**Thesis/Dissertation Template Starter**

**APA 7**

This template is meant to help you put your thesis or dissertation in order before you bring it in for a successful format check. Presented herein are all possible sections you might need for your document (i.e., Table of Contents, Table of Figures, Acknowledgements, etc). Page numbers, margins (1.5” left, 1” right), font styles (12pt Times New Roman) are already set in this template in order to help you out.

Table of Contents, List of Figures, and List of Tables can be tricky sections to complete. As such, there is a link in this document to a Table of Contents helper that should aid in the creation of these components of your final document.

If you already know how to set the style of your section headings you are ready to go. Under styles you will find: ChapterNum (**CHAPTER 1: INTRODUCTION**- CAPS, already centered), ChapSub (**1.2 Thesis Fun** - already centered), ChapSubSub (**1.2.1 Types of Thesis Fun**- always left). All section titles are 12pt font, bold, and Times New Roman. Table of Contents requires just Level Three headings.

Highlighted words are words that should be either be deleted or changed before you compose your final draft.

Delete this introduction page from your final draft. The first page should be the title page which follows on the next page.

Enjoy!

This is where your title goes. It should be capitalized AND SINGLE SPACED.

A Dissertation Presented

by

Caitlin Elizabeth Jeffrey, D.V.M.

to

The Faculty of the Graduate College

of

The University of Vermont

In Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy

Specializing in Animal and Veterinary Sciences

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ABSTRACT

This is where you place your abstract. An abstract is simply a concise (usually between ½ of a page and ¾ of a page) explanation of your document from motivation to conclusion. The abstract page is single spaced. It is officially page i but it is not labeled (you will see that there is no page number below).

The first line of each paragraph is indented ½ an inch, and double space between paragraphs.

CITATIONS

Material from this dissertation has been published in the following form:

Jeffrey, C.E., Andrews, T., Godden, S.M., Neher, D.A., Barlow, J.W. (2024). Relationship Between Facility Type and Bulk Tank Milk Bacteriology, Udder Health, Udder Hygiene, and Milk Production on Vermont Organic Dairy Farms. Journal of Dairy Science. Epub ahead of print. DOI: 10.3168/jds.2023-24576. PMID: 38908690.

AND/OR

Material from dissertation has been accepted for publication in (name of journal) on (month, day, year) in the following form:

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The caption here should match that in the body of the document exactly.

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The caption here should match that in the body of the thesis exactly.

CHAPTER 1: CHAPTER NAME (CAPS)

1.1. Section Name

Here you can write your thesis. Perhaps you would like to make a reference [1]. Or, maybe you have many references saying the same thing [4-7].

Begin a new paragraph with an indentation

1.2. Section Breaks

Below, Page Breaks will be discussed. Here, Section Breaks, a different animal than Page Breaks, will be covered. A Section Break is the tool that allowed us to switch from the List of Tables on page v to Chapter 1 on page 1. Thus, Section Breaks allow one to define distinct sections in a single document, each governed by its own formatting.

One of the beauties of the Section Break is mentioned above, specifically, the ability to stop one form of page numbering and begin another on the next page. In this template, at the end of Title page, one section ends and another section begins with the Acknowledgements page. By placing the cursor after the last line of the page, open the Insert drop-down menu (at the top of the window) and select Break… On the resulting menu, choose the “next page” option under Section Break. This will move the cursor to a new page. If the previous page had a certain numbering system, the new page can have an entirely new numbering system.

1.2.1. Page Numbering

Numbering pages can be controlled a number of ways. Most notably, to start numbering pages, use the Insert menu and select Page Numbers. This will open a menu that governs the style, starting point, location, etc of page numbers. Further, one can access this menu more directly once page numbers are placed on the page. Simply double click on the page number you wish to change and proceed to make the changes.

1.3 Figures

1.3.1. Inserting a Figure

To insert a figure, copy the desired image from its source. Then, in this document, right click on the location where you would like to place the figure, and select Copy from the menu that appears. To add a caption, right click on the figure and select Caption to insert the word “Figure” and the number of the figure. You add the caption in the document itself. The Caption menu just lets you add the word Figure, Equation, Table, etc. See below for how to reference a Figure, Table, etc. (Figure 1).

Figure 1:

*Left justify the Caption Above the Figure and Center the Figure on the Page*



(Do not leave any Widow Headings; go to next page.)

1.3.2. Tables

Place a table caption above the actual table. Do this by right clicking on the table, selecting caption, and changing the option within the caption menu.

Table 1:

*See Below for How to Create a Table Caption*

|  |  |
| --- | --- |
| Data 1 | Value 1 |
| Data 2 | Value 2 |

1.4. Creating Tables

Creating a table starts with the Table menu. Select Insert from the drop-down menu, and choose Table. Select the size and options as you require. Adding a caption is accomplished in the same manner as with figures. In order to reference the Table in the body of the text, click the Insert menu and select Reference → Cross-reference. From the menu that appears, choose Table from the Reference Type drop-down list and select how you want the reference to appear, such as just the label and the number (Table 1). Tables should only have horizontal lines as in example above.

CHAPTER 1: Literature review – Antimicrobial susceptibility of bovine staphylococcal mastitis isolates on organic vs. conventional dairy farms

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Abstract

An unfortunate consequence of any antimicrobial use is the potential to select for the emergence of resistant strains of bacteria in a population. A unique opportunity in which to assess the effect of antimicrobial use on resistance of mastitis pathogens is to compare dairy farms which are managed “conventionally” to those that are managed “organically.” Without the selective pressure of antimicrobial usage (as on organic dairies), it would be expected that resistant bacterial strains would gradually be replaced by susceptible strains if an advantage was no longer conferred by carriage of antimicrobial resistance (AMR) genes. The objective of this narrative review was to summarize studies which compared the relationship between antimicrobial usage at the farm level (organic vs. conventional) and AMR of bovine staphylococcal mastitis isolates, the predominant group of bacteria causing intramammary infections in dairy cattle globally. Other potential explanatory factors for differing antimicrobial susceptibility of staphylococci causing intramammary infections are also described. These include differences in AMR carriage between staphylococcal species and various risk factors associated with the prevalence of different species causing intramammary infections in a particular herd. Overall, studies comparing AMR of mastitis-associated staphylococci between herds under organic management and herds managed conventionally find either no difference or that isolates originating from organic farms exhibit slightly more susceptibility. Although some level of resistance was observed against a number of antimicrobials important for veterinary medicine (cephalosporins, penicillin, tetracycline), overall resistance of mastitis-associated staphylococci is generally low and the most commonly-used mastitis treatments are still effective. Studies exploring this issue varied widely in their approach, including use of differing methodology to determine susceptibility patterns and variation in sampling scheme. Most studies were carried out in either the US or Europe. This is somewhat problematic, as definitions of “organic” differ for dairies in the EU (where antimicrobial usage is still allowed, but is more tightly regulated and limited) and the US (any animal treated with antimicrobials must leave the herd). However, the overall conclusions from studies comparing the two different management systems are still informative. Directions for future work could include comparing AMR for staphylococci between these two systems while controlling for species, comparison of predominant strain types within a given species between organic and conventional farms, or long-term studies of farms transitioning from conventional to organic status to better understand what types of AMR are maintained in organic dairy herds and for how long.

Introduction

Effective antimicrobial therapy is a cornerstone of livestock veterinary medicine, maintaining the health of animals producing food and fiber to support the global population and alleviating suffering due to infectious disease. However, use of antimicrobial agents is inherently a “powerful selective force that promotes the emergence of resistant strains,” and the cumulative effect of antibiotic use in general has “clearly been to increase the prevalence of resistance in the population [of bacteria] as a whole” (Lipsitch and Samore, 2002). Resistance to antimicrobials can be acquired by bacteria in multiple ways. Spontaneously occurring genetic mutations (passed vertically to daughter cells) can confer antimicrobial resistance, but more commonly it is acquired by the horizontal transfer of mobile DNA elements from a donor cell, often another species of bacteria (Chambers, 2001; Sefton, 2002). In the case of horizontal transfer, antimicrobial resistance genes can become rapidly and widely disseminated throughout a bacterial population. This occurs either by further genetic exchanges between the newly-resistant strain and susceptible strains, or by clonal spread of the newly-resistant strain itself (Chambers, 2001). Although the interplay between development of resistance and antimicrobial use is complex and multifactorial, it is generally accepted that antimicrobial resistance (AMR) is potentially amplified in both human healthcare environments and on farms, where frequent exposure to antimicrobial compounds can select for resistant populations of bacteria (Parker et al., 2024). A direct temporal relationship between antimicrobial use and resistance has been described, both in human healthcare settings over the long-term (López-Lozano et al., 2000) and in transient increases in resistant fecal bacteria in cattle (Stabler et al., 1982; Langford et al., 2003; Berge et al., 2005; Lowrance et al., 2007). It has been suggested that antimicrobial usage in food animals could negatively affect human health by influencing the selection of drug-resistant foodborne pathogens (Yan and Gilbert, 2004). However, the risk of transmission of resistant bacteria between farm systems and humans is not fully understood; selection for resistant bacteria and transfer of AMR genes occurs through a variety of mechanisms, and is not always linked to use of a specific antibiotic (Mathew et al., 2007).

The most “obvious selection pressure for AMR” on cattle farms is the use of antimicrobials for treating sick animals (Call et al., 2008). Specifically, this can promote AMR on cattle farms by two potential mechanisms: 1) treatment with antimicrobials provides a competitive advantage for strains that carry resistance to that particular drug, allowing the relative proportion of resistant bacteria in a populations to increase; and 2) if resistance genes are harbored on horizontally transmissible elements (plasmids or conjugative transposons), strains carrying these elements can then successfully disseminate them to new, previously-susceptible bacteria (Call et al., 2008). The primary reason for antimicrobial drug usage in adult dairy cows in the US is for treatment of mastitis (Pol and Ruegg, 2007b). Bacteria belonging to the genus Staphylococcus, which broadly includes the major mastitis pathogen Staphylococcus aureus and a heterogeneous group of bacteria known as the non-aureus staphylococci and mammaliicocci (NASM), are the predominant pathogens causing intramammary infections (IMI) in dairy animals worldwide (as summarized in De Buck et al., 2021). A limited number of antimicrobials are approved for treatment of mastitis in lactating dairy cattle in the US, including various β-lactams (penicillin, cephapirin, ceftiofur, amoxicillin, hetacillin, and cloxacillin) and one lincosamide (pirlimycin) (FARM, 2020). At this time, S. aureus, NASM, and other mastitis pathogens are generally susceptible to the antibiotics currently used to treat IMI (Kolar et al., 2024; Pol and Ruegg, 2007b; with the notable exception of some studies finding S. aureus and NASM exhibiting moderate resistance against penicillin, see below). However, efforts to continue surveying and understanding the AMR patterns for these ubiquitous mastitis pathogens is warranted. The importance of S. aureus as a human pathogen is well-established (Tong et al., 2015), and virulence genes known to cause disease in both humans and animals have been demonstrated in NASM isolates from bovine IMI (Park et al., 2011; Unal and Cinar, 2012). Additionally, transmission of resistance genes between different staphylococcal species have led to the idea that NASM may act as a “reservoir” of AMR for more pathogenic staphylococcal species such as S. aureus (Cuny et al., 2017; Feßler et al., 2018; Khazandi et al., 2018).

A unique opportunity in which to assess the effect of antimicrobial use on AMR of these important mastitis pathogens is to compare dairy farm systems which are managed “conventionally” to those that are managed “organically.” Although the definition can differ by region (namely, the US and EU; see below), antimicrobial usage on “organic” dairies is usually less or non-existent when compared to “conventional’” dairy farms. When comparing bacterial isolates of bovine origin from these two types of systems, the general hypothesis is that AMR would be expected to diminish in prevalence when antimicrobial use is decreased or discontinued. Without the selective pressure of antimicrobial usage (as on organic dairies), bacterial strains containing resistance genes would gradually be replaced by susceptible strains, as selective advantage is no longer conferred by AMR carriage (assuming AMR carriage incurs a fitness cost; see below). The goal of this narrative review is to summarize studies which compared the relationship between antimicrobial usage at the farm level (organic vs. conventional) and antimicrobial susceptibility of bovine staphylococcal mastitis isolates.

Limitations and caveats for comparisons between studies

An important qualification when considering the body of work comparing resistance patterns of mastitis pathogens between management systems is that “organic” dairies differ between the US and Europe, where the majority of these studies have been carried out. Organic regulations in European countries still allow for some antimicrobial use (albeit with extended withdrawal periods and stricter veterinary oversight; EU Commission, 2024), while organic regulations in the US mandate that any animal treated with antimicrobials be permanently removed from the herd (USDA, 2024). The level of on-farm antimicrobial usage (and therefore selective pressure for resistance) therefore differs between European and US dairies, making comparisons between studies carried out under these varying regulations somewhat complicated. Specific rules for both organic dairy production certifications have evolved over time (Dimitri and Nehring, 2022; Grodkowski et al., 2023), further adding to the nuance of what is meant by “organic” dairy production in a retrospective analysis. The specific antimicrobials approved for usage in livestock varies by country, as well as which compounds are most commonly-used (e.g., for mastitis: penicillin in Finland, Taponen 2023; cephalosporins in the US, de Campos 2021). Even within the US, the amount and type of antimicrobials used in dairy cows changes over time as new products are developed or regulations around usage shift (USDA, 2009). Consequently, geographic and temporal differences can affect the type and amount of antimicrobial selective pressure experienced by mastitis pathogens on dairy farms.

Direct comparison of antimicrobial sensitivity results across studies can be problematic for a number of reasons. Importantly, the methodology used to determine the minimum inhibitory concentration (MIC) or categorization of an isolate as susceptible or resistant varies between studies. Further, inconsistencies exist between phenotypic and genotypic resistance results, due either to 1) detection of phenotypic resistance in the absence of expected genotypic determinants, or 2) phenotypic susceptibility despite the presence of genotypic determinants. For isolates of S. aureus associated with bovine mastitis, both of these types of discrepancies have been reported for penicillin resistance (Sampimon, 2009; Taponen et al., 2023). This also holds true for the other staphylococci; as summarized by Sampimon (2009), “agreement between phenotypic and genotypic test results for assessment of resistance of CNS of bovine origin to penicillin, oxacillin, and ML [macrolide] antibiotics depended on the antimicrobial compound of interest and on methods used to analyse and interpret test results, but was rarely perfect.” In a study by Taponen et al. (2023) comparing methods of testing for β-lactamase mediated resistance, overall agreement between phenotypic and genotypic resistance tests was moderate to substantial for staphylococci from bovine IMI. However, some inconsistencies were found between phenotypic susceptibility by disk diffusion method, the nitrocefin test to assess β-lactamase production, and PCR to detect the presence of the blaZ, mecA, and mecC genes encoding the β-lactamase gene. Disagreements have also been described within different methods of phenotypic determination of resistance for mastitis pathogens. A study comparing commercially-available broth microdilution plates (Sensititre Custom Plates) and agar disk diffusion for determining antimicrobial susceptibility of bovine IMI isolates found fair agreement overall (80.7%) between the two methods, but this varied based on the particular bacterial-antimicrobial combination tested (Palladini et al., 2023). No NASM species were included, but there was satisfactory agreement (89 to 100%) for S. aureus and all antimicrobial agents tested. In a study comparing Sensititre (broth microdilution) and disk diffusion for determining AMR in clinical mastitis pathogens, agreement was good for most isolate-antimicrobial MIC combinations (Saini et al., 2011). An important exception to this was that diagnostic accuracy was low when S. aureus was tested against both ceftiofur and oxacillin using either method. Low correlation was also found when S. aureus was tested against erythromycin and neomycin in another study comparing 2 dilution methods to determine MIC and disk diffusion diameters for mastitis-associated isolates (Klement et al., 2005). Further complicating comparison of AMR profiles between studies is shifting criteria for classifying an isolate as susceptible or resistant. Breakpoints for antimicrobial susceptibility testing are updated every few years, and multiple conflicting standards exist for categorization of resistant or susceptible bacteria which are dependent on geographical location (Clinical & Laboratory Standards Institute, CLSI; European Committee on Antimicrobial Susceptibility Testing, EUCAST).

Difference in sampling scheme for studies collecting milk from individual cows will affect observed prevalence of resistance in bacteria isolated from samples. Within the studies summarized in this review, sampling strategies for quartermilk and criteria for cow inclusion vary widely. Some studies included sampled cows in a herd at random or without using any specific criteria (Tikofsky et al., 2003; Bombyk et al., 2008; Garmo et al., 2010), while others used the California Mastitis test (CMT) to selectively sample cows with evidence of extant mastitis (Busato et al., 2000; Roesch et al., 2006). Bennedsgard et al. (2006) used a specific set of criteria in order to maximize their chances of sampling cows with S. aureus IMI specifically, while others sampled only multiparous cows in the herd (Pol and Ruegg, 2007a; McDougall et al., 2021). Sampling multiparous cows exclusively increases the likelihood samples collected will have an IMI, as increasing parity is a risk factor for mastitis generally (Barkema et al., 1998; Busato et al., 2000) and IMI with S. aureus specifically (Zadoks et al., 2001; Tenhagen et al., 2006). The likelihood of different NASM species causing IMI varies by parity, and resistance patterns are species-specific for NASM (see below). Therefore, sampling multiparous cows exclusively will bias which species are included and thereby the resistance profiles of mastitis pathogens described. A further consideration is whether the bacteria included were associated with cases of subclinical mastitis, clinical mastitis, or both. AMR has been shown to be more prevalent in NASM isolates associated with clinical vs. subclinical mastitis, so inclusion criteria around sample type will affect the observed AMR prevalence. Oxacillin resistance was more frequent in clinical mastitis isolates (56.5%) vs. subclinical mastitis isolates (43.9%; Frey et al., 2013), β-lactamase production was more common in subclinical vs. clinical cases (Persson Waller et al., 2011), and Wuytack et al. (2020) found carriage of the resistance gene mecA was proportionately higher in NASM isolates causing clinical vs. subclinical infection. However, as certain NASM are more likely to be associated with clinical mastitis vs. subclinical mastitis and vice versa (Persson Waller et al., 2011; although, see Condas et al., 2017b) and resistance patterns of NASM are species-specific (see below), this observed difference in AMR prevalence between sample type may ultimately result from species differences between the 2 categories. In Persson Waller et al. (2011), S. epidermidis and S. saprophyticus were more prevalent in subclinical vs. clinical mastitis, while S. hyicus was more common in clinical mastitis. The authors attribute the higher proportion of penicillin resistance in subclinical isolates to the high prevalence of S. epidermidis and S. saprophyticus in these samples, as these species demonstrated significantly more penicillin resistance when compared with other NASM. Further support that differences in AMR for NASM associated with clinical vs. subclinical mastitis is primarily a result of species differences is found in Naushad et al. (2018). In their analyses of 328 NASM isolates from samples with subclinical mastitis and 57 isolates from clinical mastitis, within the same species, no significant differences existed in the prevalence of drug-specific AMR or resistance determinants when contrasting the two sample types.

Summary of studies describing AMR of staphylococci from conventional vs. organic dairies

Nomenclature for the group of staphylococci causing bovine IMI excluding S. aureus has shifted over the past few decades, as both phylogeny and techniques for species-level identification have evolved. Some species which had been previously identified as staphylococci were recognized more recently as belonging instead to a closely related genus (Mammaliicoccus), and identification methods beyond a coagulase test have become more widely used. Although NASM is used throughout the rest of the review, the terminology used below when referring to results of a specific study is consistent with authors’ language and groupings of organisms (e.g., “coagulase-negative staphylococci,” or “CNS;” “non-aureus staphylococci,” or “NAS”). This decision was made in an attempt to be consistent with the original authors’ contemporary understanding of phylogeny and methodology.

Overall, studies comparing AMR of mastitis-associated staphylococci between herds under organic management and herds managed conventionally find either no difference or that isolates originating from organic farms exhibit slightly more susceptibility (Table 1). However, these studies vary widely in their approach to exploring this question, primarily in number of isolates included and herds sampled, as well as approach to statistical analysis. In a descriptive study from Switzerland, Busato et al. (2000) found that the proportions of S. aureus isolates from organic herds (ORG) resistant to different antimicrobials were equivalent to those from conventional herds (CON). Similarly, the proportions of resistant isolates of CNS were comparable between the two systems, with the exception of a numerically higher proportion resistant to rifamyin from organic herds. A limitation of this study is that the data describing susceptibility of staphylococci from conventional herds was from a previously unpublished survey by the authors, and not contemporaneous with analysis of the organic isolates. In another descriptive study, researchers in Norway (Garmo et al., 2010) found similar proportions of S. aureus and CNS isolates resistant to penicillin between the two herd types (S. aureus: 6/68 or 8.8% from CON, vs. 9/64 or 14.0% from ORG; CNS: 81/167 or 48.5% for CON, vs. 93/200 or 46.5% from ORG). The authors note that penicillin resistance was proportionately higher in CNS vs. S. aureus isolates, consistent with more recent work looking at the resistance of staphylococci from bovine milk samples (as summarized in Taponen et al., 2023). In a Swiss study comparing resistance profiles of NAS and S. aureus from quartermilk samples, Roesch et al. (2006) also found that NAS isolates exhibited a higher overall percentage of AMR than S. aureus isolates. For 12 antimicrobials representing either drugs used to treat mastitis in dairy herds or drugs important in human medicine, they found that percentage of AMR did not differ significantly between S. aureus and NAS isolates from cows kept on organic vs. conventional herds. Although the overall proportion of S. aureus isolates resistant to ≤1 antimicrobial was numerically higher from organic cows (16/46, 35%) vs. conventional cows (6/33, 18%), this difference was not statistically significant. The proportion of NAS isolates resistant ≤1 antimicrobial to between systems was very similar (ORG: 9/19, 47%; CON: 10/19, 53%).

In contrast, Bombyk et al. (2008) found that staphylococci causing mastitis on organic dairies were associated with more overall antimicrobial susceptibility than those from conventional farms. For this study, researchers differentiated mastitis-associated staphylococci into 3 categories: coagulase-positive Staph. (CPS), novobiocin-sensitive CNS (NSCNS), and novobiocin-resistant CNS (NRCNS). In an analysis combining all 3 groupings of staphylococci, a larger proportion of isolates from organic herds were susceptible to pirlimycin and tetracycline compared with those from conventional herds. Susceptibility to erythromycin and penicillin did not differ significantly by herd type when all staphylococci were combined (CON vs. ORG). No significant differences between organic and conventional systems were found for S. aureus, although the numbers of isolates found was fairly small compared to both categories of CNS (36 S. aureus vs. 210 NSCNS and 159 NRCNS). When each category of CNS (novobiocin-susceptible or resistant) was analyzed separately, isolates within both groups from organic herds were more likely to be susceptible to pirlimycin than CNS from conventional dairies. No difference in tetracycline, erythromycin or penicillin susceptibility was seen between herd types (CON vs. ORG) within either CNS category. A larger proportion of NSCNS vs. NRCNS (when analyzed separately for conventional and organic herds) were susceptible to tetracycline, leading the authors to suggest that management practices unrelated to antimicrobial use may contribute to the observed differences in susceptibility patterns of CNS on dairy herds.

A number of studies comparing resistance patterns of mastitis-associated bacteria between conventional and organic dairy systems have focused specifically on S. aureus. Researchers in New York and Vermont (US) found that S. aureus isolates from both types of herds showed good susceptibility to most antimicrobials used to treat mastitis, but isolates from organic herds were significantly more susceptible (Tikofsky et al., 2003). In this study, researchers took two different approaches to analyzing the data: 1) the strength of association between the proportion of susceptible and resistant isolates was evaluated by management category, and 2) numeric differences in mean zone diameter were compared for isolates from organic vs. conventional herds. When results were combined over both analyses, S. aureus isolates from organic herds were more susceptible than those from conventional herds for 7 of the 9 antimicrobials studied. Contrary to these findings, researchers comparing resistance of isolates from bulk tank milk of organic and conventional systems in both the US and Denmark found that overall, antimicrobial susceptibility was very similar for S. aureus in both countries (Sato et al., 2004). Bulk tank isolates from conventional herds in Wisconsin (US) had significantly reduced susceptibility to ciprofloxacin (vs. isolates from organic herds), and isolates from organic herds in Denmark had reduced susceptibility to avilamycin (vs. isolates from conventional herds). In a finding highlighting the importance of geography in epidemiological studies, authors point out that differences in the antimicrobial susceptibility of S. aureus isolates between organic and conventional herds were small relative to differences in resistance patterns observed between countries. In agreement with Sato et. al, Bennedsgaard et al. (2006) observed no statistically significant differences in the prevalence of cows with penicillin-resistant S. aureus mastitis or the proportion of S. aureus isolates from quartermilk resistant to penicillin between conventional and organic dairies in Denmark.

Two studies looking at bulk tank milk (BTM) focused on detection of staphylococci carrying genetic determinants conferring penicillin resistance (mecA and mecC genes), an important consideration for public health globally. In a large study with the goal of surveilling dairy-associated methicillin-resistant S. aureus (MRSA) in Germany, researchers collected BTM from 372 conventional and 303 organic herds (Tenhagen et al., 2018). Using binary logistic regression to describe association of MRSA-positive samples with herd type (conventional vs. organic), they found that the prevalence of MRSA was significantly higher in BTM samples from conventional herds (9.7%) compared with organic herds (1.7%). The model-based approach allowed researchers to control for the effects of geographical region and herd size, both of which were also significant predictors of MRSA herd status. When comparing the proportion of BTM MRSA isolates resistant to 12 different antimicrobials between conventional and organic herds, MRSA isolates from conventional farms tended to be more resistant. However, as there were a limited number of isolates from organic herds (n = 5) compared to conventional herds (n = 36), no statistical analyses were performed. A large, multistate study in the US sampled BTM from 192 organic herds and 100 conventional herds matched for geographical location and herd size (Cicconi-Hogan et al., 2014). They identified 13 isolates from BTM as methicillin resistant (mecA-positive): 7 isolates from conventional herds and 6 from organic. Using 16S rRNA and rpoB genes for species-level identification, these 13 isolates were identified as S. aureus (n = 1), S. sciuri (n = 5), S. chromogenes (n = 2), S. saprophyticus (n = 3), S. agnetis (n = 1), and Macrococcus caseolyticus (a genus closely related to staphylococci; n = 1). Surprisingly, the single methicillin-resistant S. aureus isolate was from an organic herd, for an observed 0.3% prevalence of MRSA at the herd level. Methicillin-resistant CNS were found at a prevalence of 2% in the organic population and 5% in the conventional population. The authors highlight the relatively large number of methicillin-resistant S. sciuri identified (6 out of the 12 methicillin-resistant CNS) compared with previous work, and also suggest that a potential methicillin-resistant Staphylococcus reservoir in the dairy herd population of the US may be independent of the type of production system. To this point, Walther and Perreten (2007) report the occurrence of a dairy cow on an organic farm in Switzerland that was diagnosed twice within 2 months with subclinical mastitis caused by methicillin-resistant S. epidermidis. The two strains had identical PFGE patterns of chromosomal DNA, exhibited resistance to chloramphenicol, and contained streptomycin- and trimethoprim-resistance genes but did not display phenotypic resistance against these drugs in vitro. Furthermore, the second S. epidermidis isolate contained an additional aminoglycoside-resistance gene, indicating the potential acquisition of resistance by horizontal gene transfer since isolation of the first bacterium. Similar to Cicconi-Hogan et al. (2014), the authors highlight that this finding demonstrates cows on organic farms may harbor multidrug-resistant staphylococci despite the limited use of antimicrobials under EU organic regulations.

Perhaps a limitation of the above studies comparing the resistance of staphylococci from organic and conventional dairy farms is that limited or no quantification of on-farm antimicrobial usage was calculated or presented. In order to evaluate if the level of antimicrobial usage in food animals selects for drug-resistant pathogens, an important component in a study exploring this question would be a quantification of antimicrobial use at the farm or cow level to be able to estimate the amount of selective pressure exerted on intramammary pathogens. Although all antimicrobial usage is prohibited on US organic dairies, the amount and type of antimicrobials used by conventionally-managed farms can vary widely (Pol and Ruegg, 2007b). Two of the largest-scale, statistically robust studies comparing the resistance profiles of staphylococci from quartermilk samples between conventional and organic dairies include a detailed, numeric quantification of antimicrobial usage by enrolled farms. In a 2007 study in the US, Pol and Ruegg report a standardized level of exposure to 10 different antimicrobials by calculating of the number of defined daily doses used per cow on each enrolled farm, and then categorize the 40 enrolled herds based on their respective antimicrobial usage. Herds are categorized into 3 groups: organic (no antimicrobial usage), conventional–low usage (conventional farms not using or using ≤ the first quartile of use for each drug; CON-LO), and conventional–high usage (conventional farms using > the first quartile for a particular drug; CON-HI). The authors took multiple approaches to compare resistance among isolates from the 3 antimicrobial usage groups. First, they compared the proportion of each type of isolate (CNS or S. aureus) that was susceptible or resistant in each category (CON vs. ORG) using a categorical test of association, in order to explore if proportion of susceptible isolates was independent of herd type. Secondly, they used a test of association to explore if the MIC for each type of isolate (CNS or S. aureus) was independent of herd type (CON vs. ORG). Lastly, they performed survival analysis for each type of isolate (CNS or S. aureus) based on the 3 antimicrobial usage categories (ORG, CON-LO, or CON-HI). In this last analysis of “time to event,” antimicrobial concentration in wells of the susceptibility test was considered “time,” and the “event” was inhibition of any bacterial growth. Overall, Pol and Ruegg found that isolates from organic herds were more susceptible to antimicrobials than those from conventional herds. Specifically, for S. aureus: (1) isolates from conventional herds were more likely to be resistant to ampicillin and penicillin when compared with isolates from organic herds, and herd type was not associated with the proportion of resistant isolates for the other antimicrobial drugs tested; (2) isolates from conventional herds had a higher MIC for pirlimycin and sulfadimethoxine compared with isolates from organic herds, and herd type was not associated with the MIC of the other antimicrobial drugs tested; and (3) in the survival analysis, the MIC that inhibited 90% (MIC90) of S. aureus isolates from organic herds for penicillin and pirlimycin was lower than the MIC90 of the isolates from CON-LO and CON-HI herds (MIC50, the MIC that inhibited 50% of isolates, was not different for these drugs). For CNS: (1) isolates from conventional herds were more likely to be resistant to ampicillin, penicillin, pirlimycin, and tetracycline compared with isolates from ORG herds, and herd type was not associated with the proportion of resistant isolates for the other antimicrobial drugs tested; (2) isolates from conventional herds had a higher MIC for ampicillin, pirlimycin, and tetracycline compared with isolates from organic herds, and herd type was not associated with the MIC of the other antimicrobial drugs tested; and (3) in the survival analysis, the MIC90 of CNS isolates from organic herds for ampicillin, penicillin, pirlimycin, and tetracycline was lower than the MIC90 of the isolates from CON-LO and CON-HI herds (ORG and CON-LO herds had a lower MIC50 for erythromycin than CON-HI herds, but the MIC90 did not differ by usage group). The authors highlight that although some differences were found between antimicrobial usage groups, most isolates from all farm types were inhibited at the lowest dilution tested of most antimicrobial drugs routinely used on dairy farms.

The other study comparing resistance of staphylococci between organic and conventional dairies to include a detailed quantification of antimicrobial usage enrolled 7 organic herds, 11 conventional herds using ampicillin-cloxacillin dry cow therapy (CON-AC), and 8 conventional herds using cephalonium dry cow therapy (CON-CE) in New Zealand (McDougall et al., 2021). Although the study was carried out in NZ, participating herds were all certified under the USDA National Organic Program. Conventional herds of both categories were selected on the basis that >50% of the cows were treated in each of the 3 previous years with at least 1 dry cow therapy (DCT) product. Similar to Pol and Ruegg (2007a), the authors took a multifaced approach to exploring the resistance patters of S. aureus and CNS from organic and conventional systems. Overall, the MIC of CNS from ORG herds were lower than isolates from both types of CON herd. For S. aureus, they found that the MIC50 for ampicillin and penicillin were greater by more than 1 dilution for isolates from CON-CE herds compared with CON-CA and ORG herds, but this relationship did not hold for the MIC90 of these drugs (MIC for CON-CE and ORG herds was greater than that for CON-CA herds). In a univariate analysis, the proportion of penicillin-resistant S. aureus isolates was significantly higher in CON-CE herds (76/111; 68.5%) compared to CON-CA (4/99; 4.0%) or ORG herds (32/110; 29.1%). A multilevel model (accounting for clustering of quarter within cow within herd) was made, where the 3 herd types were the main explanatory variable. Other potential variables offered to this model included age of the cow, breed, DIM at time of sampling, SCC at last test, and antimicrobial treatment history for that cow. Results from this multilevel model showed that the proportions of penicillin-resistant S. aureus isolates did not differ between the 3 herd types. For analysis of resistance to ceftiofur, sulfadimethoxine, and erythromycin, 3 different groupings of breakpoints were made for each compound. When comparing the proportion of S. aureus isolates falling into the 3 different breakpoint groups for ceftiofur resistance, the only significant difference was that there were fewer organic isolates in the middle breakpoint category (1 μg/mL); otherwise, there were no differences in the proportion of isolates falling into the different breakpoint groups from each of the 3 herd types. When comparing the proportion of S. aureus isolates falling into 3 different breakpoint groups for sulfadimethoxine resistance, the only significant difference was that there were more organic isolates in the lowest category (32 μg/mL); otherwise, there were no differences in the proportion of isolates falling into the different breakpoint groups from each of the 3 herd types. There were no significant differences between the 3 herd types when comparing the proportion of S. aureus isolates falling into 3 different breakpoint groups for erythromycin resistance. For CNS isolates, the MIC50 and MIC90 for ampicillin and penicillin were lower by more than 1 dilution for CNS isolates from organic herds compared to both types of conventional herds; otherwise, these values did not differ by more than 1 dilution between the 3 herd types for the other antimicrobials tested. In a univariate analysis, the proportion of penicillin-resistant CNS isolates was significantly greater in both types of conventional herds (CON-CE, 42/82; 51%; CON-CA, 22/74; 30%) vs. organic herds (14/84; 17%). Similar to the analyses for S. aureus, a multilevel model was made to compare penicillin resistance of CNS with herd type as the main explanatory variable. Results from this multilevel model showed that the proportion of penicillin-resistant CNS isolates was significantly greater for CON-CE herds (0.50 ± 0.07) compared to CON-CA (0.31 ± 0.06) or ORG herds (0.17 ± 0.05). When comparing the proportion of CNS isolates falling into 3 different breakpoint groups for ceftiofur resistance, the only significant difference was that there were more organic isolates in the lowest (0.5 μg/mL) and highest (2 μg/mL) categories compared to both conventional herd types; otherwise, there were no differences in the proportion of isolates falling into the various breakpoint groups from each of the 3 herd types. There were no significant differences between the 3 herd types when comparing the proportion of CNS isolates falling into 3 different breakpoint groups for sulfadimethoxine resistance. When comparing the proportion of CNS isolates falling into 3 different breakpoint groups for erythromycin resistance, the only significant difference was that there were more CON-CA isolates in the highest category (≥1 mg/mL); otherwise, there were no differences in the proportion of isolates falling into the different breakpoints from each of the 3 herd types. Importantly, the authors point out that any differences in MIC between isolates from different herd types occurred below clinical breakpoints, so therefore may not affect bacteriological cure rates. Rather unexpectedly, they found bimodal distributions of MIC for ampicillin and penicillin in S. aureus isolates from organic herds, suggesting either (1) isolates with a higher MIC are “a natural part of the bacterial population of the bovine mammary gland,” or (2) isolates with higher MIC have persisted within organic herds from a time when antimicrobials were used on the farm.

Dairy farms in the process of transitioning from conventional management to organic certification provide a unique opportunity to study patterns resistance over time after a change in the level of antimicrobial exposure. In addition to comparing conventional and organic farms, Bennedsgaard et al. (2006) followed 19 Danish herds in the process of transitioning to becoming certified organic dairies. These herds were sampled at year 0, 1, and 2 of transition, with quartermilk samples collected from 30 cows at each farm at high risk of infection with S. aureus (as determined by a score based on a history of high SCC, breed, and lactation). Herds in the “old organic” category were certified for ≥ 5 years. Antimicrobial exposure for each herd was approximated by calculating the amount of mastitis treatments used in % cows treated/cow-year. The amount of mastitis treatment used by the conventional group was significantly higher than “old organic” herds, but no other significant differences existed between “old organic” herds or the conventional herds in comparison to any of the transition groups (transition year 1, transition year 2, transition year 3) with respect to usage of antimicrobial mastitis treatments. As previously mentioned, the prevalence of penicillin resistance in S. aureus and the proportion of penicillin-resistant isolates was similar between “old organic” and conventional herds. Furthermore, no differences were seen in these measures of penicillin resistance between “old organic,” conventional, or any of the 3 transition groups. The same 19 herds were sampled repeatedly over 3 years, and the amount of penicillin resistance among S. aureus on these farms did not decrease year after year as they transitioned to organic status. This finding is somewhat unsurprising in light of the fact that antimicrobial usage also was not significantly different. In contrast, Park et al. (2012) found that β-lactam resistance rates of CNS decreased with discontinuation of β-lactam antibiotics in a study following 2 dairies through the process of converting from conventional to organic management over a 3-year period. Composite milk samples were collected from cows at the end of lactation, at freshening, and from cases of clinical mastitis during the last year of conventional dairy production, the transition year, and during the first year of organic production. While still conventional, cows with clinical mastitis were treated with an intramammary product with pirlimycin, and a product with cephapirin, streptomycin and penicillin, or novobiocin and penicillin was given to all cows at dry-off. There was a significant increase in zone diameter for mastitis-associated CNS isolates against cephalothin, cloxacillin, and penicillin when comparing the conventional vs. organic phase. There was no significant change in zone diameter of the other 8 antimicrobials tested. Interestingly, no changes in resistance patterns were seen for mastitis-associated S. aureus isolates for the 12 antimicrobials tested. Of importance to note is that the 2 farms in Park et al. were in the US, and therefore antimicrobial usage was completely discontinued at the beginning of the transition to organic status. A similar small-scale case report from Thailand compared AMR of mastitis pathogens before and after the experimental farm’s transition from conventional to organic status for 7 antimicrobial drugs used to treat mastitis (Suriyasathaporn, 2010). All cows in the herd were sampled before beginning the transition, and after 6 months of operating as an organic dairy. The frequency of antimicrobial treatment on the farm decreased from <3 cases/month to > 1 case/month during the study period. Although isolate numbers were small (7 CNS isolates from before transition, 6 from after), a significant decrease was seen in the percent of CNS isolates resistant to gentamycin. Although numeric decreases in percent of resistant CNS isolates were seen for the other 6 antimicrobials, no changes were statistically significant. Data on susceptibility was not reported for S. aureus isolates.

Additional factors explaining variation in antimicrobial susceptibility of staphylococci

Although some evidence exists that conventional vs. organic management may influence the prevalence of AMR in staphylococci causing bovine IMI, this relationship is difficult to tease out from other factors determining the resistance profiles of these mastitis pathogens. This is especially true for NASM (primarily grouped as “CNS” in these studies), where prevalence and type of AMR carriage differs by species. Herd-level management factors, cow-level factors, and geography have all been shown to influence which NASM species may be present or predominant in causing IMI in a particular herd (see below). It is therefore difficult to attribute differences in AMR prevalence of NASM without accounting for this species-level effect. Table 2 summarizes work describing the species-specific antimicrobial susceptibility of staphylococci isolates from bovine IMI. The 10 observational studies included describe phenotypic resistance profiles and are limited to work where isolates were identified to species level using genotypic techniques or MALDI-TOF.

When considered as a group, resistance to β-lactam antibiotics is the predominant type of AMR present in staphylococci. The reported proportion of NASM isolates with β-lactamase resistance can be fairly high, with 51.6% phenotypically resistant to penicillin in Argentina (Raspanti et al., 2016), 63% phenotypically resistant to penicillin in South Africa (Phophi et al., 2019), and 80% of CNS isolates positive for the blaZ gene (encoding the production of a β-lactamase enzyme) in a study from the Netherlands (Sampimon, 2009). Proportion of phenotypically penicillin-resistant NASM seems to vary geographically, with Nordic countries reporting 34% (Nyman et al., 2018), 23% (Fergestad et al., 2021), and 29% (Persson Waller et al., 2011), while a Korean study found 14% of NASM isolates were resistant to penicillin (Kim et al., 2019) and Nobrega et al. (2018) report a prevalence of 10% in Canada. β-lactam antibiotics are among the few choices for treating mastitis in the US, with first- and third-generation cephalosporins being the most commonly-used mastitis treatments (USDA, 2016; de Campos et al., 2021). Moderate resistance has been observed in NASM against tetracycline, another highly important antimicrobial frequently used in dairy herds, with 30.1%, 20.9%, and 10% of isolates reported to be resistant in Argentina, India, and Canada, respectively (Raspanti et al., 2016; Mahato et al., 2017; Nobrega et al. 2018). This marked geographic variation in resistance patterns may likely be due to differing selective pressure in dairy farm systems around the world. Which specific antimicrobials are most typically used to treat mastitis and in what amount, as well as the various regulation around their usage, varies from country to country.

Studies comparing NASM at the species level have consistently shown that AMR profile varies between species (Sampimon, 2009; Persson Waller et al., 2011; Taponen et al., 2016; Nobrega et al., 2018; Fergestad et al., 2021; Taponen et al., 2023). Overall, both phenotypic resistance and resistance genes are relatively rare in the most common species, S. chromogenes, in comparison to other NASM (Sampimon, 2009; Persson Waller et al., 2011). A notable exception is the presence of the blaZ gene, which was found in 80% of all 170 CNS isolates and 87% of S. chromogenes specifically in a Flemish study (Sampimon, 2009). β-lactamase production was significantly lower for S. chromogenes vs. S. epidermidis and S. haemolyticus in Sweden (Persson Waller et al., 2011). Although a smaller-scale study in Argentina found a relatively high proportion of S. chromogenes were resistant to penicillin (45.1%), both S. haemolyticus and S. xylosus had an even higher proportion of penicillin-resistant isolates (58.6% and 92.9%, respectively; Raspanti et al., 2016). Across a number of studies, authors report that some less-commonly isolated NASM species carried AMR profiles which were the most concerning for public health. Sampimon et al. (2011) found a high prevalence of genotypic resistance (particularly mecA) or presence of multiple resistance genes in species with relatively a low prevalence (S. cohnii, S. equorum, S. fleurettii, and S. sciuri). In Nobrega et al. (2018), resistance to quinupristin/dalfopristin (a combination used to treat serious nosocomial infections in humans) was common in S. gallinarum (98% prevalence of resistance among isolates), and S. cohnii and S. arlettae were frequently resistant to erythromycin (prevalence of 63 and 100%, respectively). The authors specifically highlight S. arlettae as worrisome in its AMR profile; it had the highest prevalence of AMR against penicillin (61%), ampicillin (23%), erythromycin (100%), pirlimycin (18%) and clindamycin (99.9%), as well as the highest prevalence of multidrug resistance. A number of studies also call attention to concerning AMR patterns for S. epidermidis, which is moderately common in the US and Canada but one of the predominant species found in Nordic countries. In Sampimon et al. (2009), S. epidermidis was the second most commonly-found species, it carried multiple resistance genes in ~50% of isolates, and phenotypic penicillin resistance was more common compared to other CNS. The proportion of penicillin-resistant isolates was highest for S. epidermidis in a Finnish study compared to other species, with S. epidermidis accounting for 6/8 NASM isolates carrying the mecA gene (Taponen et al., 2023). Similarly, β-lactamase production was higher for S. epidermidis compared to other species (Persson Waller et al., 2011), and it was one of a few species where AMR (including resistance to trimethoprim-sulfonamide) was most frequently observed in Fergestad et al. (2021). Lastly, Taponen et al. (2016) found that S. epidermidis was the most resistant among the four major species studied, several isolates were multidrug resistant, and 19% of isolates were mecA-positive (encoding for methicillin resistance). Even within a given species, AMR carriage has been linked to certain strain types. For S. aureus, carriage of methicillin resistance has been associated with particular clonal complexes both in human medicine (Smith et al., 2021; Garrine et al., 2023) and certain clusters of spa ¬type for bovine clinical mastitis isolates (Freu et al., 2022). The linkage between strain type and AMR is not as well studied for NASM, but Persson Waller et al. (2023) found that blaZ was significantly more common among S. chromogenes strains belonging to 2 specific clusters of strain types vs. strains belonging to other clusters.

As AMR carriage differs by species, the particular diversity of NASM responsible for causing IMI on a farm will partly determine the observed herd-level resistance pattern. Various regional and herd-level risk factors have been identified explaining some of the diversity and prevalence of different NASM associated with mastitis and BTM. Different times of year were associated with higher likelihood of IMI for S. chromogenes, S. haemolyticus, S. xylosus, and S. warneri in Dolder et al. (2017), and S. cohnii, S. simulans, S. sciuri in BTM in De Visscher et al. (2017). Geographical differences in NASM species diversity among quartermilk samples were found between 4 regions in Canada (Condas et al., 2017a) and 4 states in the US (Jenkins et al., 2019). It is difficult to discern whether these differences are truly a function of geographical variation, or result from farms in a region sharing a similar suite of management practices leading to similar NASM species prevalence and diversity in a herd. Although S. chromogenes is the dominant species causing IMI in many countries (as summarized in De Buck et al., 2021), S. epidermidis (closely followed by S. simulans) was the most commonly-found species in both a Finnish (Taponen et al., 2022) and a Swedish study (Nyman et al., 2018). At the herd level, facility type has been shown to explain some of the diversity of NASM species: cows from herds using a tiestall barn were more likely to have an IMI due to S. simulans, S. xylosus, S. cohnii, S. saprophyticus, S. capitis, and S. arlettae compared with other NASM species, and less likely to have an IMI due to S. epidermidis (Condas et al., 2017a). Cows from herds in Canada using a bedded pack system had a higher relative risk for IMI due to S. chromogenes and S. sciuri vs. other NASM (Condas et al., 2017a), while Adkins et al. (2022) found S. cohnii, S. hyicus, and S. pseudintermedius in BTM from sand-bedded freestalls (but not bedded packs), and S. pasteuri and S. piscifermentans were unique to BTM from bedded packs. In a study by Piessens et al. (2011), sawdust bedding material was associated with IMI due to S. xylosus and S. succinus for Belgian dairy herds. De Visscher et al. (2017) identified a number of management practices around milking protocol and hygiene associated with the presence of different NASM species in BTM. These include a decreased risk for S. xylosus, S. simulans, and S. chromogenes in BTM from herds that clip udders, a decreased risk of S. devriesei in herds with consistent glove use during milking, an increased likelihood of S. cohnii in herds sharing towels between cows when drying udders, and a decreased likelihood of S. haemolyticus, S. cohnii, and S. simulans in herds that flushed or steamed milking units after use. Hogan et al. (1987) found more IMI due to S. epidermidis in herds using no teat dip compared to herds that did, and that S. hyicus constituted a greater proportion of staphylococci IMI in herds that used teat dip vs. herds that did not. However, it should be noted that species-level identification of staphylococci in this study was performed using a biochemical test, which may have had limited typeability and accuracy for identification of bovine staphylococci isolates (Vanderhaeghen et al., 2015). Lastly, some herd-level management factors associated with NASM diversity were related to feed and water provided to dairy cows: De Visscher et al. (2017) found an increased likelihood of S. simulans in BTM if drinking water for cows was from a public supply (vs. a well), and Petzer et al. (2022) reported proportionally more IMI due to S. chromogenes from herds that were pasture-based compared to those that were fed a total mixed ration (TMR), while S. haemolyticus was more likely to cause IMI for TMR herds.

Risk factors at the cow level which affect the likelihood of IMI with different NASM have also been identified. Both Thorberg et al. (2009) and Mork et al. (2012) found that S. chromogenes was more likely to be isolated from first-lactation animals, while S. epidermidis was found more often in third-lactation and older cows. These findings are consistent with 3 other studies reporting S. chromogenes, S. xylosus, and S. simulans more commonly caused IMI in heifers vs. third-lactation and older cows (De Visscher et al., 2016; Condas et al., 2017a; Nyman et al., 2018). The most likely species to cause IMI also varies within a lactation: Dolder et al. (2017) found that S. xylosus was more commonly found in early lactation and S. warneri was isolated from mid- to late-lactation animals, while Condas et al. (2017a) report the prevalence of S. chromogenes, S. gallinarum, S. cohnii, and S. capitis to be highest at freshening, and the prevalence of S. chromogenes (after an initial decrease from levels at freshening), S. haemolyticus, S. xylosus, and S. cohnii increased throughout lactation. In Belgian herds, S. chromogenes was the predominant species causing IMI both at parturition and throughout lactation; the next most commonly seen species at freshening were S. sciuri and S. cohnii (De Visscher et al., 2016), while S. simulans, S. xylosus, S. epidermidis, and S. haemolyticus were the next most common causes for NASM IMI during lactation (Piessens et al., 2011; Supré et al., 2011). Dirty teats have been associated with an increased likelihood of IMI due to S. cohnii, S. equorum, S. saprophyticus, and S. sciuri, which the authors indicate is consistent with a likely environmental origin for these species (De Visscher et al., 2016). Even physical features of the udder and teats have been associated with different NASM species (De Visscher et al., 2016: quarters with an inverted teat end had higher odds of being infected with S. chromogenes, S. simulans, or S. xylosus; Dolder et al., 2017: udder edema was a risk factor for IMI with S. chromogenes).

In addition to unmeasured animal or management-associated risk factors, an important determinate in AMR carriage of mastitis isolates is clonal dissemination within a particular herd. Consistent with behavior of a contagious mastitis pathogen, a certain strain (or strains) of S. aureus will predominant for any given herd (Lange et al., 1999; Zadoks et al., 2000; Freu et al., 2022). If the dominant strain of S. aureus causing IMI in a dairy herd happens to carry a given AMR determinant, a high proportion of S. aureus isolates from that herd will likely exhibit phenotypic resistant against a particular antimicrobial: not solely as a result of environmental pressure and selection, but also as a consequence of phylogeny and the behavior of the pathogen itself. This dominant strain type effect can result in issues of non-independence between isolates from a particular farm (Call et al., 2008), which would be exacerbated in studies enrolling a relatively small number of herds. Pol and Ruegg (2007a) directly address this issue of statistical dependence in their study of 40 herds. In order to avoid dependence between the cow, herd, and exposure category (conventional vs. organic), the authors included only 1 isolate per cow and ≤ 20 isolates per herd in all analyses. Additionally, they report the range of isolates used per herd for each category of mastitis pathogen.

Why is AMR maintained in organic systems?

In almost all studies summarized in this review, some degree of AMR was found in isolates despite decreased (EU) or absence (US) of selective pressure of antimicrobial use; organic farms in McDougall et al. (2021) had no antimicrobial usage for a range of 7-19 years, with a median of 12 years of organic certification. Assuming there is a fitness cost to bacteria for maintaining AMR genes (Vanacker et al., 2023), this certainly begs the question of why resistance genes have been maintained to any degree in the absence of selective antimicrobial pressures. A rather extreme example of AMR persistence in cattle farms is a study comparing bacteria isolated from retail ground beef raised in conventional and “raised without antibiotics” operations. LeJeune and Christie (2004) identified resistance against chloramphenicol in isolates from both systems, an antimicrobial that had been banned from use in US food animals since 1986. Resistant bacteria remaining on organic farms long after selective pressure of antimicrobial use is gone suggests that other factors play an important role in this long-term persistence. In a study where feedlot steers were fed subtherapeutic levels of antibiotics, Alexander et al. (2008) found that ampicillin-resistant E. coli in the control group (no antibiotics) increased due to an evident clonal expansion of an environmental strain (detected by PFGE) during the latter part of this longitudinal study. This environmental strain outcompeted other strains of E. coli present in the intestinal tract of the steers in the control group, suggesting that fitness traits beyond carriage of AMR genes play an important role in the prevalence of AMR bacteria. Specifically, the authors suggest that one environmental factor related to the level of AMR was diet, as the prevalence of steers shedding tetracycline-resistant E. coli was higher in animals fed grain-based vs. silage-based diets in both treatment and control groups. Although specifically looking at commensal E. coli in dairy calves and not mastitis pathogens, one group of researchers set out to explore which factors beyond antimicrobial usage may explain the persistence of an E. coli strain (SSuT) in the GI tract which was resistant to streptomycin, sulfonamide and tetracycline (Khachatryan et al., 2004, 2006a, 2006b, 2008; as summarized in Call et al., 2008). Their first study asked if direct antimicrobial selection pressure was maintaining a high prevalence of SSuT E. coli strains in calves, and they found that it was not; a clinical trial showed that addition or removal of oxytetracycline from the diet had no effect on the prevalence of SSuT strains in fecal samples over a period of 3 months. Their next step was to ascertain if SSuT traits themselves provide a secondary but unrecognized fitness advantage to these particular strains of E. coli by generating null mutants for the SSuT traits (now susceptible to these antibiotics). On average, they found that the null mutant strains retained a competitive advantage over the other susceptible strains, and concluded that the specific genes conferring the SSuT phenotype were not responsible for providing any secondary fitness advantages. At some point between studies, the farm stopped feeding a medicated milk replacer. The researchers observed that after only a short time frame, the SSuT strain had suddenly declined in prevalence. This was unexpected, given that their previous work demonstrated that the SSuT strains had an obvious advantage compared to the susceptible strains. This unexplained decline prompted an additional study, which hypothesized that the milk supplement itself (comprised of dried milk powder, vitamin A and D) was somehow providing an advantage to the SSuT strains. When the milk supplement was reintroduced (both with and without tetracycline), the prevalence of SSuT E. coli strains nearly doubled for both groups of animals receiving the milk supplement vs. those that received none. This work highlights an example of a positive selective force (a dietary supplement) in a dairy farm system either directly or indirectly favoring strains of resistant E. coli, which was completely unrelated to antimicrobial exposure.

Call et. al (2008) summarize the 3 possible outcomes after exposure to antimicrobials in an individual animal produces a transient increase in AMR prevalence in a population of bacteria, as has been documented to occur in fecal bacteria. Once the negative selective pressure of antimicrobial usage is removed, the first possible outcome is subsidence of AMR in the population, assuming there is a fitness cost to maintaining the AMR traits. Alternatively, if there is no additional fitness cost to maintaining AMR, we would expect to see “eventual displacement in the face of natural turnover of clonal types at the level of individual animals.” A third possibility, as seen in the work from Khachatryan et al., is that there is no (or limited) change in the level of AMR prevalence after selective pressure from antimicrobials is removed. This could occur if AMR traits have been coupled with other some other locally beneficial traits which provide the bacteria possessing them an advantage in their specific environmental niche. Call et al. (2008) illustrate this with a hypothetical model illustrating the effect of antimicrobial exposure in an individual animal (Figure 1). First, a transient increase occurs in the relative number of resistant bacteria within a population after exposure to an antimicrobial. During this time of increased replication, there is an increased probability for a genetic event to occur which links AMR carriage to some other trait providing increased fitness in that specific environment. Organisms with the linked AMR carriage and locally advantageous trait survive better in the population, but in the absence of antimicrobial exposure, there is nothing to actively suppress the susceptible strains in the population. Although the relative proportion of bacteria with AMR may decline gradually over time, linkage of AMR to some other advantageous trait could also lead to a gradual increase or maintenance of a baseline prevalence of AMR, even in systems devoid of antimicrobial exposure. So far, work exploring this question has been limited to studying the effect of antimicrobials on resistant bacteria present in the GI tract of cattle. The potential exists for research focused on exploring why maintenance of AMR genes occurs in mastitis pathogens from organic dairies, years after the selective pressure of antimicrobial use has been removed.

Conclusions

Organic dairy systems provide a novel opportunity in which to identify the antimicrobial resistance patterns of mastitis pathogens experiencing decreased or no selective pressure from antimicrobial use. This narrative review aimed to summarize studies comparing antimicrobial susceptibility of bovine staphylococcal mastitis isolates on organic vs. conventional dairy farms. Numerous factors make direct comparisons of AMR results difficult between studies, including: use of various methods for antimicrobial susceptibility testing and continuously evolving or conflicting schemes for breakpoints; variation in sampling scheme (random vs. targeted sampling of cows, bulk tank milk vs. quartermilk samples, inclusion of isolates associated with clinical vs. subclinical mastitis); differing definitions of “organic” between herds in the EU (where antimicrobial usage is still allowed, but is more tightly regulated and limited) and the US (any animal treated with antimicrobials must leave the herd). Furthermore, studies including a limited number of herds may suffer from a lack of independence between observations. However, the overall conclusions from each study comparing the two different management systems are still informative, as long as the methodology is consistent within a study. Generally, studies comparing the resistance profiles of staphylococci associated with bovine milk samples show that isolates from organic farms are similar or slightly more susceptible to antimicrobials than those associated with mastitis on conventional farms. Although some level of resistance was observed against a number of antimicrobials important for veterinary medicine (cephalosporins, penicillin, tetracycline), overall resistance of mastitis-associated staphylococci is generally low and the most commonly-used mastitis treatments are still effective. A considerable amount of resistance for both NASM and S. aureus against penicillin has been described, but the majority of isolates in European and US studies remain susceptible.

Another factor influencing AMR of staphylococci causing mastitis at the herd level is the particular assortment of NASM causing IMI in a herd, as resistance profiles are species-specific. Consequently, different management factors (unrelated to antimicrobial usage) which affect the prevalence and species diversity of NASM on particular farms can indirectly affect the prevalence of observed AMR in a herd. Furthermore, as strain types within species can differ in likelihood of AMR carriage, AMR prevalence may also be a function of predominate strain type(s) in a given herd.

A consistent finding between all studies described was the persistence of resistant mastitis-associated staphylococci on dairy farms which had not used antimicrobials for many years. Some insight on this phenomenon may be gleaned from a theory put forth to explain the observed maintenance of AMR in fecal bacteria in cattle, despite the absence of antimicrobial use. In the transient expansion of a population of resistant isolates following antimicrobial treatment, the likelihood increases that an AMR gene can become linked with some other locally advantageous trait during replication. The selective advantage bestowed on the resistant bacteria could then lead to an increase in their relative abundance and maintenance of the AMR genes over the long-term, provided that the trait linked to AMR continues to afford a selective advantage.

The biggest limitation of most studies comparing resistance profiles of mastitis pathogens between organic and conventional farms is that staphylococci were not identified to the species level. Organisms were primarily grouped as either S. aureus or “coagulase-negative staphylococci.” Before MALDI-TOF became more widely available, accurate species-level identification of mastitis-associated staphylococci on a relatively large scale was prohibitively expensive and time-consuming. As resistance profile varies by species, additional work comparing AMR for NASM isolates (while controlling for species) may give further insight into whether resistance profiles differ between management systems for these bacteria. Comparison of predominant strain types within a given species causing IMI between organic and conventional farms could further our understanding of the complex interplay between phylogeny and selection pressures resulting from management factors on AMR of mastitis pathogens. Although researchers were studying fecal E. coli and not mastitis pathogens, Walk et al. (2007) found that phylogenetic groupings varied between organic and conventional dairies, suggesting there may be differences between lineages of E. coli in their ability or likelihood of acquiring resistance genes. Based on their findings, the authors conclude that “organic farming practices not only change the frequency of resistant strains but also impact the overall population genetic composition of the resident E. coli flora.” Additionally, few studies have described resistance patterns of mastitis pathogens before and after transitioning to organic status, and most were limited in both the number of herds enrolled and the amount of time farms were followed. Although likely logistically difficult and expensive, a long-term, larger study of farms transitioning from conventional to organic status would be incredibly valuable in understanding what types of AMR are maintained in organic dairy herds and for how long.

Fortunately, AMR in general remains relatively low in mastitis pathogens from dairy farms. Nevertheless, continued surveillance and further understanding of factors affecting resistance of staphylococci is warranted. Not only are they important pathogens affecting human health, staphylococci are the predominant group of bacteria responsible for mastitis in dairy animals globally. Understanding the complicated interplay of factors affecting AMR in bacterial populations on dairy farms is vital to making science-based decisions around regulations dictating antimicrobial usage. It is in the best interest of the dairy industry to maintain effective antimicrobial treatments that keep cows healthy, decrease animal suffering, minimize production expenses for livestock producers, and allow dairy cows to produce a high-quality product.

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Tables

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| Table 1**.** Summary of observational studies comparing antimicrobial susceptibility of staphylococci isolates between organically-managed (ORG) and conventionally-managed (CON) dairy herds. Most studies describe using a combination of morphology, Gram staining, coagulase and catalase test to identify bacterial isolates as *S. aureus* or non-*aureus* staphylococci (NAS)/coagulase-negative staphylococci (CNS). Additional methods for identifying staphylococci to the species level are identified where appropriate. DCT = dry cow treatment; SCC = Somatic cell count; MIC = Minimum inhibitory concentration | | | | | | |
| *Reference; Country*  *Organisms described1* | *Study design and sampling scheme* | *Herd selection considerations*  *Min. no. yr. ORG certified* | *Quantification of AM usage*  *Description of antimicrobials used on farms* | *Susceptibility method2*  *Antimicrobials tested* | *No. isolates tested* | *Selected results* |
| Busato et al., 2000; Switzerland (EU)  *S. aureus*, CNS | Longitudinal (2 herd visits/yr.: 1x on pasture, 1x in confinement); Performed CMT on each lactating cow in herd, quartermilk samples then collected from quarters with CMT >1; Isolates from subclinical mastitis | 152 ORG herds; Stratified random selection (by herd size and farm location by altitude) from herds agreeing to participate; num. herds selected within strata based on actual proportion of herds in each stratum of entire population of Swiss organic dairies  No. yr. ORG herds certified not provided | No quantification of AM usage  65% ORG herds regularly used AM DCT treatment (mostly β-lactam antimicrobials, combinations of β-lactams and other antimicrobials) | *Disk diffusion*  Ampicillin, cefalotin, chloramphenicol, ciprofloxacin, clindamycin, cloxacillin, cotrimoxacol, erythromycin, gentamicin, neomycin, penicillin, rifamycin, tetracycline | *S. aureus*: 37 ORG  CNS: 54 ORG | Data describing the proportion of staphylococci from CON herds resistant to different antimicrobials taken from a previously unpublished survey by the authors (completed 6 years prior).3  Proportions of *S. aureus* isolates from ORG herds resistant to different antimicrobials were similar to those from CON herds (no statistical comparison carried out).  Proportion of CNS isolates from ORG herds resistant to different antimicrobials were similar to those from CON herds, with the exception of a numerically higher proportion of isolates resistant to rifamyin from ORG herds (no statistical comparison carried out). |
| Tikofsky et al., 2003; US  *S. aureus* | Cross-sectional (1 visit/herd); Composite quartermilk samples from each lactating cow in herd; Not specified if isolates from clinical or subclinical mastitis | 22 ORG herds, 16 CON herds; Herds of similar size and geographic distribution selected; All CON herds used blanket DCT  ORG herds certified ≥ 3 yr. ("most much longer") | No quantification of AM usage  On CON herds, β-lactam antimicrobials used most commonly (amoxicillin and pirlimycin most common treatments administered during lactation, penicillin-novobiocin for DCT) | *Disk diffusion*  Ampicillin, cephalothin, erythromycin, novobiocin, oxacillin, penicillin, penicillin-novobiocin, pirlimycin, tetracycline, vancomycin | 261 *S. aureus*: 117 CON, 144 ORG | Strength of association between proportion susceptible/resistant and mgmt. category was evaluated, as well as differences in mean zone diameter for isolates from ORG vs. CON herds.  Differences in antimicrobial susceptibility were observed between *S. aureus* isolates from ORG and CON herds for 7 of 9 antimicrobials studied (results combined over both analyses). *S. aureus* isolatesfrom both types of herds showed good susceptibility to most mastitis antimicrobials, but isolates from ORG herds were significantly more susceptible. |
| Sato et al., 2004; US and Demark (EU)  *S. aureus* | Cross-sectional (1-2 herd visits/yr. for US herds, 1 visit/herd for Danish herds); Bulk tank milk | 30 ORG herds, 30 CON herds from US; 20 ORG herds, 20 CON herds from Denmark; In US, "neighboring" CON herd enrolled as match for each ORG herd; Danish herds chosen randomly  US: ORG herds certified ≥ 3 yr. (mean = 8 yr.); Denmark: ORG herds converted ≥ 9 yr. prior to publication date | No quantification or description of AM usage provided | *Broth microdilution (Sensititre)*  Bacitracin, cephapirin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, kanamycin, oxacillin, penicillin, streptomycin, sulphamethoxazole, quinupristin/dalfopristin, tetracycline, trimethoprim, vancomycin | 483 *S. aureus*: 229 CON, 254 ORG | Overall, antimicrobial susceptibility was very similar between *S. aureus* isolates from ORG and CON herds in both countries. Isolates from CON herds in Wisconsin had significantly reduced susceptibility to ciprofloxacin (vs. isolates from ORG herds), and isolates from ORG herds in Denmark had reduced susceptibility to avilamycin (vs. isolates from CON herds). Differences in antimicrobial susceptibility of *S. aureus* isolates between ORG and CON herds were small relative to differences in isolates observed between the US and Denmark. |
| Bennedsgaard et al., 2006; Denmark (EU)  *S. aureus* | Cross-sectional and longitudinal components; Herds converting to organic farming sampled 3x 1 year apart, CON and ORG herds sampled 1x; Quartermilk samples collected from 30 cows with "high risk of infection" (criteria: history of high SCC, breed, and lactation); Not specified if isolates from clinical or subclinical mastitis | 20 CON herds, 18 ORG herds, and 19 transitioning herds (sampled at 0, 1, 2 yr. of transition); Herds not matched  ORG herds certified ≥ 5 yr. | Estimated mastitis treatments given in % cows treated/cow-year for each of 5 herd grps  CON used more than ORG, but transitioning grps not different from either CON or ORG; Type of AM usage not described | *Blood agar plates with 1 IU penicillin/ml*  Penicillin | 749 *S. aureus* | No statistically significant differences were observed in the prevalence of penicillin resistance in *S. aureus,* or the proportion of *S. aureus* isolates resistant to penicillin between herd groups (ORG, CON, transition year 1, transition year 2, transition year 3). |
| Roesch et al., 2006; Switzerland (EU)  *S. aureus*, NAS | Cross-sectional (1 visit/herd); 5-13 lactating cows (dep. on farm size) randomly selected at 31 DIM (median); Quartermilk samples collected from quarters with CMT ≥ 2+; Isolates from subclinical mastitis | 60 ORG herds, 60 CON herds; ORG herds chosen randomly from interested pool; Matching CON herds selected based on geographic proximity, same agricultural zone (elevation), and farm size  ORG herds certified ≥ 3 yr. | No quantification of AM usage provided, but prophylactic use of AM lower for ORG herds than CON herds  Main AM used for DCT for ORG and CON herds were penicillin (40 and 66%, respectively), cloxacillin (36.5 and 37%, respectively), neomycin (23.5 and 52.7%, respectively), and gentamicin (11.8 and 2.4%, respectively) | *Broth microdilution (custom plates; Sensititre)*  Amoxicillin-clavulanic acid, ceftiofur, chloramphenicol, clindamycin, enrofloxacin, erythromycin, gentamicin, oxacillin, quinupristin-dalfopristin, penicillin, tetracycline, vancomycin | 79 *S. aureus*: 33 CON, 46 ORG  38 NAS: 19 CON, 19 ORG | Percentage of antibiotic resistance did not differ significantly between *S. aureus* and NAS isolates from cows kept on ORG and CON herds for 12 antimicrobials representing either drugs used to treat mastitis in dairy herds, or drugs important in human medicine. The proportion of resistant *S. aureus* isolates was numerically higher from ORG cows (16/46, 35%) vs. CON cows (6/33, 18%), but this difference was not statistically significant. The proportion of resistant CNS isolates was very similar from ORG cows (9/19, 47%) and CON cows (10/19, 53%). NAS isolates had a higher percentage of antibiotic resistance than *S. aureus* isolates. |
| Bombyk et al., 2007; US  Coagulase-positive *Staph.* (CPS), Novobiocin-sensitive CNS (NSCNS), Novobiocin-resistant CNS (NRCNS) | Cross-sectional (1 visit/herd); Composite quartermilk samples collected from "all healthy cows;" Not specified if isolates from clinical or subclinical mastitis | 8 ORG herds, 8 CON herds; All small dairies (20-100 cows), herds not matched  ORG herds certified ≥ 1 year under USDA National Organic Program (no AM usage for ≥ 4 yr.: 1 yr. certified, 3 yr. of transition) | No quantification of AM usage provided  CON herds reported usage of several AM drugs in the past year: cephalosporins (7 herds), penicillins (6 herds), tetracyclines (5 herds) and pirlimycin (5 herds), and 5 herds practiced blanket DCT | *Disk diffusion*  Cefoxitin, cephalothin, erythromycin, novobiocin, penicillin, pirlimycin, tetracycline, vancomycin | 36 *S. aureus*: 9 CON, 27 ORG  210 NSCNS: 55 CON, 155 ORG  159 NRCNS: 102 CON, 57 ORG | Organic dairy management was associated with more overall antimicrobial susceptibility among staphylococci than was conventional management. In an analysis combining all (3) groupings of staphylococci, a larger proportion of isolates from ORG herds were susceptible to pirlimycin and tetracycline compared with those from CON herds. Susceptibility to erythromycin and penicillin did not differ significantly by herd type when all staphylococci were combined (CON vs. ORG).  When broken down by category of CNS (novobiocin susceptible or resistant), isolates within both CNS categories from ORG herds were more likely to be susceptible to pirlimycin than CNS from CON dairies. No difference in tetracycline, erythromycin or penicillin susceptibility was seen between herd types (CON vs. ORG) within either CNS category. A larger proportion of NSCNS vs. NRCNS for both CON and ORG herds were susceptible to tetracycline, leading the authors to suggest that management practices unrelated to antimicrobial use may contribute to the observed differences in susceptibility patterns of CNS on dairy herds. |
| Pol and Ruegg, 2007; US  *S. aureus*, CNS | Cross-sectional (1 visit/herd); Quartermilk samples from a maximum of 50 multiparous cows with no signs of clinical mastitis; Multiparous cows sampled to ensure at least 1 known exposure to intramammary antimicrobial drugs (DCT); Isolates from subclinical mastitis | Herds categorized based on amount of antimicrobial exposure: 20 ORG herds (no usage); 15 conventional–low usage herds (CON-LO) herds not using or using less than or equal to the first quartile of use of each AM compound); 5 conventional–high usage herds (CON-HI) herds using more than the first quartile of a particular AM compound); All herds had 6-mo. avg. bulk tank SCC ≥250,000 cells/mL; CON herds required to have used blanket DCT for at least 5 yr.; Herds not matched  ORG herds certified ≥ 3 yr. | AM usage quantified at both herd and cow level as defined daily dose (DDD).4 Herd-level DDD was calculated by dividing the reported total dose of each drug used per year by the DDD of that AM. Number of DDD was divided by the total number of milking cows to estimate the density of use of particular AM (expressed as number of DDD per lactating cow per year)  β-Lactams, including cephapirin, penicillin, and ceftiofur, were used on the majority of the herds. Cephapirin and penicillin were used as intramammary infusions (treatment of clinical mastitis, DCT). Detailed description of AM usage by drug provided in reference | *Broth microdilution (Mastitis panel; Sensititre)*  Ampicillin, ceftiofur, cephalothin, erythromycin, oxacillin + 2% NaCl, penicillin, penicillin/novobiocin, pirlimycin, sulfadimethoxine, tetracycline | 137 *S. aureus*: 52 CON (15 herds), 85 ORG (18 herds); Range of no. isolates used per herd: CON: 1-9, ORG 1-18  295 CNS: 160 CON (20 herds), 135 ORG (19 herds); Range of no. isolates used per herd: CON: 2-16, ORG 1-16 | Authors took multiple approaches to compare resistance among isolates from the 3 antimicrobial usage groups:   1. Compared proportion for each type of isolate (CNS or *S. aureus*) that was susceptible or resistant in each category (CON vs. ORG) using χ2 test of association, in order to ask if proportion of susceptible isolates independent of herd type 2. Used χ2 test to explore if the MIC for each type of isolate (CNS or *S. aureus*) was independent of herd type (CON vs. ORG) 3. Performed survival analysis of each type of isolates (CNS or *S. aureus*) based on the 3 antimicrobial usage categories (ORG, CON-LO, or CON-HI). Antimicrobial concentrations in wells of the susceptibility test were used as “time,” and event was inhibition of bacterial growth   In order to avoid statistical dependence, only 1 isolate per cow and no more than 20 isolates per herd were included in the analysis. Overall, isolates from ORG herds were more susceptible to antimicrobials than those from CON herds. The authors stress that although some differences were found between antimicrobial groups, most isolates of both types were inhibited at the lowest dilution tested of most antimicrobial drugs.  *S. aureus:*   1. *S. aureus* isolates from CON herds were more likely to be resistant to ampicillin and penicillin compared with isolates from ORG herds. Herd type was not associated with the proportion of resistant isolates for the other antimicrobial drugs tested 2. *S. aureus* isolates from CON herds had a higher MIC for pirlimycin and sulfadimethoxine compared with isolates from ORG herds. Herd type was not associated with the MIC of the other antimicrobial drugs tested 3. In the survival analysis, the MIC that inhibited 90% (MIC90) of *S. aureus* isolates from ORG herds for penicillin and pirlimycin was lower than the MIC90 of the isolates from CON-LO and CON-HI herds (MIC50, the MIC that inhibited 50% of isolates, was not different for these drugs)   *CNS:*   1. CNS isolates from CON herds were more likely to be resistant to ampicillin, penicillin, pirlimycin, and tetracycline compared with isolates from ORG herds. Herd type was not associated with the proportion of resistant isolates for the other antimicrobial drugs tested 2. CNS isolates from CON herds had a higher MIC for ampicillin, pirlimycin, and tetracycline compared with isolates from ORG herds. Herd type was not associated with the MIC of the other antimicrobial drugs tested 3. In the survival curve analysis, the MIC that inhibited 90% (MIC90) of CNS isolates from ORG herds for ampicillin, penicillin, pirlimycin, and tetracycline was lower than the MIC90 of the isolates from CON-LO and CON-HI herds (ORG and CON-LO herds had a lower MIC50 for erythromycin than CON-HI herds, but the MIC90 did not differ by usage group) |
| Garmo et al., 2010; Norway (EU)  *S. aureus*, CNS | Cross-sectional (1 visit/herd); Quartermilk samples from all lactating cows; Isolates from subclinical mastitis | 25 CON herds, 24 ORG herds; All herds Norwegian Red cows; Matching CON herds selected based on herd size (± five cow-years) and type of housing  ORG herds certified ≥ 4 yr. | No quantification of AM usage provided  Generally, Benzyl penicillin and dihydrostreptomycin are the most common antimicrobials used for intramammary treatment in Norway | *Cloverleaf lactamase test*  Penicillin | 132 *S. aureus*: 68 CON, 64 ORG  260 CNS: 167 CON, 93 ORG | Proportions of *S. aureus* and CNS isolates from ORG herds resistant to penicillin were similar to those from CON herds, although no statistical comparison was carried out. Penicillin resistance was proportionately higher in CNS vs. *S. aureus* isolates*.*  *S. aureus:*  6 out of 68 (8.8%) isolates from CON herds were penicillin-resistant, compared with 9 out of 64 (14.0%) from ORG herds.  CNS:  81 out of 167 (48.5%) isolates from CON herds were penicillin-resistant, compared with 93 out of 200 (46.5%) from ORG herds. |
| Cicconi-Hogan et al., 2014; US  *S. aureus*, CNS | Cross-sectional (1 visit/herd); Bulk tank milk | 192 ORG herds, 100 CON herds; Matching CON herds selected based on proximity to ORG herd and herd size category (0–99, 100–199, or ≥200 adult cows)  No. yr. ORG herds certified not provided | No quantification or description of AM usage provided | *Detection of mecA gene by PCR, MRSASelect plates (Bio-Rad Laboratories Inc.)*  β-lactamase resistance (MRSA*Select* plates used to screen for methicillin resistance, and contain a proprietary combination of an unspecified β-lactam, lithium chloride, aztreonam and cycloheximide) | Not provided | 13 isolates from bulk tank milk were identified as methicillin resistant (positive for mecA gene): 7 from CON herds, 6 from ORG. Species identification of isolates from bulk tank milk was performed using 16S rRNA and rpoB genes.  These 13 isolates were identified as *S. aureus* (n = 1), *S. sciuri* (n = 5), *S. chromogenes* (n = 2), *S. saprophyticus* (n = 3), *S. agnetis* (n = 1), and *Macrococcus caseolyticus* (n = 1). The single methicillin-resistant *S. aureus* isolate was from an ORG herd, for an observed 0.3% prevalence at the herd level. The methicillin-resistant CNS prevalence was 2% in the organic population, and 5% in the conventional population.  The authors highlight the high number of methicillin-resistant *S. sciuri* identified (6 out of 12 methicillin resistant CNS) compared to previous work, and also suggest that a potential methicillin-resistant *Staphylococcus* reservoir in the dairy herd population of the United States may be independent of production system type (CON vs. ORG). |
| Tenhagen et al., 2018; Germany (EU)  *S. aureus* | Cross-sectional (1 visit/herd); Bulk tank milk | 372 CON herds, 303 ORG herds; Minimum herd size 30 lactating cows; Selection of herds based on sampling plan designed to cover German states according to their share of national CON and ORG cow population; Separate sampling plans for the 2 categories as proportion ORG herds comparatively low  No. yr. ORG herds certified not provided | No quantification or description of AM usage provided | *Broth microdilution*  Cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, kanamycin, linezolid, mupirocin, penicillin, quinupristin/dalfopristin, rifampicin, sulfamethoxazole, streptomycin, tetracycline, tiamulin, trimethoprim, vancomycin | Not provided | Genomic methods used for identifying isolates to the species level (multiplex PCR: 23S rDNA, specific for Staph; *nuc* gene, specific for *S. aureus*; *mecA* gene, β-lactam resistance)  Used a binary logistic regression to describe association of methicillin-resistant *S. aureus*-positive samples with herd type (CON vs. ORG), controlling for effect of region and herd size (both significant predictors of MRSA herd status)  The prevalence of MRSA was significantly higher in BTM samples from CON herds (9.7%) compared with ORG herds (1.7%). Proportion of methicillin-resistant *S. aureus* isolates resistant to 12 different antimicrobials tended to be higher from bulk tank milk samples of CON herds (vs. ORG herds). As there were limited number of isolates from ORG herds (n = 5) compared to CON herds (n = 36), no statistical tests were performed |
| McDougall et al., 2020; New Zealand (US organic regulations)  *S. aureus*, CNS | Cross-sectional (1 visit/herd); Quartermilk samples from cows that had had at least 1 lactation, had been treated with DCT (in herds using DCT), had not been treated with any other antimicrobial within 30 d before sample collection, and had an individual SCC of >200,000 cells/mL; Not specified if isolates from clinical or subclinical mastitis | 7 ORG herds, 11 CON herds using ampicillin-cloxacillin DCT (CON-AC), 8 CON herds using cephalonium DCT (CON-CE); CON herds selected on the basis that >50% of cows were treated in each of the 3 previous yr. with 1 DCT product; Herds not matched  ORG herds certified ≥ 3 yr. (median = 12 yr.; range = 7-19 yr.) | Herd-level use of antimicrobials estimated by extracting AM sales data for each herd for the previous 3 yr. to determine total mass of antimicrobials used per kilogram of liveweight per year for each herd, and mass of each class of AM per kg of liveweight per year  β-lactam AM most commonly used DCT products in New Zealand generally, with 25% containing ampicillin, 61% containing cloxacillin, and 13% containing cephalonium, by mass | *Broth microdilution (Mastitis CMV1AMAF; Thermo Scientific)*  Ampicillin, ceftiofur, cephalothin, erythromycin, oxacillin, penicillin, penicillin/novobiocin, pirlimycin, sulfadimethoxine, tetracycline | 320 *S. aureus*: 111 CON-CE, 99 CON-CA, 110 ORG  240 CNS: 82 CON-CE, 74 CON-CA, 84 ORG | Overall, the authors found that the MIC of CNS from ORG herds were lower than isolates from both types of CON herd. However, they point out that these differences in MIC occurred below clinical breakpoints, and therefore may not affect bacteriological cure rates. They found bimodal distributions of MIC for ampicillin and penicillin in *S. aureus* isolates from ORG herds, and suggest either (1) isolates with a higher MIC are “a natural part of the bacterial population of the bovine mammary gland,” or (2) isolates with higher MIC have persisted within ORG herds since antimicrobial usage was occurring on the farm  *S. aureus:*  The MIC50 for ampicillin and penicillin were greater bymorethan1dilutionfor *S. aureus* isolates from CON-CE herds compared with CON-CA and ORG herds, but this relationship did not hold for the MIC90 of these drugs (MIC for CON-CE and ORG herds greater than CON-CA).  In a univariate analysis, the proportion of penicillin-resistant *S. aureus* isolates was significantly higher in CON-CE herds (76/111; 68.5%) compared to CON-CA(4/99;4.0%)orORG herds (32/110; 29.1%). A multilevel model (accounting for clustering of quarter within cow within herd) was made where the 3 herd types were the main explanatory variable. Other potential variables offered to this model included age, breed, DIM, SCC, and antimicrobial treatment history for that cow.  In the multilevelmodel,proportionsofpenicillin-resistantisolatesdidnotdifferbetweenisolates from the 3 herd types.  When comparing proportion of *S. aureus* isolates falling into 3 different breakpoint groups for ceftiofur resistance, the only significant difference was that there were fewer ORG isolatesin the middle category (1 μg/mL); otherwise, there were no differences in the proportion of isolates falling into the different breakpoint groups from each of the 3 herd types.  When comparing proportion of *S. aureus* isolates falling into 3 different breakpoint groups for sulfadimethoxine resistance, the only significant difference was that there were more ORG isolatesin the lowest category (32 μg/mL); otherwise, there were no differences in the proportion of isolates falling into the different breakpoint groups from each of the 3 herd types.  There were no significant differences between the 3 herd types when comparing the proportion of *S. aureus* isolates falling into 3 different breakpoint groups for erythromycin resistance.  CNS:  The MIC50 and MIC90 for ampicillin and penicillin were lower by more than 1 dilution for CNS isolates from ORG herds compared to both types of CON herds; otherwise, these values did not differ by more than 1 dilution between the 3 herd types for the other antimicrobials tested.  In a univariate analysis, proportions of penicillin-resistant CNS isolates were significantly greater in both types of CON herds (CON-CE, 42/82; 51%; CON-CA, 22/74; 30%) than ORG herds (14/84; 17%). Similar to the analyses for *S. aureus,* a multilevel model was also made to compare penicillin resistance with herd type as the main explanatory variable. In this multilevel model, proportion of penicillin-resistant CNS isolates was significantly greater for CON-CE herds (0.50 ± 0.07) compared to CON-CA (0.31 ± 0.06) or ORG herds (0.17 ± 0.05).  When comparing proportion of CNS isolates falling into 3 different breakpoint groups for ceftiofur resistance, the only significant difference was that there were more ORG isolates in the lowest (0.5 μg/mL) and highest (2 μg/mL) categories compared to both CON herd types; otherwise, there were no differences in the proportion of isolates falling into the different breakpoint groups from each of the 3 herd types.  There were no significant differences between the 3 herd types when comparing the proportion of CNS isolates falling into 3 different breakpoint groups for sulfadimethoxine resistance.  When comparing proportion of CNS isolates falling into 3 different breakpoint groups for erythromycin resistance, the only significant difference was that there were more CON-CA isolates in the highest category (≥1 mg/mL); otherwise, there were no differences in the proportion of isolates falling into the different breakpoints from each of the 3 herd types. |
| 1 Terminology used is consistent with authors’ language and groupings of organisms (e.g., NAS vs. CNS) | | | | | | |
| 2 Manufacturer information provided when specified | | | | | | |
| 3 Unpublished survey on antibiotic resistance performed in Swiss dairy farms by the Swiss Federal Dairy Research Station (Schallibaum, 1992) | | | | | | |
| 4DDD is the maximum dose a standard animal (BW = 680 kg) would receive if it were treated following the FDA-approved label dosages | | | | | | |

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| Table 2. Observational studies describing species-specific antimicrobial susceptibility of staphylococci isolates from bovine intramammary infections. Ten studies are included which describe phenotypic resistance profiles and isolates were speciated using genotypic techniques or MALDI-TOF. NAS= non-*aureus* staphylococci; CNS = coagulase-negative staphylococci; AMR = antimicrobial resistance; CM = clinical mastitis; SCM = subclinical mastitis | | | |
| *Reference*  *Country* | *Number of isolates1*  *CM or SCM associated* | *Methodology* | *Overall findings* |
| Sampimon et al., 2009  The Netherlands | 170 CNS  Not specified | Broth microdilution; PCR for *blaZ, mecA, ermA, ermB, ermC, msrA, lnuA, msrA, mphC* | Significant differences in resistance patterns were found between CNS species. Phenotypic resistance and resistance genes were relatively rare in *S. chromogenes*, with the exception of *blaZ* (which was present in 80% of all CNS isolates).  For phenotypic resistance, *S. fleuretti* and *S. epidermidis* had the highest resistance to penicillin, oxacillin resistance was most commonly found in *S. fleurettii, S. cohnii, and S. xylosus*, and resistance to macrolide antibiotics was most prevalent in *S. cohnii*, *S. equorum*, and *S. epidermidis.* There was a high prevalence of genotypic resistance (particularly *mecA*) or presence of multiple resistance genes in species with relatively a low prevalence (*S. cohnii, S. equorum, S. fleurettii, S. sciuri*).  The authors note that the resistance profile of *S. epidermidis* was of the most concern; it was the second most commonly found species, carried multiple resistance genes in ~50% of isolates, and phenotypic penicillin resistance was more common compared to other CNS. |
| Persson Waller et al., 2011  Sweden | 154 CNS  Compares clinical and subclinical | Broth microdilution; Cloverleaf β-lactamase test | Overall, prevalence of antimicrobial resistance for CNS was low, but some variation between species was observed. β-Lactamase production was the most common resistance mechanism found, with 29% of isolates found to be positive. The prevalence isolates of producing β-lactamase varied markedly between species. β-lactamase production was significantly higher for *S. epidermidis* and *S. haemolyticus* (40%) compared to *S. simulans* and *S. chromogenes*, where none or only a few of the isolates were β-lactamase positive. Resistance to other antimicrobials besides penicillin was uncommon, and was markedly lower than previous work describing erythromycin, oxacillin and tetracycline resistance levels in CNS. |
| Frey et al., 2013  Switzerland | 408 CNS  Compares clinical and subclinical | Broth microdilution; PCR for *mecA, mecC* | Overall phenotypic resistance: oxacillin resistance (indicator of *mec* gene-mediated methicillin resistance) was the most frequently identified (47.0% of all isolates), and was more frequent in clinical (56.5%) vs. subclinical mastitis isolates (43.9%). In order, the next most common resistances to antimicrobials identified were fusidic acid (33.8% of isolates resistant), tiamulin (31.9%), penicillin (23.3%), tetracycline (15.8%), streptomycin (9.6%), erythromycin (7.0%), sulfonamides (5%), trimethoprim (4.3%), clindamycin (3.4%), kanamycin (2.4%), and gentamicin (2.4%)  Resistance to oxacillin was attributed to *mecA* gene in 9.7% of oxacillin-resistant isolates, while remaining oxacillin-resistant CNS did not contain *mecC* or *mecA1* promoter mutations. Isolates of *S. fleurettii, S. epidermidis, S. haemolyticus,* and *S. xylosus* were identified as carrying the *mecA* gene. Resistance to tetracycline was attributed to the presence of *tetK* and *tetL* genes, penicillin resistance to *blaZ*, streptomycin resistance to *str* and *ant(6)-Ia*, and erythromycin resistance to *ermC, ermB,* and *msr* genes. |
| Taponen et al., 2016  Finland | 400 CNS  Combines clinical and subclinical | Broth microdilution | *S. simulans, S. chromogenes, S. haemolyticus,* and *S. epidermidis* differed in their antimicrobial susceptibility, with penicillin resistance was the most common type of antimicrobial resistance identified. Phenotypic oxacillin resistance was found in all four species (34% of the isolates overall). Whereas the majority of *S. epidermidis* isolates were resistant to benzylpenicillin, only a few *S. simulans* isolates were penicillin-resistant. 21 isolates (5% of isolates overall) were positive for the *mecA* gene (20 *S. epidermidis*, 1 *S. sciuri*).  *S. epidermidis* was the most resistant among the four major species studied, as resistance to antimicrobials was common, several isolates were multidrug resistant, and 19% of isolates were *mecA*-positive (encoding methicillin resistance). |
| Raspanti et al., 2016  Argentina | 219 CNS  Not specified | Broth microdilution | Overall, 51.6% of isolates were resistant to penicillin. The MIC90 value for penicillin was > 8g/ml for CNS isolates included in the study, which the authors note was well above the recommended breakpoint. Fourteen percent of all CNS isolates tested were resistant to oxacillin (of which 16.7% were *mecA* positive), 29.2% to erythromycin and 30.1% to tetracycline. *S. chromogenes* and *S. haemolyticus* showed a very high proportion of isolates resistant to penicillin (45.1% and 58.6%, respectively).The proportion of penicillin-resistant isolates was smaller for *S. warneri* (4/16), and no resistance to oxacillin was observed. In *S. xylosus,* penicillin resistance was the most common among the species tested (13/14 isolates). |
| Mahato et al., 2017  India | 62 CNS  Clinical isolates | Disk diffusion; PCR for *mecA, mecC, vanA* | As a whole, CNS demonstrated a high level of resistance toward oxacillin (85.5% of isolates) and cefoxitin (83.9%), moderate resistance against rifampicin (37.1%), clindamycin (32.3%), erythromycin (25.8%), and tetracycline (20.9%), and a low level of resistance against ciprofloxacin (11.3%) and gentamycin (9.7%). All strains were susceptible to vancomycin, teicoplanin and linezolid. The methicillin resistance gene *mecA* was found in 95.16% of isolates. *S. sciuri* and *S. haemolyticus* had the highest proportion of methicillin resistant isolates. |
| Nobrega et al., 2018  Canada | 1,702 NAS  Combines clinical and subclinical | Broth microdilution (1,702 isolates); whole genome sequencing (405 isolates) | Prevalence of resistance to important antimicrobials highly important frequently used in dairy herds was relatively common (β-lactams: 10%, tetracyclines: 10%), as was resistance to erythromycin (6%), but resistance to antimicrobials critically important for human medicine (vancomycin, fluoroquinolones, linezolid and daptomycin) was rare (<1%). The most frequently identified genetic resistance determinants were mutations in the *folP* gene and MDR efflux pumps; these mutations were present in all NAS isolates and not associated with a multi-drug resistant phenotype. For NAS species intrinsically resistant to novobiocin, specific residues were found in the in *gyrB* gene. The authors were able to link the presence of *blaZ, mecA, fexA*, *erm, mphC, msrA,* and *tet* genes with drug-specific resistance.  In this study, phenotypic antimicrobial resistance patterns were “clearly species-dependent.” Resistance to quinupristin/dalfopristin was common in *S. gallinarum* (98% prevalence), and *S. cohnii* and *S. arlettae* were frequently resistant to erythromycin (prevalence of 63 and 100%, respectively). The authors highlight *S. arlettae* as particularly concerning in its AMR profile; it had the highest prevalence of AMR against penicillin (61%), ampicillin (23%), erythromycin (100%), pirlimycin (18%) and clindamycin (99.9%), as well as the highest prevalence of MDR. Species-specific patterns were also seen in the prevalence of some AMR genetic determinants. *mecA* elements had a 17% prevalence in *S. epidermidis*, but were close to zero for other species. *erm* genes (encoding rRNA adenine N-6- methyltransferases) were found only in *S. epidermidis, S. cohnii, S. equorum*, and *S. chromogenes*. |
| Fergestad et al., 2021  Belgium and Norway | 227 NAS, 45 *S. aureus*  Combines clinical and subclinical | Disk diffusion; PCR for *mecA, mecC* | Staphylococci isolates were analyzed as 3 separate collections from previous studies (1 in Norway, 2 from different regions of Belgium). Over all 3 sample groups, descriptive analyses showed that antimicrobial resistance was more widespread in several NAS species when compared with *S. aureus* isolates (not including MRSA). Resistance to penicillin was most frequently identified in the Norwegian isolate group. Regardless of sample group, AMR was frequently observed in *S. epidermidis* and *S. haemolyticus*. Resistance to trimethoprim-sulfonamide was frequently observed in *S. aureus,* *S. epidermidis*, and *S. haemolyticus*. |
| Taponen et al., 2023  Finland | 244 NAS, 260 *S. aureus*  Not specified | Disk diffusion; PCR for *mecA, mecC, blaZ* | Authors found that penicillin resistance was the only significant form of AMR from staphylococci associated with IMI in Finland, with 18.8% of all isolates (*S. aureus*: 9.3%; NAS: 28.9%) found to be resistant by disk diffusion. Genotypic potential for resistance to β-lactamases was higher, with *blaZ* found in 26.6% of all isolates (*S. aureus*: 18.5%; NAS: 35.2%). In a phenotypic test detecting production of β-lactamases (nitrocefin test), 21.5% of all isolates were positive (*S. aureus:* 11.6%; NAS: 32.0%). Species-specific differences were observed in penicillin resistance, with the proportion of penicillin-resistant being lowest in *S. simulans* and highest in *S. epidermidis*, and *S. epidermidis* accounting for 6/8 NAS isolates carrying the *mecA* gene. |
| Yang et al., 2023  China | 160 CNS, 172 *S. aureus*  Clinical isolates | Disk diffusion; PCR for *blaZ, mecA, mecC, tetK, tetM, ermA, ermB, ermC* | Overall, both phenotypic and genotypic resistance was highest amongst *S. aureus* and CNS for penicillin, followed by erythromycin and tetracycline. Phenotypically, *S. aureus* isolates showed the highest resistance rates to penicillin (58.7%), followed by erythromycin (22.1%), tetracycline (15.1%), gentamicin (10.5%), ciprofloxacin (8.7%), and chloramphenicol (5.8%). CNS isolates displayed high phenotypic resistance to penicillin (71.3%), followed by erythromycin (28.8%), tetracycline (19.4%), gentamicin (9.4%), chloramphenicol (7.9%), ciprofloxacin (2.5%), and cefoxitin (1.3%).  *blaZ* was detected in 61.0% of *S. aureus* isolates, with all penicillin-resistant S. aureus isolates positive for the gene. *tetK* and *tetM* were found in 12.2% and 9.9% of *S. aureus* isolates, respectively, with all *tetK/tetM*-positive isolates showing resistance to tetracycline. *ermC* and *ermB* were found in 22.1% and 13.4% of *S. aureus* isolates, respectively, with all erythromycin-resistant isolates carrying ermC alone or in combination with *ermB*. No *S. aureus* were positive for *mecA, mecC or ermA.* For CNS isolates evaluated, *blaZ* was found in 69.4% isolates with all showing resistance to penicillin. One each *S. equorum* and *S. saprophyticus* that were resistant against penicillin were negative for blaZ but carried *mecA*. *tetK* and *tetM* were found in 17.5% and 12.5% CNS isolates, respectively, with all *tetK*/ *tetM*-positive isolates showing resistance to tetracycline. *ermC* and *ermB* were found in 28.1% and 16.9% of CNS isolates, respectively, with all erythromycin-resistant isolates carrying *ermC* alone or in combination with *ermB*. No CNS were positive for *mecC* or *ermA*. |
| 1 Terminology used is consistent with authors’ language and groupings of organisms (e.g., NAS vs. CNS) | | | |

Figures



Figure 1.Adapted from Call et. al, 2008. A proposed model illustrating how antimicrobial resistance can be maintained in a farm environment despite the absence of antimicrobial selection pressure, primarily based on studies of resistant bacteria in the GI tract of cattle. Antimicrobial treatment of an individual animal leads to a transient expansion of AMR subpopulations within the gut, as resistant bacteria have a selective advantage. Eventually, the antimicrobial-induced expansion of the resistant population abates when the selective force of antimicrobial use is removed. If there is a fitness cost for maintenance of AMR for an organism, the relative proportion of AMR subpopulations decline in the absence of antimicrobials. However, expansion of the resistant population also increases the likelihood of a genetic event where an AMR gene is linked to another trait, one that confers a niche-specific fitness advantage to the resistant bacteria. If this selective linkage of AMR occurs, maintenance of a baseline prevalence of the AMR subpopulation may occur, despite the lack of selective pressure from antimicrobial use.

CHAPTER 2: Relationship Between Facility Type and Bulk Tank Milk Bacteriology, Udder Health, Udder Hygiene, and Milk Production on Vermont Organic Dairy Farms

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2.1 Abstract

The primary objective of this cross-sectional observational study was to determine whether bulk tank milk quality, udder health, udder hygiene and milk production outcomes were associated with facility type on organic dairies. A secondary objective was to identify other management-related risk factors associated with bulk tank milk quality, udder health, udder hygiene, and milk production on organic dairy herds in Vermont. We aimed to enroll 40 farms, to compare herds using the 2 most common systems (freestalls, tiestalls) for housing organic dairy cattle in the state with those using a bedded pack during the non-grazing season (typically November-May). Two general styles of bedded packs were observed: cultivated bedded packs and untilled deep bedded packs. Due to the limited number of herds using bedded packs to house lactating dairy cattle in Vermont, we combined untilled and cultivated bedded packs to describe udder hygiene, milk quality, and udder health on these loose-housing systems deeply-bedded with organic material. The study was completed on 21 farms (5 bedded packs, 6 freestalls, 10 tiestalls) before interruption due to the COVID-19 pandemic. Data captured from Dairy Herd Improvement Association records from the test closest to the date of the farm visit included average somatic cell score (SCS), standardized 150-day milk (pounds), % cows with current high SCS (SCS ≥4.0), % cows with newly elevated SCS (previous SCS <4.0 to current ≥4.0), and % cows with chronically elevated SCS (SCS ≥4.0 last 2 tests). Multivariable linear regression models were used to describe outcomes by facility type, but suffered from limited statistical power due to small group sample sizes. Unconditional comparisons failed to find statistically significant differences between farms grouped by facility type in metrics captured from Dairy Herd Improvement Association test data, bulk tank milk somatic cell count (BTSCC) and aerobic culture data, or udder hygiene scores. A secondary analysis was conducted using univariate linear regression to identify associations between herd management factors and outcomes for all 21 farms combined. Although not all differences found were statistically significant in this secondary analysis combining all farms, numeric differences that may be biologically important are reported showing farms with deeper bedding had a lower BTSCC, lower newly elevated SCS, lower chronically elevated SCS, lower elevated current SCS, lower average SCS, and better udder hygiene metrics. Farms with lower mean udder hygiene scores had numerically lower chronically elevated SCS, lower elevated current SCS, and lower average SCS. We could not reject the null hypothesis that milk quality and udder health outcomes did not differ by facility type, and this does not preclude the existence of biological differences in these outcomes between facility types. The current study provides insight on factors affecting bulk tank milk quality, udder health and hygiene measures on organic dairy farms in Vermont. Bedded packs may be a viable option for confinement housing during the winter non-grazing season for pasture-based herds interested in a loose-housing system in the Northeastern US, but more research such as longitudinal studies with a larger sample size is needed to test this hypothesis.

2.2 Introduction

Mastitis due to environmental pathogens, such as those commonly found in bedding material, has now become the “most common and costly form of mastitis in modern dairy herds” that have implemented standard mastitis control practices limiting the effect of contagious pathogens (Klaas and Zadoks, 2018). Teats of dairy cattle may be in direct contact with bedding materials for 40 to 60% of the day, making this an important potential source of exposure to opportunistic environmental mastitis pathogens (Tucker and Weary, 2004; Cook et al., 2005; Hogan and Smith, 2012). Work exploring how bedding materials relate to a cow’s risk of contracting mastitis has understandably focused on the most frequently used bedding materials and housing systems in the dairy industry. Currently, the most common type of dairy cattle housing for organic farms in Vermont is a tiestall barn, with freestall barns a distant second (Andrews et al., 2021). As consumer opinion about confinement housing of dairy cattle evolves and influences dairy policy, both the dairy industry and consumers are looking to move away from traditional housing systems that restrict cow movement (Barkema et al., 2015). Many smaller-scale organic dairy farmers in Vermont with aging facilities, and especially tiestall barns, may be looking to adopt a bedded pack system on their farms as a form of loose-housing (Andrews et al., 2021).

The term “bedded pack” encompasses a variety of management styles (Bewley et al., 2017), including compost bedded-packs (CBP), which utilize aerobic decomposition to break down a bedding material of fine wood sawdust or shavings, as well as “conventional,” “traditional,” or “deep bedded packs” (Thurgood, 2009; Benson, 2012; Bewley et al., 2017; The Dairyland Initiative, 2024). CBP can vary in depth, frequency and depth of aeration (tilling), type of bedding material used, and in some regions the inclusion of forced air systems to dry the bedding in situ (Leso et al., 2020). In the Northeastern U.S., some producers are using deep bedded pack systems where large volumes of straw or hay are added daily to an untilled surface and strata of bedding and waste accumulate throughout winter season (Benson, 2012). Oxygen is retained in the system by the selection of bedding material and the timing of its application (Neher et al., 2022; Thurgood et al., 2009). Some authors suggest deep bedded pack barns are synonymous with traditional straw yard housing systems (Bewley et al., 2017; Leso et al., 2020; Ferraz et al., 2020). However, we find the deep bedded packs being constructed for winter housing on organic dairy farms in the Northeastern U.S. differ from traditional straw yards where bedding material is completely removed at approximately monthly intervals and the housing is used year-round (The Dairyland Initiative, 2024; Thurgood et al., 2009; Benson, 2012).

Bedded packs (BP) are perceived to integrate well into Northeastern US pasture-based farm systems, and state and federal agencies in the U.S. are providing financial incentives for dairies to build these structures as part of manure management practices which improve water quality and contribute to soil conservation (USDA-NRCS; Andrews et al., 2021; Thurgood et al., 2009). As interest in BP grows, it is important to better understand milk quality, udder health and hygiene on farms using these housing alternatives. Understanding mastitis risk for cattle housed on BP is especially important for organic dairy farmers, as they have limited effective options for treating intramammary infections (Ruegg, 2009). As mastitis-causing bacteria may thrive in the conditions found in compost bedded-packs (Black et al., 2014), previous work studying mastitis risk and bedding would suggest BP could pose a relatively higher risk for intramammary infections. Loose-housed cows continually add manure to the pack, contributing both pathogenic bacteria (non-*aureus* staphylococci, Wuytak et. al., 2020; *E. coli*, *Klebsiella* spp., and *Enterobacter* spp., Eberhart, 1984; streptococci, Zadoks et al., 2005) and nutrients to the organic bedding material. Organic bedding material is more likely to have a higher bacterial count than inorganic bedding, such as sand, (Hogan et al., 1989; Rowbotham and Ruegg, 2016b), as it supplies nutrients and moisture which encourages bacterial growth. This could lead to higher concentrations of bacteria on teat skin for cows on BP, because: 1) organic bedding (in general) is inherently associated with a higher number of bacteria on teat ends (Fairchild et al., 1982; Rowbotham and Ruegg, 2016b), and 2) a higher concentration of bacteria in bedding is associated with a higher concentration of bacteria on teat ends (Hogan and Smith, 1997; Zdanowicz et al., 2004; Rowbotham and Ruegg, 2016b). This higher concentration of bacteria on teat ends may put the mammary gland at an increased risk of infection, although limited evidence exists for this relationship (Neave et al., 1966; Pankey, 1989; Rowbotham and Ruegg, 2016a).

Previous work describing mastitis risk and cow hygiene on BP systems includes descriptive studies of CBP (Barberg et al., 2007b; Black et al., 2013; Fávero et al., 2015; Eckelkamp et al., 2016b; Albino et al., 2018; Heins et al., 2019). However, research comparing milk quality and cow hygiene between BP and more traditional housing types has so far been limited to freestalls with sand, which is an uncommon housing type for organic farms in Vermont (Andrews et al. 2021). These include a study comparing CBP and sand-bedded freestalls for farms with a history of low bulk tank somatic cell counts (Eckelkamp et al., 2016a), work describing hygiene and bulk tank milk somatic cell count (BTSCC) for sand-bedded freestalls and CBP (Adkins et al., 2022), and a comparison of CBP and 2 types of freestall barns (Lobeck et al., 2011). It is unclear whether the herds included in these prior studies were conventionally-managed or organic dairies. To the best of our knowledge, no studies describe and compare bulk tank milk quality, udder health and hygiene for BP and tiestall barns on small to midsize organic dairies in the same geographic area.

To better inform organic dairy producers in the Northeastern US, who may be interested in using a BP for housing their cattle during the non-grazing season (i.e., for “winter housing,” typically the months of November-May), we conducted a cross-sectional, observational study on organic dairies in Vermont. This study aimed to quantify bulk tank milk bacteriology, udder health and udder hygiene measures for the 2 most common indoor housing systems (freestalls and tiestalls) and farms using a BP among organic farms in Vermont. The objectives of this project were to identify whether bulk tank milk quality, udder health and hygiene outcomes differed by facility type, with a view to determining if BP are a viable option for indoor housing of lactating cows in VT during the non-grazing season. We hypothesized that udder health, hygiene, and bulk tank milk bacteriology of BP herds are inferior to that of more traditional housing types, as has been suggested by some previous research (Barberg et al., 2007b; Lobeck et al., 2011). Therefore, our null hypothesis was that there no association between facility type and udder health, hygiene, and bulk tank milk bacteriology on organic dairy farms using BP and other systems for winter housing of lactating cow in Vermont. A secondary objective was to identify other (non-facility) management-related risk factors associated with bulk tank milk quality, udder health, udder hygiene, and milk production for organic VT dairy herds.

2.3 Materials and Methods

STROBE-VET (Strengthening the Reporting of Observational Studies in Epidemiology–Veterinary Extension) statement guidelines were followed in the reporting of this study (O'Connor et al., 2016).

2.3.1 Herd enrollment and selection

The source population for this study was the farms that responded to a survey sent to all (n = 177) certified organic dairy farms producing cow milk in Vermont in Winter 2018-2019. Certified organic dairy farms in the United States are required to allow their cows daily access to pasture during the grazing season, and cows must obtain 30% of their dry matter intake from grazing (Rinehart and Baier, 2011). In Vermont and other Northeastern US states, forage is unavailable directly from pasture during winter months and the climate necessitates use of indoor housing. When cows have no access to pasture in the winter non-grazing season, organic farms in Vermont house cows in a variety of indoor facility types. Our previous Winter 2018-2019 industry survey quantified the frequency and diversity of indoor housing and bedding types used by organic dairy farmers in the state when cows were not on pasture, and for the current study farms were recruited from the 145 respondents to this survey (Andrews et al., 2021). Dairy farms were eligible for enrollment in the current study if they: 1) responded to the initial survey in the Winter 2018-2019, 2) indicated they met the enrollment criteria of testing with the Dairy Herd Improvement Association (DHIA) at least monthly, 3) milked between 35 and 120 cows, and 4) indicated they would be interested in further participation. Eligible farms were contacted from this source population in Spring 2019 if they responded that they were using 1 of 4 categories of bedding/housing combinations for their indoor housing system: 1) freestall (FS) barn bedded with sand, 2) FS barn bedded with shavings or sawdust, 3) tiestall (TS) barn bedded with shavings or sawdust, or 4) BP. The first 3 housing and bedding combinations are the most frequently used by organic dairies in Vermont to house cows during the non-grazing season, and were compared to BP as they were the housing type of interest for this project. For the purposes of this study, the inclusive term “bedded pack” is used to encompass both CBP and deep bedded packs, and was defined as an enclosed loose housing facility deeply bedded with organic material, in which bedding and waste accumulate throughout the 6–8-month period of time when cows are housed on it and which is only removed once a year. Both CBP and deep bedded packs use carbon-rich substrates to create a clean, comfortable surface which allows animals to move freely. Urine and manure are not removed when bedding material is renewed, in contrast with other housing systems.

A convenience sample of farms was enrolled in Spring 2019 from a list of eligible farms (grouped by housing/bedding combination) using the phone number or email address provided in the 2018-2019 survey response. Our aim was to enroll 40 farms for the current study, with 10 farms from each of the 4 housing/bedding categories described above.

Prior to obtaining the 2018-2019 survey results, based on preliminary data collected by the University of Vermont Center for Sustainable Agriculture Extension group, the study was designed anticipating that it would be possible to enroll 10 organic Vermont dairies using a BP as their primary indoor housing system. However, out of the 17 farms from the 2018-2019 survey that indicated at least some use of a BP, 1 farm was not interested in any further participation, 5 did not use DHIA testing, and 6 only used a BP as a secondary housing system in conjunction with a TS barn, or cows were only on the pack a few hours a day. Because the number of farms using BP was fewer than anticipated, the eligibility requirements were relaxed to include 1 farm where cows spend the majority (two-thirds) of their time in a BP, with the remaining time in a TS with wood shavings. Additionally, 2 BP farms were included that had limited DHIA information: 1 farm did not utilize cow-level testing, and cow-level data for a second farm was limited due to their seasonal lactation schedule. As the number of BP being used in the state to house lactating dairy cattle was less than anticipated, those that were enrolled and grouped together utilized a variety of management strategies. Of the 5 enrolled farms using a BP, 2 would be classified as “compost bedded-packs,” utilizing tilling to promote aerobic decomposition to break down a bedding material of dry, fine wood sawdust or shavings (The Dairyland Initiative, 2024; Bewley et al., 2017; Endres, 2021). These 2 farms bedded solely with shavings/sawdust, adding new bedding only as needed, and cultivated the pack twice a day. Two other farms used a “traditional” or “deep bedded pack” system, where large volumes of fresh, dry straw (or poor-quality hay) sufficient to keep cows clean and dry was added daily to a mass of bedding that accumulates over the 6-8 months cows are housed indoors (The Dairyland Initiative, 2024; Thurgood, 2009; Benson, 2012). The 1 remaining farm fell somewhere between these 2 types of classically defined BP; this farm bedded with straw and woodchips and cultivated every 48 hrs., adding chopped hay and woodchips every time the pack was cultivated. All farms in the study grouped as “bedded packs” shared the qualities of being an enclosed loose housing facility, deeply bedded with organic material (0.9-1.7 meters), which accumulated over the period of time animals were housed indoors and was only removed once a year.

Of the intended 40 herds to be recruited in the study, 21 herds (1 FS bedded with sand, 5 FS bedded with wood shavings/sawdust, 10 TS bedded with wood shavings/sawdust, 5 BP) agreed to participate and farm visits were completed April-May 2019. This study was intended to study cows while they were in their winter (non-grazing months) indoor housing system, so all herds visits were completed before any grazing had begun for the season. Each herd was visited once during the study period. All herds sampled during this period were housing their cows as they would in the non-grazing season. Farm visits were suspended in mid-May 2019 as farms began turning their cows out to pasture, with the intention of resuming in April 2020 to complete the remaining 19 herds. Due to COVID-19 pandemic activity restrictions, the decision was made to not resume the study, and the final analysis included the 21 herds sampled in 2019. As there was only 1 farm sampled using a FS facility bedded with sand, the initial plan to group farms by the 4 housing/bedding combinations specified was abandoned in favor of grouping farms by the 3 facility types used. The single sand FS was combined with FS bedded with wood shavings/sawdust (FS; n = 6), there were 10 TS bedded with wood shavings/sawdust (TS), and 5 BP.

2.3.2 Questionnaire administration, sampling, and udder hygiene scoring

At each farm visit, a questionnaire was administered to collect information about housing and bedding management, as well as other practices on the farm that could impact mastitis risk (Supplemental Material - Questionnaire). The study questionnaire was largely adapted from a previously published survey (Stiglbauer et al., 2013), with additional questions specific to the current study. The questionnaire was reviewed by a social scientist experienced in gathering qualitative data and tested before use with herd managers at the University of Vermont teaching dairy. Questions about mastitis risk explored producer concerns about bedding/mastitis risk; mastitis control, identification and record keeping; milking facilities, procedures, and hygiene practices; information about diet, vitamin and mineral supplementation, and water source; typical calving and periparturient practices; and fly control. Questions about housing and bedding management included describing type of housing system used for both lactating and dry cows; classification and description of any bedding material used; and bedding management practices for each housing type used. The questionnaire also collected some basic herd information (production numbers; number of lactating, dry, and youngstock; breed; record-keeping systems). Farms using BP were asked additional questions to gather detailed information about pack construction, management, monitoring practices, and perceptions comparing BP to any previously used systems. Completion of the questionnaire required 45 minutes on average, ranging from about 30 minutes to 1.5 hours. The questionnaire and interview protocols were registered with the University of Vermont Institutional Review Board (IRB certification 19-0057). The questionnaire was created and administered on a tablet using KoboCollect software (KoboCollect, 2019).

At each farm visit, a bulk tank milk sample was collected directly from the top of the tank using a 250-mL sterile single-use vial (Blue Dippas, Dynalon Products, England) after at least 5 minutes of agitation. Samples were kept on ice in a cooler during transport until they were processed fresh for SCC measurement or were frozen and stored at −20°C in the laboratory, before being sent to a diagnostic lab for microbiological analysis. An on-farm observation sheet was completed, which collected information about the bulk tank, cow identification, a subjective assessment of air quality, and any outdoor exercise area (Supplemental Material – Observation sheet). Additionally, measurements of the housing facilities were recorded for FS and TS where appropriate (stall sizes, pen sizes, bedding depth, stocking density, trainer use), as well as observations about BP when applicable (depth, pen size, and stocking density in m2 per animal). Bedding depth of FS and TS was included as a producer reported value in the questionnaire. Bedding depth of BP facilities was measured by forcing a meter stick down to the level of the cement pad or gravel under the pack, where the pack met a cement knee wall, and recording the height of the pack at that point. Udder hygiene scoring was completed by the same researcher at all farms for a minimum of 30 cows on each farm (the first 30 able to be evaluated in a loose pen, or the first 30 encountered in a TS). A 4-point udder hygiene scoring system was used, where 1 = free of dirt, 2 = slightly dirty (2–10% of surface area), 3 = moderately covered with dirt (10–30% of surface area), and 4 = covered with caked on-dirt (>30% of surface area) (Schreiner and Ruegg, 2002). Animal use for this project was approved by the University of Vermont Institutional Animal Care and Use Committee (IACUC; protocol #PROTO202000089).

2.3.3 Herd-level udder health measurements

Herd-level DHIA test results for the test day closest in time to the farm visit (either preceding or following day of farm visit, whichever was shorter) were captured from the record processing center working with each herd (Lancaster DHIA, Manheim, PA; Dairy One Co-Op. Inc., Ithaca, NY). Information captured included test date, number of lactating cows, standardized 150-day milk production (STD 150-day milk), and test-day average cow-level somatic cell score (SCS). The following udder health measures were also captured from DHIA records: proportion of cows with an SCC ≥200,000 cells/mL on most recent test day (“elevSCS”), where elevated SCS was defined as a somatic cell score of ≥4.0; the proportion of cows with a newly elevated SCS (“newSCS”), which was defined as a SCS changing from <4.0 to ≥4.0 over the last 2 tests; and the proportion of cows with a chronically elevated SCS (“chronSCS”), which was defined as having a SCS ≥4.0 on the last 2 tests (Schukken et al., 2003).

2.3.4 Bulk tank milk culture and bulk tank somatic cell count measures

An aliquot of the bulk tank milk sample was stored at -4°C until it could be transported to the laboratory of a dairy processing plant (St. Alban’s Cooperative/Dairy Farmers of America, St. Albans, VT) within 48 hours of collection for determination of the bulk tank somatic cell count (BTSCC).

Frozen bulk tank milk samples were shipped on ice to the Laboratory for Udder Health (University of Minnesota Veterinary Diagnostic Laboratory, St. Paul) for analysis. Methodology for bulk tank milk cultures at the Laboratory of Udder Health are described elsewhere (Patel et al., 2019). Briefly, thawed, room-temperature bulk tank milk and a 10-fold dilution of each bulk tank milk sample were plated onto MacConkey, Factor (gram-positive selective agar; University of Minnesota), and Focus (selective for streptococci or strep-like organisms; University of Minnesota) media plates and incubated for 2 days at 37°C. Any lactose-fermenting colonies on MacConkey medium were counted and reported as coliform bacteria. Any β-hemolytic colonies on Focus medium were counted and identified to the species level using a MALDI Biotyper (suspect *Streptococcus agalactiae*). All remaining colonies on Focus medium that were not identified as *Strep. agalactiae* were counted and recorded as streptococci or strep-like organisms (SSLO). Hemolytic colonies on Factor medium were counted and identified to the species level using a MALDI Biotyper (suspect *Staph. aureus*). Any hemolytic colonies with a confidence score ≥2.0 for *Staph. aureus* were counted and reported as such. Remaining colonies of staphylococci on Factor media (based on colony morphology, catalase reaction, or Gram stain) were counted and reported as *Staph.* spp. Bulk tank samples were also cultured for *Mycoplasma* spp. (0.1 mL milk was swabbed across a Mycoplasma agar plate, then placed in a 7% CO2 incubator at 37°C for 7 days, after which they were examined for *Mycoplasma* spp. by a trained microbiology technician). For each bulk tank milk sample, total colony-forming units (cfu) per mL were calculated for coliform organisms, *Staph.* spp., SSLO, *Staph. aureus*, *Strep. agalactiae*, and *Mycoplasma* spp. The lower threshold of detection for bacteria in this bulk tank milk culture protocol was 5 cfu/mL, and the upper threshold was 62,500 cfu/mL.

2.3.5 Data management and analysis

Bulk tank milk culture results, BTSCC, DHIA test results, farm-level udder hygiene outcomes, questionnaire data, and farm observations were entered into an Excel database (Microsoft Corp., Redmond, WA). Udder hygiene scores for individual cows were used to calculate 2 farm-level udder hygiene measures: 1) mean udder hygiene score, and 2) proportion of cows with dirty udders (udder hygiene score ≥3), which were incorporated into the database. This Excel database was then imported into the R Statistical Programming Environment (R Development Core Team, 2023) for data cleaning, checking, and statistical analysis. The distribution of outcome variables was assessed to check for normality using a Shapiro-Wilk test with significance set at *P* ≤0.05, visual assessment of distribution and residuals, skewness, and comparison of the median and mean values. Raw bulk tank somatic cell count (BTSCC) data was log10 transformed for analyses. Descriptive statistics were calculated to evaluate the distribution of data, data integrity, and to identify missing data. Descriptive statistics generated included description of general herd characteristics and farm traits, lactating cow housing/facilities, lactating cow bedding material/bedding management practices, milking hygiene procedures, and mastitis control practices for all 21 herds included in the study.

**2.3.5.1 Objective 1. Evaluation of relationships between housing system and measures of milk quality, udder health, udder hygiene and milk production**.

As most measures of aerobic culture data were not normally distributed even after log transformation, a Kruskal-Wallis test was used to compare cfu counts of bacteria from bulk tank milk between the 3 facility types. Statistical significance for this test was declared at *P* ≤0.05. Multiple attempts were made using multivariable analysis to compare the 4 aerobic culture outcomes for bulk tank milk, but all modeling approaches suffered from over-parametrization even when data were log transformed and were not pursued further.

Independent farm-level predictors from the herd-management questionnaire offered to the multivariable models are described in Table 1. Continuous variables underwent correlation analysis to identify predictor variables that were highly correlated (correlation coefficient ≥0.60), and unconditional associations among categorical variables were evaluated using a Pearson’s chi-squared or Fischer’s Exact test as appropriate (*P* ≤0.05). An ANOVA was used to check for correlation between numeric continuous variables and categorical variables (*P* ≤0.05). When a categorical variable had multiple groups with a small number of observations in each, groups were combined when biologically reasonable to have all categories of predictor variables contain at least 5 observations. If any predictor had only 1 observation in a group and there was no way to combine groups in a logical way, it was excluded from further analysis (but listed in descriptive statistic tables, Supplemental Tables S1-S4).

Univariate linear regression was performed in R using the “stats” package to investigate the unconditional relationship between four udder health and production outcomes (BTSCC, avg. SCS, elevSCS, STD 150-day milk) and 2 hygiene outcomes (mean hygiene score, proportion of dirty udders) for each farm and the previously-described herd-level independent variables. The 2 udder hygiene metrics (proportion dirty udders and average udder hygiene score) were used as both predictor variables (in models for other outcome variables) and outcome variables in models of their own. Univariate logistic regression was also performed in R using the “stats” package to investigate the unconditional relationship between chronSCS and newSCS, and the previously-described herd-level independent variables. Any explanatory variable that was unconditionally associated with 1 or more of the 8 outcomes of interest at *P* <0.20 was then offered into a multivariable model (linear for BTSCC, avg. SCS, elevSCS, STD 150-day milk, mean hygiene score, proportion of dirty udders; logistic for newSCS and chronSCS) investigating the relationship between the udder health and production or hygiene outcome and the herd-level predictor variables. If any predictor variables were found to be correlated with each other at the previously described cut-offs, the one with the more highly significant relationship from univariate analysis was offered to the multivariable model when appropriate. The 2 udder hygiene metrics were highly correlated (derived from the same data), so whichever one had a smaller *P-*value from the univariate analysis was chosen for inclusion in the model-building process. Facility type was forced into these multivariable models, as it was the primary explanatory predictor of interest. A backward stepwise variable selection process was then used, with the least significant variables being removed one by one. Final models were selected based on lowest Akaike information criteria, and an *F-*test or likelihood ration test (as appropriate) was used to compare the final model to the model with facility type as the only predictor (significance declared at *P* ≤0.05). Overall statistical significance for facility type (the main predictor of interest) was declared at *P* ≤ 0.05. The multivariable modelling approach described above aimed to investigate the conditional relationship between facility type and the 8 outcomes of interest while controlling for different farm management practices, housing characteristics, milking procedures and mastitis control practices.

**2.3.5.2 Objective 2. Identify other (non-facility) management-related risk factors associated with bulk tank milk quality, udder health, and milk production in organic dairy herds.**

After grouping all 21 farms together, we used linear regression in the same manner as described above in Objective 1 to explore associations between the independent predictors described in Table 1 and 4 udder health and production outcomes (BTSCC, avg. SCS, elevSCS, STD 150-day milk) and 2 hygiene outcomes (mean hygiene score, proportion of dirty udders). Similarly, all 21 farms were grouped together for analysis using logistic regression to explore associations between the independent predictors described in Table 1, and the udder health outcomes newSCS and chronSCS. Unconditional relationships between the 8 outcome variables and independent predictors are reported for a significance level of *P* ≤0.20 for an F-test or Z-test (where appropriate), and only for predictor variables with group sizes of at least n = 5.

2.3.6 Power analysis

A priori sample size calculations were not performed, as group size was determined by the number of organic dairy herds housing lactating cows on BP in our region.

2.4 Results

2.4.1 Description of study herds

Of the 21 herds enrolled, 5 used a BP, 1 used a FS bedded with sand, 5 used a FS bedded with shavings/sawdust, and 10 used a TS bedded with shavings/sawdust (Supplemental Table S1). The predominant breeds on all farms were Holstein (n = 8 farms), Jersey (n = 10), and mixed Holstein-Jersey crosses/other (n = 3). The median (mean; range) number of lactating cows was 68 (64.9; 32-99). The median annual rolling herd average milk production for the farms was 6,367 (6,424; 4,082-9,618) kg. Nineteen of the 21 farms tested with DHIA monthly while their cows were in milk, 1 farm tested 5-8 times/year, and 1 tested every other month. On average, DHIA data was captured from a test day 4 days before the farm visit (range: -28 days to +33). The average depth of bedding in the 15 FS and TS where producers provided an estimate was 4.5 cm (SD: 3.5 cm; range: 1.3-12.7 cm). The average depth of bedded packs (measured by researchers) was 130 cm (SD: 31; range: 90-170 cm). Detailed descriptions further characterizing study farm management practices and housing characteristics for lactating animals (e.g., laying surface, ventilation, stocking density), and details about bedding material and bedding management practices for lactating animals (e.g., bedding depth, frequency of adding new bedding, manure removal) are provided in Supplemental Tables S1 and S2, respectively. Detailed descriptions of routine milking procedures and mastitis control practices are provided in Supplemental Tables S3 and S4, respectively.

2.4.2 Description of bulk tank milk quality, udder health measures, milk production, and udder hygiene scores

In a non-parametric, unconditional comparison, there were no statistically significant differences in cfu count between the 3 facility types for any of the 4 bacterial groups measured. However, estimated median values varied numerically between groups (Table 2). For example, the median value for streptococci and strep-like organisms among the 10 TS was 167.5 cfu/ml, compared to 32.5 and 35 cfu/ml for FS and BP, respectively. This difference is driven by greater variation in values for TS herds (range: 20 – 1250 cfu/ml). Similarly, the median values of *Staph* spp. cfu counts were numerically higher for TS herds (Table 2). None of the 21 bulk tank milk samples were positive for *Strep. agalactiae* or *Mycoplasma* spp. Sixteen of the 21 samples were negative for coliforms on aerobic culture, while 5 farms had a coliform count of 5 cfu/mL. *Staph. aureus* was found in the bulk tank milk from 13/21 herds, with a median (range) cfu/mL of 50 (15-320) when present.

Mean BTSCC, % cows with newly elevated SCS, % cows with chronically elevated SCS, % cows with elevated SCS, avg. SCS, and STD 150-day milk production were numerically similar between the 3 facility types, with overlapping 95% confidence intervals on the mean estimates (Table 3).

The overall mean (95% CI) of herd-level udder hygiene scores for all 21 farms was 2.32 (2.16-2.49). The mean hygiene score was 2.2 (1.91-2.44) for BP (n = 5), 2.5 (2.24-2.76) for TS (n = 10), and 2.15 (1.93-2.37) for FS (n = 6). The overall mean proportion of cows with dirty udders in a herd (udder hygiene score ≥3) was 40% (31-48). The mean proportion (95% CI) of cows with dirty udders was numerically higher on TS farms at 49% (35-62), compared to 32% (18-46) for BP farms, and 32% (20-44) for FS farms.

2.4.3 Objective 1. Analysis of relationship between facility type and measures of bulk tank milk quality, udder health, milk production, and udder hygiene scores

Final multivariable models are summarized in Table 4. All 21 farms were able to be included in the models for BTSCC, average hygiene score, and proportion of dirty udders. For the models exploring newSCS, chronSCS, and elevSCS, 2 BP farms did not have available DHIA data (n = 19; group sizes: FS =6, TS = 10, BP = 3). One BP farm did not have average cow-level SCS data (n = 20; group sizes: FS = 6, TS = 10, BP = 4). For STD 150-day milk, 1 BP farm and 2 TS farms were missing DHIA data (n = 18; group sizes: FS = 6, TS = 8, BP = 4). Farms with missing data for a particular outcome were excluded for the analyses of that outcome.

2.4.3.1 Bulk tank milk quality outcomes

Variables that were associated at *P* <0.20 with BTSCC in univariate analysis included predominant breed, if herds ever performed culture of mastitic milk, glove use, and herd size. The final multivariable model included facility type (forced) and herd size. Facility type was not associated with BTSCC in the final model (Table 4).

2.4.3.2 Udder health outcomes

Herd size category, use of bedding amendment, air quality as assessed by researcher, glove use at milking, and clinical mastitis record keeping practices were offered to a multivariable model for newSCS. The final multivariable model included facility type (forced), air quality and glove use. Facility type was not associated with newSCS in the final model (Table 4).

Variables that were associated at *P* <0.20 with chronSCS in univariate analysis included feeding additional supplemental selenium, use of a bedding amendment, clipping/flaming udder hair, clinical mastitis record keeping practices, use of injectable selenium and vitamin E product and proportion of dirty udders. The final multivariable model included feeding a supplemental selenium product, use of bedding amendment, clipping/flaming udders, proportion of dirty udders and facility type (forced). Facility type was not found to be a significant predictor of the outcome chronSCS (Table 4).

Bedding amendment use and mean hygiene were offered to a multivariable model for elevSCS. Facility type (forced), bedding amendment, and mean hygiene were retained in the final multivariable model. Facility type was not associated with elevSCS in the final model (Table 4).

Feeding additional supplemental selenium, use of bedding amendment, OMRI-listed intramammary product at dry-off, injectable selenium and vitamin E product, and mean hygiene were offered to a multivariable model for herd average SCS. The final multivariable model for avg. SCS included facility type (forced), use of bedding amendment, dry product, injectable selenium, and mean hygiene score. Facility type was not found to be a significant predictor of avg. SCS (Table 4).

**2.4.3.3 Milk production outcome**

Variables that were associated at *P* <0.20 with STD 150-day milk included use of injectable selenium and vitamin E product, whether producers cultured high SCC cows, and herd size group. All 3 variables and facility type (forced) remained in the final multivariable model (Table 4). Facility type was not associated with STD 150-day milk in the final model (Table 4).

**2.4.3.4 Udder hygiene outcomes**

Air quality assessed by researcher was offered to the multivariable model for proportion of dirty udders. The final multivariable model included only facility type (forced), which was not associated with proportion of dirty udders.

Variables that were associated at *P* <0.20 with average hygiene score included whether the producer ever cultured quarter milk samples and whether they checked for cases of clinical mastitis by both examining the udder and forestripping. The final multivariable model included facility type (forced), and how the producer checked for clinical mastitis. Facility type was not associated with the outcome of mean udder hygiene (Table 4).

2.4.4 Objective 2. Analysis of farm management factors (non-facility) associated with bulk tank milk quality, udder health, milk production, and udder hygiene scores for all farms combined

Selected results of univariate linear regression models identifying management factors beyond facility type which were unconditionally associated with bulk tank milk quality, udder health, milk production and hygiene outcomes for all farms combined (n = 21) at *P* <0.20 are presented in Table 5. We report the results of these univariate regression models as they may be biologically important, even though many failed to reach threshold for declaring statistical significance at *P* ≤0.05, possibly due to our small sample size.

The depth of bedding in stalls for FS and TS herds was unconditionally associated with multiple udder health outcomes. As the depth of bedding in FS and TS herds increased, multiple udder health measures improved, including lower avg. SCS, BTSCC, elevSCS, chronSCS and newSCS. Similarly, comparing farms where cows were on deep bedding (i.e., grouping all herds reporting deeply-bedded stalls plus BP herds) to herds that had stalls with a smaller amount of bedding on top of a mattress or concrete, farms with deep bedding had a numerically lower BTSCC.

Udder hygiene measures were associated with several udder health outcomes. Higher mean hygiene scores and proportion of udders scored ≥3 were associated with higher chronSCS, elevSCS, and average SCS. A few specific management practices were also found to be unconditionally associated with udder health outcomes: consistent glove use was associated with lower newSCS and BTSCC, clipping or flaming udders and parenteral supplementation of vit. E/selenium were associated with fewer chronSCS, and both parenteral supplementation of vit. E/selenium and use of an OMRI-listed intramammary product at dry-off were associated with lower average SCS and higher STD 150-day milk.

Both udder hygiene outcomes were unconditionally associated with the same predictors, most of which were related to the depth of bedding for cows. For the 5 herds using a BP, deeper bedding was associated with lower average hygiene scores and lower proportion of dirty udders. Farms with cows housed on some type of deep bedding (i.e., grouping the 3 FS and TS reporting deeply-bedded stalls, plus the 5 BP herds) had numerically lower average udder hygiene scores and proportion dirty udders compared to cows on stalls with bedding over a mattress or concrete surface. For the fifteen TS and FS reporting bedding depth in stalls, increased bedding depth was associated with lower mean udder hygiene score and a numerically lower proportion of dirty udders.

2.5 Discussion

This work presents the results of our observational study exploring the relationship between facility type and udder health and hygiene metrics, BTM quality (SCC and microbiology), and milk production on organic dairy farms in Vermont. The current study is to the authors’ knowledge the first direct comparison of milk quality, udder health and udder hygiene on BP farms to both TS and FS herds of similar size and management styles, for a population of entirely small to midsize organic dairy farms. The major objective was to identify if milk quality, udder health and hygiene outcomes were associated with facility type, thereby exploring if BP systems are a viable option for housing in Vermont during the non-grazing season compared to the 2 most common indoor housing systems in the state (FS, TS). This study is also the first to describe udder health and hygiene during winter on BP in the Northeastern US, which is significant as the performance of these systems can be greatly influenced by climatic and seasonal factors. We compared BTM bacteriology, udder health and hygiene metrics, and milk yield between BP, TS, and FS herds. There was insufficient evidence to reject our null hypothesis that these metrics do not differ by facility type. However, due to small sample size and limited statistical power, the lack of finding any statistical differences does not rule out the potential existence of biologically important differences between facility types. In fact, for a number of outcomes we found BP herds achieved better results compared to TS herds in the same region. Our findings, while limited due to small sample size, provide observed data to design future studies exploring differences in milk quality outcomes on organic dairy herds using different bedding and housing systems.

2.5.1 Objective 1: Comparison of bulk tank milk quality, udder health, milk production, and udder hygiene measures by facility type

Although there is a substantial body of work describing udder health and milk quality for cows housed in straw yards (Astiz et. al, 2014; Fregonesi and Leaver, 2001; Fregonesi and Leaver, 2002; Ward et. al 2002; Peeler et al. 2000), description of these outcomes in the literature is limited for static deep bedded packs. As such, the focus of the discussion will compare BP in the current study (both static and composting) to the more recent body of work on compost bedded-pack farms. Previous work describing bulk tank milk aerobic culture data for farms using a BP system has primarily been descriptive studies of compost bedded-pack herds (Barberg et al., 2007b; Shane et al., 2010), with one study directly comparing bacterial counts between CBP and FS barns (Lobeck et al., 2012). Although farms in these previous studies used a similar array of bedding materials to those in the current study (wood sawdust, wheat straw by-product, Lobeck et al. 2012; wood sawdust, Barberg et al. 2007; “alternative” organic materials, Shane et al. 2010), the sampling period for these previous works differed from the present in seasonality, compounding the difficulty of direct comparison for milk quality outcomes (Pantoja et al., 2009). Barberg et al., 2007b evaluated milk culture results across the summer months, while Lobeck et al., 2012 sampled year-round; the current study focused solely on sampling during the winter, when organic pasture-based herds are primarily housed inside in Vermont.

The *Staph.* spp. count for the 5 BP farms included in this study was comparable to previous work describing bulk tank milk quality for CBP in Minnesota during the winter months. Lobeck et al. 2012 found a mean of 26.1 cfu/mL (95% CI: 2-443) and Shane et al. (2010) found a range of 0-108 cfu/mL for *Staph.* spp. from BTM in the winter months from 6 CBP farms. Within this highly heterogenous group of bacteria, some species are considered primarily host-adapted (colonizing the skin or udder), while others have been associated with stall surfaces, air, and unused sawdust (Piessens et al., 2011), different facility types (Condas et al., 2017), and environmental contamination and poor teat hygiene at milking time (De Visscher et al., 2016; De Visscher et al., 2017). In general, the use of pre- and post- milking teat dip decreases contamination of bulk tank milk both by commensal skin organisms and environmental contamination at milking time (Hogan et al., 1987, Pankey et al., 1985; Pankey et al., 1987; Quirk et al., 2012). All but 1 farm in the current study would fall into the “low” category for BTM *Staph.* spp. counts (Jayarao et al., 2004), which is consistent with all 21 herds using both pre- and post-dip consistently at milking time.

Streptococci and strep-like organisms counts in BTM for BP in the current study were much lower than those from Minnesota CBP in the winter (98-48,400 cfu/mL, Shane et al. 2010; mean: 911 cfu/mL, 95% CI: 138-6,01, Lobeck et al. 2012). Work from Barberg et al. (2007) describing milk quality on CBP in Minnesota noted that 6 of 12 farms sampled had “high” levels of SSLO. The overall SSLO count for all 21 farms included in the current study was lower than that for the overall *Strep.* count for all facility types studied in Lobeck et al. 2012 (445 cfu/mL, 95% CI: 116-1704). We speculate that milking and bedding hygiene practices among herds included in the current study may best explain this difference in BTM pathogen profiles compared to herds enrolled in prior studies (Jayarao and Wolfgang, 2003), and our results support prior industry guidelines for limiting environmental mastitis pathogen exposure, including monitoring and maintaining udder clealiness (Schreiner and Ruegg, 2003).

All farms had low levels of coliforms in bulk tank milk, indicating excellent hygiene practices at milking time (Jayarao and Wolfgang, 2003). The low BTM coliform counts for BP in the current study are similar to those found for 3 CBP farms in Brazil (2.8 cfu/mL; Fávero et al. 2015). This is in contrast with previous work describing BTM quality for this kind of facility in the U.S. (15-1,128 cfu/mL, Shane et al., 2010; mean: 63.7 cfu/mL, 95% CI: 6-735, Lobeck et al. 2012), although direct comparison of coliform counts between studies may be potentially problematic due to variation in duration of freezer storage (Schukken et al., 1989). Barberg et al. 2007 found that 5 of 12 BP sampled during the summer months had “high” levels of coliforms in BTM, contributing to their conclusion that “special attention to cow preparation procedures at milking time are a must for achieving satisfactory milk quality when cows are housed in compost dairy barns.” Season of the year also effects BTM coliform counts, which are higher in summer seasons (Gillespie et al., 2012). Sampling in late winter may partially explain our findings of lower coliform counts for herds in this study compared to previous studies.

Prevalence of *Staph. aureus* was similar between the 5 VT BP farms in the current study and the 6 described in Lobeck et al. 2012 (6.2 cfu/mL, 95% CI: 1.3-30.1). Farm-level prevalence of *Staph. aureus* was also fairly low for BP studied in Shane et al. 2010 (3 of 6 farms BTM negative) and Barberg et al. 2007 (only 1 of 12 farms with a “high” level of *Staph. aureus*). Overall, the population of all 21 farms in the current study had a higher amount of *Staph. aureus* in BTM than the 18 Minnesota farms described in Shane et al. 2010 (median: 30 cfu/mL, range: 0-320; vs. 17.3 cfu/mL, 95% CI: 3.3-91.2). Although it is not clear how many herds included in previous work on BP were certified organic, this higher prevalence of *Staph. aureus* on organic farms in the current study is consistent with work comparing organic and conventional dairy systems (Pol and Ruegg, 2007).

Analysis of a single bulk tank milk sample from a farm is a simple, convenient, and relatively inexpensive way to capture a snapshot of current milk quality and animal health on a farm, and can be a highly specific (albeit poorly sensitive) screening test for major contagious mastitis pathogens (*Staph. aureus* and *Strep. agalactiae;* Godkin and Leslie 1993). Our bulk tank sampling strategy (collecting a single sample) differed from previous work describing the bacteriology of milk from BP farms, where 4 or 5 consecutive bulk tank milk pickups were collected and then pooled for analysis (Barberg et al., 2007b; Shane et al., 2010; Lobeck et al., 2012). We acknowledge that analysis of a single BTM sample in the current study comes with limitations. Bacterial groups traditionally considered to be primarily environmental in origin (non-*ag. Strep., Staph* spp*.,* coliforms), may enter BTM from cows with an intramammary infection, but also may originate from non-specific contamination (teat and udder skin, bedding, manure, or other environmental sources; Elmoslemany et al., 2009). Furthermore, a single bulk tank sample does not give insight into long-term, consistent patterns of a particular farm’s milk quality as is possible from repeated BTM samplings (Jayarao and Wolfgang, 2003). With the financial constraints of research on commercial dairy farms, the limitations inherent in performing analysis of a single bulk tank milk sample from each farm were a trade-off for the ability to get a picture of milk quality on a larger number of farms included in the study.

The estimates from multivariable models of udder health outcomes included in the current study (percent cows with elevSCS, percent cows with chronSCS, percent cows with newSCS, BTSCC, and avg. SCS) were not statistically different between facility types (Table 4). For BTSCC, BP were numerically lower than the other 2 facility types; the difference in BTSCC for BP vs. FS and BP vs. TS equated to an increase of 34,628 and 28,105 cells/mL, respectively, which could amount to an important difference in the bulk tank milk quality premiums under some systems. The odds of a new infection (newSCS) for FS were 10% lower than that for BP; similarly, the odds of a new infection on TS farms were 6% lower than that for BP. Although these estimates represent the relative odds of infection between different facility types and not a proportion of the herd infected, which is often used for industry guidance. Ruegg and Pantoja (2013) propose a benchmark of having <8% of cows developing a new subclinical mastitis infections per month, and Schukken et al. (2003) suggest <10%. Seventy-eight percent of herds, including 3 of the BP herds, enrolled in this study were below those thresholds. The odds of a chronic infection (chronSCS) for FS were 20% higher than that for BP, while the odds of a chronic infection on TS were approximately equivalent to those for BP (1% lower). Although these estimates again represent the relative odds of a chronic infection by facility type, an industry benchmark is to have <10% of cows with chronic subclinical mastitis infections carrying over month to month (U. Minnesota Extension Dairy Team), so the numeric difference seen between BP and FS for this outcome may be biologically important. ElevSCS was numerically lowest for TS herds, while FS herds had a higher proportion of cows with an SCS ≥ 4.0 on current test compared to BP farms. The relative magnitude of the difference for these estimates when compared to BP may be biologically significant (1.8% for FS, -2.4% for TS), as a suggested goal for herds is to have a <15% prevalence for cows with subclinical mastitis (Ruegg and Pantoja, 2013). With regards to numeric difference in avg. SCS, BP farms performed slightly better than FS, and were approximately equivalent to TS. The increase in estimated avg. SCS for FS equates to an increase of roughly 16,250 cells/mL at the cow level, which represents a slight to modest increase in SCC. Although some numeric differences for outcomes were observed in the current study between facility types for newSCS, chronSCS, elevSCS, and avg. LS, given the proportionately large standard errors for all estimates, interpretation of the effect of facility type for these outcomes is challenging.

Some previous work has found BTSCC to be elevated for CBP farms (425,000 cells/mL over all 4 seasons, Black et. al 2013; 325,000 cells/mL during summer, Barberg et. al 2007b). Other groups have found udder health and milk quality measures on CBP farms are similar to farms using more traditional facility types. Specifically, in a study of 18 commercial dairy farms, subclinical mastitis prevalence levels was not statistically different between CBP and 2 types of FS housing in Minnesota and South Dakota, where the percent of cows in a herd with an SCC on test day ≥200,000 cells/mL was 33.4, 26.8, and 26.8% for CBP, cross-ventilated FS, and naturally-vented FS, respectively (Lobeck et al., 2011). Like our study, the sample size of this and other prior studies may have influenced the ability to detect a statistical difference when the observed differences are biologically relevant. Eckelkamp et. al (2016a) found no significant difference in subclinical mastitis prevalence in 8 CBP vs. 7 sand-bedded FS in Kentucky with a history of low BTSCC (21.8 and 19.4%, respectively), as well as no difference in BTSCC between the 2 facility types (229,582 and 205,131 cells/mL, respectively). Subclinical mastitis prevalence was 27.7% for 12 CBP farms in Minnesota (Barberg et. al 2007b), which may be more representative of the general population of BP farms in that state as there were no inclusion criteria around maintaining a low SCC prior to the start of the study. The prevalence of subclinical mastitis for herds in our current study is similar to previous work in the U.S. In contrast, Fávero et. al (2015) found a much higher prevalence of subclinical mastitis (43.8%) and percent new infections (20.9%) for 3 BP farms in Brazil than our study (26 and 7% respectively, for 3 BP with available data).

Cows on BP farms numerically made slightly more milk than those in TS, and were equivalent to those in FS. This increase of 1.7 pounds for BP over TS represents roughly 3% of the average STD 150-day milk production for herds in the study, which is a relatively modest increase in milk production. However, the comparatively large standard errors for both STD 150-day milk estimates make it difficult to interpret the effect of facility type for this metric. Previous research has found no significant differences in various production metrics of cows housed on BP vs. in FS barns (Lobeck et al., 2011; Eckelkamp et al., 2016a; Costa et al., 2018). Varying production metrics for cows housed on BP have been reported previously (kg/cow/day, fat-corrected milk/cow/day, average L/cow/day, ME-305, rolling herd average, energy-corrected milk), preventing direct comparisons of milk production between the BP in the current study and other work. Additionally, many variables play a role in determining milk production (nutrition, breed, seasonality, DIM), so teasing out the effect of facility type alone on production in an observational study is difficult. However, as Leso et. al (2020) point out, “results in the literature indicate that high levels of milk production are possible in CBP.” As BP potentially improve cow comfort, one might expect improved milk production compared to traditional confinement housing systems (Calamari et al., 2009; Ruud et al., 2010).

TS farms had numerically higher proportion of dirty udders and avg. udder hygiene score, while FS and BP systems were similar. However, interpretation of these numerical differences is difficult, given that the standard errors for all 4 estimates are large relative to the coefficient estimates. Previous work found that cow hygiene on BP systems was comparable to traditional facility types in the Upper Midwestern U.S., Southeastern U.S., and Brazil (Barberg et al., 2007b; Shane et al., 2010; Black et al., 2013; Eckelkamp et al., 2016b; a; Costa et al., 2018; Adkins et al., 2022; Andrade et al., 2022). Black (2013) and Eckelkamp (2016a) reported that increased pack moisture allows wet bedding material and manure to adhere more easily to animals, meaning that cow hygiene is dependent on conditions of the BP. This sentiment was echoed by the BP producers in the current study, who shared that keeping their cows clean during periods of wet or humid weather could be a challenge. However, all BP in the current study had an average udder hygiene score of less than 2.5, and the farm with the lowest mean average udder hygiene score overall was a BP. Although Cook (2002) identified challenges of comparing dairy cattle hygiene between different facility types, we chose to focus on gathering observations of udder hygiene. The relationship between udder hygiene and health is well-studied, and was a tractable observation to make during non-grazing season farm visits where individual animals were often roaming freely in a pen, or confined in a TS barn.

2.5.2 Objective 2: Analysis of farm management factors (non-facility) associated with bulk tank milk quality, udder health, milk production, and udder hygiene scores for all farms combined

One finding from the univariate analysis combining all 21 farms is that farms with deeper bedding had more favorable udder hygiene metrics. When comparing farms that housed cows with a deep bedding system (deeply-bedded stalls or a BP) to those that housed cows on stalls with a smaller amount of bedding (over a mattress or concrete surface), the deeply-bedded systems tended to have better hygiene scores. This agrees with previous observational field studies of FS barns, including: Cook et al. 2016 (prevalence of dirty udders 13% lower for farms using deep bedding vs. stalls with mats), de Vries et al. 2015 (deep-bedding vs. mat/mattress reduced the likelihood of a cow having a dirty hindquarter by half), and Robles et al. 2020 (farms with mattress-based stalls had a higher prevalence of cows with dirty upper legs/flanks vs. those using a deep bedding system, often inorganic sand). In contrast, an experimental study looking at the effect of bedding depth in TS over 28-day periods found no difference between leg, flank, and udder hygiene of cows using deeply-bedded stalls (14 cm) and the control treatment (2-3 cm; Wolfe et al., 2018).

Beyond comparing udder hygiene of cows housed on a deep-bedding system to cows that were not, there was a linear association between bedding depth (depth of BP, depth of bedding in FS and TS) and hygiene score. As the measured height of bedding got deeper (height of BP, or amount of bedding material in stall), cows tended to have cleaner udders. To the best of our knowledge, work exploring this direct relationship between measured bedding depth and hygiene is limited to a single study by de Vries et al. 2015, who found no relationship between prevalence of dirty hindquarters and 3 different FS bedding height groups (<0.56 cm, 0.56–1.75 cm, >1.75 cm). This relationship between bedding depth and udder hygiene was especially strong for BP in particular, although our sample size was limited at 5 herds. To the best of our knowledge, this specific association has not previously been explored for BP herds. There is opportunity for future research looking at this relationship between increased amount of bedding used in deep-bedded systems (or more deeply-bedded stalls) and the benefit of improved udder hygiene and milk quality.

Multiple measures of udder health in this study were associated with udder hygiene, in accordance with the well-supported tenet that better cow hygiene is associated with better milk quality. The association between hygiene and udder health has been well-documented, both at the cow level (for IMI presence: de Pinho et al. 2012; for SCS/SCC: Reneau et al. 2005, Dohmen et al. 2010, and Sant’anna et al. 2011; for both SCS and IMI: Schreiner and Ruegg, 2003) and at the herd-level (BTSCC: Barkema et al. 1998; new IMI rate: Cook et al. 2002; average herd SCC, incidence clinical mastitis, and % new high SCC: Dohmen et al. 2010). Of particular relevance to the current work, a study carried out on 3 BP farms in Brazil found the odds of a new case of subclinical mastitis (SCC ≥200,000 cells/mL) and of a cow having subclinical mastitis on test day increased 32% and 16%, respectively, for each 1-unit increase in leg cleanliness score, (Fávero et al., 2015). Although leg cleanliness score was associated with both mastitis outcomes on 3 Brazilian BP, udder hygiene score was not, which should not be interpreted to suggest a better biological significance of either of these alternative hygiene scoring systems.

We also found from the univariate regression results farms using deeper bedding had better milk quality outcomes. Although there is an established recommendation of 15 cm for deep bedding of FS (Bickert, 2000; Cook, 2002) and limited studies exploring ideal bedding material depth for TS barns (Tucker and Weary, 2004; Tucker et al., 2009), these prior studies focused on cow comfort outcomes. As stated in a literature review by McPherson (2020), "very little research has investigated the effect of bedding depth on cow cleanliness” or considerations around udder health outcomes. We speculate that the relationship between deeper bedding and better udder health outcomes seen in our current work is mediated through the presumed causal pathway of (1) deeper bedding leading to improved hygiene, and (2) improved hygiene resulting in better udder health. Although recommending a particular depth may prove difficult as there are many contributing factors which are particular to a producer’s barn and bedding source, the opportunity still exists for research exploring optimal stall bedding depths of different organic materials with a focus on mastitis and udder health outcomes.

As for any observational study, there is the potential for bias to have influenced the observed results. Most importantly, participating herds were not a random sample of organic farms in the state, possibly resulting in selection bias. Participating herds were a convenience sample of a subset who responded to our initial survey in Winter 2018-2019 (source population). The potential exists that producers who volunteered to participate in the current study are systematically different in some way with regards to their management practices compared to the general population of organic farms in Vermont. In 2021, there were 147 organic dairy farms in Vermont selling milk, with an average herd size of 87 cows making 6,627 kg milk/cow/year (USDA, 2022). Herds in the current study were slightly smaller, averaging 65 cows per farm, but with higher-producing cows (7,828 kg milk/cow/year, estimated from captured DHIA records). For comparison, the average dairy cow in the U.S. produced an average of 10,926 kg of milk in 2022 (Progressive Dairy, 2017). It is important to acknowledge that organic cows on average produce less milk (Stiglbauer et al., 2013), and with decreased milk production comes decreased susceptibility to mastitis (Grohn, 2000). This relationship may in part explain the relatively low prevalence of mastitis occurring on these farms in comparison to the general population of dairy farms. Lastly, cross-sectional studies are unable to demonstrate causality for associations presented between management practices and outcomes. However, these limitations are inherent to every observational study, and all attempts were made to control for potential confounding with the multivariable models presented.

Perhaps the biggest limitation of the current study is the small number of farms in each facility type, which limited statistical power. As state agencies had been promoting the use of BP systems for years in Vermont, we had anticipated it would be feasible to enroll 10 farms using this system to house their lactating animals. This turned out not to be the case; the Winter 2018-2019 survey showed that many dairy farms were instead using these systems for non-lactating animals (heifers, dry cows; Andrews et al. 2021). Furthermore, the COVID-19 pandemic precluded resumption of the study in Spring 2020, limiting the number of farms included to herds sampled in 2019, and not all farms had DHIA data for every outcome of interest. A related limitation is that well-established mastitis control practices were widely adapted by participating herds, so we were unable to analyze associations between certain practices and BTM quality, udder health, and hygiene. A large body of work exists showing consistent udder health benefits from using these and other practices, so lack of association between these fundamental mastitis control practices and desirable outcomes in the current study should not be taken as evidence that they provide no benefit. As group sizes for each facility type were limited, we would caution against making inferences from the findings beyond the source population of this study. The potential still exists for future studies with a larger number of farms enrolled to further characterize milk quality and udder health on BP systems in the Northeastern US. By enrolling farms from a larger geographic area, future studies may be able to enroll a larger number of BP farms, increasing the statistical power needed to identify particular management factors which are beneficial or detrimental on BP specifically. Our data may be used to inform new hypotheses and power calculations for future study design.

While BP systems are not common for housing lactating cows in Vermont, farms using this system in the state are using both compost bedded-packs managed with daily cultivation and untilled deep bedded pack systems. As untilled and cultivated bedded pack systems differ in numerous regards (Leso et al., 2020), the initial goal was to enroll enough farms using each type and treat them as separate groups in the analysis. As the relatively small number of BP used in our state to house lactating dairy cattle created a challenge for enrolling 10 herds using this kind of system in our observational study, it was necessary to combine both types of system in order to achieve our objective of describing udder hygiene, milk quality, and udder health on these loose-housing systems deeply-bedded with organic material. While we acknowledge that grouping them together is not ideal, this diversity is a reflection of how the target population (small-medium, pasture-based organic dairy farms) are actually using them in the Northeastern U.S. (Benson, 2012). Despite this limitation, including bedded pack farms managed in a variety of ways sheds light on a broader spectrum of options used within this loose-housing system. Our current study demonstrates that farms can achieve excellent milk quality using either an untilled, deep bedded pack system or a tilled aerobically composting bedded pack system for indoor housing; 3 of the 5 BP farms had a BTSCC ≤99,000 cells/mL, and the remaining 2 were ≤160,000 cells/mL. Furthermore, the lowest BTSCC in the study (54,000 cells/mL) was a static BP farm using woodchips and straw. This low BTSCC was not achieved by selectively dumping milk from high-SCC cows; this farm also had the lowest overall % cows with elevated SCS (8.6%; data not shown).

BP systems have a number of advantages for producers considering updating their facilities, including a smaller initial investment when compared to a new FS or TS barn (Barberg et al., 2007a; Janni et al., 2007; Black et al., 2013), although the cost year-over-year for bedding is substantial (Shane et al., 2010). Bedded packs are designed for cow comfort (Barberg et al., 2007b; Bewley et al., 2012), and prevalence of lameness, foot, and leg injuries in these systems has been found to be less than TS and FS barns (Barberg et al., 2007b; Lobeck et al., 2011; Burgstaller et al., 2016). Lastly, manure management and environmental stewardship is a top concern for both dairy producers and the general public (Holly et al., 2018). Anecdotally, the BP producers enrolled in the study were pleased with their systems of manure management, viewing their used bedding material and manure as a valuable soil amendment and an integral part of their nutrient management plan. Bedded pack systems decrease the amount of liquid manure waste when compared to conventional barns, and the used bedding with manure is more easily composted before use as a soil amendment. As aged pack material is drier before it is spread on fields, it poses less of a risk for run-off into waterways, increases soil infiltration of nutrients, and creates flexibility around timing of manure application to fields (Rushmann, 2023). Bedded packs may be a good housing option for small, pasture-based farms in the Northeastern U.S. when properly managed on farms with excellent milking hygiene practices already in place. However, more research is needed to explore how udder health, milk quality, udder hygiene and milk production compares to more traditional housing systems.

2.6 Conclusion

In an observational study of 21 organic dairy herds in Vermont we found no statistical differences in milk quality and udder health outcomes between herds using different bedding and housing management systems. For 5 of the 6 studied udder health and production metrics, and both udder hygiene measures, BP either performed slightly better numerically or were approximately equivalent in comparison to the most commonly-used facility types for organic dairy cows in Vermont. However, the relatively large standard errors for most of these estimates and the lack of finding a statistical difference does not rule out possible biologically important effects of facility type for these outcomes. Our results may be due to the small group size for each facility type. Bedded packs may therefore be a viable option for pasture-based herds looking for a loose-housing system, but future studies enrolling larger number of farms using each type of housing are needed to more definitively explore these relationships. Findings from the secondary analysis of results supported the established tenets that better cow hygiene is associated with better milk quality, and farms with deeper bedding had more favorable udder hygiene metrics. Additionally, farms using deeper bedding had better milk quality outcomes, which may likely be mediated through improved hygiene resulting in better udder health outcomes.

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2.9 Tables

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| **Table 2.1** Predictors offered to multivariable models for each of the 8 different outcomes of interest along with facility type (forced) | | | |
| Predictor | | | Level of parameter, if categorical: |
| Farm demographics/lactating cow housing | | |  |
|  | Facility type | | Bedded pack; Freestall; Tiestall |
|  | Predominant breed | | Holstein; Jersey/Other |
|  | Herd size (lactating cows) | |  |
|  | Herd size group (lactating cows) | | 30-55; 56-69; 70-100 |
|  | Subjective assessment of air quality (producer) | | Excellent; Good; Fair/Poor |
|  | Subjective assessment of air quality (researcher) | | Good; Fair |
|  | Age of facility (years) | |  |
|  | Feed supplemental vit. E and selenium | | Yes; No |
| Lactating bedding management practices | | |  |
|  | Lying surface for cows1 (deeply-bedded vs. not) | | Deeply-bedded stalls or bedded pack; Stalls with bedding on a mattress or concrete surface |
|  | *If use shavings/sawdust/*  *woodchips for bedding material:* | |  |
|  |  | Moisture-content | Kiln-dried; Fresh/raw |
|  | Bedding amendment (e.g., hydrated lime) used on surface | | Yes; No |
|  | *If facility is freestall or tiestall:* | |  |
|  |  | Freq. adding new bedding to stalls (times per week) |  |
|  |  | Freq. scraping stalls (times per week) |  |
|  |  | Depth bedding in stalls (cm) |  |
| Mastitis control and milking hygiene practices | | |  |
|  | Clip/flame udder hair | | Yes; No |
|  | Keep record of clinical mastitis events | | Always; Sometimes/Temp.; Never |
|  | Routinely culture mastitic milk | | Always/Sometimes; Never |
|  | Routinely culture high somatic cell count cows | | Always/Sometimes; Never |
|  | Ever perform culture of mastitic cows | | Yes; Never culture |
|  | Use intramammary product at dry-off (OMRI-listed) | | Yes; No |
|  | Parenteral supplementation with vit. E and selenium | | All lactating cows regularly/ Occasionally as needed; No |
|  | Glove use at milking | | All milkers consistently; Inconsistently/No |
|  | Check for clinical mastitis by noticing abnormal cow/abnormal udder and forestripping | | Yes; No |
|  | Type of milking system used3 | | Parlor; Tiestall |
| Farm-level udder hygiene metrics | | |  |
|  | Average udder hygiene score | |  |
|  | Prop. dirty udders (%; udder hygiene score ≥3) | |  |
| 1 If freestall or tiestall, producer asked if used deeply-bedded stalls | | | |
| 2 OMRI: Organic Materials Review Institute | | | |
| 3 One freestall farm used an automated milking system | | | |

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| **Table 2.2** Objective 1: Descriptive and univariable results for bulk tank milk aerobic culture outcomes by facility type [median (range)]. *P-*value is for Kruskal-Wallis test by facility type grouping | | | | | |
| Bacteria group (cfu/mL) | Overall (n = 21) | Bedded packs (n = 5) | Tiestalls (n = 10) | Freestalls (n = 6) | *P-*value |
| *Staph.* spp. | 65 (0-665) | 40 (0-130) | 85 (15-665) | 67.5 (5-125) | 0.62 |
| *Strep.* and strep-like orgs. | 45 (10-1250) | 35 (10-80) | 167.5 (20-1250) | 32.5 (25-260) | 0.10 |
| *Staph. aureus* | 30 (0-320) | 0 (0-30) | 47.5 (0-320) | 42.5 (0-100) | 0.19 |
| Coliforms | 0 (0-5) | 0 (0-5) | 0 (0-5) | 0 (0-5) | 0.82 |

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| **Table 2.3** Objective 1: Descriptive results for milk quality, udder health and production outcomes by facility type [mean (95%CI)] | | | | |
| Outcome | Overall | Bedded packs | Tiestalls | Freestalls |
| BTSCC (log10cells/mL) | n = 21 | n = 5 | n = 10 | n = 6 |
|  | 5.13 (5.06-5.20) | 5.00 (4.84-5.17) | 5.14 (5.05-5.23) | 5.21 (5.09-5.33) |
| % newly elevated SCS1 | n = 19 | n = 3 | n = 10 | n = 6 |
|  | 5.7 (4.2-7.3) | 7.0 (2.8-11.2) | 5.4 (3.0-7.8) | 5.6 (3.0-8.3) |
| % chronically elevated SCS1 | n = 19 | n = 3 | n = 10 | n = 6 |
|  | 13.6 (11.2-16.1) | 14.5 (5.4-23.7) | 14.3 (11.9-16.7) | 12.0 (6.7-17.3) |
| % SCS ≥ 4.0 current test1 | n = 19 | n = 3 | n = 10 | n = 6 |
|  | 24.9 (21.6-28.3) | 26.0 (12.6-39.3) | 25.4 (22.1-28.6) | 23.7 (16.9-30.5) |
| Avg. SCS2 | n = 20 | n = 4 | n = 10 | n = 6 |
|  | 2.44 (2.26-2.62) | 2.38 (1.84-2.91) | 2.45 (2.31-2.59) | 2.50 (2.00-2.93) |
| Standardized 150-day milk (pounds)3 | n = 18 | n = 4 | n = 8 | n = 6 |
|  | 50.0 (45.7-54.3) | 46.9 (39.8-53.9) | 49.4 (43.1-55.7) | 53.0 (43.5-62.5) |
| 1 DHIA data not available for 2 bedded pack farms | | | | |
| 2 DHIA data not available for 1 bedded pack farm | | | | |
| 3 DHIA data not available for 1 bedded pack farms and 2 tiestall farms | | | | |

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| **Table 2.4** Objective 1: Final multivariable models describing the relationship between facility type (forced) and milk quality, udder health, production, and udder hygiene outcomes | | | | |
| Outcome | Explanatory variable | Group (sample size) | Coefficient estimate (SE) | *P-*value |
| BTSCC (log10cells/mL) | |  |  |  |
|  | Intercept |  | 4.8 (0.15) |  |
|  | Facility type (forced) | Freestall (n = 6) | 0.19 (0.09) | 0.05 |
|  |  | Tiestall (n = 10) | 0.16 (0.08) | 0.07 |
|  |  | Bedded pack (n = 5) | Ref. | Ref. |
|  | Herd size | All herds (n = 21) | 0.003 (0.002) | 0.15 |
| % newly elevated SCS | |  |  |  |
|  | Intercept |  | -3.8 (0.55) |  |
|  | Facility type (forced) | Freestall (n = 6) | -0.11 (0.40) | 0.79 |
|  |  | Tiestall (n = 10) | -0.07 (0.38) | 0.86 |
|  |  | Bedded pack (n = 3) | Ref. | Ref. |
|  | Subjective assessment air quality (researcher) | Good (n = 14) | 0.99 (0.43) | 0.02 |
|  |  | Fair (n = 5) | Ref. | Ref. |
|  | Glove use at milking1 | Never/Inconsistently (n = 9) | 0.63 (0.30) | 0.03 |
|  |  | Always (n = 9) | Ref. | Ref. |
| % chronically elevated SCS | |  |  |  |
|  | Intercept |  | -2.8 (0.37) |  |
|  | Facility type (forced) | Freestall (n = 6) | 0.18 (0.43) | 0.68 |
|  |  | Tiestall (n = 10) | -0.01 (0.32) | 0.97 |
|  |  | Bedded pack (n = 3) | Ref. | Ref. |
|  | Feed supplemental vit. E and selenium2 | Yes (n = 11) | 0.20 (0.29) | 0.50 |
|  |  | No (n = 7) | Ref. | Ref. |
|  | Use bedding amendment | Yes (n = 5) | 0.55 (0.32) | 0.08 |
|  |  | No (n = 14) | Ref. | Ref. |
|  | Clip/flame udder hair | Yes (n = 5) | -0.55 (0.31) | 0.07 |
|  |  | No (n = 14) | Ref. | Ref. |
|  | % udder hygiene scores ≥3 | Herds with available data (n = 19) | 1.8 (0.61) | 0.003 |
| % SCS ≥ 4.0 current test | |  |  |  |
|  | Intercept |  | 0.85 (10.6) |  |
|  | Facility type (forced) | Freestall (n = 6) | 1.8 (5.7) | 0.75 |
|  |  | Tiestall (n = 10) | -2.4 (5.3) | 0.66 |
|  |  | Bedded pack (n = 3) | Ref. | Ref. |
|  | Use bedding amendment | Yes (n = 5) | 8.0 (4.2) | 0.07 |
|  |  | No (n = 14) | Ref. | Ref. |
|  | Mean hygiene | Herds with available data (n = 19) | 9.8 (4.7) | 0.06 |
| Avg. SCS | |  |  | 20 |
|  | Intercept |  | 0.93 (0.44) |  |
|  | Facility type (forced) | Freestall (n = 6) | 0.38 (0.21) | 0.09 |
|  |  | Tiestall (n = 10) | 0.03 (0.19) | 0.86 |
|  |  | Bedded pack (n = 4) | Ref. | Ref. |
|  | Use intramammary product at dry-off (OMRI-listed) | Yes (n = 5) | -0.30 (0.16) | 0.08 |
|  |  | No (n = 15) | Ref. | Ref. |
|  | Use bedding amendment | Yes (n = 5) | 0.52 (0.16) | 0.007 |
|  |  | No (n = 15) | Ref. | Ref. |
|  | Parenteral supplementation vit. E/selenium | Regularly or occasionally (n = 9) | -0.36 (0.14) | 0.02 |
|  |  | No supplementation (n = 11) | Ref. | Ref. |
|  | Mean hygiene | Herds with available data (n = 20) | 0.64 (0.19) | 0.005 |
| Standardized 150-day milk (pounds) | |  |  | 18 |
|  | Intercept |  | 41.2 (6.1) |  |
|  | Facility type (forced) | Freestall (n = 6) | -0.06 (7.0) | 0.99 |
|  |  | Tiestall (n = 8) | -1.7 (6.6) | 0.80 |
|  |  | Bedded pack (n = 4) | Ref. | Ref. |
|  | Parenteral supplementation vit. E/selenium | Regularly or occasionally (n = 7) | 7.0 (5.2) | 0.20 |
|  |  | No supplementation (n = 11) | Ref. | Ref. |
|  | Culture high SCC cows | Always/Sometimes (n = 8) | 9.3 (5.9) | 0.14 |
|  |  | Never (n = 10) | Ref. | Ref. |
|  | Herd size grp. (lact. cows) | 70-100 (n = 8) | -0.18 (7.3) | 0.98 |
|  |  | 56-69 (n = 5) | 10.3 (6.2) | 0.12 |
|  |  | 30-55 (n = 5) | Ref. | Ref. |
| % udder hygiene scores ≥3 | |  |  |  |
|  | Intercept |  | 0.32 (0.08) |  |
|  | Facility type (forced) | Freestall (n = 6) | 0.002 (0.11) | 0.99 |
|  |  | Tiestall (n = 10) | 0.17 (0.10) | 0.12 |
|  |  | Bedded pack (n = 5) | Ref. | Ref. |
| Avg. udder hygiene score | |  |  |  |
|  | Intercept |  | 2.3 (0.17) |  |
|  | Facility type (forced) | Freestall (n = 6) | -0.04 (0.21) | 0.84 |
|  |  | Tiestall (n = 10) | 0.33 (0.19) | 0.11 |
|  |  | Bedded pack (n = 5) | Ref. | Ref. |
|  | Check for clinical mastitis by noticing abnormal cow/abnormal udder and forestripping | Yes (n = 8) | -0.25 (0.16) | 0.14 |
|  |  | No (n = 13) | Ref. | Ref. |
| 1 One farm used automatic milking system | | | | |
| 2 One farm unable to provide response | | | | |

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| **Table 2.5** Objective 2: Selected models of univariate analysis identifying (non-facility type) factors unconditionally associated with milk quality, udder health, production, and udder hygiene outcomes at *P* <0.20 | | | | | |
| Outcome | Explanatory Variable | Group (sample size) | Coefficient estimate (SE) | *P-*value | Intercept |
| BTSCC (log10cells/mL) | |  |  |  |  |
| Model 1 | Lying surface | Mattress or concrete (n = 13) | 0.12 (0.07) | 0.12 | 5.1 |
|  |  | Deep bedding (n = 8) | Ref. | Ref. |  |
| Model 2 | Depth of bedding in stalls (cm)1 | Tiestalls and freestalls (n = 15) | -0.02 (0.01) | 0.11 | 5.2 |
| Model 3 | Glove use at milking2 | Never/Inconsistently (n = 9) | 0.10 (0.07) | 0.19 | 5.1 |
|  |  | Always (n = 11) | Ref. | Ref. |  |
| % newly elevated SCS3 | |  |  |  |  |
| Model 4 | Glove use at milking | Never/Inconsistently (n = 9) | 0.58 (0.29) | 0.05 | -3.1 |
|  |  | Always (n = 9) | Ref. | Ref. |  |
| Model 5 | Depth of bedding in stalls (cm)1 | Tiestalls and freestalls (n = 15) | -0.13 (0.07) | 0.06 | -2.4 |
| % chronically elevated SCS3 | |  |  |  |  |
| Model 6 | Clip/flame udder hair | Yes (n = 5) | -0.37 (0.25) | 0.13 | -1.8 |
|  |  | No (n = 14) | Ref. | Ref. |  |
| Model 7 | Parenteral supplementation vit. E and selenium | Regularly or occasionally (n = 8) | -0.31 (0.19) | 0.11 | -1.7 |
|  |  | No supplementation (n = 11) | Ref. | Ref. |  |
| Model 8 | % udder hygiene scores ≥3 | Herds with available data (n = 19) | 1.26 (0.48) | 0.01 | -2.4 |
| Model 9 | Avg. udder hygiene score | Herds with available data (n = 19) | 0.63 (0.25) | 0.01 | -3.3 |
| Model 10 | Depth of bedding in stalls (cm)1 | Tiestalls and freestalls (n = 15) | -0.05 (0.04) | 0.17 | -1.7 |
| % SCS ≥ 4.0 current test3 | |  |  |  |  |
| Model 11 | Depth of bedding in stalls (cm)1 | Tiestalls and freestalls (n = 15) | -1.2 (0.42) | 0.01 | 30 |
| Model 12 | % udder hygiene scores ≥3 | Herds with available data (n = 19) | 13.6 (8.5) | 0.13 | 19.6 |
| Model 13 | Avg. udder hygiene score | Herds with available data (n = 19) | 7.7 (4.3) | 0.09 | 7.1 |
| Average SCS4 | |  |  |  |  |
| Model 14 | Parenteral supplementation vit. E and selenium | Regularly or occasionally (n = 9) | -0.27 (0.18) | 0.15 | 2.6 |
|  |  | No supplementation (n = 11) | Ref. | Ref. |  |
| Model 15 | Use intramammary product at dry-off (OMRI-listed) | Yes (n = 5) | -0.29 (0.21) | 0.18 | 2.5 |
|  |  | No (n = 15) | Ref. | Ref. |  |
| Model 16 | Depth of bedding in stalls (cm)1 | Tiestalls and freestalls (n = 15) | -0.05 (0.03) | 0.10 | 2.6 |
| Model 17 | % udder hygiene scores ≥3 | Herds with available data (n = 20) | 0.75 (0.45) | 0.12 | 2.1 |
| Model 18 | Avg. udder hygiene score | Herds with available data (n = 20) | 0.39 (0.23) | 0.11 | 1.5 |
| Standardized 150-day milk (pounds)5 | |  |  |  |  |
| Model 19 | Parenteral supplementation vit. E and selenium | Regularly or occasionally (n = 7) | 9.0 (4.5) | 0.06 | 46.5 |
|  |  | No supplementation (n = 11) | Ref. | Ref. |  |
| Model 20 | Herd size | Herds with available data (n = 18) | 0.26 (0.14) | 0.07 | 33.1 |
| % udder hygiene scores ≥3 | |  |  |  |  |
| Model 21 | Depth of bedded pack (m) | Bedded pack herds (n = 5) | -0.5 (0.06) | 0.004 | 0.97 |
| Model 22 | Lying surface | Mattress or concrete (n = 13) | 0.17 (0.08) | 0.06 | 0.30 |
|  |  | Deep bedding (n = 8) | Ref. | Ref. |  |
| Model 23 | Depth of bedding in stalls (cm)1 | Tiestalls and freestalls (n = 15) | -0.02 (0.02) | 0.13 | 0.54 |
| Avg. udder hygiene score | |  |  |  |  |
| Model 24 | Depth of bedded pack (m) | Bedded pack herds (n = 5) | -0.96 (0.15) | 0.008 | 3.4 |
| Model 25 | Lying surface | Mattress or concrete (n = 13) | 0.33 (0.16) | 0.06 | 2.1 |
|  |  | Deep bedding (n = 8) | Ref. | Ref. |  |
| Model 26 | Depth of bedding in stalls (cm)1 | Tiestalls and freestalls (n = 15) | -0.06 (0.03) | 0.07 | 2.6 |
| 1 Stall bedding depth for freestalls and tiestalls bedded with wood shavings or sawdust | | | | | |
| 2 One farm used automatic milking system | | | | | |
| 3 DHIA data available for n = 19 herds. | | | | | |
| 4 DHIA data available for n = 20 herds. | | | | | |
| 5 DHIA data available for n = 18 herds. | | | | | |