., 1999), Bovine Virus Diarrhea virus (Renshaw et al., 2000), a modified mastitis bacteria count (Hogan et al., 1999) and antibodies to *Mycobacterium avium* subspecies *paratuberculosis* (Johne's disease) (Paracheck®, Prionics, Zurich, Switzerland). Aliquots of these samples were then couriered to Dairy One Cooperative (Ithaca, NY) and tested for milk quality including somatic cell count, fat percentage, protein percentage, standard plate count, *E. coli* coliform count and lab pasteurized

count (Wehr and Frank, 2004). Bulk tank SCC and SPC information were collected on the farm from 3 months prior to the herd visit and compared to the collected sample to help ensure accuracy of gathered information. Prospective data forms and mastitis samples were returned to the investigators by the farmer after a reminder phone call. Individual quarter mastitis samples from Wisconsin and Oregon study herds were analyzed at University of Wisconsin-Madison's Dairy Science laboratory. Mastitis samples from New York herds were analyzed at Quality Milk Production Services at Cornell University.

Chapter Concepts

While all the chapters contained in this thesis are derived from a common theme, assessing the characteristics of organic and conventional dairy production in the United States, each chapter is dedicated to a specific topic within that theme. **Chapter 2** assesses the differences between organic, conventional non-grazing and conventional grazing dairy farms in the US. It also briefly delves into the attitudes and use of outside resources by dairy farms included in the study. **Chapter 3** is focused on the bulk tank SCC of the farms in the study. We used information from the questionnaire to determine what management variables, procedures and farm characteristics were associated with bulk tank SCC. This information also allowed us to build on the outside resource information from Chapter 2 to develop a more complete way to assess external support on dairy farms. **Chapter 4** aims to assess management and farm variables that are associated with high bulk tank SPC, high coliform counts and the presence of *S. aureus* in the bulk tank. The goal of **Chapter 5** was to assess the prevalence of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative *Staphylococcus* using a combination of genotypic and phenotypic methods in organic and conventional populations.

Chapter 6 is a discussion on each of the chapters and how they relate to each other to make a complete story on bulk tank milk quality on organic and conventional dairy production in the US.

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CHAPTER TWO

Assessment of herd management on organic and conventional dairy farms in the United States.

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INTERPRETIVE SUMMARY

Assessment of herd management on organic and conventional dairy farms in the United States.

Stiglbauer, Cicconi-Hogan and Richert et al. The organic dairy industry is one of the fastest growing agricultural sectors in the United States, making it an appropriate choice for current dairy research. The goal of our study was to evaluate management and herd health characteristics on organic and similarly sized conventional farms across the U.S. A key finding was that organic farmers were much less likely to use support from external sources in regard to management and health advice than their conventional counterparts.

ABSTRACT

The objective of this study was to evaluate management characteristics on organic and similarly sized conventional dairy farms located in New York, Wisconsin and Oregon. Data from 192 organic farms (ORG), 64 conventional non-grazing farms (CON-NG) and 36 conventional-grazing (CON-GR) farms were collected during farm visits and were size-matched and analyzed. The average lactation of animals on ORG and CON-GR farms was 2.6 lactations, which was greater than CON-NG farms, at 2.3 lactations. A greater percentage of first lactation heifers were found on conventional farms than ORG farms. Facilities used by adult animals, including housing and milking facilities, were not different among the grazing systems. Cattle on conventional farms were fed approximately twice as much grain as cattle on ORG farms, and had greater milk production. Little difference was found for the average reported somatic cell count (SCC) and standard plate count (SPC), suggesting that milk quality is not dependent on grazing system. Milking procedures were similar across all three grazing systems, indicating that there is now an industry standard for milking, and that milk quality problems will need to be addressed with other management problems in mind. While some disease prevention measures were commonly utilized on ORG farms, such as keeping a closed herd and having a written record of treatments administered to the animals, the use of outside support and vaccinations were found to be less prevalent on organic farms than conventional farms.

Key words: dairy, organic, management, comparative study

INTRODUCTION

Over the past 10 years, the demand for organic agricultural products has increased rapidly in the United States and worldwide. Sales of organic foods have reached \$25 billion in the United States, and account for approximately 3.7% of total U.S. food sales. Organic dairy products comprise 15% of total organic sales, while organic fruits and vegetables account for 38% of total sales, making them the top two sectors of organic food (Organic Trade Association, 2010). From 2000 to 2008, the number of certified organic cows in the U.S. increased from 38,196 to 249,766 (Economic Research Service, 2008). Based on the current growth rate of organic food consumption, it is predicted that the demand for organically produced dairy products will continue to increase (Batte et al., 2007). The increase in the number of certified organic dairy animals and the associated production of organic milk products is consumer driven, as many consumers are concerned with the animal welfare and the environmental impact of conventional dairy farming (Sundrum, 2001); however, there is a lack of scientific data on management methods and herd health on organic farms to validate or refute these concerns.

The National Organic Program (NOP) defines U.S. standards for the transition to organic production (National Organic Program, 2007). From the starting point of a completely conventional dairy system, the land must be transitioned to organic status over the course of three years without the use of any substances prohibited by the NOP. In the third year, the dairy animals must be managed 100% organically and be fed a diet comprised of only certified organic feed. All animals that will constitute the organic herd must be on the farm at the beginning of the third year of transition, with no additions from non-organic farms during that year. Calves that are entering an organic farm must be raised organically from the last third of gestation (NOFA, 2009). Animals certified as organic may not receive NOP prohibited substances, including

antimicrobials or synthetic reproductive drugs. While there is some overlap between US and European organic standards, there are some key differences. Antimicrobials are allowed as a disease treatment on organic farms in the European Union (EU), with the involvement and diagnosis of a veterinarian. Any animal given an antimicrobial must have their milk kept out of the tank or their meat kept from sale for double the withholding time as conventional herds. However, these animals can return to the herd and do not lose their organic status (CEC, 1999). On organic dairy farms in the US, animals or products from animals given NOP prohibited substances may no longer be sold as organic, and these animals must then be removed from the herd. However, producers are prohibited from withholding necessary treatment from a sick or injured animal (National Organic Program, 2007). Organic management in the US therefore heavily promotes preventative measures to handle disease through measures such as vaccination (Ruegg, 2008).

As of 2008, most organic dairies in the US were former conventional farms that transitioned to organic management within the past 10 years (Ruegg, 2008). Organic farms receive a higher price for their milk, which has encouraged many smaller farms to transition to organic production. However, some economic analyses have indicated that net profits of conventional and organic farms in some regions are similar (Dalton et al., 2008; Cook et al., 2010), thus more studies about the economic sustainability of organic dairy farming are needed. Due to the recent surge in the number of organic farms, the need for established best management practices and overall herd health information on organic dairy farms is growing, but the industry lacks sufficient scientific information based on contemporary organic practices. This lack of scientific information on best management practices is particularly true in the US, as organic management has been studied in more detail on European organic farms (Nauta et al.,

2006; Ellis et al., 2007; Padel et al., 2009; Bennedsgaard et al., 2010). There have been several comparisons of antimicrobial use and mastitis management on organic and conventional dairy operations in the US (Zwald et al., 2004; Pol and Ruegg, 2007; Ruegg, 2008), but no large scale studies have been conducted that investigate the broader health care practices and the resulting well-being of cows on size and location matched organic and conventionally managed herds.

The primary objective of this manuscript was to assess health, management and herd characteristics among size-matched conventional non-grazing farms (**CON-NG**), conventional grazing farms (**CON-GR**) and organic (**ORG**) dairy herds across New York, Wisconsin and Oregon.

MATERIALS AND METHODS

Recruitment and Herd Selection

A total of 192 organic (ORG) and 100 conventional (CON) herds were recruited between March 2009 and May 2011 from dairy herds located in New York (NY), Oregon (OR), and Wisconsin (WI). In the NY subset of farms, one ORG farm from Vermont and one CON farm from Pennsylvania (matched to a similarly sized NY organic farm within a 50 mile radius) were included due to their proximity to the NY border. Because of herd demographics within each state, herds were matched by dairy production system (ORG vs. CON) with differing ratios by state. The ratios were: 3 ORG to 1 CON (NY), 1 ORG to 1 CON (OR), and 2 ORG to 1 CON (WI). Recruitment letters were sent to producers in all three states. Organic herds were identified initially by listings from organic certifying organizations, county extension agents, and personal contacts. A list of potential CON herds was compiled by obtaining a list of licensed dairy farms

from each state's department of agriculture. Recruitment letters for the study were sent to all ORG and randomly selected CON farms in the same county. Each recruitment letter included a participation reply postcard requesting further contact information and basic demographic information. Positive respondents were contacted by phone or mail to determine eligibility for the study. Non-responders were sent multiple mailings in order to increase participation. In NY, 400 ORG farms were contacted through recruitment letters with approximately 80 positive responses; in OR, 32 ORG farms were contacted with 24 positive responses; in WI, 600 ORG farms were contacted with 120 positive responses.

Herd Inclusion and Exclusion Criteria

To fulfill study entrance criteria, ORG farms had to have been certified organic and shipping organic milk for at least 2 full years prior to their participation in the study, and have a minimum of 20 lactating cows. Conventional herds included in the study were required to have a minimum of 20 cows and must have been shipping milk for at least 2 years. Farms with less than 20 adult cows were not included in the study, as the goal of the study was to assess management strategies on established commercial dairy operations, and including farms with less than 20 cows would open the study up to 'hobby' farms. Conventional farms were within a 50-mile radius of enrolled organic herds and were matched based on herd size, characterized into three groups: 20 – 99 adult cows, 100 – 199 adult cows, and ≥ 200 adult cows.

Questionnaire

The study questionnaire (available at <http://milkquality.wisc.edu/organic-dairies/project-cow/>) was adapted from previously published surveys (Zwald et al., 2004; Pol and Ruegg, 2007).

It was reviewed by professional questionnaire developers, and tested before use with pilot ORG and CON dairy farmers at each site. Information regarding questionnaire format and specifics are listed in detail in Richert et al., accepted. The Institutional Review Board at Oregon State University approved the use of human subjects for the questionnaire.

Data Collection

Data collection has been described in detail in Richert et al., (accepted). In brief, a single member of the study team conducted all interviews within each state. Before herd visits began, all study personnel were trained together on administration of the questionnaire and scoring systems used in the study. In most herds, the individual directly responsible for animal health and farm management was interviewed. Farmers were asked to refer to all available records to ensure accuracy of answers. Recall was frequently limited to the 12 months prior to the herd visit or less. Questionnaire information addressed several questions of each of the following themes: animal health personnel, herd inventory, production, reproduction, housing, feed and water, milking procedures, routine procedures, disease definitions and treatment, mastitis, Johne's disease, veterinarian involvement, calves and heifers, and DHIA information (if applicable). Retrospective treatment data including calf and adult cattle disease, mastitis, culling, vaccinations and veterinarian use was collected for the 60-day period before the visit using on-farm records or farmer recall. The farmer was asked to record all herd health events that occurred in the 60-day period after the herd visit on predefined recording forms. Farmers were also asked to take milk samples from cases of subclinical or clinical mastitis during the 60-day prospective period. Prepaid mailers were left to return recording forms and milk samples. If no prospective

data was received after the 60-day period had lapsed, the farmer was called and reminded to send in the information.

Samples of bulk milk collected by study personnel were sent to Quality Milk Production Services at Cornell University and tested for foodborne pathogens (*Salmonella spp.*, *Listeria monocytogenes*, Shiga toxin *E. coli*), *Mycoplasma bovis*, Bovine Virus Diarrhea virus (BVD), and *Mycobacterium avium* subspecies *paratuberculosis* (Johne's disease). Samples were couriered to Dairy One Cooperative (Ithaca, NY) and tested for milk quality including somatic cell count, fat percentage, protein percentage, standard plate count, *E. coli* coliform count and lab pasteurized count. Bulk tank SCC and SPC information were collected from 3 months prior to the herd visit and compared to the collected sample to help ensure accuracy of gathered information. Prospective data forms and mastitis samples were returned to the investigators by the farmer after a reminder phone call. Individual quarter mastitis samples from Wisconsin and Oregon study herds were analyzed at University of Wisconsin-Madison's Dairy Science laboratory. Mastitis samples from New York herds were analyzed at Quality Milk Production Services at Cornell University.

Statistical Analysis

Study Variables. Dairy production system (ORG and CON) and grazing information were combined to create a new explanatory variable, grazing system, which had three levels 1) ORG, 2) CON-GR, and 3) CON-NG. Grazing was defined as herds where $\geq 30\%$ of DMI of lactating cows was obtained from pasture during the grazing season. Outcome study variables were analyzed as continuous, binary or categorical variables.

Continuous outcome variables (Table 2.1), were: number of years operating a dairy farm; milk per cow per day (kg); age of housing (years); percentage of lactating cows that have milk segregated from the bulk tank and are untreated; mean reported SCC for the 3 months prior to the herd visit (x 1,000 cells/mL); calving interval in days (obtained from herd record systems or estimated by adding 60 days to the estimated lactation length); mean lactation number; percentage of first lactation animals; number of days the animals had spent grazing in the past year; and the amount of grain fed per lactating cow per day (kg).

Binary outcome variables, (Table 2.2), were: predominant breed (more than 50% of Holstein, Jersey or other breed); written documentation of treatments; written documentation of herd health events; use of DHIA (defined as use of the full service, including milk quality, breeding, production, calving and inventory information); use of a dedicated calving area (use of a dedicated area, no dedicated area); entering replacement stock brought onto the farm in the past year; use of a quarantine unit at milking; use of a California Mastitis Test; use of automatic take offs; use of pre-dipping; use of post-dipping; use of gloves as part of a regular milking routine; use of a written milking routine; use of rotational grazing; regular use of a nutritionist to formulate ration; regular veterinary visits; utilization of vaccinations for cows (defined as yes if the farmer reported any specific vaccination for cows); utilization of vaccinations for calves (defined as yes if the farmer reported any specific vaccination for calves); use of footbaths on dry cows; use of footbaths on lactating cows; use of rotational grazing; and if cows are transferred to another farm.

Categorical outcome variables (Table 2.3), were: method used to breed cows (AI only, some natural service, all natural service); method used to breed heifers (AI only, some natural service, all natural service); mean reported SPC of three months prior to the herd visit (0 – 7,000

cells/mL, 8,000 – 32,000 cells/mL, $\geq 33,000$ cells/mL); mean percentage of DMI from pasture across the previous grazing season for cows ($\geq 50\%$, 51 – 75%, 76 – 100%); mean percentage of DMI from pasture across the previous grazing season for heifers ($\geq 50\%$, 51 – 75%, 76 – 100%); number of milking units (0-10, 11-20, ≥ 20); use of a blanket treatment at dry off (antimicrobial exclusively, internal or external sealant, a combination of antimicrobial and sealant treatments, other treatment, no treatment); and milking facility (double side pit parlor, flat parlor, tie stall or stanchion, other type of parlor). To determine how frequently a farmer used advice from an outside source, the following 3 variables were used to create the explanatory variable Outside Support Score (**OSS**): 1) utilization of a nutritionist, 2) utilization of DHIA, and 3) utilization of regular veterinary visits. Outside Support Score was additive, as a positive response for any of the OSS variables was given a single point, with a minimum score of 0 (no outside help from any of the given sources) to a maximum score of 3 (utilization of regular veterinary visits, DHIA, and a nutritionist).

Categorical herd characteristics that were included as explanatory variables in the analyses were: Grazing system, herd size category (20 - 99 cows, 100 - 199 cows, and ≥ 200 cows) and site (NY, OR and WI). All farms were included in the analyses of every variable, with the exception of the percentage of herds that transfer cows to another farm. Only farms in NY and WI were analyzed for this variable, as the question was not interpreted the same way by the OR investigator.

Statistical Procedures. All statistical analysis was performed using SAS version 9.2 (SAS Institute, Inc., 2008). PROC UNIVARIATE was used to analyze individual continuous variables and assess normality of the variable distributions. Variables with a non-normal

distribution were categorized into three groups based on the 25th and 75th percentiles (0 – 25, 26 – 75, 75 – 100). Statistical significance was $P \leq 0.05$ for all variables.

Associations between continuous outcome variables and grazing system were assessed individually by linear regression using PROC MIXED (SAS Institute, Inc., 2008) with class statements for grazing system, herd size category and site. The regression model is formulated as:

$$Y = \beta_0 + \beta_1 \text{Grazing System} + \beta_2 \text{HerdSizeCategory} + \beta_3 \text{Site} + \varepsilon$$

where Y = continuous outcome variable, β_0 = intercept term, β_i = regression coefficient and ε = error term. Herd size and site were corrected for in the models that analyzed continuous variables, as these were design characteristics. The exception to this was the estimated calving interval variable, which had the use of DHIA also included into the model to account for the estimation bias of those farms not on DHIA.

Associations between categorical outcome variables and grazing system were assessed using PROC FREQ (SAS Institute Inc., 2008) by a chi-square test. For these variables, herd size and site were not corrected for in the analysis. In case of statistical significance, dominant cells were identified using the contribution of each cell to the chi-square statistic.

Associations between binary outcome variables and grazing system were assessed individually by logistic regression using PROC LOGISTIC (SAS Institute, Inc., 2008) with class statements for grazing system, herd size category and site. The regression model is formulated as:

$$\ln \left[\frac{Y}{1-Y} \right] = \beta_0 + \beta_1 \text{Grazing System} + \beta_2 \text{HerdSizeCategory} + \beta_3 \text{Site} + \varepsilon$$

where Y = binary outcome variable, β_0 = intercept term, β_i = regression coefficient and ε = error term. Herd size and site were corrected for the model, as these were design variables.

For the continuous and binary outcome variables, the least squares means between the grazing systems were assessed using multiple comparisons with the Bonferroni correction. The multiple comparison was implemented using the PDIF statement of SAS (SAS Institute Inc., 2008). Values reported are least squares means.

To assess differences among farms that utilized grazing, a sub-analysis was performed with data from only CON-GR and ORG farms. The only outcome variables included in this analysis were: the average number of days spent grazing, the use of rotational grazing, the mean percentage of DMI from pasture for heifers and the mean percentage of DMI from pasture for adult cows.

The association between OSS and utilization of vaccination was assessed through logistic regression using the regression equation shown above for binary outcome variables. The association between OSS and average reported SCC was assessed through linear regression using the regression equation shown above for continuous variables.

RESULTS

General Farm and Herd Characteristics

The mean percentage of first lactation heifers on the farm at the time of the visit was 37.3% on CON-NG farms, 33.9% on CON-GR farms and 31.6% on ORG farms ($P = 0.002$), as shown in Table 2.1. The mean lactation number for cows on the farm at the time of the visit was lesser on CON-NG farms ($P < 0.001$), with a mean lactation number of 2.3 as compared to 2.6 lactations on CON-GR and ORG farms (Table 2.1). The distribution of the mean lactation

number of the different grazing systems also indicates a trend of younger cows on conventional farms and older cows on ORG farms, as demonstrated in Figure 2.1. There was no difference in the number of years spent farming among CON-NG (27.9 years), CON-GR (23.7 years) or ORG farmers (24.1 years) ($P = 0.099$; Table 2.1). The age of the primary housing facilities did not differ by grazing system ($P = 0.994$; Table 2.1).

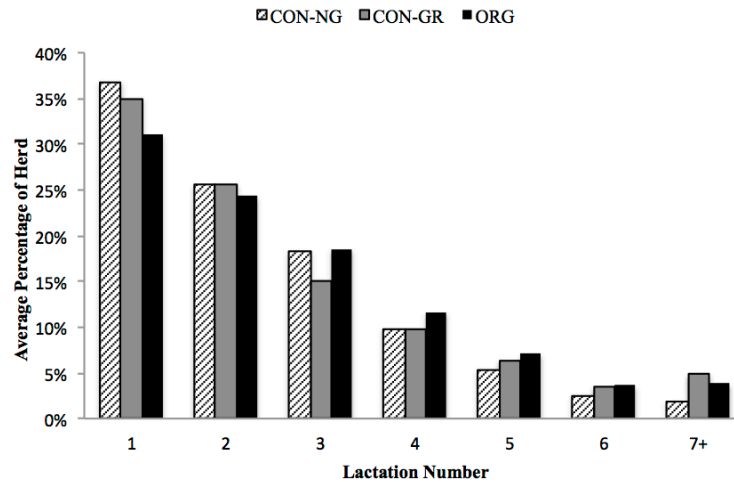


Figure 2.1. Mean percentage of all herds in lactation 1 to 7+ for conventional non-grazing (CON-NG; n = 64), conventional grazing (CON-GR; n = 36), and organic (ORG; n = 192) dairy farms in New York, Wisconsin and Oregon. Each bar represents the mean percentage of animals that fall into each lactation category of a given grazing system.

Holstein cows were the predominant breed ($\geq 50\%$) on all three grazing systems, with numbers of crossbred and other breeds on ORG and CON-NG farms being the dominant cells, as 27% of ORG farms reported having primarily crossbred animals, as compared to only 9% of crossbred CON-NG herds ($P < 0.001$; Table 2.3). Distribution of primary milking facility types was similar across the three grazing system ($P = 0.751$; Table 2.3).

Table 2.1 Least squares means of continuous farm characteristics, herd performance indicators and nutrition variables analyzed among conventional non-grazing (CON-NG; n = 64), conventional grazing (CON-GR; n = 36), and organic (ORG; n = 192) dairy farms. The linear model always consisted of the variable of interest (grazing system), farm size category (0 – 99 adult cows, 100 – 199 adult cows, ≥ 200 adult cows), and study site (New York, Wisconsin, Oregon). Multiple comparisons were assessed using the Bonferroni correction.

Variable	Grazing System			<i>P</i> -value ¹
	CON-NG Mean	CON-GR Mean	ORG Mean	
Farm Characteristics				
Mean percentage of first lactation animals	37.3 ^a	33.9 ^a	31.6 ^b	0.002
Mean lactation number	2.3 ^a	2.6 ^{a,b}	2.6 ^b	< 0.001
Number of years in dairy business	27.9	23.7	24.1	0.099
Mean age of housing (years)	36.8	37.2	36.5	0.994
Herd Performance Indicators				
Estimated calving interval (days) ²	406	411	404	0.556
Milk per cow per day (kg)	27.9 ^a	24.5 ^b	19.5 ^c	< 0.001
Percentage of the herd segregated from the tank	1.0 ^a	1.0 ^a	4.0 ^b	< 0.001
Mean reported bulk tank SCC from previous 3 months (x 1,000 cells/mL)	213	208	221	0.707
Nutrition and Grazing				
Grain fed (kg/day)	9.0 ^a	8.8 ^a	5.2 ^b	< 0.001
Mean number of days spent grazing	—	182 ^a	190 ^b	0.041

¹P-value for the continuous variables represents an F-test on the Type III sum of squares of the indicated variable across the three grazing systems.

²Estimated calving interval had the use of DHIA also forced into the model to correct for the estimation bias of those farms not on DHIA.

^{a,b,c}Means within a within a row with different superscripts differ ($P < 0.05$).

Herd Performance Characteristics

The estimated calving interval was similar among grazing systems ($P = 0.556$; Table 2.1). The mean milk per cow per day was significantly different among all three groups. Conventional non-grazing farmers reported a mean of 27.9 kg, CON-GR farmers reported a mean of 24.5 kg and ORG farmers reported a mean of 19.5 kg ($P < 0.001$; Table 2.1). Conventional non-grazing and CON-GR farms had a lesser percentage of lactating cows segregated from the tank (1.0% for both) as compared to milk produced on ORG farms (4.0%, $P < 0.001$). Reported bulk milk SCC was not different among the three groups, as shown in Table 2.1 ($P = 0.707$). The reported SPC category was not different based on grazing system ($P = 0.349$; Table 2.3).

As shown in Table 2.3, ORG and CON-GR farmers were more likely to use natural service for both non-lactating heifers and lactating cows as compared to CON-NG ($P < 0.001$ for both). Farmers on 45% of ORG farms reported using natural service exclusively to breed their non-lactating heifers, as compared to 13% of CON-NG farmers and 16% of CONGR farmers. Farmers on only 2% of CON-NG farms reported using exclusively natural service for their adult cows, while 20% of ORG and 14% of CON-GR farmers reported using only natural service for breeding.

Table 2.2. Least squares means of binary nutrition and grazing information, milking procedures and preventative management variables analyzed among conventional non-grazing (CON-NG; n = 64), conventional grazing (CON-GR; n = 36), and organic (ORG; n = 192) dairy farms in New York, Wisconsin and Oregon. Percentages represent a positive response. Multiple comparisons were assessed using the Bonferroni correction.

Variable	Grazing System			<i>P</i> -value ¹
	CON-NG	CON-GR	ORG	
Nutrition and Grazing				
Regular use of a nutritionist	97% ^a	89% ^a	46% ^b	<0.001
Use of rotational grazing	_____	81% ^a	95% ^b	< 0.001
Milking Procedures:				
Use of written milking routine	17%	14%	15%	0.996
Glove usage	80%	53%	66%	0.076
Pre-dipping	88%	81%	84%	0.654
Post-dipping	95%	97%	90%	0.267
Use of Automatic Take Offs	50%	44%	33%	0.568
Routine use of California Mastitis Test or cow side SCC test	61%	78%	75%	0.067
Preventative Management				
Regular Veterinarian Visits	77% ^a	56% ^a	36% ^b	< 0.001
Vaccination of Adult Cows	97% ^a	100% ^a	64% ^b	< 0.001
Vaccination of Calves	98% ^a	100% ^a	67% ^b	< 0.001
Records kept of herd health events	94%	92%	95%	0.745
Records kept of treatments	30% ^a	28% ^a	79% ^b	< 0.001
Use of DHIA ²	70% ^a	69% ^a	53% ^b	< 0.001
Entering replacement stock from outside sources ³	36% ^a	36% ^a	15% ^b	0.001
Use of dedicated calving area	41%	44%	24%	0.117
Use of quarantine unit at milking	31%	27%	32%	0.464
Percent of herds that transfer cows to another farm ⁴	6%	0%	4%	0.631
Use of footbaths – lactating cows	48%	36%	18%	< 0.001

Use of footbaths – dry cows	13%	14%	5%	0.033
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¹*P*-value for the binary variables represents an F-test on the Type III sum of squares of the indicated variable across the three grazing systems.

²DHIA is defined as use of the full service, including milk quality, breeding, production, calving, and inventory information.

³Analysis included information on weaned heifers and lactating cows only.

⁴Analysis included farms from NY and WI only.

^{a,b}Means within a within a row with different superscripts differ ($P < 0.05$).

Nutrition and Grazing

Conventional farmers fed more grain than ORG farmers ($P < 0.001$; Table 2.1), with ORG farmers feeding approximately 45% less grain than CON-NG and CON-GR farmers. Organic farmers reported grazing for a greater number of days during the year prior to the herd visit (190 days) as compared to CON-GR farmers (182 days; $P = 0.041$; Table 2.1).

Conventional farmers were more likely to use a nutritionist for ration and feeding advice as compared to ORG farmers ($P < 0.001$; Table 2.2). Organic farmers were more likely to employ rotational grazing throughout the grazing season as compared to CON-GR farmers, (95% and 81%, respectively, $P < 0.001$; Table 2.2).

Organic and CON-GR farmers reported similar percentages of DMI from pasture for their non-lactating heifers ($P = 0.058$; Table 2.3). For their adult cows, however, ORG farmers reported a greater mean percentage of DMI from pasture than CON-GR farmers, as 25% of the ORG farms fell into the high DMI category, as compared to 0% of the CON-GR farms ($P = 0.003$).

Table 2.3. Categorical farm characteristics, herd performance indicators, nutrition and grazing information, milking procedures and preventative management variables analyzed among conventional non-grazing (CON-NG; n = 64), conventional grazing (CON-GR; n = 36), and organic (ORG; n = 192) dairy farms in NY, WI and OR.

Variable	Level	Grazing System			<i>P</i> - <i>value</i> ¹
		CON-NG	CON-GR	ORG	
Farm Characteristics					
Predominant Breed	≥ 50% Holstein	86%	72%	63%	< 0.001
	≥ 50% Jersey	5%	17%	10%	
	≥ 50% Cross or other Breeds	9%	11%	27%	
Milking Facility	Double Side Pit	38%	47%	32%	0.751
	Parlor	3%	3%	6%	
	Flat Parlor	50%	47%	51%	
	Tie Stall or Stanchion	9%	3%	11%	
	Other				
Herd Performance Indicators					
Percentage of farms using natural service for heifers	None	56%	44%	34%	< 0.001
	Some	31%	39%	21%	
	All	13%	16%	45%	
Percentage of farms using natural service for lactating cows	None	78%	69%	49%	< 0.001
	Some	20%	17%	31%	
	All	2%	14%	20%	
Mean reported Bulk tank Standard Plate Count (cfu/mL)	0 – 7,000	27%	28%	36%	0.349
	8,000 – 32,000	48%	42%	45%	
	≥ 33,000	25%	30%	19%	
Nutrition and Grazing					
Mean percentage DMI from pasture: Heifers	≥ 50%	_____	33%	21%	0.058
	51 – 75%	_____	14%	7%	
	76 – 100%	_____	53%	72%	
Mean percentage DMI from pasture: Adult cows	≥ 50%	_____	69%	51%	0.003
	51 – 75%	_____	31%	24%	
	76 – 100%	_____	0%	25%	
Milking Procedures					
Number of milking units	0 – 10	64%	72%	75%	0.509
	11 – 20	30%	25%	20%	
	≥ 20	6%	3%	5%	
Preventative Management					

Dry Treatment	Blanket	36%	36%	0%	< 0.001
	Antimicrobial				
	Sealant ²	9%	25%	4%	
	Combination ³	25%	15%	0%	
	Other ⁴	0%	0%	2%	
	No blanket intervention	30%	22%	94%	

¹ *P*-value for the categorical variables represents the Chi-Square test of the indicated variable across the three grazing systems.

² 'Sealant' dry treatments include internal and external teat sealants (e.g. Orbeseal, tHexx).

³ 'Combination' is representative of antimicrobial with a sealant treatment.

⁴ 'Other' dry treatments include dry treatment specific teat dip and alternative treatments.

Milking Procedures

Milking procedures and characteristics were similar across the three grazing systems. The use of a written milking routine, glove use at milking, the use of a pre-dip or post-dip solution at milking, the use of automatic takeoffs and the use of a California Mastitis Test or other cow side SCC test did not differ among grazing systems (Table 2.2). The number of units used during milking also was not different among the grazing systems ($P = 0.509$; Table 2.3).

Herd Health Management Practices

Preventative Procedures. Organic farmers were less likely to have regular veterinary visits (36%) than CON-NG farmers (77%) or CON-GR farms (56%), shown in Table 2.2 ($P < 0.001$). Only 64% of ORG farmers reported vaccinating adult cows, as compared to 100% of CON-GR and 97% of CON-NG farmers ($P < 0.001$; Table 2.2). Similarly, 67% of ORG farmers reporting vaccinating calves as compared to 100% of CON-GR farmers and 98% of CON-NG farmers ($P < 0.001$; Table 2.2). There were no differences among conventional and ORG farmers to keep written records of herd health events ($P = 0.745$), but ORG farmers were more likely to keep records of treatments given to animals as compared to conventional farmers ($P < 0.001$;

Table 2.2). Organic farmers were less likely to use the full DHIA service than conventional farmers ($P < 0.001$), with 53% of ORG farmers reporting use of DHIA as compared to 69% and 70% of CON-GR and CON-NG farmers, respectively (Table 2.2). Conventional grazing and CON-NG farmers were more likely to have entering replacement stock from outside sources (36% for both) as compared to 15% of ORG farmers ($P = 0.001$; Table 2.2). No difference was found regarding the use of a dedicated calving area ($P = 0.117$) or a quarantine unit at milking ($P = 0.464$; Table 2.2). Organic farmers in NY and WI were not found to be any more likely to transfer cows to another farm than conventional farmers ($P = 0.631$; Table 2.2). Footbaths were used more frequently on conventional farms for both lactating and dry cows than ORG farms ($P < 0.001$ and $P = 0.033$, respectively).

Dry-off Treatment. The majority of ORG farmers reported no blanket dry-off treatment of any kind (94%; Table 2.3). Conventional non-grazing farmers were equally likely to utilize traditional blanket intramammary antimicrobial treatment (36%), a combination treatment of blanket intramammary antimicrobials and internal or external sealant products (25%) or no blanket treatment (30%), while the remaining 9% reported using a sealant product exclusively (Table 2.3). Conventional grazing farmers reported more variation in their use of dry treatments (Table 2.3), indicating use of traditional blanket antibiotic treatment (36%), combination therapy (15%), sealant product only (25%) and no blanket dry treatment (22%; $P < 0.001$).

Use of External Support for Herd Health Management. Grazing system was associated with utilization of vaccinations and veterinarians ($P < 0.001$; Figure 2.2). The percentages of farmers who reported no vaccination categorized by their Outside Support Scores were as follows: OSS = 0, 60%; OSS = 1, 29%; OSS = 2, 17%; OSS = 3, 1% (data not shown). Outside Support Score was also associated with mean reported SCC, as shown in Figure 2.3, as farms

with a lower OSS had an overall higher SCC ($P = 0.014$). Outside Support Score is associated with grazing system, as 95% of CON-NG farms and 80% of CON-GR scored 2 or 3, compared with 40% of ORG farms, shown in Figure 2.4.

DISCUSSION

The primary objective of this study was to assess health, management and herd characteristics of similarly sized conventional and organic dairy herds across New York, Wisconsin and Oregon. This manuscript provides a concise, descriptive summary of some the key differences and similarities between size and region matched grazing systems. While there have been several studies that have compared organic and conventional dairy management and disease in the United States (Zwald et al., 2004; Pol and Ruegg, 2007; Sato et al., 2005) as well as many in Europe (Ellis et al., 2007; Haskell et al., 2009; Langford et al., 2009), this is the first large scale study in the U.S. that has investigated management procedures in different parts of the country while matching based on herd size and geographical location. It is not straightforward to compare results from organic dairy studies in the U.S. to those done in Europe, as the standards for organic dairy production are considerably different (Ruegg, 2008).

This study was designed to enroll herds that were a representative sample of the organic dairy community in NY, WI and OR in order to better understand the management practices of organic dairy farms on a national level. Approximately 30% of eligible ORG herds in NY and WI, and 66% of eligible ORG herds in OR were enrolled in the study. Conventional herds in the study were included based on their interest, location and approximate herd size. However, we recognize the possibility of selection bias in this study. Herds were recruited through written

letters and personal contacts, with bulk tank testing and results of milk samples as compensation. Potentially, herds that did not have a high management standard or did not adhere closely to organic standards may have chosen not to participate in the study. Therefore, we have a study population of herds that is somewhat self-selected, as with most survey research. Since this study was primarily a cross-sectional study, we recognize that there are several limitations to our research. Further research that would allow investigators to follow farms over a longer period of time would be justified and useful.

The milk quality of organic farms in comparison to their conventional counterparts is a topic of interest for many scientists as well as consumers. Despite the consumer perception that organic milk is of higher quality (Hill and Lynchehaun, 2002) scientific studies do not support this perception. Little difference has been found in the bulk tank somatic cell count between organic and conventional dairy farms (Rosati and Aumaitre, 2004; Zwald et al., 2004; Sato et al., 2005). It is generally accepted that bulk milk SCC is the primary indicator of milk quality in dairy herds and is associated with management practices on the farm. (Dohoo, 1982; Barkema et al., 1998; Schukken et al., 2003). Our study results have supported these conclusions, based on the mean reported SCC and SPC from 3 months prior to the herd visit. In our data, little difference exists in the milk quality of organic and conventional herds, when matched by size and location.

When aiming to improve upon milk quality on dairy farms in general, milking procedures are generally considered a critical control point for maintaining milk quality (Fenlon et al., 1995; Jayarao et al., 2004; Hutton et al., 1990). Our study shows that there is no relationship between grazing system and milking procedures, suggesting that there is an industry standard for milking on dairy farms that is not affected by differences in production system. It also suggests that

future troubleshooting on farms with a high SCC will need to be addressed through management techniques that are not production system specific. Previous work evaluating organic and conventional dairy farms suggested that organic farms typically house their animals in older housing during the winter months than conventional farms, which is considered a risk factor for disease (Ruegg, 2008). In this study population, there was no indication that age of housing varied based on grazing system when the herds were matched by region and size.

While our data suggests that there is a significant difference between the number of days spent grazing between ORG and CON-GR farms, the difference is 8 days, which is unlikely to be biologically significant. Conventional farmers fed more grain, while ORG cows and heifers got a higher percentage of DMI from pasture than CON-GR cows, which was also expected, since ORG regulations require a significant amount of DMI from pasture (NOFA, 2009). With the higher expense of organic grain and other input costs, it is possible that organic farmers may be feeding less grain as an economic management strategy; however, it is beyond the scope of this paper to fully understand the economic or other drivers of behavior in organic and conventional farms. Our results show that ORG farmers are more likely to employ rotational grazing than CON-GR farms. As organic farmers are well trained in the use of grazing and this may be reflected in their more likely use of rotational grazing methods. Organic farmers reported using footbaths less than conventional farms. Since grazing is present in a large part of the year, the reduced use of footbaths may be expected on organic dairies.

Our data indicates that ORG and CON-GR farms have higher percentages of older cows compared to CON-NG farms. Older cows are typically associated with an increased risk for milk fever, mastitis, lameness and other age-related illnesses (Dohoo et al., 1984). Our data supports the common perception that organically managed dairy farms are more likely to have older cows.

The results show a 5% lower cull rate on ORG farms, which, while relatively modest, is still a significant difference in regard to overall cull rates. A possibility for a higher percentage of older animals may be the purchasing of organically raised cows onto the farm. Buying cows opens the herd to non-endemic diseases. Because of limited USDA-approved treatments on organic farms, the introduction of new animals poses a relatively larger risk compared to conventional farms, suggesting that the lower numbers of imported replacements may be a disease management strategy or alternatively, the result of a limited supply of organic animals for purchase. The hesitation to purchase on ORG farms is further supported by the percentage of conventional farms that bring in replacement stock from outside sources, which is significantly higher than on ORG farms (Table 2.2).

Preventative management practices are important on any dairy farm, but especially on organically managed farms, as the availability of USDA approved products to treat disease is limited. As demonstrated in our results, ORG farmers were more likely to keep written records than conventional farmers, although this is likely to be biased by grazing system, as organic regulations require keeping a written record of all treatments administered to the animals. Organic farmers in the study were more likely to segregate milk from the bulk tank than conventional farmers (Table 2.1). While this may be a method to keep the SCC low, the greater proportion may also be a response to the need for milk for calves, as no organic milk replacer is currently commercially available. Blanket antimicrobial dry treatments reported among conventional were lower than expected, as previous studies reported 87% of farmers using dry treatment with antimicrobial infusion in Sato et al., (2005) and 88% using treatment with antimicrobial infusion in the 1996 NAHMS study. In our population, only 60% of CON-NG and 53% of CON-GR reported use blanket antimicrobial with or without the use of a sealant (Table

2.3). This difference may be due to the fact that the question presented to the farmer only asked about routine procedures for most or all cows in the dry cow group, and was not inclusive of selective treatment of dry cows. Vaccinations are allowable by national organic standards and are generally considered an efficacious method to prevent various diseases in cattle (LeBlanc et al., 2006); however, only 64% of ORG farms in the study reported using vaccines on their adult cows, compared to 97% of CON-NG and 100% of CON-GR farms. This trend was also present in vaccinations of dairy calves, as 67% of ORG farms in the study reported administering vaccines to their calves, compared to 98% of CON-NG and 100% of CON-GR. The difference in vaccine use at a farm level between organic and conventional dairy establishments is a point of interest, and an area for future research.

Fewer ORG farmers reported regular veterinarian visits as compared to conventional farmers in this study (Table 2.3), a finding that is expanded further in Richert et al., accepted. For this manuscript, we first evaluated the association between veterinarian use and vaccination on the farms. Figure 2.2 shows the percentage of each grazing system that reported both regular veterinarian use and vaccination, which suggests that ORG farmers are less likely to utilize vaccination and a veterinarian.

We expanded on this analysis to consider the use of various outside resources as management tools, and how they affected the percentage of farmers who reported vaccination. The variables that we considered were use of a nutritionist, regular veterinarian visits and use of DHIA to calculate Outside Support Score (OSS). Outside Support Score is associated with the average reported SCC, as farms with a lower OSS reported overall higher SCC (Figure 2.3).

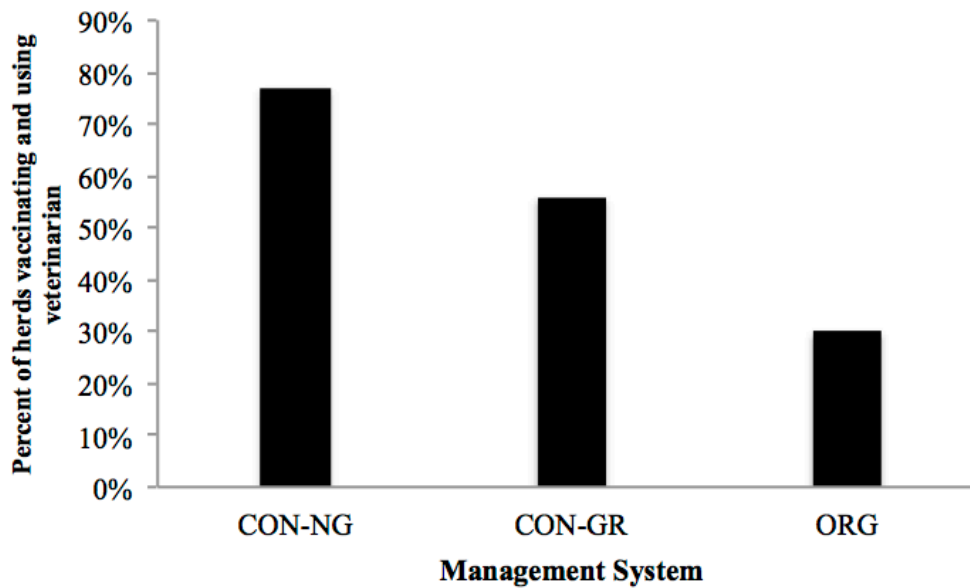


Figure 2.2. Percentage of conventional non-grazing (CON-NG; $n = 64$), conventional grazing (CON-GR; $n = 36$), and organic (ORG; $n = 192$) dairy farms in New York, Wisconsin and Oregon that reported both vaccinating their adult cows and regular visits from the veterinarian. Each management group adds up to 100% (farms reporting no vaccinations not shown). Significantly more CON-NG and CON-GR farmers reported utilization of both a veterinarian and vaccination than ORG farmers ($P < 0.001$).

The least squares means of OSS are not different between CON-NG and CON-GR farms, who likely to score a 2 or 3, but are both different than ORG farms, who were more likely to score a 0 or 1 (Figure 2.4).

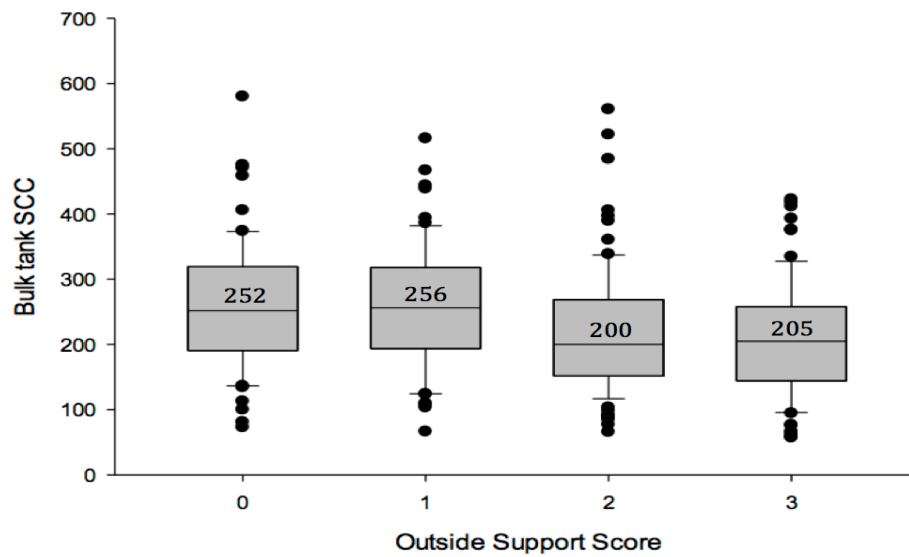


Figure 2.3. Average reported SCC of dairy farms in the study by Outside Support Score. Outside Support Score (OSS) is additive, defined as: a) utilization of a nutritionist, b) utilization of DHIA, and c) utilization of regular veterinary visits. A positive response for any of the OSS variables was given a single point, with a minimum score of 0 (no outside help from any of the given sources) to 3 (utilization of regular veterinary visits, DHIA, and a nutritionist). Farms included in the analysis categorized as conventional non-grazing (CON-NG; n = 64), conventional grazing (CON-GR; n = 36), and organic (ORG; n = 192) in New York, Wisconsin and Oregon. Somatic cell count units shown are x 1,000 cells/mL. Numbers reported in boxplots represent median SCC for each group. Outside Support Score was also associated with mean reported SCC, with farms with a lower OSS having a higher SCC ($P = 0.014$).

More research on the use of vaccines and other preventative measures in organic dairy farming would be of value, with a particular need for socio-economic research on the reasons for non-adoption of these common preventative practices in the organic community which may be driven by economics and philosophy.

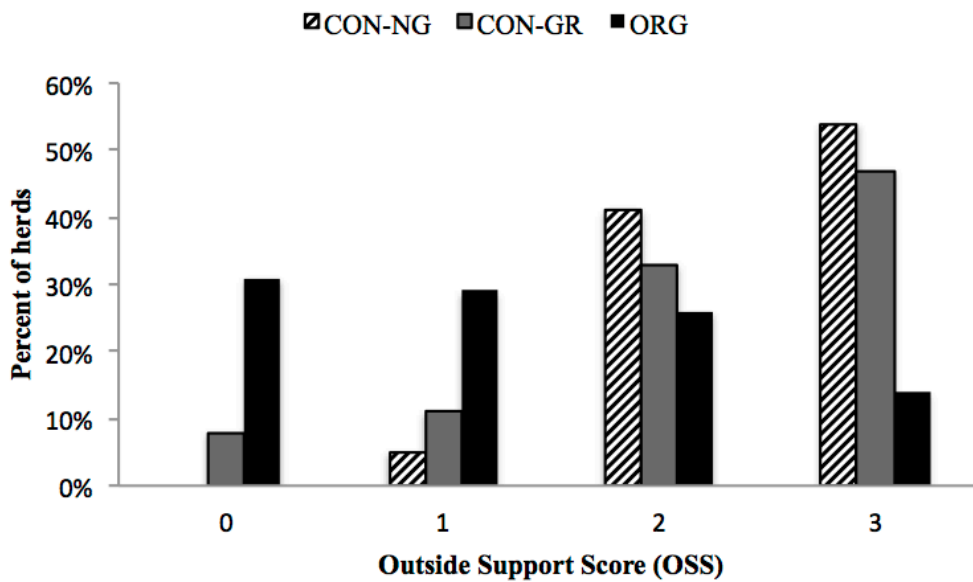


Figure 2.4. Percentages of conventional non-grazing (CON-NG; n = 64), conventional grazing (CON-GR; n = 36), and organic (ORG; n = 192) dairy farms in New York, Wisconsin and Oregon by each Outside Support Score (OSS). Outside Support Score is additive, defined as: a) utilization of a nutritionist, b) utilization of DHIA, and c) utilization of regular veterinary visits. A positive response for any of the OSS variables was given a single point, with a minimum score of 0 (no outside help from any of the given sources) to 3 (utilization of regular veterinary visits, DHIA, and a nutritionist).

CONCLUSION

Consumer and scientific interests have increased the demand for knowledge regarding milk quality, animal welfare and management on organic farms in regard to conventional farms. Our research indicates that there are some profound differences between the production systems, but that as a whole, the ORG and CON dairy communities are quite similar in nature, likely explained by most organic dairy farms having transitioned from being conventional.

Organic and conventional dairy farms of the same size tend to employ similar milking procedures, be housed in facilities of comparable age, be managed by individuals with corresponding years of dairy experience and have similar milk quality results. As expected,

conventional farms tend to produce more milk, feed more grain, have longer calving intervals and have younger cows than their organic counterparts. Organic farms are more likely to keep records of treatments given to the animals, have more non-treated animals segregated from the bulk tank than conventional farms. Organic farms reported a lower use of veterinary support, DHIA, nutritionists and vaccinations, in comparison to conventional dairies. Organic farms also report a lower use of AI on the farms. Further research on the non-adoption of preventative measures and the use of external resources for management support by the organic community should be investigated in the future.

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CHAPTER THREE

Associations of risk factors with somatic cell count in bulk tank milk on organic and conventional dairy farms in the United States.

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INTERPRETATIVE SUMMARY

Associations of risk factors with somatic cell count in bulk tank milk on organic and conventional dairy farms in the United States. Cicconi-Hogan et al. Because the organic dairy industry is one of the fastest growing agricultural sectors in the United States, research on the best management practices is especially useful. The goal of this study was to evaluate the somatic cell count on organic and conventional dairy farms and determine what management and herd characteristics were most influential. A key result was that farms that employed more intensive management strategies tended to have lower somatic cell counts.

ABSTRACT

The objective of this study was to evaluate the association of bulk tank milk somatic cell count with management characteristics on organic and conventional dairy farms in New York, Oregon and Wisconsin. Data from similarly sized organic farms ($n = 192$), conventional non-grazing farms ($n = 64$) and conventional grazing farms ($n = 36$) were collected at a single farm visit. Of the 292 farms visited, 290 bulk tank milk samples were collected. Overall, no difference in somatic cell count (SCC) was observed between the conventional and organic grazing systems. Two models were created to assess the effects of various management and herd characteristics on the \log_{10} transformation of the SCC (LSCC), one using data from all herds and one using data from organic herds only. From the Total Herd Model, more grain fed per cow per day was negatively associated with LSCC, while a positive bulk tank culture for *Staphylococcus aureus* (*S. aureus*) and years that a farmer reported being in the dairy business were both positively associated with LSCC. In the Organic Herd Model, there was a seasonal effect that indicated LSCC tended to increase in the summer and decrease in the winter. Grain fed per cow per day, the use of anionic salts in transition cow diets, the use of gloves during milking and regular use of a quarantine unit at milking were all negatively associated with LSCC. Like the Total Herd Model, a *S. aureus* positive bulk tank culture was positively associated with LSCC in the organic model. Standard Plate Count was also positively associated with LSCC in the organic model. Several variables that were associated with management using external resources were combined to create an External Input Score. In the Total Herd model, use of more external resources was negatively associated with LSCC. Conventional herds in the study tended to use outside management resources than organic herds.

Key words: dairy, somatic cell count associations, management, organic

INTRODUCTION

Udder health is an essential component of quality milk production and cow well-being. Mastitis is the most costly and common disease found in dairy herds in the United States. Clinical mastitis is often responsible for a decrease in milk production and milk discard due to low quality and a rare risk of antimicrobial residues from treatment (Van Schaik et al., 2002). Subclinical mastitis results in a lower milk production and a reduced milk quality due to increased somatic cell count (SCC) in milk (Roesch et al., 2007). Testing bulk milk SCC is an internationally recognized method to determine the quality of the milk and the udder health status of the cattle in the herd. Many management practices have been associated with an increased disease risk and with a subsequent higher bulk milk SCC (Dohoo, 1982; Schukken et al., 2003; Barkema et al., 1998).

Interest in quality food production, animal welfare and environmental sustainability has increased in recent years. This holds true for the dairy industry across both organic and conventional dairy production systems. A large body of literature reports associations between management of dairy farms and animal health, as well as milk quality (Zwald et al., 2004; Pol and Ruegg, 2007; Dufour et al., 2011). Consumers have become increasingly curious about the source of their food, and many have become interested in organic or local food sources (Yiridoe and Bonti-Ankomah, 2005). The associated increase in certified organic dairy animals and production of organic dairy products is a direct result of consumers' interest in animal welfare and the environmental impact of conventional dairy farming (Sundrum, 2001). However, there is a lack scientific data on management methods and herd health on organic farms to determine the impact of these methods on animal well-being.

Organic management often places an emphasis on preventative measures such as vaccination to control disease (Ruegg, 2009). On organic dairy farms in the U.S., animals or products from animals given substances prohibited by the National Organic Program may not be sold as organic, and these animals must be removed from the herd following treatment. However, producers are prohibited from withholding necessary treatment from a sick or injured animal (National Organic Program, 2007). From 2000 to 2008, the number of certified organic cows in the U.S. increased from 38,196 to 249,766 (Economic Research Service, 2008). Due to the recent surge in the number of organic dairy animals, the need for established best management practices and overall herd health information on organic dairy farms is growing, but the industry currently lacks sufficient scientific information to define best organic management practices. Antimicrobial use and mastitis management have been compared between organic and conventional dairy operations in several U.S. studies (Zwald et al., 2004; Pol and Ruegg, 2007; Ruegg, 2009), but few studies have included conventional herds matched on size and location to organic herds. Recent publications from our project have described management on organic and conventional farms (Stiglbauer, Cicconi-Hogan and Richert et al., in press), as well as the use and role of veterinarians within the same population (Richert et al., in press). There is a perception among consumers that organically produced milk is healthier or of better quality (Yiridoe and Bonti-Ankomah, 2005). However, previous research has found little difference in SCC between organic and conventional dairy farms (Rosati and Aumaitre, 2004; Sato et al., 2005; Zwald et al., 2004) and a review of published literature on organic products lacked any strong evidence for a nutritional advantage of organic foods (Smith-Spangler et al., 2012).

The objective of this study is to evaluate the relationship between management practices and SCC for all herds involved in the study and specifically for the subset of organic herds. The

aim is to identify a set of management variables for the overall dairy community, as well as specifically to the organic community, that are associated with a lower SCC and can be used to define best management practices for conventional as well as organic dairy farms.

MATERIALS AND METHODS

Recruitment and Herd Selection

Herd inclusion and recruitment criteria are as described by Stiglbauer, Cicconi-Hogan and Richert et al., (in press) and Richert et al., (in press). Briefly, 292 dairy herds were visited in New York (NY), Oregon (OR), and Wisconsin (WI) between March 2009 and May 2011. A total of 192 organic (**ORG**) herds and 100 conventional (**CON**) herds were frequency matched based on herd size and location. In New York, 72 ORG and 25 CON farms were visited; in Wisconsin, 96 ORG and 51 CON farms were visited; and in Oregon, 24 of each ORG and CON farms were visited. Organic herds were initially identified by listings from certifying organizations, county extension agents and personal contacts. Conventional herds were identified by compiling a list of licensed dairy farms from each state's department of agriculture. Non-responders received multiple mailings to increase participation. In order to be eligible for the study, ORG herds were required to have a minimum of 20 adult cows and had been shipping certified organic milk for at least 2 years. Conventional herds were required to have a minimum of 20 adult cows and must have been shipping milk for at least 2 years.

Questionnaire and Data Collection

The study questionnaire was adapted from previously published survey instruments with input from veterinarians familiar with the dairy industry (Zwald et al., 2004; Pol and Ruegg,

2007) (available at <http://milkquality.wisc.edu/organic-dairies/project-cow/>). It was reviewed by professional survey developers, and pre-tested with organic and conventional dairy farmers in each state. Farmers were asked to refer to all available records to ensure accuracy of answers. Recall was frequently limited to the 12 months prior to the herd visit or less. Questionnaire information addressed questions under each of the following themes: animal health and personnel, herd inventory and expansion, milk production, breeding and reproductive information, housing, feed and water systems, milking and other routine procedures, disease definitions and treatment, mastitis, management of Johne's disease, veterinarian involvement, calf and heifer information, and DHIA information (if applicable).

In each state, a single member of the study team conducted all interviews. Throughout the data collection period, monthly conference calls were held to discuss questions and help ensure standardization of data collection among states. Data collection methods were consistent across the three states, as described by Richert et al, (in press). In most herds, the individual directly responsible for animal care was interviewed.

Body condition scoring, udder hygiene and hock scoring was done by the interviewer on all farms. Scoring on farms with more than 50 adult cows was assessed for a minimum of 50 lactating and 20 dry cows, or 20% of each group, whichever was larger. All adult cows were scored from herds with less than 50 cows. Body condition was evaluated using an accepted scoring method with provided guidelines for examining the thurl line, hooks, pins, sacral and tailhead ligaments (Elanco Animal Health, 1997; Ferguson et al, 1994). Udder hygiene was assessed on a 4-point system (Schreiner and Ruegg, 2003). Hock scores were assessed using a 3-point system developed by Cornell University (2007)

(<http://www.ansci.cornell.edu/prodairy/pdf/hockscore.pdf> accessed September 2012). All scoring

forms can be found at <http://milkquality.wisc.edu/wp-content/uploads/2011/10/Reference-Guides-for-Scoring.pdf>.

Bulk Tank Milk Sample Collection and Testing

Samples of bulk milk were collected by study personnel at 290 of the study farms and sent to Quality Milk Production Services at Cornell University for analysis. All samples were taken directly from the bulk tank with a sterile sampler after a minimum of 5 minutes of agitation, immediately put on ice and transported to the testing facilities. Two farmers in the study requested that their bulk tank milk not be analyzed and were therefore not included in the analyses for this manuscript.

The samples were analyzed using real-time PCR for foodborne pathogens *Salmonella* species (detecting the presence of the *invA* gene) (Nam et al., 2005), *Listeria monocytogenes* (detecting the gene *hly*) (Jothikumar et al., 2003), and Shiga toxin producing *E. coli* (detecting for the presence of the *stx1* or *stx2* gene) (Reischl et al., 2002; Manning et al., 2007). Samples were also tested for *Mycoplasma bovis* (Hogan et al., 1999), Bovine Virus Diarrhea virus (Renshaw et al., 2000), a modified mastitis bacteria count (Hogan et al., 1999) and antibodies to *Mycobacterium avium* subspecies *paratuberculosis* (Johne's disease) (Paracheck®, Prionics, Zurich, Switzerland). Samples were couriered to Dairy One Cooperative (Ithaca, NY) and tested for SCC, standard plate count (SPC), lab pasteurized count (LP), coliform and *Escherichia coli* (*E. coli*) count, and butterfat and protein percentages (Wehr and Frank, 2004).

Statistical Analysis

Study Variables. All statistical analysis was performed using SAS version 9.2 (SAS Institute, Inc., 2008). Descriptive analysis of bulk tank milk was performed using PROC UNIVARIATE for: SCC (x 1,000 cells/mL), SPC (x 1,000 cfu/mL), LP count (x 100 cfu/mL), protein (%), butterfat (%), coliform count (cfu/mL), *E.coli* count (cfu/mL). The presence of food borne pathogens, *Staphylococcus aureus* (*S. aureus*) and *Streptococcus agalactiae* (*S. agalactiae*) were also included in the analysis. Somatic cell count and SPC were transformed to \log_{10} cells/ml and \log_{10} cfu/mL, respectively and reported as geometric means using the antilog to back transform the parameters. Due to a large number of negative results, LP and *E. coli* were dichotomized. The descriptive analysis of LP and *E. coli* reports the percentage of each grazing system with a positive result (Table 3.1).

Dairy production system (ORG, CON) and grazing information were combined to create a new predictor variable, grazing system, which had three levels 1) organic, which therefore requires grazing (ORG), 2) conventional grazing (CON-GR), and 3) conventional non-grazing (CON-NG). Grazing was defined as herds where $\geq 30\%$ of DMI of lactating cows was obtained from pasture during the grazing season. Grazing system, herd size category (0-99 cows, 100-199 cows, ≥ 200 cows), and site (NY, OR, WI) were associated with the design of the study and were forced into the modeling process.

Predictor variables considered for inclusion in the SCC model were selected based on similarity to variables described in the meta-analysis of Dufour et al. (2011) and were then separated into the following groups: 1) General farm information, 2) Management information, 3) Milking procedures and 4) Nutrition. The variables considered within each group were as follows: 1) General farm information: Average age of primary adult housing in years, percentage

of first lactation cows on the farm, mean lactation number, number of years the farmer had been in the dairy industry, seasonality of herd visit (computed using the formulas $\sin(2\Pi*(\text{day of year}/365))$ and $\cos(2\Pi*(\text{day of year}/365))$, where $\Pi = 3.14$), percent of problem breeders in the past 12 months (defined as animals that had been removed from their due to failure to conceive, animals that had been removed from the breeding population by labeling her “do not breed” because of failure to conceive or animals that were 9 month post-partum and not yet pregnant), estimated calving interval in days (provided by herd record systems or calculated by adding 60 days to the estimated lactation length), amount of milk produced per cow per day (kg), type of milking facility (pit parlor, flat or walkthrough parlor, tie stall or stanchion, other type of facility), presence of *S. aureus* (yes, no), presence of *S. agalactiae* (yes, no), mean body condition score, mean hock score, mean udder hygiene score, and average reported SPC of milk shipped in the 3 months prior to herd visit (0 – 7,000 cfu/mL, 8,000 – 32,000 cfu/mL, $\geq 33,000$ cfu/mL); 2) Management information: use of natural service for non-lactating heifers (none, some, all), use of natural service for adult cows (none, some, all), use of DHIA (yes, no), use of a dedicated calving area separate from all other cows (yes, no), farmer reported improved laneways (yes, no), use of a segregation unit or bucket milker during milking (yes, no), clinical mastitis culturing (performed regularly, performed infrequently, not ever performed), number of people who treat mastitis, written herd health and treatment records (yes, no), regular veterinarian visits (yes, no), use of vaccinations in adult cows (yes, no), use of a nutritionist (yes, no), use of anionic salts in transition cow diets (yes, no), frequency of bulk tank cultures taken per year (never, monthly, quarterly, other times per year), use of a blanket dry treatment (categorized as a blanket antimicrobial, internal or external sealant for all cows, combination of antibiotic and sealant for all cows, other blanket treatment, no blanket treatment), routinely

checking postpartum cows (yes, no), use of a California Mastitis Test (yes, no), keeping sick and healthy cows separate (yes, no), use of a separate location to transfer cows (yes, no), introduction of new dairy cows or heifers in the past 12 months (yes, no), percent of herd with 3 or fewer teats and the percent of the herd that had at least one quarter segregated from the bulk tank; 3) Milking procedures: number of times milked per day, use of a pre-dipping solution (yes, no), use of a post-dipping solution (yes, no), use of gloves during milking (yes, no), use of automatic take offs (yes, no), number of milkers on the farm, fore-stripping prior to milking (yes, no), and number of milking units (0-10, 11-20, ≥ 20); 4) Nutrition: Use of grazing (yes, no), use of TMR (yes, no), and amount of grain fed per cow per day (kg). The following variables were specific to the organic model, and not analyzed in the total herd model: number of acres used for pasture, average percentage of DMI from pasture, percent improved pasture from 7 years prior to the herd visit, the number of years certified organic, number of days grazing in the last grazing season and the use of rotational grazing (yes, no).

Use of External Input. In order to assess the level of external support a farmer was using, several variables were combined to create a new variable, named External Input Score (**EIS**). It was additive of the following variables, several of which were changed to binary to allow for a 0 (no) or 1 (yes) scale: use of nutritionist (no, yes), regular use of a veterinarian (no, yes), use of DHIA (no, yes), use of anionic salts in transition cow diets (no, yes), vaccination of cows (no, yes), vaccination of calves (no, yes), regular pregnancy checks (no, yes), a written record of herd health events (no, yes), any use of AI in non-lactating heifers (no, yes), and any use of AI in lactating cows (no, yes). The resulting additive scale ranged from 0 to 10. Because of the statistical and logical correlation of these variables to one another, the EIS was used in the

modeling process in replacement of the individual variables that remained in the final models prior to the backward stepwise process.

Statistical Models. We analyzed data from 290 of the 292 farms included in the study due to missing bulk tank information from 2 Oregon farms. The primary outcome variable was the SCC on the day of the herd visit. Due to the heavily right skewed distribution of the SCC variable, it was log-transformed to \log_{10} cells/mL prior to model construction, (**LSCC**). PROC UNIVARIATE was used to analyze individual continuous variables and assess normality of the variable distributions. Variables with a non-normal distribution were categorized into 3 groups based on the 25th and 75th percentiles (0 – 25%, 26 – 75%, 76 – 100%).

Two linear regression models were constructed to determine the relationship between LSCC and the individual predictor variables using different subsets of data: 1) a model constructed using data from all herds in the study, hereinafter referred to as the Total Herd Model, and 2) a model constructed using data from all organic herds in the study, hereinafter referred to as the Organic Herd Model. Variable selection was done according to the method described by Dohoo et al., (2010).

The general form of the linear model used for the Total Herd Model was as follows:

$$\text{LSCC} = \beta_0 + \beta_1 \text{ Grazing System} + \beta_2 \text{ Herd Size Category} + \beta_3 \text{ Site} + \beta_k X_k + \dots + \epsilon$$

where β_0 = intercept term, β_i = regression coefficient, X_k = predictor variable and ϵ = error term.

Model selection was performed in 3 steps. First, each individual predictor variable was screened for unconditional associations with LSCC by linear regression using PROC MIXED. Then, unconditionally associated variables from each of the 4 groups of predictor variables (general dairy information, management, nutrition and milking procedures), in addition to all

design variables, were included for selection for 4 multivariable sub-models using a $P \leq 0.25$ cutoff. Finally, all predictor variables that remained in the sub-models ($P \leq 0.25$) were selected for a final multivariable regression model, analyzed using PROC GLM. Biologically relevant first order interactions were assessed in the final model. The final multivariable model was constructed using backward selection techniques. Predictor variables significant at $P \leq 0.1$ and all design variables remained in the final multivariable model.

The model building process used for the Organic Herd Model was identical to the process used for building the Total Herd Model except that grazing system was not included as a design variable, and grazing specific variables were included.

RESULTS

Bulk Tank Results

The geometric mean SCC of the overall study population was 191,000 cells/mL, ranging from 41,000 cells/mL to 725,000 cells/mL (Table 3.1). When the back transform of LSCC was stratified by grazing system, the geometric mean SCC were 182,000 cells/mL, 166,000 cells/mL and 195,000 cells/mL, for CON-NG, CON-GR and ORG farms, respectively (Table 3.1). The overall geometric mean SPC was 6,800 cfu/mL, and stratified means were 8,800 cfu/mL, 5,600 cfu/mL and 6,500 cfu/mL for CON-NG, CON-GR, and ORG farms, respectively. Total coliform counts among CON-NG, CON-GR and ORG farms had medians of 11 cfu/mL, 12 cfu/mL, and 4 cfu/mL, respectively, with an overall median of 5 cfu/mL. Due to a high number of negative results (0 cfu/mL), the descriptive analysis of LP and *E.coli* variables reports the percentage of each grazing system with a positive result (≥ 1 cfu/mL) (Table 3.1). Lab pasteurized count results were positive on 45% of CON-NG farms, 39% of CON-GR farms and 34% of ORG

farms, and on 37% of the total population. Twenty-eight percent of bulk tanks on both CON-NG and CON-GR farms were positive for *E.coli*, while 19% of ORG farms and 22% of all herds' milk tested positive. Mean butterfat (%) on CON-NG, CON-GR and ORG farms was 3.91%, 3.87% and 4.02%, respectively. The mean protein percentages from the bulk tank milk were 3.13%, 3.19% and 3.12% for CON-NG, CON-GR and ORG, respectively. The prevalence of food borne pathogens *Listeria monocytogenes* and Shiga toxin producing *E.coli* was low (Table 3.1). *Salmonella* was not detected in any of the samples. Bovine Viral Diarrhea virus and *Mycoplasma bovis* were rare in the bulk milk of the study population, with only 1% of total samples testing positive for each.

Contagious mastitis pathogens from the bulk tank that were assessed for differences between grazing systems were *S. aureus* and *S. agalactiae*. The percentage of ORG bulk tanks with a *S. aureus* positive culture was 61%, as compared to 42% of CON-NG and 43% of CON-GR bulk tanks (Table 3.1), with an overall percentage of 55%. The prevalence of *S. agalactiae* was low, with only 2% of bulk tanks testing culture-positive for *S. agalactiae*.

Univariate Analysis

Of the approximately 60 predictor variables analyzed using univariate linear regression analysis, 29 were unconditionally associated ($P < 0.25$) with LSCC and selected for inclusion in the final model. The association of average reported SCC and LSCC were analyzed to ensure accuracy and reassurance of the bulk tank SCC (Figure 3.1; $R = 0.76$, $P < 0.001$), but the average reported SCC variable was not included in the model building process.

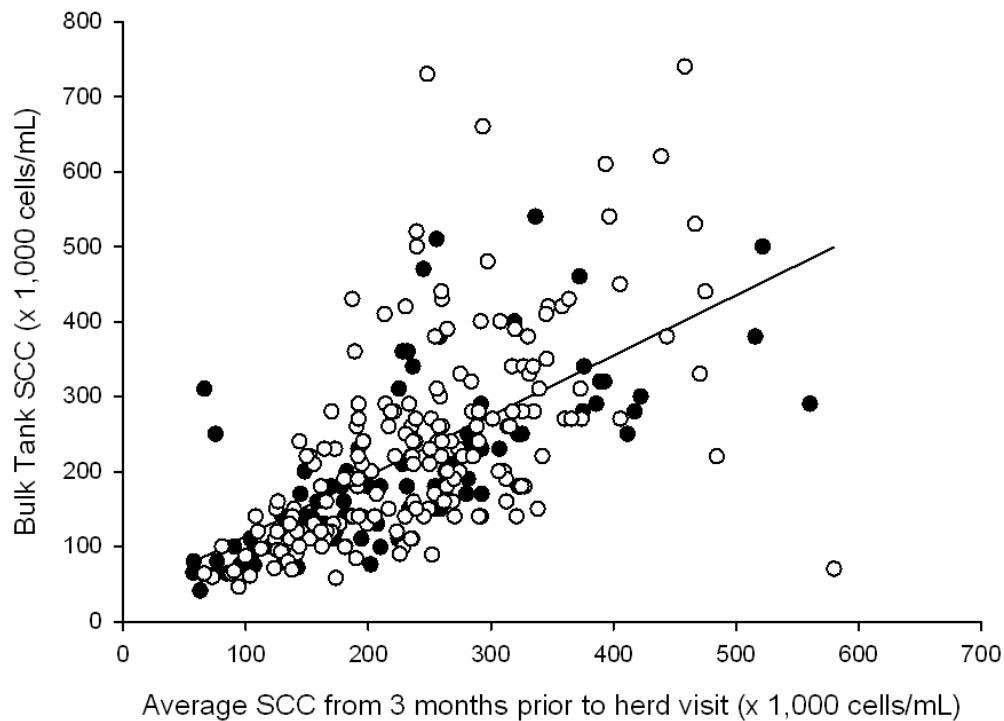


Figure 3.1. Measured SCC (x 1,000 cells/mL) from bulk tank milk on the day of collection plotted against the average somatic cell count from 3 months prior to the collection date from 187 organic (open circles) and 96 conventional (closed circles) farms in New York, Oregon and Wisconsin. SCC information was unavailable for 4 organic and 3 conventional farms. Data was collected between March 2009 and May 2011. $R = 0.76$.

Total Herd Model Building. Continuous general farm characteristic variables that were unconditionally associated ($P \leq 0.25$) with LSCC in the total herd dataset were the number of reported years in the dairy business, percentage of first lactation cows on the farm and the milk production per cow per day. The cosine and sine seasonality variables were also associated with LSCC; summer months tended to have an increase in LSCC. Differences and associations with LSCC among binary and categorical variables are presented in Table 3.2.

Table 3.1. Descriptive analysis of measures of central tendency for milk quality variables from bulk tank samples collected from dairy farms in New York, Oregon and Wisconsin from March 2009 to May 2011 analyzed among grazing systems.

Milk Quality Variable	Measures of Central Tendency			
	All Herds N = 290	CON-NG ¹ N = 63	CON-GR ¹ N = 36	ORG ¹ N = 191
SCC (x 1,000 cells/mL) ²	191 (41 – 725)	182 (61 – 501)	166 (40 – 616)	195 (45 – 724)
SPC (x 1,000 cfu/mL) ²	6.8 (1 – 4,000)	8.8 (1 – 4,000)	5.6 (1 – 110)	6.5 (1 – 1,700)
LP (x 100 cfu/mL) ⁴	<1 (<1 – 300)	<1 (<1– 54)	<1 (<1 – 11)	<1 (<1 – 300)
Coliform (cfu/mL) ⁴	5 (0 – TNTC ⁵)	11 (0 – TNTC ⁵)	12 (0 – TNTC ⁵)	4 (0 – TNTC ⁵)
<i>E.coli</i> (cfu/mL) ³	22% (0 – 150)	28% (0 – 22)	28% (0 – 150)	19% (0 – 29)
Butterfat (%)	3.94 (2.86 – 5.80)	3.91 (3.14 - 4.96)	3.87 (2.86 – 5.24)	4.02 (2.90 – 5.80)
Protein (%)	3.13 (2.44 – 3.89)	3.13 (2.73 – 3.89)	3.19 (2.61 – 3.89)	3.12 (2.44 – 3.86)
<i>Salmonella spp.</i> ⁶	0%	0%	0%	0%
<i>Listeria monocytogenes</i> ⁶	1%	3%	0%	1%
Shiga toxin <i>E.coli</i> ⁶	2%	0%	3%	1%
Bovine Viral Diarrhea ⁶	1%	2%	3%	1%
<i>Mycoplasma</i> ⁶	1%	0%	3%	2%
<i>Staph. aureus</i> ⁶	55%	61%	42%	43%
<i>Strep. agalactiae</i> ⁶	2%	2%	0%	3%

¹Conventional non-grazing (CON-NG), conventional grazing (CON-GR), organic (ORG)

²Geometric mean and range reported.

³Percentages of each group that tested positive. Range presented in parentheses in cfu/mL. Lab pasteurized count and *E.coli* counts are represented as positive results (≥ 1 cfu/mL).

⁴Analyzed variable had non-normal distribution, median and range reported.

⁵Testing was done on Petrifilm, which had an upper limit of 150 cfu/mL.

Table 3.2. Least square means of the geometric mean of the bulk tank milk of the log transformation of SCC (\log_{10} cells/mL) among 290 farms in New York, Wisconsin and Oregon. Variables shown are the categorical and binary general farm characteristics, management, milking and nutrition and grazing variables analyzed for unconditional association. Models did not include design variables grazing system, herd size or location.

		Variable Level	Geometric Mean SCC (x 1,000 cells/mL)	95% CI (x 1,000 cells/mL)	P-value (p ≤ 0.25)
Variable					
General Farm Characteristics					
Grazing System		CON-NG ¹	182	158 – 209	0.171
		CON-GR ¹	166	138 – 200	
		ORG ¹	195	182 – 214	
Site		New York	195	174 – 219	0.007
		Oregon	151	128 – 178	
		Wisconsin	200	186 – 219	
Average Reported Standard Plate Count (cfu/mL)		<1 – 7,000	178	159 – 200	0.243
		8,000 – 32,000	200	182 – 219	
		≥ 33,000	200	174 – 229	
Bulk tank culture – <i>Staph. aureus</i>		No	162	41 – 724	0.209
		Yes	214	63 – 724	
Management					
DHIA		No	209	190 – 229	0.005
		Yes	174	158 – 191	
% Bull Bred - Heifers		0-25%	166	155 – 182	<0.001
		26-75%	182	151 – 224	
		76-100%	229	204 – 252	
% Bull Bred – Adult Cows		0-25%	174	162 – 191	<0.001
		26-75%	224	182 – 269	
		76-100%	246	209 – 282	
Calving Area		Dedicated Area	174	155 – 200	0.092
		No Dedicated Area	200	182 – 214	
Improved Laneways		No	204	182 – 224	0.127
		Yes	182	166 – 200	
Use of Quarantine Unit		No	186	182 – 214	0.069
		Yes	174	155 – 195	
Regular Vet Visits		No	204	186 – 224	0.039
		Yes	178	162 – 195	
Routine Fresh Cow Checks		No	214	178 – 251	0.203
		Yes	186	174 – 200	
Use of Nutritionist		No	209	186 – 229	0.044

Use of Blanket Dry Treatment	Yes	182	162 – 195	0.031
	Antibiotic	182	159 – 209	
	Barrier	141	110 – 182	
	Other	148	85 – 257	
Vaccinate Cows	None	200	186 – 219	0.002
	No	229	200 - 257	
	Yes	182	166 – 195	
Milking Procedures				
Gloves	No	209	186 – 234	0.042
	Yes	182	170 – 200	
Use of ATOs ²	No	204	186 – 224	0.015
	Yes	174	155 – 191	
Use of Post-Dip	No	219	178 – 275	0.195
	Yes	191	178 – 200	
Nutrition				
Use of TMR	No	200	182 – 219	0.167
	Yes	182	162 – 200	
Use of Anionic Salts	No	195	182 – 209	0.046
	Yes	155	123 – 191	
Use of Grazing	No	166	132 – 209	0.191
	Yes	195	182 – 209	

Table 3.3 Least square means of the geometric mean of the bulk tank milk of the log transformation of SCC (\log_{10} cells/mL) among 191 organic farms in New York, Wisconsin and Oregon. Variable shows are the categorical and binary general farm characteristics, management, milking and nutrition and grazing variables analyzed for unconditional association. Models did not include design variables grazing system, herd size or location.

	Variable Level	Geometric Mean SCC (x 1,000 cells/mL)	95% CI (x 1,000 cells/mL)	P-value (p ≤ 0.25)
Variable				
General Farm Characteristics				
Average Reported Standard Plate Count (cfu/mL)	<1 – 7,000	178	155 – 200	0.059
	8,000 – 32,000	214	191 – 240	
	≥ 33,000	209	174 - 251	
Bulk tank culture – <i>Staph. aureus</i>	No	159	141 – 182	< 0.0001
	Yes	229	209 – 251	
Bulk tank culture – <i>Strep. agalactiae</i>	No	195	182 – 214	0.169
	Yes	269	174 – 427	
Management				
DHIA	No	209	186 – 229	0.197
	Yes	186	166 – 214	
% Bull Bred - Heifers	0-25%	170	148 – 191	< 0.001
	26-75%	182	141 – 234	
	76-100%	234	209 – 257	
% Bull Bred – Adult Cows	0-25%	178	162 – 194	0.001
	26-75%	229	191 – 282	
	76-100%	240	204 – 282	
Use of Quarantine Unit	No	214	195 – 234	0.020
	Yes	174	151 – 200	
Regular Vet Visits	No	209	191 – 234	0.071
	Yes	182	159 – 204	
Use of Blanket Dry Treatment	Antibiotic	363	182 – 794	0.147
	Barrier	145	93 – 200	
	Other	148	85 – 257	
	None	200	186 – 219	
Vaccinate Cows	No	229	200 – 263	0.009
	Yes	182	166 – 204	
Milking Procedures				
Gloves	No	219	191 – 251	0.112
	Yes	191	174 – 209	
Use of Post-Dip	No	229	185 – 295	0.206
	Yes	195	178 – 214	

Nutrition

Use of Rotational Grazing

No

269

191 – 380

0.076

Yes

195

182 – 214

Use of Anionic Salts

No

204

186 – 219

0.023

Yes

138

98 – 191

The general farm characteristic predictor variables that were selected for inclusion in the final multivariable LSCC model prior to backward stepwise regression using a cutoff value of $P \leq 0.25$, were the number of years in the dairy industry, seasonality, presence of *S. aureus* in the bulk tank and average reported SPC.

In the total herd dataset, EIS was the only continuous management variable that was unconditionally associated ($P \leq 0.25$) with decreased LSCC. Differences and associations with LSCC among binary and categorical variables are presented in Table 3.2. The management predictor variables that were selected for inclusion in the final multivariable LSCC model prior to backward stepwise regression, using a cutoff value of $P \leq 0.25$, were the EIS and the percentage of heifers bred using natural service.

The number of milking technicians on the farm was the only continuous milking variable that had an unconditional association with increased LSCC in the total herd dataset. Differences and associations with LSCC among binary and categorical variables are presented in Table 3.2. The use of gloves and the number of milking technicians on the farms were selected for inclusion in the final multivariable LSCC model prior to backward stepwise regression, using a cutoff value of $P \leq 0.25$.

The amount of grain fed per cow per day was the only continuous nutritional variable that was unconditionally associated with LSCC in the total herd dataset. Differences and associations with LSCC among binary and categorical variables are presented in Table 3.2. The nutritional variables that were selected for inclusion in the final multivariable LSCC model prior to backward stepwise regression, using a cutoff value of $P \leq 0.25$, were the amount of grain fed per cow per day and the use of anionic salts in transition cow diets.

Selected Total Herd Model. Backward stepwise linear regression modeling resulted in the final model that, in addition to the forced design variables (grazing system, herd size and location) included 4 predictor variables (Table 3.4). The variables representing the percentage of heifers on the farm bred using natural service and the use of anionic salts in transition cow diets were removed from the model, as they were a part of the EIS score calculation. EIS score was negatively associated with LSCC ($P = 0.008$), as was amount of grain fed per cow per day ($P = 0.036$). Bulk tank milk samples that tested positive for *S. aureus* typically had a higher LSCC ($P < 0.001$). As the number of years that a farmer reported being in the dairy industry increased, the LSCC also increased ($P < 0.001$).

Table 3.4. Final total herd linear regression model showing the association of significant variables ($P \leq 0.1$) variables and the bulk tank using the log transformation of SCC (\log_{10} cells/mL) among conventional non-grazing (CON-NG; $n = 63$), conventional grazing (CON-GR; $n = 36$), and organic (ORG; $n = 191$) farms. Grazing system, location (New York, Wisconsin and Oregon), and herd size were always included in the model as design variables. Estimates provided are the β coefficient for the given variable.

Variable	Level	Estimate	Final model <i>P</i> -value
Intercept		2.192	<0.001
Grazing System	CON-NG	0.059	0.286
	CON-GR	0.049	
	ORG	Reference	
Location	NY	0.086	0.093
	WI	0.098	
	OR	Reference	
Herd Size	≥ 200 cows	0.055	0.048
	100 – 199 cows	0.099	
	20 – 99 cows	Reference	
External Input Score ¹	Continuous (0 - 10)	- 0.019	0.008
Amount of Grain Fed	Continuous	- 0.011	0.036
Cow/Day (kg)			
Bulk Tank Culture – <i>S. aureus</i>	Yes	0.111	< 0.001
	No		
Number of years in the dairy industry	Continuous	0.004	< 0.001

¹External Input Score is a continuous, additive variable from 0 - 10. It consists of the following variables and their scoring schemes: use of nutritionist (no, yes; yes = 1), regular use of a veterinarian (no, yes; yes = 1), use of DHIA (no, yes; yes = 1), use of anionic salts in transition cow diets (no, yes; yes = 1), vaccination of cows (no, yes; yes = 1), vaccination of calves (no, yes; yes = 1), regular pregnancy checks (no, yes; yes = 1), a written record of herd health events (no, yes; yes = 1), any use of AI for non-lactating heifers (no, yes; yes = 1) and any use of AI for lactating cows (no, yes; yes = 1).

Organic Herd Model Building. In the organic herd dataset, continuous general farm characteristics that were associated with LSCC were the number of years in the dairy business, the number of years a farm has been certified organic and milk production per cow per day. The cosine seasonality variable was associated with LSCC, with a peak in the summer months and a low in the winter months of LSCC. Differences and associations with LSCC among binary and categorical variables are presented in Table 3.3.

In the organic herd model, the number of years a farmer had spent in the dairy business, the number of years the farm was certified organic, the average SPC and a *S. aureus* positive result from the bulk tank were the general farm characteristic predictor variables that were included in the multivariable LSCC model prior to backward stepwise regression, using a cutoff value of $P \leq 0.25$.

The EIS was the only continuous management variable that was unconditionally associated with LSCC the organic dataset. Differences and associations with LSCC among binary and categorical variables are presented in Table 3.3. The management predictor variables that were included in the multivariable LSCC model prior to backward stepwise regression, using a cutoff value of $P \leq 0.25$, were the EIS, the use of a segregation unit or bucket milker at milking, regular visits from the veterinarian and vaccinations of adult cows.

The number of milking technicians on the farm was the only continuous milk variable unconditionally associated with increased LSCC in the organic herd dataset. Differences and associations with LSCC among binary and categorical variables are presented in Table 3.3. The use of gloves was the only milking predictor variable that was selected for inclusion in the final multivariable LSCC model prior to backward stepwise regression, using a cutoff value of $P \leq 0.25$.

The continuous nutritional variables that were unconditionally associated with LSCC in the organic herd dataset were the average percentage of DMI from pasture and the number of days grazing per year and the amount of grain fed per cow per day. Differences and associations with LSCC among binary and categorical variables are presented in Table 3.3. The nutritional predictor variables included in the multivariable LSCC model prior to backward stepwise

regression using a cutoff value of $P \leq 0.25$, were the amount of grain fed per cow per day, the use of rotational grazing and the use of anionic salts in transition cow diets.

Selected Organic Herd Model. Backward stepwise linear regression model resulted in the final model that contained the two required design variables (herd size and location) and eight other variables (Table 3.5). An increase in the number of years in the dairy industry was associated with a higher LSCC ($P = 0.024$). There was a seasonal effect on LSCC ($P = 0.066$), which indicated a peak in the summer months and a depression in the winter months (Figure 3.2). A *S. aureus* positive bulk tank culture was associated with a higher LSCC ($P < 0.001$). Farmers that reported use of a segregation unit or bucket milker during milking tended to have a lower LSCC ($P = 0.002$), as did the use of gloves during milking ($P = 0.089$). Feeding more grain per cow per day was associated with a lower LSCC ($P = 0.062$). Higher average reported SPC from three months prior to the herd visit was associated with a higher LSCC ($P = 0.071$). Feeding anionic salts in transition cow diets was associated with a lower LSCC ($P = 0.035$).

Table 3.5. Final organic herd linear regression model showing the association of significant variables ($P \leq 0.1$) variables and the bulk tank using the log transformation of SCC (\log_{10} cells/mL) organic (n = 191) farms. Grazing system, location (New York, Wisconsin and Oregon), and herd size were always included in the model as design variables. Estimates provided are the β coefficient for the given variable.

Variable	Level	Estimate	Final model <i>P</i> -value
Intercept		2.239	< 0.001
Location	NY	0.054	0.625
	WI	0.032	
	OR	Reference	
Herd Size	≥ 200 cows	0.157	0.026
	100 – 199 cows	0.091	
	20 – 99 cows	Reference	
Years in the dairy industry	Continuous	0.003	0.024
Seasonal Effect ¹	Continuous	- 0.045	0.066
Bulk Tank Culture – <i>S. aureus</i>	Yes	0.174	< 0.001
	No	Reference	
Use of Segregation	Yes	- 0.112	0.002
Unit or Bucket Milker	No	Reference	
Amount of Grain Fed Cow/Day (kg)	Continuous	- 0.020	0.002
Reported SPC	$\geq 33,000$ cfu/mL	0.060	0.071
	8,000 – 32,000 cfu/mL	0.080	
	<1 – 7,000 cfu/mL	Reference	
Use of gloves during milking	Yes	- 0.057	0.089
	No	Reference	
Use of anionic salts in transition cow diets	Yes	- 0.148	0.035
	No	Reference	

¹Seasonal Effect was computed using the formulas $\sin(2\Pi*(\text{day of year}/365))$ and $\cos(2\Pi*(\text{day of year}/365))$ where $\Pi = 3.14$. We are defining it here as a continuous variable.

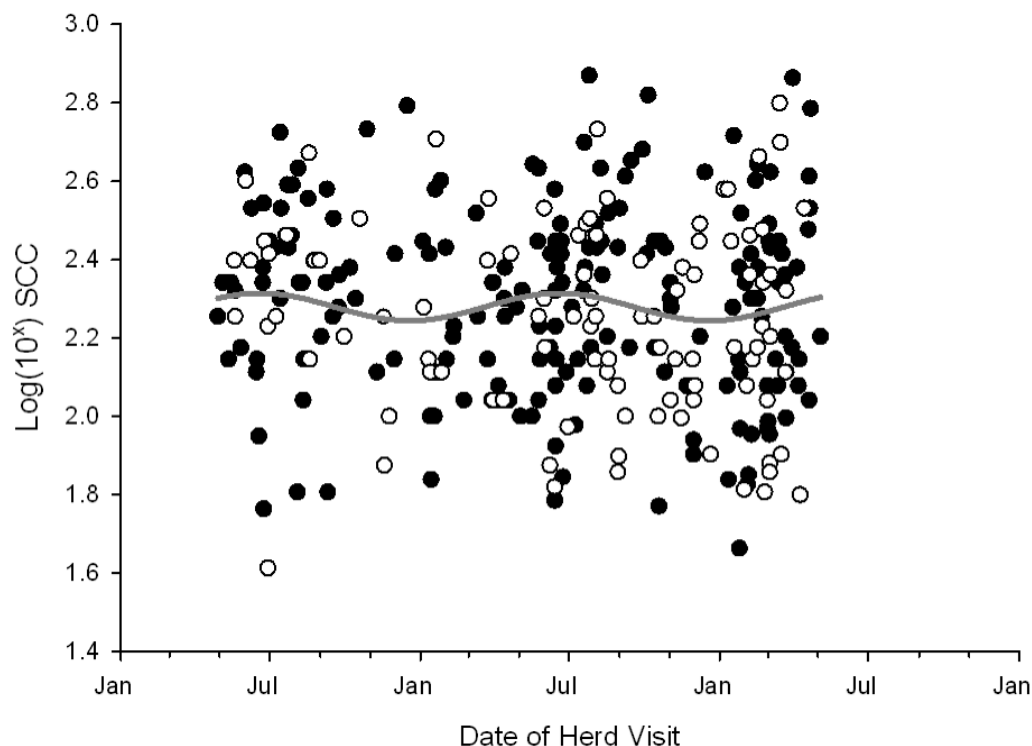


Figure 3.2. Seasonal trend of the \log_{10} transformation of SCC¹ (cells/mL) by dairy production system of 290 farms in New York, Wisconsin and Oregon. Closed circles represent organic farms ($n = 191$), open circles represent conventional farms ($n = 99$). Trend shown (gray line) is the cosine ($P = 0.091$), indicating peaks in SCC in the summer months and depression in the winter months. Date of herd visit spans from March 2009 to May 2011.

¹ The \log_{10} transformation of the SCC was on the 10^2 form of the variable.

DISCUSSION

This study was a component of a larger project with an overarching goal to assess the herd health, management practices and herd characteristics of conventional and organic dairy farms in New York, Oregon and Wisconsin. The purpose of this manuscript is to provide an analysis of the milk quality of bulk tank milk, as well as to determine the impact of selected management variables on bulk tank SCC.

All ORG farms in our study were matched with CON farms of similar size and location, so that comparisons would be as accurate as possible. Also, the wide range of management

methods, and subsequently milk quality results, are represented in our population. Approximately 30% of the organic population across the three states was visited, which is a significant portion of organic industry. This study was primarily a cross-sectional study with a longitudinal component and we recognize that there are several limitations to this method of data collection and research. While the study was strong internally, care should be taken when interpreting the results and applying to a group outside of the study demographic.

Results of this study confirmed previous research indicating that grazing system does not impact SCC (Stiglbauer, Cicconi-Hogan and Richert, et al., in press). Prevalence of food borne pathogenic bacteria in our sample of herds was lower than reported in previous studies (Van Kessel et al., 2011). This may be due to the differences in methodology between the two studies. The number of farms sampled in the 2011 study was much larger than ours and samples were also taken from milk filters, instead of just from the bulk tank. Our study population consisted of primarily small farms (≤ 100 adult cows), where as samples collected for Van Kessel et al. (2011) came from wide range of farm sizes. These differences should be kept in mind when interpreting the prevalence of food-borne pathogens.

The presence of *S. aureus* in the bulk tank milk was higher on ORG farms (61%) than CON-NG (42%) and CON-GR (43%), which was not unexpected. Because *S. aureus* is a contagious pathogen that is difficult to treat, the typical management strategy is to cull the infected animal, or alternatively, milk her last to avoid transmitting the bacteria to the rest of the herd via the milking machinery. The higher prevalence of *S. aureus* on ORG farms may be due to the difficulty in replacing culled animals that are organically certified. It may also but due to the reluctance of ORG farms to bring in adult animals from outside sources (Stiglbauer, Cicconi-Hogan and Richert, et al., in press). Approximately 30% of our ORG herds use a segregation

milking unit or a bucket milker, which was found to be associated with a lower SCC. An increased implementation of a bucket milker use by farms that are harboring *S. aureus* cows may be a relatively easy and financially sound method to improve milk quality on organic farms. In addition, our research indicates that ORG farms largely do not use blanket dry cow therapy, while conventional farms tend to use some form of blanket dry cow therapy. Dry cow therapy plays an important part in eliminating many early subclinical *S. aureus* infections. However, the cause of a higher prevalence of *S. aureus* on ORG farms is a matter that should be further explored in future research.

The Total Herd Model shows a relationship between grain feeding and decreased LSCC, which may be caused by a dilution effect. Because animals that are fed more grain are likely to produce more milk, it has been suggested that increased production is associated a slightly decreased SCC (Green et al., 2006), although the strength of this effect is low, as shown by the parameter estimate (Table 3.4). The presence of *S. aureus* in bulk tank milk and its association with a higher SCC is not unexpected (Olde Riekerink et al., 2006; Barkema et al., 1999), as subclinical mastitis caused by *S. aureus* is associated with increased SCC. More years in the dairy industry was associated with a higher SCC. This could be interpreted as younger farmers, or farmers that have recently gotten into the industry, being more aggressive in their management and treatments. However, the regression coefficient is quite small, and a change of 10 years only predicts a SCC difference of 15,000 to 30,000 cells/mL. Research more focused on management reasoning may provide a more complete picture of the relationship between SCC and the number of years in the dairy business.

A point of interest is that of the eight variables from the Organic Herd Model determined to be significant, three of the variables were also included in the Total Herd Model. This suggests

that while many of the variables that affect milk quality on organic farms are the same as those that are significant in the general dairy population, the organic demographic may use different methods and face different challenges than the conventional population.

Of interest from both models are variables that relate to the farmer's use of external management resources. In our previous paper, Stiglbauer, Cicconi-Hogan and Richert et al. (in press), we created a scale that allowed us to assess how much outside support was being utilized on the farm, and how it related to various management variables. We determined that more use of outside support and management sources was related with a lower SCC. From that scoring system, in conjunction with the multiple correspondence analysis results from Richert et al. (in press), we combined several variables to create a more complete scoring system, referred to as the EIS score. Some of the EIS variables by themselves did not have any logical relationship with LSCC, but still presented an association, such as high percentages of bull-bred heifers on the farm. This score was designed to help measure how many different outside sources the farmers were using to help manage the farm. Our EIS score was inclusive of many different overarching management decisions and factors, but is by no means exhaustive. We believe that many of the 'traditional' variables that were used to troubleshoot IMIs and the resulting high SCCs are less useful, as many farmers are either already practicing these procedures, or know that this is an 'expected' response. As described in Figure 3.3, we believe that the various EIS is indicative not only of how farmers utilize outside sources, but also serves as a proxy for precision of the management procedures. Figure 3.4 also indicates that a higher EIS was associated with CON management among size and location matched farms (simple regression, analysis not shown, $P < 0.001$), which supports the findings from Stiglbauer, Cicconi-Hogan and Richert et al. (in press). Future research should include more specific questions aimed at outside

management support used on the farm in order create a more complete picture of management on the farms and determine why the use of external resources was lower on organic farms.

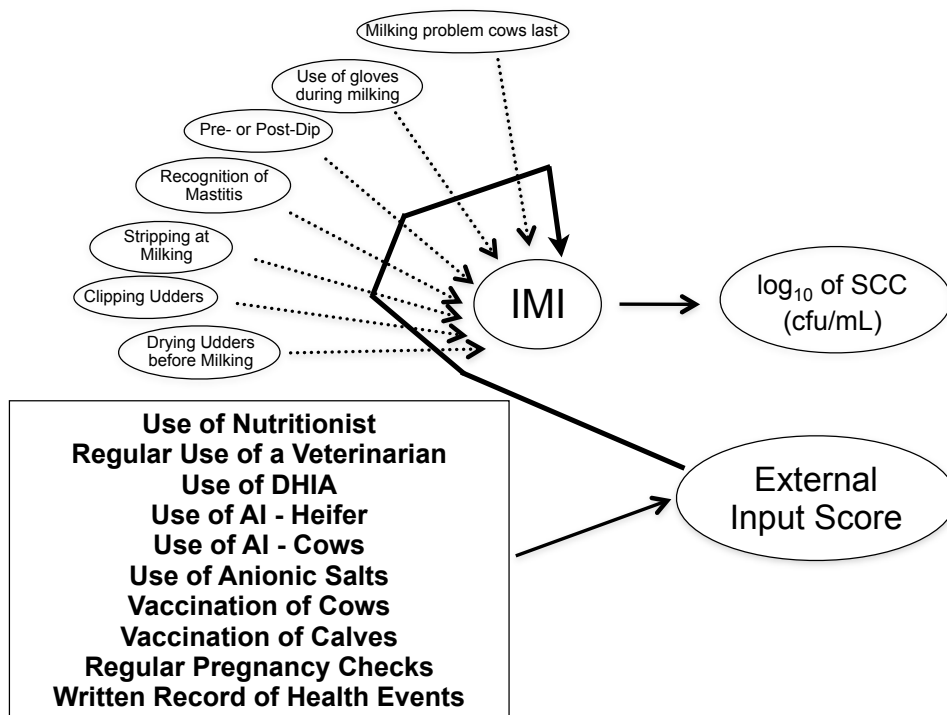


Figure 3.3. Relationship of External Input Score with IMI of dairy animals and the resulting somatic cell count. External Input Score is an additive score of several variables that indicate more involvement of outside sources in dairy farm management, which may be representative of the precision of various management procedures.

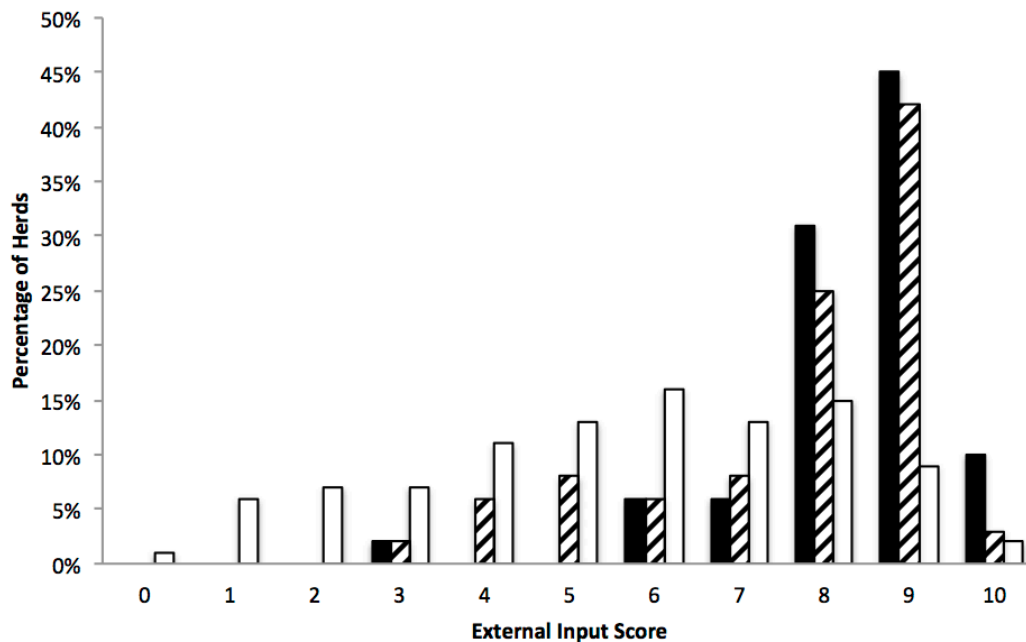


Figure 3.4. Percentage of dairy farms in each grazing system in New York, Oregon and Wisconsin distributed by the External Input Score. Black bars represent conventional non-grazing farms (n = 63), striped bars represent conventional grazing farms (n = 36) and white bars represent organic farms (n = 191). The External Input Score is an additive score (0 to 10) of several variables that indicate more involvement of outside sources in dairy farm management. The score consists of the following variables, using a 0 (no) or 1 (yes) scale: use of nutritionist (no, yes), regular use of a veterinarian (no, yes), use of DHIA (no, yes), use of anionic salts in transition cow diets (no, yes), vaccination of cows (no, yes), vaccination of calves (no, yes), regular pregnancy checks (no, yes), a written record of herd health events (no, yes), any use of AI in non-lactating heifers (no, yes), and any use of AI in adult cows (no, yes). The overall finding was that organic farms use less external resources than their conventional counterparts ($P < 0.001$).

Future studies that would allow investigators to follow farms over a longer period of time would be a useful way to build on the information we currently have available. The research and information from this manuscript contain variables and information that could be used to enhance milk quality improvement programs to maximize utility for the growing organic portion of the industry.

CONCLUSION

The increase in the demand for organic dairy products has produced the need for more knowledge and research on organic dairy management. Our research indicates that while each production system has unique challenges, the milk quality of organic and conventional dairy farms are not different.

The LSCC of the population of all herds included in this study were most associated with the amount of grain fed, the presence of *S. aureus* in the bulk tank, the number of years in the dairy industry and the EIS score. The LSCC of the organic population of the study was influenced by similar variables, with the exception of EIS. Other factors specific to the organic model included the use of a bucket milker at milking, the reported bulk tank SPC, the use of gloves during milking, the use of anionic salts in transition cow diets and a seasonal effect.

The EIS score offered an interesting analysis, as it provided insight into how the different grazing systems use external resources. Our analysis found that ORG farms were more likely to have a lower EIS score than size and location matched CON farms in this study. Further research should be done with the use of external management resources in mind, as well as to create a more exhaustive EIS scale.

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CHAPTER FOUR

Risk factors associated with bulk tank standard plate count, bulk tank coliform count and the presence of *Staphylococcus aureus* in the bulk tank on dairy farms in the United States.

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INTERPRETIVE SUMMARY

Risk factors associated with bulk tank standard plate count, bulk tank coliform count and the presence of *Staphylococcus aureus* in the bulk tank on dairy farms in the United States. Cicconi-Hogan et al. The organic dairy industry is one of the fastest growing agricultural sectors in the United States. More detailed information on factors associated with milk quality and best management practices in the organic dairy industry are needed. The goal of this study was to associate management variables with bulk tank SPC, *S. aureus* and coliform counts on organic and conventional dairy farms.

ABSTRACT

The purpose of this study was to assess the association of bulk tank milk standard plate counts, bulk tank coliform counts (CC) and the presence of *Staphylococcus aureus* (*S. aureus*) in bulk tank milk with various management and farm characteristics on organic and conventional dairy farms throughout New York, Wisconsin and Oregon. Data from size matched organic farms (n = 192), conventional non-grazing farms (n = 64) and conventional grazing farms (n = 36) were collected at a single visit for each farm. Of the 292 farms visited, 290 bulk tank milk samples were collected. Statistical models were created using data from all herds in the study, as well as exclusively for the organic subset of herds. Due to missing data, 267 of 290 herds were analyzed for the total herd modeling, and 173 of 190 organic herds were analyzed for the organic herd modeling. Overall, there were more bulk tanks from organic farms that had *S. aureus* cultured from them, while fewer organic herds had a high CC than conventional farms in the study. A high log₁₀ standard plate count (x 1,000 cfu/mL) was associated with decreased body condition score of adult cows and decreased milk production in both models. There were a number of variables that were only significant in one of the two models. The presence of *S. aureus* in the bulk tank milk was associated with fewer people treating mastitis, increased housing age and a higher percentage of cows with 3 or fewer teats in both the organic and total herd models. The total herd *S. aureus* model also showed an association with fewer 1st lactation animals, higher hock scores and less use of automatic takeoffs at milking. High bulk tank CC, defined as ≥ 50 cfu/mL, was associated with feeding a total mixed ration and using natural service in non-lactating heifers in both models. Overall, attentive management and use of outside resources seemed to be particularly useful with regard to CC on organic farms. In almost every model, with the exception of the organic CC model, there was an association with the average

reported somatic cell count from 3 months prior to the herd visit, indicating that many of the regularly tested milk quality parameters are interconnected.

Key words: dairy, milk quality associations, management, organic

INTRODUCTION

The rapid growth of the organic dairy industry has made research regarding organic milk quality and management practices essential. The resulting increase in certified organic dairy animals and production of organic dairy products is a consequence of a surge in consumer interest concerning animal welfare and the environmental impact of conventional dairy farming (Sundrum, 2001). There is a perception among consumers that organically produced milk is healthier or of better quality (Yiridoe and Bonti-Ankomah, 2005), but previous research has found little difference regarding milk quality between organic and conventional farms (Rosati and Aumaitre, 2004; Sato et al., 2005). Our research team has collected longitudinal and cross-sectional data on a large number of organic and frequency matched conventional dairy farms and reported on management characteristics (Stiglbauer, Cicconi-Hogan and Richert et al., in press), the use and role of veterinarians within these populations (Richert et al., in press), and associations of management with SCC (Cicconi-Hogan et al., submitted for publication).

Examining and monitoring bulk tank milk on a dairy farm is a useful and efficient method of assessing the quality of milk that the farm is producing. High levels of bacteria in raw milk can adversely affect the quality and shelf life of pasteurized milk (Schukken et al., 2003; Jayarao et al., 2006). Several tests are regularly used to assess the quality of bulk tank milk, such as SCC, SPC, laboratory pasteurized count, and coliform count (CC). Dairy processors use these tests to determine if their clients' products are high quality, and to evaluate whether milk is adequate for distribution and consumption.

The SPC, also known as the plate loop count, is a test that assesses the bacterial density in the milk and estimates the number of aerobic bacteria present per mL of milk. A high bulk tank SPC can be the result of bacteria from unclean milking equipment, milk from cows with

subclinical or clinical mastitis or contamination from dirty udders (Murphy and Boor, 2000). The US regulatory cut-off for SPC is 100,000 cfu/mL, which is also in accordance with the European Union Standards. *Staphylococcus aureus* (*S. aureus*) is a contagious mastitis pathogen that has a major impact on milk production and bulk tank SCC (Keefe, 2012). *Staphylococcus aureus* is known to increase the bulk tank SCC and spreads easily from animal to animal in a number of ways - by milking units, though improper milking or handling, or lack of glove use, to name a few. While methicillin-resistant *S. aureus* is the one of the most well-known human pathogens, recent research has shown that it does not appear to be a major issue in bulk tank milk in the US (Cicconi-Hogan, et al., personal communication; Haran et al., 2012). Coliform bacteria found in bulk tank milk, such as *Escherichia coli* and *Klebsiella species*, are usually indicative of fecal contamination, often from soiled udders or teats (Hogan and Smith, 2003) and may occasionally be from a mastitis cow shedding high counts of these coliform bacteria. When the bacterial count of a sample is below the regulatory limits, most coliforms will be removed from the milk by pasteurization before the milk is consumed. Occasionally, in the case of raw milk consumption or a pasteurization failure, coliforms in the milk can lead to severe human disease. Shiga toxin-producing *Escherichia coli* O157:H7, which has been isolated from bulk tank milk (Karns et al., 2007), can cause severe hemorrhagic diarrhea in humans.

Previous analyses from our research determined that CC and *S. aureus* were different among grazing systems (Stiglbauer, Cicconi-Hogan and Richert et al., in press; Cicconi-Hogan et al., submitted for publication), and thus, were natural choices for further investigation. While several studies have been done that assess bacterial milk quality, as well as determine associations of bacterial counts with management factors, few have focused on the organic dairy population in the United States. Standard plate count is an excellent way to assess management

and milk quality, as reported in previous research and milk quality management recommendations (Schroeder, 2009; van Schaik et al., 2002; Jayarao et al., 2004).

The objective of this study was to evaluate the relationship between management practices and bulk tank SPC, CC and the presence of *S. aureus* for all herds involved in the study and specifically for the subset of organic herds. The aim is to identify management characteristics that are associated with good bacterial milk quality, and can be used to define best management practices for either conventional or organic dairy farms or both.

MATERIALS AND METHODS

Recruitment and Herd Selection

Recruitment and herd inclusion criteria are described by Stiglbauer, Cicconi-Hogan and Richert et al. (in press), Cicconi-Hogan et al. (submitted for publication), and Richert et al. (in press). Dairy herds in New York (NY), Oregon (OR), and Wisconsin (WI) were visited between March 2009 and May 2011. A total of 192 organic (**ORG**) herds and 100 conventional (**CON**) herds were frequency matched based on herd size and location. In New York, 72 ORG and 25 CON farms were visited; in Wisconsin, 96 ORG and 51 CON farms were visited; and in Oregon, 24 of both ORG and CON farms were visited.

Questionnaire and Data Collection

The study questionnaire was modified from previously published survey instruments with input from dairy professionals (Zwald et al., 2004; Pol and Ruegg, 2007). It was reviewed by professional survey developers, and tested with organic and conventional dairy farmers in each

state prior to the beginning of the study. Farmers were asked to refer to all available records to ensure accuracy of answers. Recall was generally limited to the 12 months prior to the herd visit or less. More detailed questionnaire information can be found in Stiglbauer, Cicconi-Hogan and Richert, et al. (in press).

In each state, a single member of the study team conducted all interviews. Standardization of data collection among the locations was ensured by monthly conference calls. Data collection methods were consistent across the three sites, as described by Richert et al. (in press). For most of visited herds, the individual directly responsible for animal care was interviewed.

Body condition scoring, udder hygiene and hock scoring was done by the interviewer on all farms. The process for choosing what animals were scored was described in detail by Cicconi-Hogan, et al. (submitted for publication). Body condition was evaluated using an accepted scoring method with provided guidelines for examining the hooks, pins, sacral and tail head ligaments (Ferguson et al., 1994). Udder hygiene was assessed on a 4-point system (Schreiner and Ruegg, 2003). Hock scores were assessed using a 3-point system developed at Cornell University (2007) (<http://www.ansci.cornell.edu/prodairy/pdf/hockscore.pdf>, accessed September 2012). All scoring forms can be found at <http://milkquality.wisc.edu/wp-content/uploads/2011/10/Reference-Guides-for-Scoring.pdf>.

Bulk Tank Milk Sample Collection and Testing

Bulk tank milk samples were collected by study personnel at 290 farms. Bulk tanks were agitated for a minimum of 5 minutes and samples were taken directly from the tank with a sterile

sampler. Samples were put on ice and transported to Quality Milk Production Services at Cornell University for analysis. Two farmers in the study requested that their bulk milk not be analyzed and therefore were not included in the bulk tank milk analysis.

The milk quality methods and associated references are described in more detail in Cicconi-Hogan et al. (submitted for publication). The samples were analyzed for the presence of foodborne pathogens *Salmonella spp.*, *Listeria monocytogenes*, and Shiga toxin producing *E. coli*. Samples were also tested for the presence of *Mycoplasma bovis*, Bovine Virus Diarrhea virus, a modified mastitis bacteria count and antibodies to *Mycobacterium avium* subspecies *paratuberculosis* (Johne's disease). Samples were couriered to Dairy One Cooperative (Ithaca, NY) and tested for SCC, SPC, lab pasteurized count, CC, *E. coli* count, butterfat and protein percentages.

Statistical Analysis

Predictor Variables. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., 2008). Dairy production system (ORG, CON) and grazing information were combined to create a new predictor variable, grazing system, which had three levels 1) ORG, 2) conventional grazing (**CON-GR**), and 3) conventional non-grazing (**CON-NG**). Grazing was defined as herds where lactating cows obtained $\geq 30\%$ of DMI from pasture during the grazing season. Grazing system, herd size category (0-99 cows, 100-199 cows, ≥ 200 cows), and location (NY, OR, WI) were associated with the design of the study and were forced into all of the multivariable models.

Predictor variables assessed for inclusion in the models were selected based on similarity to variables described in the meta-analysis of (Dufour et al., 2011) and the SCC analysis of the

current study as reported by Cicconi-Hogan et al. (submitted for publication). General farm characteristics that were assessed were as follows: Average age of primary adult housing in years; percentage of first lactation cows on the farm; number of years the farmer had been in the dairy industry; seasonality (computed using the formulas $\sin(2\Pi*(\text{day of year of herd visit}/365))$ and $\cos(2\Pi*(\text{day of year of herd visit}/365))$, where $\Pi = 3.14$); percent of problem breeders in the past 12 months (defined as animals that had been removed from the herd due to failure to conceive, animals that had been removed from the breeding population by labeling her “do not breed” because of failure to conceive or animals that were 9 month post-partum and not yet pregnant); estimated calving interval in days (provided by herd record systems or estimated by adding 60 days to the estimated lactation length); amount of milk produced per cow per day (kg); type of milking facility (pit parlor, flat or walkthrough parlor, tie stall or stanchion, other type of facility); presence of *S. aureus* in the bulk tank at the time of the visit (yes, no); average reported SCC of milk shipped in the 3 months prior to the herd visit; and average reported SPC of milk shipped in the 3 months prior to herd visit ($<1 - 7,000$ cfu/mL, $8,000 - 32,000$ cfu/mL, $\geq 33,000$ cfu/mL). General management variables were as follows: Mean body condition score, mean hock score, mean udder hygiene score, use of natural service for non-lactating heifers (none versus at least some use of artificial insemination), use of natural service for adult cows (none versus at least some use of artificial insemination), use of a dedicated calving area separate from all other cows (yes, no), farmer reported improved laneways (yes, no), use of a segregation unit or bucket milker or during milking (yes, no), number of people who treat mastitis, written herd health and treatment records (yes, no), regular veterinarian visits (yes, no), use of vaccinations in adult cows (yes, no), use of vaccinations in calves under 90 days of age (yes, no), use of a nutritionist (yes, no), use of DHIA (yes, no), use of anionic salts in transition cow diets (yes, no), frequency of

bulk tank cultures taken per year (never, monthly, quarterly, other times per year), routinely checking postpartum cows (yes, no), use of a California Mastitis Test (yes, no), housing sick and healthy cows separate (yes, no), transferring cows to an alternate location (yes, no), introduction of new dairy cows or heifers in the past 12 months (yes, no), percent of herd with 3 or fewer teats, percent of the herd that had at least one quarter of which milk was segregated from the bulk tank, number of times milked per day, use of a pre-dipping solution (yes, no), use of a post-dipping solution (yes, no), use of gloves during milking (yes, no), use of automatic take offs (yes, no), number of milkers on the farm, fore-stripping prior to milking (yes, no), and number of milking units (0-10, 11-20, ≥ 20), use of TMR (yes, no) and the amount of grain fed per cow per day (kg). Grazing specific variables that were assessed for the organic models only were as follows: number of acres used for pasture, use of grazing (yes, no), average percentage of DMI from pasture, percent of pasture that has been improved in the past 7 years, and use of rotational grazing (yes, no).

To measure the level of external support a farmer was using, several variables were combined to create a new variable, referred to as External Input Score (**EIS**), described in more detail in Cicconi-Hogan et al. (submitted for publication). The EIS variable was created by adding the scores of several management variables, resulting in a scale that ranged from 0 to 10. The variables used for the EIS score are: use of nutritionist (yes, no), regular use of a veterinarian (yes, no), use of DHIA (yes, no), use of anionic salts in transition cow diets (yes, no), vaccination of cows (yes, no), vaccination of calves (yes, no), regular pregnancy checks (yes, no), a written record of herd health events (yes, no), any use of natural service in non-lactating heifers (yes, no), and any use of natural service in lactating cows (yes, no).

Statistical Model Building. Data was analyzed from 290 out of 292 farms. Due to missing explanatory variable information, 267 of 290 herds were analyzed in the total herd models, and 173 of 190 organic herds were analyzed for the organic herd models. Outcome variables analyzed were SPC from the bulk tank, the presence of *S. aureus* in the bulk tank and the CC of the bulk tank milk. The presence of *S. aureus* was analyzed as a binary variable (yes, no). The distribution of the SPC and CC were both truncated on the left at 0 and skewed to the right. The SPC was transformed to \log_{10} cfu/mL, hereinafter referred to as **LSPC** and reported as a geometric mean. The CC was dichotomized, based on previous research and milk quality recommendations (Elmoslemany et al., 2010; Schroeder, 2009; Jayarao et al., 2004), at either ≤ 50 cfu/mL (=0) or > 50 cfu/mL (=1).

PROC UNIVARIATE (SAS Institute, Inc., 2008) was used to analyze the distributions of the individual predictor variables. When appropriate, predictor variables were categorized into 3 groups based on the 25th and 75th percentiles (0 – 25%, 26 – 75%, 75 – 100%). Two different subsets of data were used in creating six regression models, one using information from all herds with complete information and one using only information from organic herds with complete information, to test for associations with various predictor variables. Three of the models used data from all herds in the study (SPC Total Herd Model, *S. aureus* Total Herd Model, and Coliform Total Herd Model) and 3 of the models used data from all organic herds in the study (SPC Organic Herd Model, *S. aureus* Organic Herd Model, and Coliform Organic Herd Model). Variable selection was done according to the method described by Dohoo et al. (2010).

The *S. aureus* models and the CC models were analyzed by logistic regression, using PROC LOGISTIC (SAS Institute, Inc., 2008). The general logistic regression model for the Total Herd Models is formulated as:

$$\ln \left[\frac{Y}{1-Y} \right] = \beta_0 + \beta_1 \text{Grazing System} + \beta_2 \text{HerdSizeCategory} + \beta_3 \text{Site} + \beta_k X_k + \dots + \varepsilon$$

where Y = binary outcome variable, β_0 = intercept term, β_k = regression coefficient, X_k = predictor variable and ε = error term.

The LSPC Models were analyzed by linear regression, using PROC MIXED (SAS Institute, Inc., 2008). The general form of the linear model used for the LSPC models is formulated as:

$$\text{LSPC} = \beta_0 + \beta_1 \text{Grazing System} + \beta_2 \text{Herd Size Category} + \beta_3 \text{Site} + \beta_k X_k + \dots + \varepsilon$$

where β_0 = intercept term, β_k = regression coefficient, X_k = predictor variable and ε = error term.

Model selection was performed in 3 steps. First, each predictor variable was assessed for unconditional associations by logistic regression for CC or the presence of *S. aureus*, or by linear regression for LSPC. Then, unconditionally associated variables (using a cutoff of $P \leq 0.25$) were separated into groups (general farm characteristics, general management characteristics) and were included for selection for multivariable sub-models. Predictor variables with a $P \leq 0.25$ were selected for inclusion in the final model, in addition to the design variables (grazing system, herd size category and site) that were always included in the multivariable models. Finally, all predictor variables that remained in the sub-models were selected for a final multivariable model, analyzed using PROC LOGISTIC or PROC MIXED (SAS Institute, Inc., 2008). The final multivariable model was constructed using backward selection techniques, with the design variables grazing system, site and herd size category forced into the model. Predictor variables significant at $P \leq 0.1$ and all design variables remained in the final multivariable model. The

model building process used for the organic herd models were identical to the process used for building the total herd models, excluding grazing system as a design variable, and including the specified grazing variables for analysis.

RESULTS

Descriptive Outcome Variable Results. Descriptive information of the outcome variables is shown in Table 4.1. The geometric mean of the SPC (x 1,000 cfu/mL) was 5.19, with a range of 1 – 3981 cfu/mL. A total of 55% of all herds had a bulk tank that was positive for *S. aureus*. 62% of all ORG herds, compared with 41% of CON-NG and 42% of CON-GR herds were positive for *S. aureus*. 29% of CON-NG and 25% of CON-GR farms had a high CC, compared with 12% of ORG herds.

Table 4.1. Descriptive information about outcome variables SPC (the back transform of \log_{10} cfu/mL using the antilog), the presence of *Staphylococcus aureus* (*S. aureus*) and coliform counts from 290 bulk tank milk samples from March 2009 to May 2011. Samples were from conventional non-grazing (CON-NG; n = 63), conventional grazing (CON-GR; n = 36) and organic (ORG; n = 191) in New York, Oregon and Wisconsin.

Outcome Variable	Mean	SD	Range	95% Confidence Interval
SPC (x 1,000 cfu/mL)	5.19	4.39	1 – 3981	4.38 – 6.15
<i>S. aureus</i>	% positive All herds	% positive CON-NG	% positive CON-GR	% positive ORG
	55%	41%	42%	62%
Coliform	% of all herds with high CC ¹	% high CC ¹ CON-NG	% high CC ¹ CON-GR	% high CC ¹ ORG
	17%	29%	25%	12%

¹ High coliform counts are defined as bulk tanks with coliform counts ≥ 50 cfu/mL.

Total Herd SPC Model Building. General farm characteristics that were unconditionally associated with LSPC in the total herd subset were the age of housing ($P = 0.16$), percent problem breeders ($P = 0.09$), milk production per cow per day ($P = 0.01$), a positive *S. aureus* bulk tank culture ($P = 0.18$), average reported SCC ($P < 0.001$) and average reported SPC ($P < 0.001$). General management variables that were unconditionally associated with LSPC were estimated calving interval ($P = 0.05$), mean body condition score ($P = 0.005$), mean udder hygiene score ($P = 0.23$), any use of AI in non-lactating heifers ($P = 0.005$), any use of AI for lactating cows ($P = 0.02$), keeping written records of herd health events ($P = 0.19$) and treatments ($P = 0.04$), transferring cows to an alternate farm ($P = 0.23$), bringing heifers or adult cows onto the farm from outside sources ($P = 0.15$), the percentage of animals with 1 or more teats segregated from the bulk tank ($P = 0.23$), the number of milkers on the farm ($P = 0.01$) and the EIS ($P = 0.08$). Variables that were selected for final model inclusion prior to backward stepwise regression were milk production per cow per day, average reported SCC, estimated calving interval, mean body condition score, written treatments, transferring cows, number of milkers and the EIS.

Final SPC Total Herd Model. Backwards stepwise logistic regression modeling resulted in a model that, in addition to the forced design variables (grazing system, herd size category and location), associated 4 predictor variables with LSPC (Table 4.2). A higher average reported SCC was associated with an increased LSPC ($P = 0.004$). Higher milk production was associated with a lower LSPC ($P < 0.001$). Farms that had a lower bulk tank LSPC tended to have cows with a higher mean body condition score ($P = 0.001$). As the number of milkers on the farm increased, the LSPC increase ($P = 0.03$).

Table 4.2. Final total herd linear regression model showing the association of significant variables ($P \leq 0.1$) with the \log_{10} transformation of SPC (cfu/mL) among conventional non-grazing (CON-NG; $n = 59$), conventional grazing (CON-GR; $n = 35$), and organic (ORG; $n = 173$) farms. Grazing system, location (New York, Wisconsin and Oregon), and herd size were included in the model as design variables. Only farms with complete information were included in the analysis. Estimates provided are the β coefficient for the given variable.

Variable	Level	Estimate	Final model P -value
Intercept		2.29 (0.49)	<0.001
Grazing System	CON-NG	0.53 (0.11)	<0.001
	CON-GR	0.19 (0.12)	
	ORG	Reference	
Location	NY	0.12 (0.14)	0.59
	WI	0.05 (0.13)	
	OR	Reference	
Herd Size	≥ 200 cows	0.24 (0.13)	0.04
	100 – 199 cows	0.25 (0.11)	
	20 – 99 cows	Reference	
Milk production per cow per day (kg)	Continuous	- 0.03 (0.01)	< 0.001
Average reported SCC from 3 months prior to herd visit	Continuous	0.01 (0.01)	0.004
Average body condition score	Continuous (1 – 5)	- 0.53 (0.16)	0.001
Number of milkers	Continuous	0.10 (0.05)	0.034

Organic Herd SPC Model Building. General farm characteristics that were associated with LSPC in the organic subset of data were the age of housing ($P = 0.05$), the number of years in the dairy industry ($P = 0.13$), percentage of problem breeders ($P = 0.14$), the amount of milk produced per cow per day ($P < 0.001$), the average reported SPC ($P < 0.001$), and the average reported SCC ($P < 0.001$). The general management characteristics that were associated with LSPC in the organic subset were estimated calving interval ($P = 0.13$), the mean body condition score ($P = 0.01$), the mean udder hygiene score ($P = 0.1$), the mean hock score ($P = 0.06$), any use of AI in non-lactating heifers ($P = 0.002$), any use of AI in lactating cows ($P = 0.01$), the use of a segregation unit or bucket milker for mastitis cows ($P = 0.1$), the number of people treating

mastitis on the farm ($P = 0.02$), a written record of treatments ($P = 0.19$), the percentage of the herd with 1 or more teats segregated from the bulk tank ($P = 0.04$), the number of times milking per day ($P = 0.12$) and the EIS ($P = 0.03$). Variables that were selected for final model inclusion prior to backward stepwise regression were the age of housing, years in the dairy industry, milk production, average reported SCC, percent of problem breeders, mean body condition score, mean udder hygiene score, mean hock score, use of a segregation unit, number treating mastitis cases and the EIS.

Final SPC Organic Herd Model. Backwards stepwise logistic regression modeling resulted in a model that, in addition to the forced design variables (herd size category and location), associated 7 predictor variables with LSPC (Table 4.3). Higher mean body condition was associated with a lower LSPC ($P = 0.08$), while a higher mean udder hygiene score was associated with a higher LSPC ($P = 0.07$). Use of a segregation unit or a bucket milker for milking mastitis cows was associated with a lower LSPC ($P = 0.09$). As the number of people on the farm treating mastitis increase, the LSPC increased ($P = 0.03$). Higher milk production was associated with a decrease in LSPC ($P = 0.001$). A higher LSPC was associated with a higher average reported SCC ($P = 0.002$).

Table 4.3. Final organic herd linear regression model showing the association of significant variables ($P \leq 0.1$) with the \log_{10} transformation of SPC (cfu/mL) among organic farms ($n = 173$). Location (New York, Oregon and Wisconsin) and herd size were included in the model as design variables. Only farms with complete information were included in the analysis. Estimates provided are the β coefficient for the given variable.

Variable	Level	Estimate (SE)	Final model P -value
Intercept		1.55 (0.63)	0.02
Location	NY	0.06 (0.19)	0.09
	WI	- 0.23 (0.18)	
	OR	Reference	
Herd Size	≥ 200 cows	0.11 (0.18)	0.41
	100 – 199 cows	- 0.14 (0.15)	
	20 – 99 cows	Reference	
Average body condition score	Continuous (1 – 5)	- 0.32 (0.18)	0.08
Average udder hygiene score	Continuous (1 – 5)	0.18 (0.10)	0.07
Use of a segregation unit or bucket milker for mastitis cows	Yes vs. No	- 0.17 (0.10)	0.09
Number of people treating cases of mastitis in a given month	Continuous	0.09 (0.04)	0.03
Age of housing (years)	Continuous	- 0.01 (0.01)	0.08
Milk production per cow per day (kg)	Continuous	- 0.03 (0.01)	0.001
Average reported SCC from 3 months prior to herd visit	Continuous	0.01 (0.01)	0.002

Total Herd *S. aureus* Model Building. General farm characteristic variables that were unconditionally associated with the presence of *S. aureus* in the bulk tank in the total herd subset of data were milk production per cow per day ($P = 0.05$), average reported SCC ($P < 0.001$), age of housing ($P < 0.001$), the primary milking facility on the farm ($P < 0.001$) and the percentage of 1st lactation cows on the farm ($P = 0.004$). General management characteristics that were unconditional associated with the presence of *S. aureus* in the bulk tank were the mean hock score ($P = 0.03$), any use of AI in non-lactating heifers ($P = 0.01$), any use of AI in lactating cows ($P = 0.04$), the use of a dedicated calving area ($P = 0.003$), the number of people treating mastitis ($P = 0.03$), written treatment records ($P = 0.03$), regular veterinarian visits ($P = 0.002$), vaccinations of cows ($P = 0.01$) and calves ($P = 0.005$), keeping sick cows separate from the rest of the herd ($P = 0.04$), the percentage of herd with 3 or fewer teats ($P = 0.01$), the use of pre-dip

($P = 0.03$) and post-dip ($P = 0.05$) during milking, the use of automatic take offs ($P < 0.001$), feeding a TMR ($P = 0.002$), the EIS ($P < 0.001$) and the amount of grain fed ($P = 0.009$).

Variables that were selected for final model inclusion prior to backward stepwise regression were the average reported SCC, the percentage of 1st lactation cows on the farm, the age of housing, the mean hock score, primary milking facility, use of automatic take offs, the number of people treating mastitis, regular veterinarian visits, the percentage of cows with 3 or fewer teats and feeding a TMR.

Final S. aureus Total Herd Model. Backwards stepwise logistic regression modeling resulted in a model that, in addition to the forced design variables (grazing system, herd size category and location), associated 6 predictor variables with a bulk tank that were cultures positive for *S. aureus* (Table 4.4). A positive *S. aureus* bulk tank milk culture result was associated with a higher reported SCC ($P < 0.001$), a lower percentage of 1st lactation cows on the farm ($P = 0.02$), older housing ($P = 0.01$), a higher mean hock score of scored adult animals ($P = 0.02$), a lower likelihood of using automatic takeoffs ($P = 0.07$), and a higher percentage of the herd with 3 or fewer teats ($P = 0.09$).

Table 4.4. Final total herd logistic regression model showing the association of significant variables ($P \leq 0.1$) and bulk tanks that had *Staphylococcus aureus* cultured on conventional and organic farms. Grazing system (conventional non-grazing (CON-NG; n = 59), conventional grazing (CON-GR; n = 35), and organic (ORG; n = 173)), location (New York, Oregon and Wisconsin) and herd size were included in the model as design variables. Only farms with complete information were included in the analysis. Estimates provided are the β coefficient for the given variable.

Variable	Level	Estimate (SE)	Odds Ratio ¹	95% odds ratio confidence limit	Final model <i>P</i> -value
Intercept		- 3.42 (1.13)			0.01
Grazing system	CON-NG	- 0.68 (0.40)	0.51	0.23 – 1.10	0.19
	CON-GR	- 0.47 (0.47)	0.62	0.25 – 1.57	
	ORG	Reference			
Location	NY	0.73 (0.55)	2.07	0.70 – 6.08	0.36
	WI	0.76 (0.54)	2.14	0.74 – 6.20	
	OR	Reference			
Herd Size	≥ 200 cows	0.72 (0.54)	2.05	0.71 – 5.94	0.27
	100 – 199 cows	- 0.23 (0.47)	0.80	0.32 – 2.02	
	20 – 99 cows	Reference			
Average reported SCC	Continuous	0.01 (0.01)	1.01	1.00 – 1.01	< 0.001
Percent of 1 st Lactation cows	Continuous	- 0.03 (0.01)	0.97	0.94 – 0.99	0.02
Age of Housing	Continuous	0.01 (0.01)	1.01	1.00 – 1.02	0.01
Mean Hock Score	Continuous	1.63 (0.67)	5.11	1.37 – 18.99	0.02
Use of automatic takeoffs	Yes vs. No	- 0.62 (0.34)	0.54	0.28 – 1.04	0.07
Percent of animals with 3 or fewer teats	Continuous	0.05 (0.03)	1.05	0.99 – 1.11	0.09

¹Odds of having a *Staphylococcus aureus* positive bulk tank.

Organic Herd *S. aureus* Model Building. General farm characteristic variables that were unconditionally associated with the presence of *S. aureus* in the bulk tank in the organic herd subset of data were age of housing ($P = 0.004$), average reported SCC ($P < 0.001$) and the primary milking facility on the farm ($P = 0.002$). General management characteristics that were unconditionally associated with the presence of *S. aureus* in the bulk tank in the organic herd subset of data were the mean body condition score ($P = 0.15$), mean hock score ($P = 0.15$), any use of AI for non-lactating heifers ($P = 0.05$), any use of AI for lactating adult cows ($P = 0.15$),

the number of people treating mastitis ($P = 0.02$), regular visits from the veterinarian ($P = 0.03$), vaccinations of adult cows ($P = 0.08$) and calves ($P = 0.05$), regular checks of postpartum cows ($P = 0.25$), transferring cows to an alternate farm ($P = 0.05$), percentage of the herd with 3 or fewer teats ($P = 0.01$), use of rotational grazing ($P = 0.24$), the use of a nutritionist ($P = 0.19$), use of a pre-dip ($P = 0.13$), use of a post-dip ($P = 0.21$), the number of units used during milking ($P = 0.11$), use of automatic take offs ($P = 0.008$), and the EIS ($P = 0.01$). Grazing specific variables that were unconditionally associated with the presence of *S. aureus* in the bulk tank in the organic subset were the number of acres on the farm ($P = 0.13$), the total percentage of DMI from pasture ($P = 0.18$), and the use of anionic salts in transition cow diets ($P = 0.25$). Variables that were selected for inclusion prior to backward stepwise regression were the average reported SCC, age of housing, primary milking facility, use of automatic take offs, number of milking units, mean body condition score, mean hock score, any use of AI in non-lactating heifers, regular veterinarian visits, percentage of the herd with 3 or fewer teats, number of people treating mastitis and transferring cows to an alternate farm.

Final S. aureus Organic Herd Model. Backwards stepwise logistic regression modeling resulted in a model that, in addition to the forced design variables (size category and location), associated 4 predictor variables with a bulk tank that cultured positive for *S. aureus* (Table 4.5). A positive *S. aureus* bulk tank milk culture result was associated with a higher reported SCC ($P < 0.001$), older housing for adult animals ($P = 0.03$), a higher percentage of the herd with 3 or fewer teats ($P = 0.03$), and fewer people on the farm treating mastitis ($P = 0.05$).

Table 4.5. Final organic herd logistic regression model showing the association of significant variables ($P \leq 0.1$) and bulk tanks that had *Staphylococcus aureus* cultured on organic farms (n = 173). Location (New York, Oregon and Wisconsin) and herd size were included in the model as design variables. Only farms with complete information were included in the analysis. Estimates provided are the β coefficient for the given variable.

Variable	Level	Estimate (SE)	Odds ratio ¹	95% odds ratio confidence limit	Final model P -value
Intercept		- 2.90 (0.97)			0.002
Location	NY	0.94 (0.72)	2.55	0.62 – 10.52	0.39
	WI	0.60 (0.71)	1.83	0.46 – 7.33	
	OR			Reference	
Herd Size	≥ 200 cows	0.35 (0.68)	1.41	0.37 – 5.41	0.78
	100 – 199 cows	- 0.20 (0.59)	0.82	0.26 – 2.58	
	20 – 99 cows			Reference	
Average reported SCC	Continuous	0.01 (0.01)	1.01	1.00 – 1.01	< 0.001
Age of Housing	Continuous	0.01 (0.01)	1.01	1.00 – 1.02	0.03
Percentage of the herd with 3 or fewer teats	Continuous	0.08 (0.04)	1.08	1.01 – 1.16	0.03
Number of people treating mastitis in a typical month	Continuous	- 0.32 (0.16)	0.73	0.53 – 1.00	0.05

¹Odds of having a *Staphylococcus aureus* positive bulk tank.

Total Herd Coliform Model Building. General farm characteristic variables that were unconditionally associated with a high CC in the bulk tank in the organic herd subset of data were the age of housing ($P = 0.19$), the number of years in the dairy industry ($P = 0.1$), the presence of *S. aureus* in the bulk tank ($P = 0.11$), average reported SPC ($P = 0.003$), and the average reported SCC ($P < 0.001$). The general management variables that were unconditionally associated with a high CC were mean udder hygiene score ($P = 0.02$), use of AI in non-lactating heifers ($P = 0.13$), any use of AI in lactating adult cows ($P = 0.19$), keeping a written record of treatments ($P = 0.01$), vaccinations of adult cows ($P = 0.17$) and calves ($P = 0.11$), use of a California Mastitis Test ($P = 0.25$), use of gloves during milking ($P = 0.17$), the number of milkers on the farm ($P = 0.08$) and feeding a TMR ($P = 0.06$). Variables that were selected for inclusion prior to backward stepwise regression were age of housing, years dairying, the

presence of *S. aureus*, average reported SPC, average reported SCC, mean udder hygiene score, any use of AI in non-lactating heifers, use of gloves during milking, feeding a TMR and the number of milkers on the farm.

Final Total Herd Coliform Model. Backwards stepwise logistic regression modeling resulted in a model that, in addition to the forced design variables (size category, grazing system and location), associated 4 predictor variables with a bulk tank that had a high CC (Table 4.6). Any use of AI in non-lactating heifers was associated with a lower CC ($P = 0.02$). Feeding TMR was associated with a high CC ($P = 0.081$). High CC was associated with a lesser likelihood of culturing *S. aureus* from the bulk tank ($P = 0.01$). High CC was also associated with higher reported SCC ($P < 0.001$).

Table 4.6. Final total herd logistic regression model showing the association of significant variables ($P \leq 0.1$) and bulk tanks that had a high coliform count (≥ 50 cfu/mL). Grazing system (conventional non-grazing (CON-NG; $n = 59$), conventional grazing (CON-GR; $n = 35$), and organic (ORG; $n = 173$)), location (New York, Oregon and Wisconsin) and herd size were included in the model as design variables. Only farms with complete information were included in the analysis. Estimates provided are the β coefficient for the given variable.

Variable	Level	Estimate (SE)	Odds ratio ¹	95% odds ratio confidence limit	Final model P -value
Intercept		- 3.61 (0.87)			< 0.001
Grazing System	CON-NG	0.92 (0.47)	2.51	1.01 – 6.24	0.03
	CON-GR	1.28 (0.56)	3.59	1.20 – 10.77	
	ORG			Reference	
Location	NY	- 0.27 (0.73)	0.76	0.19 – 3.16	0.22
	WI	0.52 (0.68)	1.69	0.45 – 6.40	
	OR			Reference	
Herd Size	≥ 200 cows	0.42 (0.62)	1.52	0.45 – 5.13	0.54
	100 – 199 cows	- 0.38 (0.60)	0.69	0.21 – 2.24	
	20 – 99 cows				
Any use of AI in non-lactating heifers	Yes vs. No	- 1.00 (0.43)	0.37	0.16 – 0.86	0.02
Feeding a TMR	Yes vs. No	0.79 (0.45)	2.20	0.91 – 5.33	0.08
Presence of <i>S. aureus</i> in the bulk tank	Yes vs. No	- 1.00 (0.40)	0.37	0.17 – 0.81	0.01
Average reported SCC	Continuous	0.01 (0.01)	1.01	1.00 – 1.01	< 0.001

¹Odds of having a high coliform count in the bulk tank (≥ 50 cfu/mL).

Organic Herd Coliform Model Building. General farm characteristic variables that were unconditionally associated with a high CC in the bulk tank in the organic herd subset of data were the age of housing ($P = 0.14$), the number of years in the dairy industry ($P = 0.14$), milk production per cow per day ($P = 0.1$), the primary milking facility ($P = 0.13$), average reported SPC ($P = 0.02$), percent of 1st lactation cows in the herd ($P = 0.22$) and average reported SCC ($P = 0.03$). General management variables that were unconditionally associated with a high CC in the bulk tank in the organic herd subset of data were the number of units used during milking ($P = 0.14$), use of a nutritionist ($P = 0.19$), feeding a TMR ($P = 0.04$), the number of milkers on the farm ($P = 0.07$), keeping sick cows separate from the rest of the herd ($P = 0.23$), keeping a

written record of all treatments ($P = 0.08$), the number of people treating mastitis ($P = 0.13$), any use of AI on non-lactating heifers ($P = 0.16$), and the mean udder hygiene score ($P = 0.04$).

Variables that were selected for inclusion prior to backward stepwise regression were the primary milking facility, mean udder hygiene score, age of housing, number of years in the dairy industry, average reported SPC, average reported SCC, percent of 1st lactation cows on the farm, any use of AI in non-lactating heifers, use of a written record of all treatments, the number of milkers on the farm, feeding a TMR and use of a nutritionist.

Final Organic Herd Coliform Model. Backwards stepwise logistic regression modeling resulted in a model that, in addition to the forced design variables (size category and location), associated 8 predictor variables with a high bulk tank CC (Table 4.7). Lower CC was associated with any use of AI in non-lactating heifers ($P = 0.02$), having a written record of treatments ($P = 0.02$), and using a nutritionist ($P = 0.07$). As the age of housing and the number of years spent dairying increased, the CC also increased ($P = 0.05$ and $P = 0.03$, respectively). High CC was also associated with higher average reported SPC ($P = 0.04$), a lower percentage of 1st lactation cows on the farm ($P = 0.05$) and feeding a TMR ($P = 0.003$).

Table 4.7. Final organic herd logistic regression model showing the association of significant variables ($P \leq 0.1$) and bulk tanks that had a high coliform count (≥ 50 cfu/mL) in the organic population ($n = 173$). Location (New York, Oregon and Wisconsin) and herd size category were included in the model as design variables. Only farms with complete information were included in the analysis. Estimates provided are the β coefficient for the given variable.

¹Odds of having a high coliform count in the bulk tank (≥ 50 cfu/mL).

Variable	Level	Estimate	Odds ratio ¹	95% odds ratio confidence limit	Final model <i>P</i> -value
Intercept		- 0.58 (1.76)			0.74
Location	NY	- 0.42 (1.13)	0.66	0.17 – 6.00	0.65
	WI	0.19 (1.03)	1.21	0.16 – 9.11	
	OR	Reference		Reference	
Herd Size	≥ 200 cows	0.17 (0.97)	1.18	0.18 – 7.95	0.05
	100 – 199 cows	- 1.53 (1.00)	0.22	0.03 – 1.55	
	0 – 99 cows	Reference		Reference	
Age of housing	Continuous	- 0.02 (0.01)	0.98	0.96 – 1.00	0.05
Years dairying	Continuous	0.06 (0.03)	1.06	1.01 – 1.12	0.03
Mean reported SPC	$\geq 33,000$ cfu/mL	2.32 (0.93)	10.24	1.65 – 63.46	0.04
	8,000 – 32,000 cfu/mL	1.33 (0.84)	3.77	0.73 – 19.48	
	$<1 - 7,000$ cfu/mL	Reference		Reference	
Percent 1st lactation cows	Continuous	- 0.06 (0.03)	0.95	0.90 – 1.00	0.05
Any use of AI in non-lactating heifers	Yes vs. No	- 1.49 (0.63)	0.23	0.07 – 0.77	0.02
Written record of treatments	Yes vs. No	- 1.63 (0.67)	0.20	0.05 – 0.73	0.02
Total mixed ration fed	Yes vs. No	2.26 (0.76)	9.61	2.18 – 42.32	0.003
Use of Nutritionist	Yes vs. No	- 1.26 (0.69)	0.28	0.07 – 1.09	0.07

DISCUSSION

This study was an element of a larger project with the principal goal of assessing the management practices, herd health and characteristics of conventional and organic dairy farms in New York, Oregon and Wisconsin. The purpose of this report is to determine the impact of selected management variables on bulk tank SPC, CC and *S. aureus* presence in the bulk tank.

All ORG farms in our study were matched with CON farms of similar size and location, allowing comparisons to be as precise as possible. A varied range of management methods, and consequently milk quality results, are represented in our population. Approximately 30% of the

total number of organic dairy farms across the three states was visited, representing a large portion of the organic dairy industry. This study was primarily a cross-sectional study, and we recognize that there are limitations to this method of data collection and research. While the study has solid internal validity, interpreting the results and applying to a group outside of the study demographic should be done with care, with the study design and population in mind. The cross-sectional nature of the study does not allow us to extrapolate information about the organic industry over an extended period of time. In addition to this, due to the large dataset size and our liberal significance value of $P \leq 0.1$, there is a risk of finding inaccurate results is greater. We regard our study as well designed, but also we acknowledge these potential pitfalls.

A common variable through all the models, with the exception of the organic CC model, was the average reported SCC from 3 months prior to the herd visit. It may be debated whether high SCC was influencing the increase in LSPC, *S. aureus* and CC, or whether the increase of bacteria as measured by these parameters was driving the SCC increase. To assess this, alternative models were built with SCC removed and compared to the original models. With the exception of the total herd CC model, there were no significant changes in the remaining models. In the total herd CC model, when SCC was removed, the use of gloves during milking, the number of years in the dairy industry and average reported SPC remained in the model, all of which are risk factors for high SCC. Therefore, SCC was left in as a predictor variable for all models, except the total herd CC model. Figure 4.1 shows the association of the bulk tank \log_{10} SCC (x 1,000 cells/mL) with LSPC (x 1,000 cfu/mL), indicating that a low SCC and low SPC have a clear relationship.

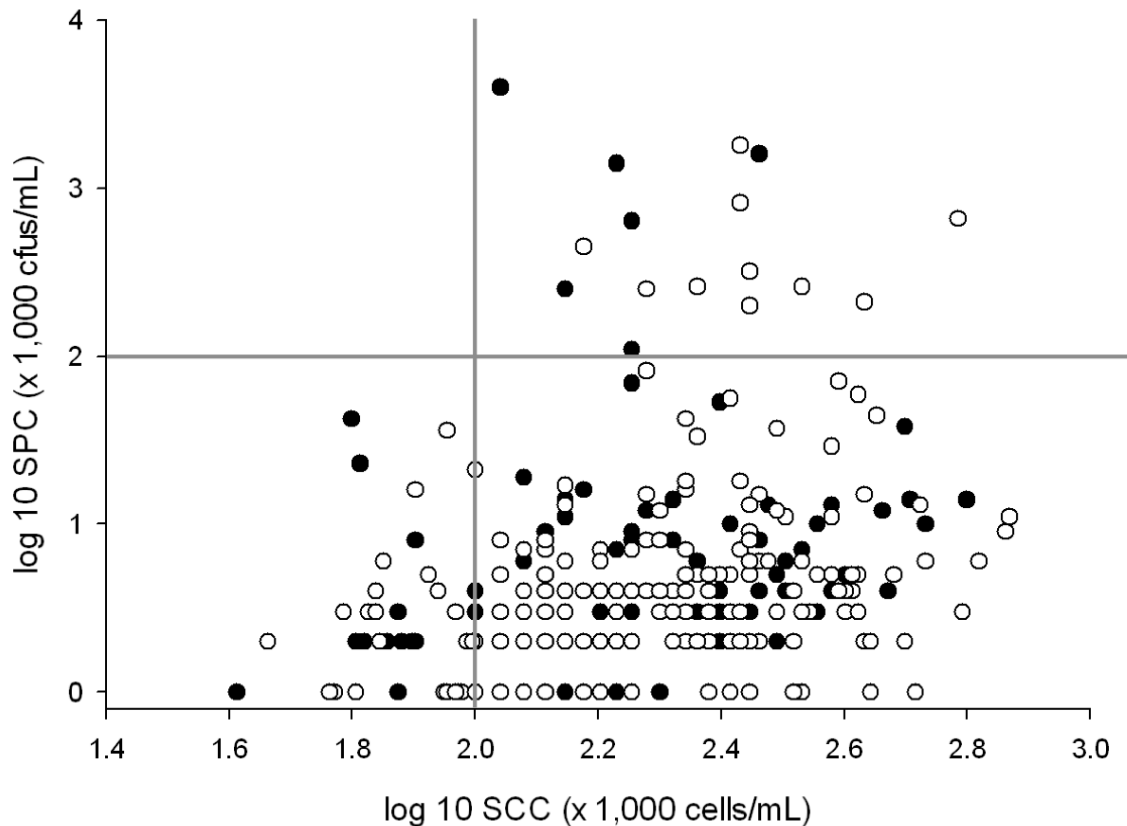


Figure 4.1. Scatterplot showing the relationship of bulk tank $\log_{10}\text{SCC}$ ($\times 1,000$ cells/mL) and $\log_{10}\text{SPC}$ in organic (white circles; $n = 190$) and conventional (black circles; $n = 100$) dairy farms in New York, Oregon and Wisconsin from March 2009 to May 2011.

The models represented in this manuscript were built based on results from previous analysis from the same project (Stiglbauer, Cicconi-Hogan and Richert et al., in press; Cicconi-Hogan et al., submitted for publication). Previously, CC and *S. aureus* were found to be different among grazing systems in univariate analyses, and were natural choices for further model building. Each model had a unique set of variables that presented an association with the various outcome parameters, but general management and management precision was the overarching theme that was common to them all. Both the organic and total herd LSPC models presented an association of high LSPC with lower milk production, higher SCC and lower mean body

condition scores. A higher SCC, a lower mean body condition score and low milk production are likely the result of a high LSPC, as the higher bacterial counts are potentially associated with more intramammary infections, many of which cause systemic effects, such as a decrease in body condition and milk production (Zadoks et al., 2011; De Vliegher et al., 2012). Alternatively, a high LSPC and under-conditioned cows may both be associated with inadequate management and a less clean environment. From the information gathered, the causality is uncertain. The associated increase in milkers with increase LSPC in the total herd model may be a result of inconsistency among larger numbers of milkers, resulting in less precision or consistency in milking procedures. Similarly, previous research has shown that frequent use of part time milkers on a farm increases the risk of residues in the milk (McEwen et al., 1991), supporting the conclusion that consistency in milking is important to milk quality. The total herd model also indicated that LSPC is significantly lower in the ORG population than conventional farms. The organic LSPC model showed that the ORG community has its own challenges, as higher udder hygiene scores, more people on the farm treating or managing mastitis cases and less usage of a segregation unit or bucket milker were associated with higher LSPC. These variables are likely indicative of the precision of management on the farm, as dirty udders, a lack of milk segregation and possible inconsistency in treatment or management protocols among large numbers of people on the farm could cause increased bacterial load. Improvement in these areas, for example through written standard operating procedures, would likely result in a decrease in the SPC of the milk, and subsequently, result in better milk quality.

The total herd and organic models assessing the associations with the presence of *S. aureus* showed an increased SCC, older housing and a higher percentage of adult animals with 3 or fewer teats on the farm in common. Increased SCC was associated with the presence of *S.*

aureus infections, so the association was expected, and further supported by Figure 4.2, showing the relationship of bulk tank SCC to the presence of *S. aureus*.

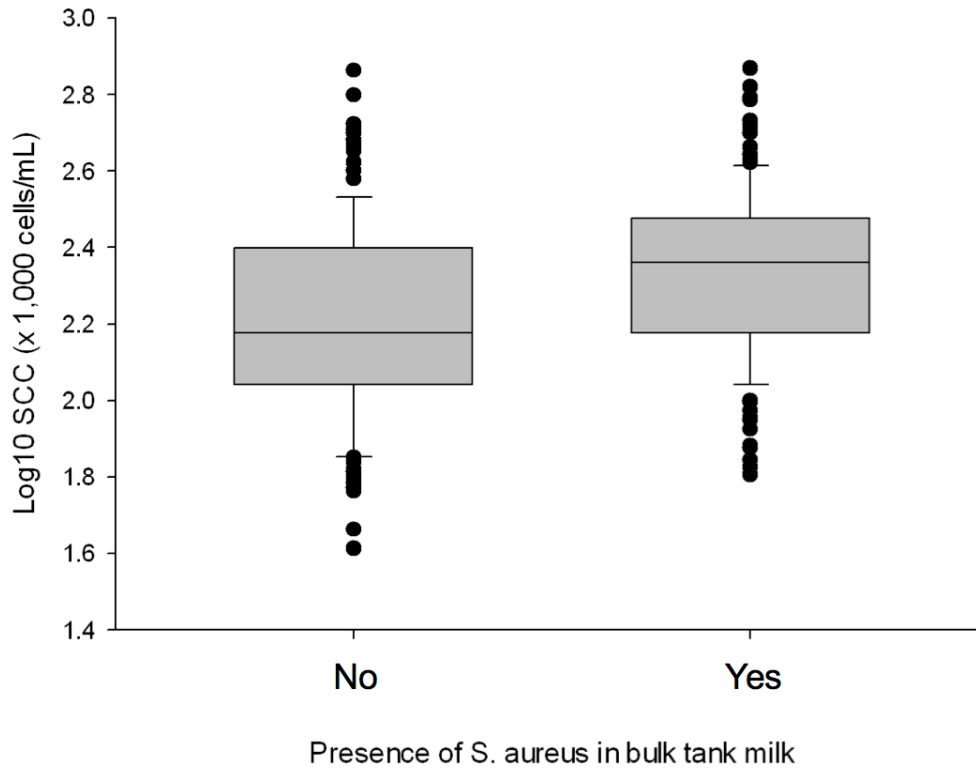


Figure 4.2. Box plot of the relationship between the presence of *Staphylococcus aureus* (*S. aureus*) and the log₁₀ SCC (x 1,000 cells/mL) in the bulk tank milk of 190 organic and 100 conventional dairy farms in New York, Oregon and Wisconsin from March 2009 to May 2011.

Older housing may be more difficult to keep clean, be associated with older milking equipment, and therefore allow the *S. aureus* bacteria to propagate more easily than in newer housing. The percentage of animals with 3 or fewer teats on the farm is likely representative of a herd that has known mastitis problem and is drying off teats from specific problem animals as a form of mastitis management. In the total herd model, the association of low numbers of 1st lactation animals on the farm with *S. aureus* in the bulk tank is indicative of more older animals in the herd, who are more likely to be chronically infected with *S. aureus* and more difficult to cure than their younger herd-mates (De Vliegher et al., 2012; Barkema et al., 2006). Higher mean hock scores, which are representative of lesions and dirty hocks may relate to more skin infections with *S. aureus* and transmission of these infections to the udder. The lack of automatic takeoffs were associated with *S. aureus* presence in the model, and may indicate over-milking, which is likely without take-offs, and is related to intramammary infections (Hillerton et al., 2002) such as *S. aureus*. The organic *S. aureus* model had only one variable that was not included in the total herd model. In the organic subset, fewer people treating mastitis on the farm was associated with the presence of *S. aureus* in the bulk tank, which may simply be a product of the lack of mastitis treatments for organic farms.

The total herd and organic herd coliform models shared only two variables. Using only natural service to breed heifers and feeding a TMR were associated with a higher CC in both models. The use of some AI to breed animals is likely a proxy for attentive management practices and use of external support, such as a nutritionist, (Cicconi-Hogan et al., submitted for publication) and not directly or causally related to an increased CC. In the total herd model, a high CC was associated with a lower odds of being positive for *S. aureus*. This negative correlation between *S. aureus* and coliform count is not unexpected, as herds with a contagious

mastitis problem due to *S. aureus* are less likely to also harbor a problem with coliform intramammary infections (Barkema et al., 1998). Organic farms had significantly lower CC, indicating that the ORG population was not as susceptible to coliform problems as the conventional population. However, as seen in the organic CC model, it is clear that the organic population that does have coliform problems has its own set of risk factors. More years in the dairy industry has been a fairly consistent finding in our data with regard to lower milk quality, as seen in Cicconi-Hogan et al. (submitted for publication), and we believe this is indicative of younger farmers, or farmers new to the dairy industry, being more assertive in their management and treatments of the herd. However, the regression coefficient is quite small, as we found previously, and likely does not predict a major change in CC. An increase in average reported SPC was associated with a high CC, which may be expected, as both parameters can be representative of possible contamination of the bulk tank from outside sources or from intramammary infections. Lack of written records of treatments and not using a nutritionist are both suggestive of less precise or intensive management of the farm, which could lead to higher CC. The association of newer housing and a high coliform count is an unexpected outcome, and we believe that it may be a variable that ended up in the model due to chance. While the average reported SCC did not have a clear association with high CC in the organic model, there was an association in the total herd model, supported further by Figure 4.3.

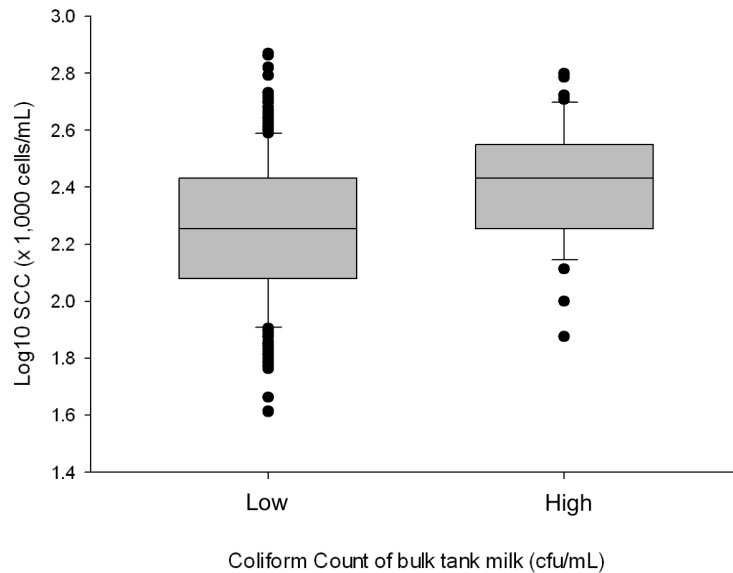


Figure 4.3. Box plot of the relationship between coliform counts and the \log_{10} SCC (x 1,000 cells/mL) in the bulk tank milk of 190 organic and 100 conventional dairy farms in New York, Oregon and Wisconsin from March 2009 to May 2011. Low coliform counts were defined as < 50 cfu/mL, high coliform counts were defined as ≥ 50 cfu/mL.

Overall, the results from this manuscript, in conjunction with previous results from the project, indicate that management practices are of the utmost importance to milk quality on both organic and conventional dairy farms. Somatic cell counts are associated with SPC, *S. aureus* and CC in our models, indicating that many of the parameters used to measure milk quality are connected. It is useful to farmers to monitor several of the parameters, to get an overall picture of their milk quality, which has a direct effect on the pay price of the milk, and thus, the economic success of the dairy operation. As expected, mastitis management had an impact on milk quality, as variables such as the number of people treating mastitis, udder hygiene, the number of people milking on the farm and the percentage of the herd with 3 or fewer teats were a common theme across several of the models. Both organic and conventional farms could benefit from more attentive management of their mastitis cases and keeping consistent records on milk quality. Both of these suggestions would engage the farmer more heavily in the health of the animals, as well as improve the bulk tank milk quality.

Our research indicates that there is no single set of variables that can predict good milk quality. However, there are many simple changes and management procedures that can contribute to bettering the milk on a farm. Both the organic and conventional farming communities can benefit from the results of this research, as this manuscript contains information that can be used to enhance milk quality improvement programs for the entire dairy industry.

CONCLUSION

The need for more research on organic dairy production and management is largely consumer driven. While our previous research has shown that the differences between organic

and conventional dairy production systems are not profound, the quest for high quality milk requires different management choices depending on said production system.

There was not one single set of variables that predicted a high LSPC, the presence of *S. aureus* or a high CC. Attention to mastitis and mastitis management was an overarching theme across most of the models, although the variables in the models were often different. The EIS score, which was found to have an impact on SCC in previous, did not appear to have an effect on the outcome variables presented here. The average reported SCC, however, did come out in most of the models, indicating that there is a clear relationship between various parameters of milk quality assessment. Both the organic and conventional dairy communities could benefit from more intense mastitis management and consistent milk quality monitoring.

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CHAPTER FIVE

A phenotypic and genotypic approach to assessing the presence of methicillin resistance and a *mecA* homologue in coagulase-negative *Staphylococcus* species and *Staphylococcus aureus* in bulk tank milk samples from organic and conventional dairy herds in New York, Oregon and Wisconsin.

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ABSTRACT

The objective of this study was to evaluate the presence of methicillin resistance in bulk tank milk samples from 288 organic and conventional dairy farms located in New York, Wisconsin and Oregon from March 2009 to May 2011. Bulk tank samples were collected directly from the bulk tanks with a sterile sampler and shipped to Quality Milk Production Services in Ithaca, NY for testing. The goal was to assess the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative *Staphylococcus* (MR-CNS) in the studied population. Due to recent publications reporting the presence of a *mecA* homologue that would not be detected by traditional *mecA*-based PCR methods, a combination of genotypic and phenotypic approaches were used to enhance the recovery of methicillin-resistant organisms as much as possible. For genotypic identification, we used the traditional *mecA* and *nuc* PCR. For phenotypic identification, individual colonies were identified on solid media plates following a two-step enrichment method and plating on a selective media, MRSASelect, to identify methicillin resistant organisms. In total, 14 isolates were identified as being methicillin-resistant: *S. aureus* (n = 1), *S. sciuri* (n = 6), *S. chromogenes* (n = 2), *S. saprophyticus* (n = 3), *S. agnetis* (n = 1), and *Macrococcus caseolyticus* (n = 1). The single MRSA isolate was identified from an organic farm in New York, for an observed 0.3% prevalence at the farm level. The MR-CNS prevalence was 4% in the organic population and 7% in the conventional population. We did not identify the tested *mecA* homologue in any of the isolates from our population. One of the isolates recovered was identified as *S. agnetis*, a new species published in late 2012, isolated from European dairy cows with subclinical or clinical mastitis. Too few isolates have been processed to understand whether the methicillin resistance of this organism is novel to our isolate or if it is typical of this species. Of interest was the high number of methicillin-resistant *S. sciuri* recovered, as the number of isolates from our study was

considerably higher than those recovered from other recent studies that also assessed milk samples. Our research suggests that the presence of methicillin-resistant *Staph* reservoirs in milk, and likely the dairy farm population in the US, is irrespective of organic or conventional production system.

Keywords: *Staphylococcus aureus*, *Staphylococcus spp.* methicillin-resistance, organic bulk tank milk

INTRODUCTION

The organic dairy industry has seen exponential growth in the past decade (Economic Research Service, 2008). Organic milk quality, management and animal health have been carefully assessed in comparison to these characteristics in conventionally managed dairy farms (Stiglbauer, Cicconi-Hogan and Richert, et al., in press; Cicconi-Hogan et al., submitted for publication; Zwald et al., 2004; Ruegg, 2009). Antimicrobial resistance in agriculture has become a major concern among consumers and scientists alike, as it presents potential health risks to animals and humans. Thus, the presence of various antimicrobial-resistant microorganisms in the dairy community, milk in particular, is of interest. This is especially true of the organic dairy industry, as there is curiosity regarding the prevalence of antimicrobial resistance despite the lack of antimicrobial use.

On dairy farms, and particularly on organic dairy farms, *Staphylococcus aureus* (*S. aureus*) is a major mastitis-causing pathogen. This is an especially serious issue on organic dairy farms, as they are unable to use the traditional antimicrobial agents used to treat *S. aureus* infections. Methicillin-resistant *S. aureus* (**MRSA**) is a major concern in the human population, as it is very difficult to treat. The *mecA* gene confers methicillin resistance by encoding a penicillin-binding protein (PBP-2 α). It is located on a highly mobile element called staphylococcal cassette chromosome (**SCCmec**), which allows other species of *Staphylococcus* to easily pick up the methicillin resistance. Although detection of MRSA in bulk tank milk has traditionally been performed with molecular methods searching for a single conserved cassette (Virgin et al., 2009; Haran et al., 2012), recent research has found a variant that is undetectable through these methods. A divergent homologue, *mecA*_{LGA251} has been described in García-Álvarez et al. (2011) and in Paterson et al. (2012), that only presents 70% similarity at the DNA level to the traditional

mecA gene. This homologue has been recovered from humans, rats, dogs, sheep and dairy cattle, among others, in Europe (García-Álvarez et al., 2011; Paterson et al., 2012).

Methicillin-resistant *S. aureus* has been isolated from bulk tank and quarter milk samples in Europe (Kreausukon et al., 2012; Spohr et al., 2010). These MRSA strains recovered from milk may be the livestock-associated MRSA strain, ST398, often found on swine farms throughout Europe (Crombé et al., 2012; Agersø et al., 2012). However, little MRSA has been found in bulk tank milk in the United States (Virgin et al., 2009; Haran et al., 2012). Due to the mobile nature of *SCCmec*, assessing the prevalence of methicillin-resistant coagulase-negative *Staphylococcus* (**MR-CNS**) in bulk tank milk is of interest, as it could be a genetic reservoir and pose a risk for new MRSA strain evolution in areas such as the US dairy cattle industry.

The object of this study was to assess for the presence of MRSA and MR-CNS in bulk tank milk samples from New York, Wisconsin and Oregon. This study, part of a larger project designed to assess differences between organic and conventional dairy farms, will also allow us to understand if there are any differences in MRSA or MR-CNS prevalence among production systems and geographical location, using both genotypic and phenotypic methods.

MATERIALS AND METHODS

Herd Selection and Farm Sampling. Herd inclusion and recruitment criteria are as described by Stiglbauer, Cicconi-Hogan and Richert et al., (in press) and Richert et al., (in press). Certifying agencies and extension organizations in each state identified a list of organic herds to be contacted. Conventional herds were selected from a list of licensed dairy herds from each state's department of agriculture. All farms received an informational letter and a return postcard indicating interest and basic herd demographics. Participation was voluntary for all farms

included in the study. Following a recruitment and matching period, 292 organic and conventional farms in New York, Oregon and Wisconsin were visited between March 2009 and May 2011. Of the 292-farm total, 192 of the farms were organic. The remaining 100 conventional farms were included in the study based on proximity to the organic farms and were matched based on herd size category (0 – 99 adult cows, 100 – 199 adult cows, ≥ 200 adult cows). In New York, 72 organic and 25 conventional farms were visited; in Wisconsin, 96 organic and 51 conventional farms were visited; and in Oregon, 24 of each organic and conventional farms were visited. Eligibility criteria for organic farms included shipping organically certified milk for ≥ 2 years and having a minimum of 20 adult cows on the farm. Conventional farms had been shipping milk for ≥ 2 years and had a minimum of 20 adult cows. Farms were compensated with bulk tank milk testing and testing of clinical mastitis samples. At the time of the visit, a questionnaire was administered to the person primarily responsible for farm management and animal health. More detailed information on the questionnaire can be found in Stiglbauer, Cicconi-Hogan and Richert, et al. (in press) and Richert et al. (in press).

Six bulk tank milk samples were collected from each farm at the time of the visit. After the bulk tank has been agitated for a minimum of 5 minutes, all samples were taken directly from the bulk tank with a sterile sampler put on ice and transported to Quality Milk Production Services in Ithaca, NY for testing. Of the 292 farms visited, two farmers requested that their bulk tanks not be sampled or analyzed, leaving a total of 290 samples. Five of the 6 samples from 1 farm fell during transport, and the remaining sample was used for customer testing and was thus unavailable for further research. Another farm needed to have the cultures set up twice due to an equipment failure, and did not have sufficient milk preserved to perform the assays described

here. The total number of bulk tank samples tested for this study was 288. All samples were kept frozen at -20°C until testing.

Genotypic and phenotypic testing for MRSA and MR-CNS. Two parallel assays were performed to assess the presence of methicillin-resistant organisms in the bulk tank milk. The genotypic approach from Virgin et al. (2009) was initially used to determine if the *nuc* gene (encodes the thermostable nuclease of *S. aureus*; Brakstad et al., 1992) and a 174-bp amplicon from the *mecA* gene (to detect methicillin resistance; Martineau et al., 2000) were present in the isolates cultured from the bulk tank milk. Approximately 20 µL of milk was swabbed onto trypticase soy agar with 5% sheep blood and 0.1% esculin (bioMérieux, Inc., Durham, NC), and incubated at 37°C. Plates were assessed for growth at 24 and 48 hours. Colonies were initially identified as *S. aureus* by appearance and hemolysis, and were then isolated on blood agar plates. Up to 10 colonies per sample were isolated for further testing. DNA templates from all colonies were obtained using a Qiagen DNA Mini Kit (Valencia, CA), according to the procedure for gram-positive bacterial organisms. Primers used are found in Table 5.1. PCR reactions were done on an iCycler (BioRad Laboratories, Hercules, CA) run at a total volume of 25 µL: 2 µL of DNA template, 12.5 µL GoTaq Green (Promega), 0.125 µL of each primer (100 µM) and 10 µL of nuclease free dH₂O. The protocol, previously described in Virgin et al. (2009), is as follows: denaturation at 94°C for 15 minutes; 37 cycles of 94°C for 60 seconds, 55°C for 30 seconds and 72°C for 60 seconds; followed by the final extension step of 72°C for 10 minutes. Two negative controls were included in each run, a negative lysate preparation and a negative for the PCR reaction. The positive control used was strain QMP S1-027 (Virgin et al., 2009), a MRSA isolate from heifer milk that was *mecA* and *nuc* positive, confirmed by PCR (a gift of John Barlow, University of Vermont, Burlington).

Table 5.1. List of all gene targets, laboratory primer names, sequences, target sizes and references for all primers used in this manuscript.

Gene Target	Primer Name	Primer Sequence	Target Size	Reference
<i>nuc</i>	nucF	GCGATTGATGGTGATACGGTT	447 bp	Brakstad et al., 1992
	nucR	AGCCAAGCCTTGACGAACTAAAGC		
<i>mecA</i>	mecA174F	AACAGGTGAATTATTAGCACTTGTAAG	174 bp	Martineau et al., 2000
	mecA174R	ATTGCTGTTAATATTTTTTGAGTTGAA		
16S rRNA	16S-P5SH	TGAAGAGTTTGATCMTGGCTCAG	1500 bp	Greisen et al., 1994
	16S-DG74	AGGAGGTGATCCAACCGCA		
<i>rpoB</i>	CNSrpoBF79	CAATTC ATG GAC CAA GC	588 bp	Drancourt and Raoult, 2002
	CNSrpoBR667	GCIACITGITCCATACCTGT		
<i>femB</i>	femB1F	CATGGTTACGAGCATCATGG	533 bp	Pérez-Roth et al., 2001
	femB1R	AACGCCAGAAGC AAGGTTTA		
<i>mecA</i> homologue	mecA2F	CATTAAAATCAGAGCAGGC	188 bp	Paterson et al., 2012
	mecA2R	TGGCTGAACCCATTTTTGAT		

A phenotypic approach was also used to enhance the recovery of MRSA and MR-CNS from the milk and to identify methicillin resistant genotypes other than identified with the previously described PCR protocol. Samples were defrosted overnight at 4°C. To encourage the growth of *Staphylococcal spp.*, a two-step enrichment method was used. First, 10 mL of milk was added to 40 mL of Mueller Hinton broth with 6.5% NaCl, and the samples were incubated for 24 hours at 37°C. Then, 1 mL of the initial broth was added to 9 mL of phenol red mannitol broth (with 75 mg/mL aztreonam and 5 mg/mL cefoxitin), and was again incubated at 37°C for 24 hours. 500 µL of the resulting culture was spread onto MRSASelect plates (BioRad Laboratories, Redmond, WA) and incubated for 24 hours at 37°C. The plates were then assessed for pink colonies, indicating MRSA, or off-white colonies, indicating MR-CNS. All resulting colonies were replated on MRSASelect plates to confirm growth and on blood agar plates to re-evaluate *Staph*-like colonies for appearance. Isolates were tested for catalase and coagulase production. DNA templates from all colonies were obtained using a Qiagen DNA Mini Kit (Valencia, CA), following the procedure for gram-positive bacterial organisms. Negative

controls used were *S. aureus* ATCC 25923, a negative PCR reaction and negative lysates prepared using no DNA. Positive controls used were MRSA strain ATCC 33591 and MRSA strain ATCC BAA-2312 (to account for the divergent *mecA* homologue).

All isolates from both approaches were identified by 16S rRNA and *rpoB* speciation. Primers used are in Table 5.1. PCR reactions were run on a T-100 Thermocycler (BioRad, Hercules, CA) at a final volume of 50 μ L, consisting of the following (Greisen et al., 1994): 2 μ L of template, 0.25 of primer 16S-P5SH (100 μ M), 2 μ L of primer 16S-DG74 (12.5 μ M), 25 μ L GoTaq Green (Promega) and 20.75 μ L nuclease free dH₂O. The PCR protocol used for 16S rRNA speciation was as follows: initial denaturation for 4 minutes at 94°C; 40 cycles of 94°C for 60 seconds, 50°C for 60 seconds and 72°C for 90 seconds; and a final extension step of 72°C for 5 minutes. Primers used are specified in Table 5.1. PCR reactions for *rpoB* were run on a T-100 Thermocycler (BioRad, Hercules, CA) at a final volume of 25 μ L, consisting of the following: 2 μ L of template, 0.25 of both primers (100 μ M), 12.5 μ L GoTaq Green (Promega) and 10 μ L nuclease free dH₂O. The PCR protocol used for *rpoB* speciation was as follows: initial denaturation for 15 minutes at 94°C; 30 cycles of 94°C for 60 seconds, 48°C for 60 seconds and 72°C for 60 seconds; with a final extension step of 72°C for 7 minutes (Drancourt and Raoult, 2002). All sequencing was done at Cornell University Core Laboratories Center using Sanger/3730XL DNA sequencing. Sequences were analyzed using Lasergene© SeqMan software (DNASTar, Inc., Madison, WI), then compared for sequence similarity to other sequences using BLAST (NCBI).

All DNA confirmed as *S. aureus* or other *Staph. spp.* were then tested to reconfirm the presence of the *mecA* gene using the protocol described previously. This was further confirmed using primers to test for the *femB* gene (encodes proteins which influence the level of methicillin

resistance of *Staphylococci*). Primers used are described in Table 5.1. The protocol used, previously described in Pérez-Roth et al. (2001), was as follows: denaturation for 5 minutes at 94°C; 10 cycles of 94°C for 30 seconds, 64°C for 30 seconds and 72°C for 45 seconds; 25 cycles of 94°C for 45 seconds, 50°C for 45 second and 72°C for 1 minute; with a final extension step of 72°C for 10 minutes. Testing for the newly described *mecA* homologue was done using the following primers from Paterson et al. (2012) (Table 5.1). The PCR protocol used is as follows (García-Álvarez et al., 2011): an initial denaturation step at 94°C for 5 min; 30 cycles of denaturing at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min; and a final extension at 72°C for 5 min.

RESULTS

The farm ID, isolate identity, farm location, organic status, and testing results of all the isolates described below are shown in Table 5.2. Isolates shown are those were positive through genotypic or phenotypic testing, or both.

MRSA prevalence. One MRSA isolate was detected from 288 bulk tank milk samples, a 0.3% prevalence in our total population of dairy herds. The isolate grew as a bright pink colony on *MRSASelect*, and was coagulase and catalase positive, indicating a possible *S. aureus* identity. The identity was confirmed as *S. aureus* by *rpoB* sequencing, with a 100% identity similar to the *rpoB* reference genes. The presence of *femB* confirmed the speciation (Figure 5.1) and the presence of the *mecA* gene confirmed methicillin resistance (Figure 5.2). Taken together, these results indicate a MRSA isolate present in the bulk tank milk sample. The isolate was obtained from a bulk tank on a small organic farm in New York State.

Table 5.2. Descriptive information on the MRSA and MR-CNS isolates; results of *rpoB* sequencing, sequence identity percentage and microbiological testing. The isolates presented here were recovered from 288 bulk tank milk samples gathered on organic (ORG) and conventional (CON) farms throughout New York, Wisconsin and Oregon from March 2009 to May 2011. Methicillin resistance was assessed using a *mecA* PCR and MRSASelect selective media.

Farm ID	Isolate	Location	Organic Status	<i>rpoB</i> BLAST Result	Sequence Identity Percentage	Catalase	Coagulase	Growth on MRSASelect
1	A	Wisconsin	CON	<i>S. sciuri</i>	99%	+	-	No
2	B	Oregon	CON	<i>S. sciuri</i>	99%	+	-	No
3	C	Oregon	ORG	<i>S. sciuri</i>	99%	+	-	No
3	D	Oregon	ORG	<i>S. chromogenes</i>	99%	+	-	No
3	E	Oregon	ORG	<i>S. chromogenes</i>	99%	+	-	No
3	F	Oregon	ORG	<i>S. sciuri</i>	99%	+	-	No
4	G	New York	CON	<i>S. sciuri</i>	98%	+	-	No
4	H	New York	CON	<i>S. agnetis</i>	99%	+	-	No
4	I	New York	CON	<i>S. sciuri</i>	99%	+	-	No
5	J	New York	ORG	<i>S. aureus</i>	100%	+	+	Yes
6	K	New York	ORG	<i>S. saprophyticus</i>	99%	+	-	Yes
7	L	New York	CON	<i>S. saprophyticus</i>	99%	+	-	Yes
8	M	Wisconsin	ORG	<i>Macrococcus caseolyticus</i>	99%	+	-	Yes
9	N	Wisconsin	CON	<i>S. saprophyticus</i>	99%	+	-	Yes

MR-CNS prevalence. Twelve MR-CNS isolates were detected, a prevalence of 4%. Of all the MR-CNS isolates, 4 were isolated from a single farm (Table 1, Farm 3, Isolates C – F) and 3 were isolated from another (Table 1, Farm 4, Isolates G – I), and the remaining isolates were from unique farms. One methicillin-resistant *Macrococcus caseolyticus* isolate was also recovered. Isolates were identified as follows, with a minimum of 98% of 100% sequence identify to *rpoB* reference genes: *S. sciuri* (n = 6), *S. chromogenes* (n = 2), *S. saprophyticus* (n = 3), and *S. agnetis* (n = 1). All isolates were catalase positive and coagulase negative. They were also negative for *femB* (Figure 5.1) and positive for *mecA* (Figure 5.2). Four MR-CNS isolates and the *M. caseolyticus* isolate grew as off-white colonies on MRSASelect. The remaining colonies did not grow on the selective media.

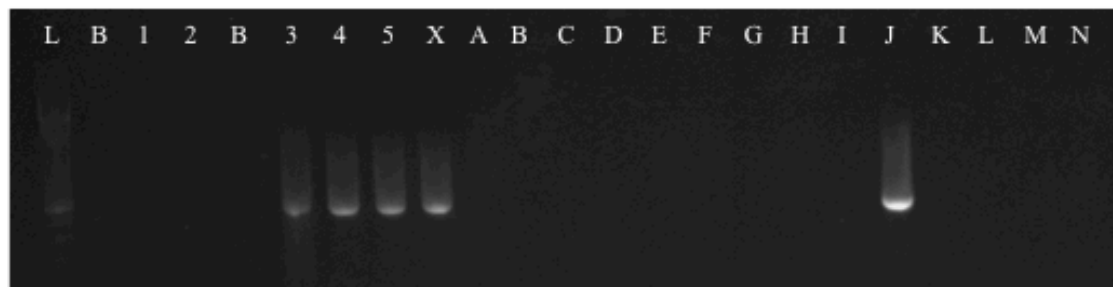


Figure 5.1. Agarose gel electrophoresis showing single PCR amplification products for the *S. aureus femB* gene. Lane L, 100-bp DNA molecular size marker; lanes B, blank; lanes 1 and 2, negatives testing set-up environments; lane 3 *S. aureus* positive control; lane 4, MRSA positive control; lane 5, divergent *mecA* homologue positive control; lane X, suspect field isolate determined to be *mecA* negative; lanes A – N (labeled from Table 5.1), bulk tank isolates. Bands were present in all *S. aureus* isolates, at approximately 533 bp.



Figure 5.2. Agarose gel electrophoresis showing single PCR amplification products for the methicillin-resistance *mecA* gene. Lane L, 100-bp DNA molecular size marker; lanes B, blank; lanes 1 and 2, negatives testing set-up environments; lane 3, *S. aureus* positive, *mecA* negative control; lane 4, MRSA positive control; lane 5, divergent *mecA* homologue positive control; lane X, suspect field isolate determined to be *mecA* negative; lanes A – N (labeled from Table 5.1), bulk tank isolates. Bands were present in all tested isolates, at approximately 174 bp.

Divergent *mecA* homologue testing. All isolates were assessed for the divergent *mecA* via genotypic testing with the SCC*mec* element XI primers (Table 5.1). No isolates tested contained the divergent *mecA* element, shown in Figure 5.3.



Figure 5.3. Agarose gel electrophoresis showing single PCR amplification products for the methicillin-resistance *mecA* homologue gene. Lane L, 100-bp DNA molecular size marker; lanes B, blank; lanes 1 and 2, negatives testing set-up environments; lane 3 *S. aureus* positive control; lane 4, divergent *mecA* homologue positive control; lane 5, MRSA positive control lane X, *mecA* negative tested *S. aureus* isolate; lanes A – N (labeled from Table 5.1), bulk tank isolates. A band was present at only the divergent *mecA* positive control (indicated by the white arrow), lane 4, at 188 bp. All tested isolates were negative.

RAPD-PCR of MRSA-positive isolate. To rule out potential contamination of the MRSA-positive isolate (Isolate J; Table 5.2.) we used a random amplification of polymorphic DNA (**RAPD**) protocol to determine strain differences, described by Gurjar et al., 2012. Figure 5.4 shows clear differences in the banding patterns of the MRSA positive control (Figure 5.4, Lane 3) and our MRSA suspect field isolate (Figure 5.4, Lane J), indicating that they are not the same strain. Randomly chosen isolates (Lanes X, D, E) were included to show differences in banding patterns among other isolates.

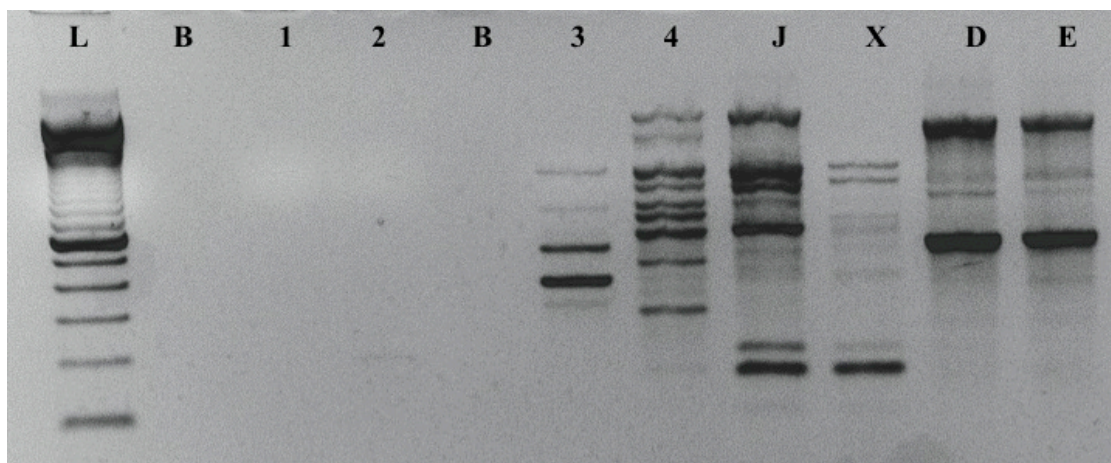


Figure 5.4. Agarose gel electrophoresis showing *Staphylococcus species* RAPD. Lane L, 100-bp DNA molecular size marker; lanes B, blank; lanes 1 and 2, negative testing set-up environments; lane 3, MRSA positive control; lane 4, divergent *mecA* homologue positive control; lane J (Table 5.1), bulk tank isolate J, MRSA suspect; lane X, *S. aureus* field isolate; lanes D-E (Table 5.1), CNS field isolates. Band patterns showed clear differences between the MRSA control (Lane 3) and the MRSA suspect field isolate (Lane J).

DISCUSSION

Methicillin-resistant *S. aureus* and MR-CNS are well known organisms in the human and animal population alike. While both are recognized as important mastitis pathogens in the dairy community, CNS is generally associated with intramammary infection and subclinical mastitis (Schukken et al., 2009), while *S. aureus* is often the cause of chronic intramammary infections – with clinical flare ups (Barkema et al., 2006). Methicillin-resistant *S. aureus* has not been commonly detected in surveys of bulk tank milk in the US (Virgin et al., 2009; Haran et al., 2012). Additionally, little research has been done to assess prevalence of MR-CNS in US bulk tank milk, although some research has been done assessing quarter milk samples in US farms (Sampimon et al., 2011). No research has specifically targeted MRSA or MR-CNS in organically produced milk at a cow or whole herd level.

Our approach to assessing methicillin resistance was to both use traditional molecular methods, as well as phenotypic methods. Due to the recent publications describing a *mecA*

homologue that is unable to be detected by the traditional *mecA* PCR method, we also used the described phenotypic method to recover isolates that produced any methicillin-resistance. We used a two-step enrichment method to encourage *Staphylococcal* growth and a selective growth medium as a method to easily screen the milk samples for methicillin-resistant colonies. While MRSASelect plates have been established as an effective selective media for isolating methicillin-resistant *Staph. spp.* (Carson et al., 2009), there are some potential pitfalls. Chromogenic agars are generally subjective, as the individual reading the plates must assess the color of the colonies. As the MRSASelect medium has a proprietary composition, it is difficult to assess the mechanism of selective growth. Henceforth, it is possible that the previously identified *mecA* homologue may not produce the same type of coloration as a MRSA isolate, resulting in a dismissal for further identification. We also employed a traditional microbiological method to identify *Staphylococcal* isolates by their morphological characteristics. We recognize the potential for missing MRSA isolates using this method, as several hundred *Staph* colonies may be present on a single plate. To minimize the risk of missing MRSA isolates, we isolated a minimum of 10 colonies from each plate so that the probability of obtained a methicillin-resistant isolate was increased. Despite these potential drawbacks, we believe that we have designed an effective screening method for assessing the prevalence of MRSA and MR-CNS in bulk tank milk.

As only one MRSA isolate was detected from 288 bulk tanks, our research supports the results of a recent study by Haran et al. (2012), which showed a low prevalence of MRSA recovered from bulk tank milk, as well as the results from the NAHMS Dairy 2007 study (Virgin et al., 2009). Because our study was largely cross-sectional, bulk tank samples were taken a single time on the day of the herd visit, and thus, may not be representative of all animals on the farm. The single MRSA isolate was recovered from a small organic farm in New York. Despite

this, due to the low prevalence reported here, we have no reason to believe that the organic dairy industry harbors substantially more MRSA than their conventional counterparts. The MRSA-positive farm reported never using gloves during the pre-milking or milking procedures. Because MRSA has been reported to be found on human hands (Mulligan et al., 1993; Shimamura and Murata, 2011), it is possible that this is the reservoir. However, the source cannot be identified without more in-depth analysis of the genotype of the isolate.

We also isolated 12 MR-CNS isolates and 1 methicillin-resistant *Macrococcus caseolyticus* isolate. All of the various species found have previously been assessed as bovine mastitis pathogens (Sampimon et al., 2011; Taponen et al., 2012). The presence of *S. agnetis* in our group of isolates is of interest, as it was recently recognized as a new species (Taponen et al., 2012). There is no mention of the antimicrobial susceptibility to methicillin in the publication, so we do not know if the methicillin-resistance of our isolate is typical of the species or if this is a novel case.

The species *Macrococcus caseolyticus* is a close relative to the *Staphylococcal* family, has previously been found in bovine milk samples and has had a *mec*-like element identified (Tsubakishita et al., 2010a), so the presence of this organism was not unexpected. Of the 12 MR-CNS isolates, 9 did not grow on MRSASelect, despite a positive *mecA* PCR. This phenotypic susceptibility and genotypic positive result combination have been previously reported in CNS (Sampimon et al., 2011). Another study reported that *S. sciuri* contains a *mecA* homologue that is closely related to the *mecA* gene typically found in MRSA, but is not located on a mobile genetic element that is often associated with the gene, and may not produce phenotypic methicillin resistance (Tsubakishita et al., 2010b). An interesting finding was the predominance of *S. sciuri* among our MR-CNS isolates (6 isolates out of 12 MR-CNS). This is a higher number of

methicillin-resistant *S. sciuri* isolated from milk than in previous studies (Sampimon et al., 2011; Piessens et al., 2011). However, it must be noted that Sampimon et al. (2011) was assessing clinical mastitis isolates, and not bulk tank samples. Piessens et al. (2011) found several isolates of *S. sciuri*, but they were predominantly in environmental samples. Note that all six *S. sciuri* isolates were only detected using the genotypic method and none of the six isolates grew on the MRSASelect agar. The relevance of a *mecA*-like genetic element that does not result in the same phenotypic bacterial growth on selective media as the *mecA* gene identified in phenotypically resistant isolates is currently unknown. However, this result is a key reason that we decided to use both a genotypic and phenotypic approach to assessing methicillin-resistant prevalence in *Staph. spp.* identified in bulk tank milk.

Overall, 4 organic farms and 5 conventional farms harbored MR-CNS in their bulk tank milk; a prevalence of 4% in organic farms and 7% in conventional farms. There is therefore no indication of methicillin-resistant *Staph spp.* being more prevalent on either organic or conventional dairies, based on this study. With such a low prevalence of methicillin-resistant *Staph*, the power to detect significant differences is low. However, the power to detect large differences in our study was substantial (10-15%; Ruegg, 2008). Our study analyzed a large portion of the organic industry, and assessing large numbers of samples for a specific cohort such as this industry is no easy task. In general, we conclude that the prevalence of methicillin resistance in bulk tank milk is low, and moreover, the prevalence of MRSA in bulk tank milk is even lower.

CONCLUSION

The recent surge in the organic industry and demand for organic products has created a need for more in depth research, especially for organic dairy products. Recent interest regarding antimicrobial use in agriculture, combined with the interest regarding organic dairy products has produced a curiosity concerning antimicrobial resistance on organic dairy farms. Our research was primarily concerned with assessment of methicillin-resistance *Staph* spp. in bulk tank milk.

Using a combination of phenotypic and genotypic approaches, we assessed that our population of 288 bulk tank samples had a low prevalence of both MRSA and MR-CNS. The total prevalence of MRSA at the farm level was 0.3%, with one positive sample from a New York organic herd. The prevalence of MR-CNS was 4% in our organic population and 7% in our conventional population, and was predominantly *S. sciuri* (n = 6). We conclude that the prevalence of both MRSA and MR-CNS in US bulk tank milk is low, and is no more prevalent in organic or bulk tank milk, based on this study.

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CHAPTER SIX

DISCUSSION OF FINDINGS

Assessment of Project C.O.W.

Project C.O.W. was designed with the intention of assessing the organic industry in the United States in a manner that allowed differences in herd size and farm location to be controlled for. The choice to visit farms in New York, Wisconsin and Oregon was made so as to include the organic demographic in the three major dairy producing regions of the country – the east coast, mid-west and west coast. Approximately twice as many organic herds were included in the study as conventional for two reasons – to allow for an adequate comparison of organic to conventional farms, as well as to allow for analysis within in the organic demographic. By matching conventional farms to organic farms in the same herd size strata and within a 50-mile radius, we were able to get frequency-matched controls for the organic farms.

The questionnaire was an assessment of the management and herd health on the farm. It touched on all the major management procedures and information that provide a snapshot of how the farm is run. Information was also gathered on recent health and treatment events, as well as cow body condition, udder hygiene and hock condition for a large portion of the cows. A bulk tank sample, taken the day of the herd visit, was analyzed for milk quality. Information was collected for 60-days prior to and after the herd visit regarding treatments, culling and general health of the herd. The farmers sent in clinical mastitis samples for the 60-day prospective period to their respective laboratories. While all these data collection mechanisms provided a large amount of information, a more longitudinal study would have also been useful, especially in assessing management and its relationship to bulk tank milk quality. A single herd visit only

provides a look at the farm at the time of the visit. Due to human and animal variability, answers given, milk quality and cow information can change on a daily basis. Moreover, management may have changed based on recent bulk milk results and therefore true causal conclusion cannot be drawn from such predominantly cross-sectional studies (Kleinbaum et al., 1982). It is important that all results provided in this thesis are considered with that in mind.

Bulk Tank Milk Quality

Prior research has shown no evidence that organically produced milk is of higher quality than conventionally produced milk (Rosati and Aumaitre, 2004; Sato et al., 2005; Zwald et al., 2004), despite consumer perception to the contrary (Sundrum, 2001). The perceived higher quality of organic products may have contributed to the exponential growth of the organic dairy industry (Economic Research Service, 2008). We assessed both the SCC and SPC of bulk tank milk at the time of the visit, as well as from bulk tank records from the 3 months prior to the herd visits. Our results support prior research, as no significant difference was found among production system (accounting for herd size and location) in the SCC in prior records ($P = 0.65$) or from the bulk tank sample taken ($P = 0.25$). A lack of significance was also found in regard to the bulk tank SPC ($P = 0.76$ for analysis of prior records, $P = 0.26$ for the bulk tank sample taken the day of the visit). The LP ($P = 0.93$), butterfat percentage ($P = 0.11$), protein percentage ($P = 0.97$) and food borne pathogen percentages were not significantly different among grazing system groups (*Salmonella* – no positives; *Listeria monocytogenes* – $P = 0.57$; Shiga toxin producing *E. coli* – $P = 0.23$). Coliform counts and *E. coli* counts were significantly lower on organic farms ($P = 0.009$ and $P = 0.008$, respectively), while percentage of farms with *S. aureus* in the bulk tank was significantly higher among organic farms ($P = 0.09$).

Organic and Conventional Farm Comparisons

The goal of Chapters 2, 3 and 4 was, in the most essential sense, to assess differences between organic and conventionally managed dairy farms across the three states. The outcomes of interest included milk quality, farm management and herd health parameters. The purpose of the assessment was not necessarily to pit the management systems against each other, but to do a complete comparison and determine management characteristics that would characterize the most optimal herd management for all farms, as well as management characteristics related to health and milk quality that were production system specific.

Initial analysis evaluated only the differences between organic and conventional dairy farms. However, it was decided to split the conventional farms into two groups of farms, conventional grazing and non-grazing, after it became apparent that there were a relatively large number of conventional farms that were grazing their animals on a regular basis and using pasture as their primary housing and source of DMI in the summer months. This group of 36 farms presented some interesting findings and was a relevant comparison group to organic farms. The farms included in the conventional grazing group did not have the same characteristics as the organic or conventional non-grazing farms, and seem to have their own set of challenges and successes. The results of farms using conventional grazing generally coincide with the conventional non-grazing population of farms, when variables pertaining to herd health and mastitis management were analyzed. This was expected, since they are able to use conventional treatments, such as mastitis antimicrobials and blanket antimicrobial dry treatment. A surprising result was that despite their grazing status, similar percentages of conventional grazing and non-grazing farmers reported feeding a TMR, which was significantly different from the percentage

of organic farms reporting feeding a TMR. However, since the feedstuffs of conventional grazing farms do not need to be organically certified, as is obviously the case for organic farms, it is easier to feed a TMR on conventional grazing farms.

An interesting finding, addressed in Chapter 2, is the difference in the distribution of lactations in cows on organic farms compared to conventional farms. Overall, organic farms had a significantly lower percentage of 1st lactation animals and a higher mean lactation number. Previous studies in Europe have reported that organically managed cows tend to be older (Reksen et al., 1999; Hardeng and Edge, 2001). During the herd visits, we observed that organic herds had cows with much higher lactation numbers compared to conventional farms, and often we identified a few cows with lactation numbers in the double digits. We suspect that this is due to the difficulty in purchasing organically certified animals, in combination with hesitancy or a lack of a perceived need of organic dairy farmers to cull animals. In our study, we found that organic farms had a 5% lower cull rate than the conventional farms in the study, a finding further supported by Reksen et al. (1999) and Hardeng and Edge (2001). Organic farms tend to frequently be closed herds and rarely buy animals from outside the herd, a suggested disease management strategy (Vaarst, 2006). The biosecurity risk of buying animals from outside the herd opens the herd up to new infections and diseases, which may be difficult to eliminate without conventional antimicrobial treatments. As a result, organic farms may be less likely to remove animals from the herd for a health problem that is inconvenient, but not life threatening to the animal. In conjunction with this, organic farmers may see their heifers as particularly valuable, since they are born organically certified and without the biosecurity risks of a purchased animal. In Chapter 4, we discuss the presence of *S. aureus* and the association of having a *S. aureus* positive bulk and higher lactation cows. As older cows are more likely to

have a chronic *S. aureus* infection and more difficult to cure than the younger animals in the herd (De Vliegher et al., 2012; Barkema et al., 2006), organic farmers' reluctance to cull animals may contribute to the higher percentage of organic farms with *S. aureus* in the bulk tank.

The conventional grazing and organic populations also had some similarities. The mean lactation number of cows on conventional grazing farms tended to be higher, similar to the organic population. Despite the statistical significance of a difference in the number of days spent grazing during the grazing season between organic and conventional grazing farms, the difference of 8 days is unlikely to be biologically significant. The number of years in the dairy business was similar between organic and conventional grazing farms, less than the conventional non-grazers. Interestingly, there were several similarities across all three groups of farms in the study. Milking procedures – use of gloves, pre-dipping, post-dipping, use of automatic takeoffs – were not significantly different. Several management procedures, such as the use of a dedicated calving area, use of a bucket milker for mastitis cows and keeping records of herd health events, were consistent across the three groups. The age of the primary housing and the kind of milking facility used were also similar. The similarities and differences shown here indicate that all three groups were indeed unique, and that splitting the conventional farms into grazing and non-grazing cohorts was a valid choice for our modeling.

In future studies, it may be interesting and useful to specifically include a cohort of conventional farmers that adhere to the organic grazing regulations. Use of grazing as a major source of DMI causes a decreased milk production, a result of a less concentrated diet (Hardeng and Edge, 2001; Zwald et al., 2004; Roesch et al., 2005; Sato et al., 2005). Because the conventional grazing farms are still receiving conventional milk prices, less milk production would equate to less income. Our research supports this assumption of a decrease in milk

production, as the milk per cow per day amount was significantly lower than the conventional non-grazing farms, but also significantly higher than the organic population (Table 2.1; Stiglbauer, Cicconi-Hogan and Richert, et al., in press). It would be interesting to know what the primary purpose of employing grazing is for conventional grazing population and how it is specifically affecting the farm, both in regard to financial matters and in herd health.

Milk Quality Associations

As SCC is one of the most highly recognized and useful parameter to assess bulk tank milk quality (Schukken et al., 2003; Barkema et al., 1998), it was an obvious choice for our modeling. The goal was to assess associations that were useful to the organic community specifically, as well as to the dairy community as a whole. Both models were fairly straightforward and no variables were truly unexpected. An interesting finding from both models was that an increase in the years spent in the dairy industry was associated with a higher SCC. We believe that this is related to younger farmers with more aggressive management techniques entering the dairy industry, although we cannot say this with certainty, as demographic and educational information was not collected and there is little research on the topic of age and farmer motivation. One of the primary findings from our SCC modeling was the External Input Score (Chapter 3). According to our model, a higher EIS score (indicating more use of external resources) was associated with a lower SCC, suggesting that using outside resources to help manage a farm, regardless of whether that farm is organic or conventional, may better the quality of the milk on that farm.

Standard plate count and coliform counts are also frequently cited as indicators of milk quality and the shelf life of pasteurized milk (Schukken et al., 2003; Jayarao et al., 2006). While

we again chose to model these variables with two subsets of the data, organic farms only and all herds in the study, there was much more variability in these models than the SCC models. There was not a clear set of indicators from these models that would predict good milk quality.

Consistency in milking and mastitis management techniques were predictors of low SPC and coliform counts, although these observations are not immediately obvious without careful consideration of the models.

The presence of *S. aureus* variable was extrapolated from the modified bacteria count, and made into a binary variable. Our decision to model this variable was due to the difference in percentages of organic, conventional non-grazing and conventional grazing farms that had bulk tanks with *S. aureus* present (Table 3.1). Our goal was to use the various management variables to help explain the difference of *S. aureus* presence between production systems. Mastitis management variables were seen in both models, which was expected as it is a major mastitis pathogen. The percent of the herd with 3 or fewer teats was associated in both *S. aureus* models. A higher percentage of animals with 3 or fewer teats is likely indicative of a farm with known mastitis problem that is using the drying off of teats as a method for disease management. In the organic herd model, a lower number of people treating mastitis over a typical month was associated with *S. aureus* in the bulk tank. Initially, this may seem counterintuitive, as we have previously mentioned that large numbers of workers leading to inconsistency of management and treatments, and thus, lower milk quality. However, this result may actually be simply a product of the small farm demographic of our organic population, and therefore, having fewer people working on the farm, in conjunction with the high percentage of organic farms with bulk tank cultures positive for *S. aureus* (Table 4.1).

Use of External Resources by Production System

An interesting finding that came from this project was the use of external resources by the farmers. An initial analysis, shown in Chapter 2, determined that the use of a veterinarian was lower on organic farms (36%) than on conventional grazing (56%) or non-grazing farms (77%; $P < 0.001$). This was further supported by the findings in Richert et al (in press), which found that organic management was associated with less frequent veterinarian usage, conventional grazing farms tended to have moderate veterinarian usage and conventional non-grazing farms were associated with frequent veterinarian usage. Our initial scoring system - using only the variables that described use of DHIA, use of a nutritionist and use of a veterinarian - showed that organic farmers were less likely to use these specified outside resources than their conventional counterparts (Figure 2.4). It also showed that farmers that used 2 or 3 of the resources indicated were more likely to have a lower SCC (Figure 2.3).

The scoring system describe in Chapter 2 was further expanded on in Chapter 3, where a 10-variable score was developed using a number of other management variables describing input onto the farm from outside sources (see Chapter 3). The expansion of the scoring was based the findings of Richert et al. (in press), which used multiple correspondence analysis to assess the relationships of levels of several categorical variables, and determine variables that are highly associated. Variables representing intensive management, such as vaccinations, use of pregnancy checks and exclusive use of AI for breeding cows were associated with use of a veterinarian. Since use of a veterinarian is indicative of use external resource, using the results in the analysis from Richert et al. (in press) to create an external resource scoring system was a logical next step. The goal of the development of this scoring was to use variables that were representative of the management on the farm as a whole, and had little to do directly with mastitis management. The

reason for this was that several of the variables typically associated with mastitis management and milk quality maintenance, such as use of gloves during milking, pre-dipping and post-dipping (Fenlon et al., 1995; Jayarao et al., 2004), did not have any association with SCC in our models. However, several other variables that on superficial investigation had no logical connection with milk quality – such as use of natural service for heifers and cows – did have an association with SCC in the models.

We postulate that the variables with no logical initial connection to SCC, in conjunction with other management variables representing the management of the farm, are actually proxies for the precision of the direct mastitis management variables. In general, most farmers are aware of what procedures are useful and effective in preventing mastitis on their farms. There is a possibility that when answering the questions regarding milking procedures and good mastitis management, the farmers answered as they felt they should, or how they perceived the procedures should be done, instead of answering how things are actually done on the farm. In that same vein, larger farms often employ large numbers of workers, and cannot check to see that the procedures are being done carefully and correctly for every animal at every milking.

The difference in external resource use by production system was striking. Organic farms span scores from 0 (no external resource use) to 10 (use of all defined external resources) with no clear trend, while most conventional farms are clustered in the upper range of the scores (Figure 3.4). Of particular interest is the use of vaccinations of both adult cows and calves, which was a part of the external resource score system. Only 64% of our organic herds reported vaccinating their adult cows, compared with 97% of conventional non-grazing herds and 100% of conventional grazing herds (Table 2.2). Results for vaccinations of calves were similar (Table 2.2). Vaccinations are an efficacious and organically allowed method of disease prevention

(LeBlanc et al., 2006), so the lack of use by the organic subset of farms was surprising and largely unexpected. We did not collect any demographic, religious or educational background information on our participants, and no research has been done exploring the reasoning for lack of vaccine use on organic farms. Therefore, it is difficult to speculate the reason for such deviation in regard to use of external resources. Some research has found that farmer attitude is a good measure of predicting frequency of diseases, such as mastitis (Jansen et al., 2010). Other research suggests that the decision to vaccinate may be related to economic motives or a perception (or lack thereof) of disease (Elbers et al., 2010). Unfortunately, the attitude and motivation of a person is often difficult information to gather and quantify, and it is subject to extreme variability. Despite this, future studies could benefit from gathering farmer motivational information, provided that it could be assessed in a factual manner.

One major question that comes from the results provided in this thesis is the connection between use of external resources and SCC. We have previously mentioned that organic farmers are more likely to have a lower EIS score than the conventional farmers in this study, indicating that they use fewer outside resources for management. We have also found that a higher EIS score, indicating more use of outside resources, is associated with a lower SCC. Finally, results from Chapters 2 and 3 show that there is no difference in SCC between organic and conventional production systems in our study (Stiglbauer, Cicconi-Hogan and Richert, et al., in press; Cicconi-Hogan, et al., submitted for publication). So the question becomes – Are organic farmers producing milk of the same quality as conventional farmers, despite using fewer resources and preventative measures? The answer, for farms in this study, seems to be yes. Table 3.4 shows that production system is not significant in a model including our design variables (herd size and location) and the EIS score, along with other variables. When EIS is removed from the model,

the parameter estimates and standard deviations of production system change very little, while the P -values actually increase slightly (Table 6.1) This indicates the EIS is not a proxy for production system, and that there is little statistical association between EIS and production system. The EIS (or it's predecessor from Chapter 2) were significant in 2 of our manuscripts, but it was not significant in any of the models reported in Chapter 4. This is a curious finding, as our results suggest that many milk quality parameters are connected or have an impact on one another.

Table 6.1. Final total herd linear regression model showing the association of significant variables ($P \leq 0.1$) and the log transformation of bulk tank SCC (\log_{10} cells/mL) among conventional non-grazing (CON-NG; $n = 63$), conventional grazing (CON-GR; $n = 36$), and organic (ORG; $n = 191$) farms, without the inclusion of the EIS. Grazing system, location (New York, Wisconsin and Oregon), and herd size were always included in the model as design variables. Estimates provided are the β coefficient for the given variable.

Variable	Level	Estimate	Final model P -value
Intercept		2.111	<0.001
Grazing System	CON-NG	0.032	0.640
	CON-GR	0.033	
	ORG	Reference	
Location	NY	0.085	0.075
	WI	0.105	
	OR	Reference	
Herd Size	≥ 200 cows	0.004	0.078
	100 – 199 cows	0.119	
	20 – 99 cows	Reference	
Amount of Grain Fed Cow/Day (kg)	Continuous	- 0.017	0.001
Bulk Tank Culture – <i>S. aureus</i>	Yes	0.119	< 0.001
	No		
Number of years in the dairy industry	Continuous	0.004	< 0.001

Farmers that use fewer resources, theoretically, would be spending less financially on management, as they would not be paying for vaccinations, veterinarians or artificial

insemination. Organic farmers get higher compensation per cwt for their milk, as well as more generous premiums for high quality milk (NOFA, 2009). However, because organic dairy production is pasture-based with relative small amounts of concentrate, milk production is less than that of then conventional farmers in the study, as mentioned previously. So while it seems as though organic farmers would financially be significantly better off than conventional farmers, research suggests that this may not be the case (Cook et al., 2010). The organic community could benefit from an in depth management and economic survey, to fully assess the financial impact of milk quality in combination with an external resource use assessment.

Methicillin Resistant *S. aureus* and Coagulase Negative Staph

Our research focusing on bulk tank MRSA and MR-CNS both corroborates previous research, as well as provides a new set of findings. Previous screenings for MRSA in US dairy herds have found little or no MRSA in bulk tank milk (Virgin et al., 2009; Haran et al., 2012). However, MRSA had not previously been screened for specifically in an organic population. Other recent research has shown the presence of a *mecA* homologue in isolates obtained from clinical cases as identified in livestock populations in Europe (García-Álvarez et al., 2011; Paterson et al., 2012), however, this *mecA* homologue has yet to be screened for or found in the US.

We also recovered and assessed isolates of MR-CNS from the bulk tank milk. Due to the highly mobile nature of the SCC*mec* (staphylococcal cassette complex), and its ability to move across species, it is possible for otherwise methicillin-susceptible *S. aureus* or CNS to pick up this element and become methicillin-resistant (Katayama et al., 2000). Methicillin-resistant organisms may also be an issue in regard to raw milk consumption or inadequately pasteurized

milk, as MRSA contaminated animal products have previously been the culprit of an outbreak of gastrointestinal illness (Jones et al., 2002). However, due to the low prevalence of both MRSA and MR-CNS in our population of milk samples, in combination with previous research (Virgin et al., 2009; Haran et al., 2012) we do not believe that methicillin-resistance is currently a major problem in the bulk tank milk of US conventional or organic farms. However, we believe that our combination of a genotypic and phenotypic approach to assessing methicillin resistance is valid and useful for future research, as it allows us to assess organisms that are genotypically resistant, but phenotypically susceptible to methicillin, and vice versa.

Global Findings

Our goal for this body of work was to understand the differences between organic and conventional dairy farms. More specifically, we aimed to assess bulk tank milk quality predictors and specific antimicrobial resistance in the population, as a way of relating that information to the rest of the dairy population. The organic population of dairy farms in the US had not been assessed in any detail or with a large sample size of herds prior to our research. The research presented in this thesis aims to provide a more complete picture of the distinctions and similarities among production systems, the various challenges that each production system faces in the quest for high quality milk, and how profoundly management ultimately affects the quality of the milk.

Overall, our models suggest that the organic and conventional production systems are not all that different from one another with regard to the epidemiology and pathobiology as it relates to milk quality and mastitis. Milk quality is very similar among the organic and conventional dairy farms in the study, suggesting that organic management does not seem to be positively or

negatively affecting the quality of bulk tank milk prior to pasteurization. Similar risk factors for milk quality and general herd characteristics are apparent throughout all the models.

Management, however, did seem to be a common theme in regard to differences between organic and conventional farms. It is unfortunate that more demographic information was not collected regarding management choice and decision, but it is something that can be achieved in future research. The similar methicillin resistance prevalence of CNS and *S. aureus* among our organic and conventional farms indicates that the use of antimicrobials on our dairy farms, or a lack thereof, does not necessarily mean a high prevalence of methicillin-resistant *Staph* in milk.

However, as we did not assess any other antimicrobial resistance factors in this study, we cannot make any overarching statements regarding the overall antimicrobial presence in bulk tank milk.

In closing, we believe that we have achieved a thorough assessment of a portion of the organic industry in the United States. The papers included in this body of work aim to make a controversial, and sometimes confusing, subject more clear and concise. We recognize that not all questions regarding organic dairy production have been answered, as the research on conventional dairy in the US is far more extensive. However, we hope to have provided a basis for future work in the field.

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