**Interpretive summary**

**Graphical abstract**

**Running head:**

**Working title**

Caitlin E. Jeffrey,1 XXXXX, John W. Barlow1

1Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT 05405

Corresponding author: John Barlow

Department of Animal and Veterinary Sciences,

202 Terrill Building,

University of Vermont,

Burlington, VT 05405

Phone: 802-656-1395

Email: [john.barlow@uvm.edu](mailto:john.barlow@uvm.edu)

**Abstract**

A cross-sectional observational study on organic dairies had the objective of identifying whether bulk tank milk quality, udder health and hygiene outcomes were associated with facility type, and whether bedded pack systems are a viable option for winter housing in VT. We aimed to collect bulk tank milk samples, udder hygiene scores, and complete a survey on mastitis risk and bedding management on 40 farms, in order to compare the two most common winter housing systems in the state (freestalls, tiestalls) with those using a bedded pack. The survey was completed on 21 farms (5 bedded packs, 6 freestalls, 10 tiestalls) before interruption due to the pandemic. DHIA information captured included avg. linear score (LS), standardized 150-day milk (STD 150-day milk), % cows with high SCC (“elSCC;” LS ≥4.0), % cows with newly-elevated SCC (“neSCC,” LS <4.0 to ≥4.0), and % cows with chronically-elevated SCC (“ceSCC,” LS ≥4.0 last 2 tests). There were no significant differences between bulk tank udder health measures, aerobic culture data, milk production, and hygiene scores between facility types. As sample sizes were limited, a multivariable model to describe outcomes by facility type was abandoned in favor of univariate linear regression to identify associations between management factors and outcomes for all farms combined. Farms with deeper bedding showed a tendency (p ≤ 0.20) toward a lower bulk tank SCC, lower % neSCC, lower % elSCC, lower average LS, and improved hygiene metrics. Farms with lower mean udder hygiene scores tended towards having lower % chronically-elevated SCC, lower % elevated SCC, and lower average LS. Increased bedding depth measures tended to be associated with improved udder hygiene metrics. Although statistical power was limited, the current study provided insight on factors affecting bulk tank milk quality, udder health and hygiene measures on organic dairy farms in Vermont. Additionally, outcomes for bedded packs were comparable to more commonly used winter housing systems, and are therefore a viable option for pasture-based herds interested in a loose-housing system in VT.

**Keywords:** Mastitis, organic dairy cattle, (composting) bedded pack, hygiene, bulk tank milk quality…

**Introduction**

Teats of dairy cattle may be in direct contact with bedding materials for 40 to 60% of the day, making bedding materials an important potential source of exposure to mastitis pathogens (Cook, 2005; Hogan, 2012; Tucker, 2004). 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 which limit the effect of contagious pathogens (Klaas, 2018). Work exploring how bedding materials relate to a cow’s risk of getting mastitis has understandably been focused on the most commonly-used bedding materials and housing systems in the dairy industry. However, there is growing interest and adoption amongst dairy producers of a bedded pack system to house dairy cattle. Smaller-scale dairy farmers with aging facilities may be looking to implement a bedded pack system on their farms as a sustainable, cost-effective, loose-housing option which integrates well into pasture-based production systems, as both the dairy industry and consumers look to move away from traditional housing systems that restrict cow movement (Barkema et al., 2015). As interest in this type of facility grows, it will be important to better understand how milk quality, udder health and hygiene looks for farms using a bedded pack system. Previous work describing mastitis risk and hygiene for actively-managed, composting bedded packs specifically have comparable milk quality and udder health to more traditional housing systems (Favero, 2015; Albino, 2018; Eckelcamp 2016 “Understanding…”; Black, 2013; Barberg, 2007; Heins, 2018). However, direct head-to-head comparisons of milk quality, udder health, and hygiene on bedded pack farms to other more traditional housing systems of similar size and management style are limited. To date, this research has only directly compared actively-managed composting bedded packs and sand-bedded freestalls, and primarily conventionally-managed dairy farms (Eckelcamp 2016 “Sand…”; Lobeck 2011).

Organic farms in the Northeast have expressed particular interest in bedded pack systems, as many of them are pasture-based and these loose-housing structures integrate well into their farm systems. Currently, the most common type of dairy cattle housing for organic farms in Vermont is a tiestall barn, followed by freestall barns as a distant second (Andrews 2021). As attitudes about confinement housing of dairy cattle evolve amongst the general public, many small to mid-size dairy farms in the Northeast are looking to replace their aging tiestall facilities. Many are considering converting to a bedded pack system, as the initial investment is smaller than a new freestall barn (Bewley 2012). Understanding mastitis risk for bedded packs will be especially important for organic dairy farmers, as they have limited options for treating intramammary infections. Furthermore, as previous research directly comparing bedded packs to other housing systems has been limited to freestalls with sand (an uncommon housing type of organic farms in the Northeast), a direct comparison of udder health and hygiene on bedded pack systems and tiestall barns would be a more readily useful tool for organic farms in the Northeast deciding whether or not the facility may be a good option for them.

In order to better inform organic dairy producers in the Northeast who may be interested in using a bedded pack barn, we carried out a cross-sectional, observational study on organic dairies in Vermont which aimed to compare udder health and hygiene measures for the two most common housing systems in the state (freestalls, tiestalls) with farms using a bedded pack. The objectives of this project were to (1) Identify whether bulk tank milk quality, udder health and hygiene outcomes were associated with facility type; and (2) Determine if bedded pack systems are a viable option for winter housing in VT when cows are not turned out to pasture.

**Materials and Methods**

1. **Herd enrollment and selection**

The source population for this study was the 145 farms that responded to a survey sent to all certified organic dairy farms producing cow milk in Vermont (n = 177) which aimed to quantify the frequency and diversity of winter housing and bedding types used by organic dairy farmers in the state, as previously described in Andrews et. al (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 of 2018-2019, 2) indicated they met the enrollment criteria of testing with the Dairy Herd Improvement Association (DHIA) at least monthly and milking between 35 and 120 cows, and 3) indicated they would be interested in further participation. Eligible farms were contacted from this source population if they responded that they were using 1 of the 4 categories of bedding/housing combinations for their winter housing system of interest to the current study: 1) a freestall system with bedded with sand, 2) a freestall system bedded with shavings/sawdust, 3) a tiestall system bedded with shavings/sawdust, or 4) a loose housing system deeply bedded with organic material (hereafter, “bedded pack”). The first three housing and bedding combinations were found to be the top three used by organic dairies in the state to house cows over the non-grazing season, and bedded packs were the primary housing style of interest for this project.

A list of eligible farms was made by housing/bedding combination, and were haphazardly/by convenience (?) contacted by phone or email provided in the previous survey in Spring 2019.

**OR:**

A convenience sample was enrolled from a list of eligible farms (grouped by housing/bedding combination) using the phone number or email address provided in the previous survey in Spring 2019.

The aim was to complete the survey and sampling at 40 farms total, with 10 farms from each housing/bedding style: 1) freestall bedded with sand (FS), 2) freestall bedded with wood shavings or sawdust (FW), 3) tiestall bedded with wood shavings (TW), or 4) bedded pack system (BP). As this preliminary study design to select 10 farms of each type was outlined before getting the full results from the initial survey, it was anticipated that it would be possible to select enough organic dairies in Vermont using a bedded pack system as their primary winter housing system. However, out of the 17 farms from 2018-2019 survey that indicated at least some use of a bedded pack system, 1 farm was not interested in any further participation, 5 did not use DHIA testing at all, and 6 only used a bedded pack system as a secondary housing system in conjunction with a tiestall barn, or cows were only on the pack a few hours a day. As the number of farms using the bedding system of interest was significantly smaller than anticipated, the eligibility requirements were relaxed to include a farm where cows spend the majority (two-thirds) of their time in a bedded pack, with the remaining time in a tiestall with wood shavings. The survey was intended to study cows while they were in their winter housing system, so all herds visits were completed before any grazing had begun for the season.

Of the intended 40 herds to be recruited in the study (10 each from each of 4 housing/bedding combination categories: freestalls with sand, freestalls with wood shavings/sawdust, bedded packs, and tiestalls with wood), 21 herds that were contacted agreed and the survey and sampling were completed throughout April-May, 2019. All herds sampled during this period were housing their cows as they would in the winter months. Completion of the survey and sampling was suspended in mid-May, as farms began turning their cows out to pasture, with the intention of resuming in Spring 2020 to complete the remaining 19 herds. However, due to the COVID-19 pandemic, the decision was made to not resume the survey and sampling, and the final analysis included herds only completed in 2019 (n = 21). As there was only 1 farm sampled using a freestall facility bedded with sand, the initial plan to group farms by the four housing/bedding combinations specified was abandoned in favor of grouping farms by the three facility types used (tiestall, freestall, and bedded pack).

1. **Survey administration, sampling, and udder hygiene scoring**

At each farm visit, a survey was administered by the primary researcher at every visit which collected information which aimed to: 1) get a comprehensive understanding of factors potentially related to a cow’s mastitis risk on that particular farm, and 2) acquire a comprehensive understanding of housing and bedding management and related practices on the farm. The survey is included in its entirety in the provided in Supplemental Data (XXX). The survey was created and administered on a tablet using KoboCollect software, a free and open-source suite of tools for field data collection (http://www.kobotoolbox.org). The section about mastitis risk included 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. The section 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 survey also collected some basic herd information (production numbers; number of lactating, dry, and youngstock; breeds; record-keeping systems). As the focus of this study was the use of bedded pack systems by organic dairy producers in Vermont, some additional questions were asked of these farms to gather more detailed information about management, monitoring, impressions comparing them to previously used systems, and initial construction of the pack. Completion of the survey took about 45 minutes on average, but ranged from roughly 30 minutes to 1.5 hours.

While the producer and primary researcher completed the survey, a technician collected a bulk tank milk sample was collected directly from the top of the bulk tank after agitating the milk for at least 5 minutes, using a 250-mL sterile single-use vial (Blue Dippas™, Dynalon Products, England). Samples were kept on ice in a cooler until they could be frozen and stored at −20°C in the laboratory before being sent to a diagnostic lab for analysis. Also during this time, the technician completed an on-farm observation sheet, which collected information about the bulk tank information, cow identification, air quality, and any outdoor exercise area. Additionally, measurements of the housing facilities were recorded for freestalls and tiestalls (stall sizes, pen sizes, stocking density, trainer use), as well as observations about bedded packs when applicable (temperature, depth, sq. ft per animal). Hygiene scoring was completed by the same technician on the day of every visit, with a minimum of 30 randomly selected cows housed in the same pens from which used bedding samples were collected. A 4-point scoring system described by Schreiner and Ruegg 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). Materials were left with producers to record and collect milk samples of cows with clinical mastitis in the 30 days following the farm visit, but participation in this aspect of the study was too low to include in any analyses.

1. **Bulk tank milk culture**

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 have been thoroughly 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, 2016), and Focus (University of Minnesota, St. Paul, selective for SSLO bacteria) media plates and incubated for 2 days at 37°C. Any lactose-fermenting (pink-colored) 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*), while β-Hemolytic colonies on Factor medium were counted and identified to the species level using a MALDI Biotyper (suspect *Staph. aureus*). Any β-Hemolytic colonies with a confidence score ≥2.0 for *Staph. aureus* were counted and reported as such. All remaining colonies on Focus medium that were not identified as *Strep. agalactiae* were counted and recorded as streptococci or strep-like organisms, and non-hemolytic colonies on Factor media of *Staphylococcus* spp. (based on colony morphology, catalase reaction, or Gram stain) were counted and reported as NAS. 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, counts were generated for coliform organisms, non-*aureus* staphylococci (NAS), streptococci and strep-like organisms (SSLO), *Staph. aureus*, *Strep. agalactiae*, and *Mycoplasma* spp. as total colony-forming units per mL. The lower threshold of detection for this bulk tank milk culture protocol was 5 cfu/mL, and the upper threshold was 62,500 cfu/mL.

1. **Herd-level udder health measurements**

Herd-level DHIA test results for the test day closest in proximity to the farm visit (less than 30 days) 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 dates, number of lactating cows, standardized 150-day milk production, and test-day average cow-level linear score (LS). 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 (“elSCC”), where infection was defined as a linear score of ≥4.0; the proportion of cows with a newly-elevated SCC (“neSCC”), which was defined as a LS changing from <4.0 to ≥4.0 over the last 2 tests; and the proportion of cows with a chronically-elevated SCC (“ceSCC”), which was defined as having a LS ≥4.0 on the last two tests.

1. **Data management and analysis**

Survey data collected through KoboCollect software (KoboCollect 2019) was downloaded as an Excel worksheet (Microsoft Corp., Redmond, WA), which contained the information from the questionnaire covering herd information, description of housing, bedding, and bedding management, as well as milking hygiene and mastitis control practices. Udder hygiene scores for individual cows were used to calculate two farm-level udder hygiene measures: 1) mean udder hygiene score, 2) proportion of cows with dirty udders (udder hygiene score ≥3). Bulk tank milk culture data from the U. Minnesota Veterinary Diagnostic lab, DHIA test results, and farm-level udder hygiene outcomes were entered into an Excel database, and the accompanying data for each farm from the questionnaire was then entered into this database to combine the outcomes and possible predictor variables for each of the 21 farms. This Excel database containing questionnaire data, udder health, hygiene, and bulk tank milk findings was then imported into the R Statistical Programming Environment (R Core Team, 2023) for data cleaning, checking, and statistical analysis. The distribution of outcome variables was visually assessed in R to check for normality, and descriptive statistics were calculated to evaluate the distribution and data integrity and to identify missing data (means, variances, percentiles for numeric continuous variables, frequencies tabulations and percentages for categorical variables). Descriptive statistics generated included description of general herd characteristics/farm traits, lactating cow housing/facilities, lactating cow bedding/bedding management practices, milking hygiene procedures, and mastitis control practices for all 21 herds included in the study. Additionally, descriptive statistics were also produced to describe udder hygiene, bulk tank milk culture, and DHIA udder health outcomes, both for all herds (n = 21) and stratified by facility type (freestall, bedded pack, tiestall). Distribution of the raw somatic cell count (SCC) data, log2 transformed SCC data, and log10 transformed SCC data was assessed, and all were found to be similarly close to being normally distributed; therefore, raw SCC data was chosen for ease of interpretation.

Unconditional comparisons of bulk tank udder health measures, aerobic culture data, and hygiene scores by facility type were then carried out using an appropriate test. Bulk tank somatic cell count, % neSCC, % ceSCC, % elSCC, STD 150-day milk, mean hygiene and proportion dirty udders met the assumptions for a One-way Fisher’s ANOVA (no outliers, normality using a Shapiro-Wilk test (p ≤ 0.05), and homogeneity of variances using Levene’s test and visual assessment of a residuals vs. fitted values plot). The Tukey method was used for adjusting p-values for multiple comparisons (“TukeyHSD” function of the “stats” package in R). As most measures of the aerobic culture data were not normally distributed even after log transformation, a Kruskal-Wallis test was used to compare CFU counts between the three facility types. Welch’s ANOVA was used for analysis of average LS, as the data were normally distributed but had unequal variances. Statistical significance for ANOVA and Kruskal-Wallis tests were declared at p ≤ 0.05.

Continuous variables underwent correlation analysis to identify variables that were highly associated (R2 ≥ 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 many categories with a small number of observations in each, categories were combined when biologically plausible/reasonable in an attempt 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 discluded from further analysis (but listed in descriptive statistic tables).

Univariate linear regression was performed in R using the “lme4” package to investigate the unconditional relationship between the six udder health and production (BTSCC, avg. LS; neSCC, elSCC, ceSCC, STD 150-day) and two hygiene outcomes of interest (mean hygiene score and proportion of dirty udders for each farm) and the previously-described herd-level independent variables. Any explanatory variable that was unconditionally associated with 1 or more of the outcomes of interest at p < 0.20 was then offered into a multivariable model investigating the relationship between the udder health or hygiene outcome and the herd-level predictor variables. Facility type was forced into these multivariable models, as it was the primary explanatory predictor of interest. A backward stepwise variable selection process was used, with the least significant variables being removed one by one until all remaining predictors had P ≤ 0.10.

The initial multivariable modelling approach aimed to investigate the conditional relationship between facility type and the eight outcomes of interest while controlling for different farm management practices, housing characteristics, milking procedures and mastitis control practices. This approach was abandoned in favor of reporting the univariate analysis results for all farms combined together (n = 21). … ??due to over parametrization and lack of statistical significance due to limited sample size/statistical power limitations. Instead, biologically plausible results of the ing is reported for predictors with group sizes of at least n = 5.

**Results**

1. **Description of study herds**

A total of 21 herds were enrolled: 5 used a bedded pack system (23.8%), one used a freestall bedded with sand (4.8%), 5 used a freestall bedded with shavings/sawdust (23.8%), and 10 used a tiestall bedded with shavings/sawdust (47.6%) (Supplemental Table S1). Two bedded pack farms bedded with shavings/sawdust and cultivated 2 times a day to promote aerobic composting, 1 farm bedded with straw and woodchips and cultivated 2 times a week, and 2 bedded pack farms bedded mainly with straw, adding woodchips as needed, and did not till the pack at all. The predominant breeds for each farm were Holstein (n = 8), 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 rolling herd average for the farms was 14,037 (14,163; 9,000-21,204) pounds. Nineteen of the 21 farms tested with DHIA monthly while their cows were in milk, with one farm testing 5-8 times/year and one testing every other month. 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.

1. **Comparison of bulk tank milk aerobic culture data**

None of the 21 bulk tank milk samples were positive for *Strep. agalactiae* or *Mycoplasma* spp. Results of aerobic culture are presented in Table 1. Sixteen of the 21 bulk tank milk 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 (mean; range) cfu/mL of 50 (70; 15-320) when present. The median (mean; range) *Staph.* spp. count found in the 21 bulk tank milk samples was 65 (96; 0-665) cfu/mL, while the median non-*ag. Strep.* count was 45 (156; 10-1,250) cfu/mL.

1. **Comparison of bulk tank milk udder health measures by facility type**

The mean (SD; range) raw somatic cell count for the 21 bulk tank milk samples collected was 144,286 cells/mL (53,934; 54,000-250,000) (Table 2). For the 19 herds with available DHIA test-day data, the mean percent of cows with newly-elevated SCC was 5.7 (3.7; 0-12.3), the mean percent of cows with chronically-elevated SCC was 13.6 (5.8; 2.9-23.1), and the mean percent of cows with elevated SCC was 25 (7.8; 8.6-36.9). For the 18 herds with available data, the mean standardized 150-day milk was 50 pounds (10.1; 33.5-68). For the 20 herds with available cow-level test data, the average LS was 2.44 (0.42; 1.7-3.3).

1. **Comparison of hygiene scores by facility type**

The overall mean (95% CI) of herd-level hygiene scores for all 21 farms was 2.32 (2.16-2.49). The mean (95% CI) hygiene score was 2.2 (1.91-2.44) for bedded pack farms (n = 5), 2.5 (2.24-2.76) for tiestall farms (n = 10), and 2.15 (1.93-2.37) for freestall farms (n = 6). A one-way Fisher’s ANOVA revealed that there was not a statistically significant difference in mean hygiene score of cows in a herd between any of the three facility types (F(2,18) = [2.307], p = 0.13).

The overall mean (95% CI) proportion of cows with dirty udders in a herd (udder hygiene score ≥ 3) was 40% (31-48%). The proportion of cows with dirty udders (95% CI) was 32% (18-46%) for bedded pack farms (n = 5), 49% (35-62%) for tiestall farms (n = 10), and 32% (20-44%) for freestall farms (n = 6). A one-way Fisher’s ANOVA revealed that there was not a statistically significant difference proportion of cows with dirty udders in a herd between any of the three facility types (F(2,18) = [2.1031], p = 0.15).

1. **Results of univariate analyses for udder health and hygiene outcomes**

Selected results of univariate analysis identifying factors unconditionally associated with udder health and hygiene outcomes at p < 0.20 are presented in Table 3.

The depth of bedding in stalls for freestall and tiestall herds was unconditionally associated with multiple udder health outcomes. As the depth of bedding in stalls increased, udder health measures improved (lower LS, BTM SCC, percent elSCC/neSCC). Similarly, herds where cows were on deep bedding had a lower BTM SCC compared to herds that had stalls with a smaller amount of bedding on top of a mattress or concrete.

Udder hygiene measures were also associated with numerous udder health outcomes. Higher mean hygiene scores and proportion of udders scored ≥ 3 were each associated with higher percentages of ceSCC, elSCC, and average LS. Additionally, a few specific management practices were also found to be unconditionally associated with udder health outcomes: consistent glove use was associated with a lower percentage of neSCC, clipping or flaming udders was associated with fewer ceSCC, and both parenteral supplementation of a vit. E/selenium product and use of an approved intramammary product at dry-off were associated with lower average LS.

Both udder hygiene outcomes were unconditionally associated with the same predictors, all of which were related to the depth of bedding for cows. For herds using a bedded pack, deeper bedding was associated with cleaner udders. For cows in tiestalls and freestalls, cleaner udders were associated increasing depth of bedding in stalls. For all herds, cows on deep bedding (bedded packs or deeply-bedded freestalls) had better udder hygiene than cows on a smaller amount of bedding on top of a mattress or concrete.

**Discussion**

**Presents results of our observational study exploring winter housing, mastitis risk,**

There were no differences in bacterial counts from bulk tank milk for *Staph.* species, coliforms, non-*ag Strep.,* or *Staph aureus* between the three facility types (Table 2). The absence of any difference in bulk tank milk bacterial counts between facility types suggests that milk from cows housed in bedded packs is not more likely to have higher amounts of these mastitis pathogens, despite cows being loose-housed on deeply-bedded organic materials.

1. **Bulk tank milk aerobic culture data by facility type**

Previous work describing bulk tank milk aerobic culture results has been limited to descriptive studies enrolling only composting bedded pack herds (2 studies), and only one has compared bacterial counts directly between conventional composting bedded packs and freestall barns (2 in minnesota? Maybe Kentucky as well). This is the first study the authors are aware of directly comparing bacterial counts of bulk tank milk between bedded packs and tiestall barns, and the first one to describe a population of exclusively organic dairies. In contrast to previous work, which evaluated milk culture results across different seasons (check this), the current study focused solely on sampling during the winter months, as this is the time of year organic farms are housing animals inside and not on pasture.

The *Staph.* spp. count for the five bedded pack farms included in this study (53 CFU/mL, 95% CI: 10-96) was similar to previous work describing bulk tank milk quality for composting bedded packs in Minnesota which also collected samples during the winter months. The six farms included in Lobeck et al. (2012) used mainly wood sawdust as a bedding source (with one using a wheat straw by‐product), which is similar to the current study (three of five bedded packs using a combination of woodchips/shavings and straw/hay, two using exclusively sawdust/shavings). The six farms included in Shane et al. (2010) bedded with a variety of “alternative” organic materials, including straw by-products, soybean stubble, and oat hulls. Lobeck et al. 2012 found a mean of 26.1 CFU (95% CI: 2-443) and Shane 2010 et al. found a range of 0-108 CFU/mL for *Staph.* spp. from BTM collected during the winter from six composting bedded pack farms.

Non-*ag* *Strep.* counts in BTM for bedded packs in the current study were much lower than those found from Minnesota composting bedded packs in the winter. Shane et al. 2010 reported a range of non-*ag Strep.* counts of 98-48,400 CFU/mL for six farms, and Lobeck et al. 2012 reported a mean of 911 CFU/mL (95% CI: 138-6,011). The mean non-*ag Strep.* counts for bedded pack farms included in the current study was 39 CFU/mL (95% CI: 17-61). Work from Barberg et al. (2007) describing milk quality on composting bedded packs in Minnesota noted that 6 of 12 farms sampled had “high” levels of non-*ag Strep*. The overall non-*ag Strep.* count for all 21 farms included in the current study (156 CFU/mL, 95% CI: 42-271) was much lower than that for the overall *Strep.* count for all three facility types studied in Lobeck et al. 2012 (445 CFU/mL, 95% CI: 116-1704). As the overall non-*ag Strep*. counts for all farm types included in the Minnesota studies are higher than that found for all 21 farms in the current study, better milking and bedding hygiene amongst herds included in this study may best explain this difference in BTM pathogen profiles (Jayarao and Wolfgang, 2003).

All farms included in the current study had low levels of coliforms in bulk tank milk (1.2 CFU/mL, 95% CI: 0.3-2.1), indicating excellent hygiene practices at milking time (Jayarao and Wolfgang, 2003). Bedded pack farms in the current study had very low coliform counts in BTM (1 CFU/mL, 95% CI: 0-3), similar to those found for three compost bedded pack farms in a Brazilian study (2.8 CFU/mL; Fávero et. al, 2015). However, these low coliform counts are in contrast with previous work describing BTM quality for this kind of facility in the United States. Coliform counts for bedded packs in Minnesota in the winter ranged from 15-1,128 CFU/mL (Shane et al. 2010), and had a mean of 63.7 CFU/mL (95% CI: 6-735) for the six bedded packs included in Lobeck et al. 2012. However, direct comparison of coliform counts between studies may be potentially problematic due to variation in duration of freezer storage (Schukken et al., 1989). Although sampled during summer months, Barberg et al. 2007 found that 5 of 12 bedded packs sampled 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.”

Although not statistically significant, the mean *Staph. aureus* count for bedded pack farms was numerically smaller than that for tiestalls and freestalls. The bedded pack category had the highest proportion of farms with BTM negative for *Staph. aureus* (60% for BP, vs. 33.3% for both TS and FS). As *Staph. aureus* is categorized as mainly a contagious mastitis pathogen, on-farm prevalence more likely is a function of milking procedure hygiene and other management practices associated with limiting cow-to-cow transmission than environmental factors (i.e., bedding material type and hygiene; Jayarao and Wolfgang, 2003; although, see Leuenberger et. al, 2019). The lower prevalence of *Staph. aureus* on bedded pack farms in the current study may be a function of confounding. Producers using this less-traditional housing type (bedded packs) are inherently/by definition more innovative/open to newer technologies. More progressive dairy farmers (younger in age, had children with higher education levels, more likely to invest in the future for their farm) were more likely to have a lower bulk tank milk somatic cell count (Barkema et. al, 1999). An extension of this relationship is that more progressive dairy farmers using a bedded pack may be more likely to have implemented stricter control programs for contagious mastitis pathogens such as *Staph. aureus*. Prevalence of *Staph. aureus* was similar between the five VT bedded pack farms in the current study (9 CFU/mL, 95% CI: 0-21) and the six bedded packs 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 bedded packs 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 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 (43.6 CFU/mL, 95% CI 14-73; vs. 17.3 CFU/mL, 95% CI: 3.3-91.2).

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 the major contagious mastitis pathogens *Staph. aureus* and *Strep. agalactiae* (Godkin and Leslie, 1993). Although the bulk tank sampling strategy differs slightly between the current study and previous work… describe other studies using multiple samples. However, analysis of BTM also comes with limitations. Bacterial groups traditionally considered to be primarily environmental in origin (non-*ag. Strep.,* non-*aureus Staph.,* coliforms), may enter into BTM from cows with an intramammary infection, but also may originate from non-specific contamination (teats and udder skin, bedding, manure, or other environmental sources). Additionally, a single bulk tank sample does not provide information about milk quality and mastitis at the individual cow level, nor does it give any insight into the long-term, consistent patterns of that farm’s milk quality as repeated BTM samplings may do (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.

1. **Bulk tank milk udder health and hygiene measures by facility type**

One theme emerging from this work is that farms with deeper bedding had more favorable udder hygiene. Increased bedding depth measures also tended to be associated with lower mean udder hygiene scores. Cows housed on some type of deep bedding (deeply-bedded stalls or bedded pack) had lower average hygiene scores than those housed on stalls with a smaller amount of bedding over a mattress or concrete surface (p = 0.06). This agrees with previous work in freestall barns, from Cook et al 2016 (prevalence of dirty udders was 13% lower for farms using deep bedding verses stalls with mats), de Vries et al 2015 (deep-bedding vs. mat/mattress reduced 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). However, limited work studying the effect of bedding depth in tiestalls found no difference between leg, flank, and udder hygiene of cows between deeply-bedded stalls (14 cm) and the control treatment (2-3 cm; Wolfe et al, 2018). For freestall and tiestall barns included in this study, there was a negative correlation between the depth of bedding in a stall and average udder hygiene score (p = 0.07). de Vries et al (2015) found no relationship between prevalence of dirty hindquarters and three different freestall bedding height groups (<0.56 cm, 0.56–1.75 cm, >1.75 cm), but there is much more opportunity for research to explore the relationship between stall bedding height and cow hygiene, especially in tiestall systems. Although sample size for the bedded pack group in this study was limited (n = 5), we found a negative correlation between depth of bedded pack and average udder hygiene score (p < 0.01), a relationship which has previously not been studied to the author’s knowledge.

Multiple measures of udder health in this work were related to udder hygiene, in accordance with the well-supported tenet that better cow hygiene is associated with better milk quality. Farms with lower mean udder hygiene scores and lower proportion of dirty udders tended to have lower % ceSCC (p = 0.05 and p = 0.05), lower % elSCC (p = 0.09 and p = 0.13, respectively), and lower average LS (p = 0.11 and p = 0.12, respectively). Additionally, farms that clipped or flamed udders on a regular basis tended to have fewer % ceSCC (p = 0.16). This association between an animal’s hygiene and udder health is been well-documented, both at the cow level (for IMI presence: de Pinho 2012; for LS/SCC: Reneau, 2005, Dohmen, 2010, and Sant’Anna, 2011; for both LS and IMI: Schreiner and Ruegg, 2003) and at the herd-level (BTSCC: Barkema 1998; new IMI rate: Cook, 2002, average herd SCC, incidence clinical mastitis, and % new high SCC: Dohmen, 2010). Of particular interest to the current study, Fávero et al (2015) found that in a study carried out on 3 bedded pack farms in Brazil, the odds of an incident case of subclinical mastitis (SCC ≥ 200,000 cells/mL), and of a cow having a prevalent case of subclinical mastitis, increased 32% and 16% for each one-unit increase in leg cleanliness score, respectively. Curiously, although leg cleanliness score was associated with both mastitis indices, udder hygiene score was not found to have a significant association.

A third predominant theme to emerge from the univariate regression results in this work is that farms using deeper bedding had better milk quality outcomes. As stall bedding depth in tiestalls and freestalls increased, farms showed a tendency toward having a lower bulk tank somatic cell count (p = 0.17), lower % neSCC (p =0.02), lower % elSCC (p = 0.01), and a lower average LS (p = 0.10). Additionally, farms that used deep-bedded stalls or a bedded pack tended to have a lower BTSCC than those using stalls with mattresses or a concrete base (p = 0.14). Although there is an established recommendation of 15 cm for deep bedding of freestalls (Bickert, 2000; Cook, 2002), but this depth appears to be based on optimizing cow comfort in deep-bedded freestalls with no relevance for udder hygiene or health considerations. There is very limited work exploring ideal bedding material depth for tiestall barns (Tucker, 2004; Tucker, 2009), and this is again solely focused on the important consideration of cow comfort. As is the experience of the authors, and is stated elsewhere 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. It is likely that the effect seen in the current work of deeper bedding and better udder health outcomes is mediated through the preestablished effects of (1) deeper bedding leading to improved hygiene, and (2) improved hygiene resulting in better udder health. Therefore, the opportunity exists for research exploring optimal stall bedding depths of different organic materials in tiestall barns focusing on mastitis and udder health outcomes. How deep do we need to bed tiestalls to keep moisture, and therefore bacteria, away from the udder as much as possible? However, it may be that recommending a particular depth of bedding to use for different types of organic material may not prove feasible, as the ideal amount would vary with many factors particular to a producer’s barn and bedding source (type of stall surface, presence/type of stall mat used, type of organic material, particle size, compressibility, percent dry matter, etc.).

We found no difference in udder hygiene measures (proportion of udders scored ≥3, average udder hygiene score) between the three facility types included in the study (tiestalls, freestalls, bedded packs). This finding is in accordance with previous work, which found that cow hygiene on bedded pack systems was comparable to traditional facility types primarily in Minnesota, Kentucky, and Brazil (Andrade, 2022; Eckelamp, 2016a; Eckelkamp, 2016b; Black, 2013; Costa, 2018; Shane, 2010; Barberg, 2007; Lobeck, 2011). In comparison to some previous work comparing hygiene of dairy cows between different facility types, only udder hygiene was measured in the current study. Other researchers studying this issue have taken a more thorough/comprehensive evaluation of dairy cow hygiene, including having separate scores for different body regions, including lower legs, upper legs, udder, flank, and belly. Cook (2002) has pointed out the challenges of comparing dairy cattle hygiene between different facility types; namely, cows in freestall barns tend to have dirtier lower legs from walking through alleyways, whereas tiestall cows are likely to have dirtier upper legs and flanks, and loose-housed cows may have a different pattern of manure deposition on their bodies depending on the cleanliness of their lying surface. Despite the limitation of only observing udder hygiene (and the limited sample size of farms in each group), we feel confident that udder hygiene of cows housed on bedded pack systems in the current study was comparable to the more conventional housing systems for dairies in the Northeast. In fact, the farm with the lowest mean average udder hygiene score was a bedded pack farm, and all bedded packs had an average udder hygiene score of less than 2.5.

STD 150-day milk production did not differ between facility type in the current study (BP 46.9 lbs., TS 49.4 lbs., FS 53.0 lbs.). This aligns with previous studies comparing milk production of cows housed on bedded packs vs. in freestall barns (Eckelkamp et al. 2016, Sand bedded…; Lobeck et. al, 2011; Costa et. al, 2018), which found no significant differences in production metrics between bedded packs and more traditional housing systems. Different studies have reported varying production metrics for cows housed on bedded packs (kg/cow/day, fat-corrected milk/cow/day, average L/cow/day, ME-305, rolling herd average, energy-corrected milk), preventing direct comparison of milk production between the bedded packs in the current study and previous work. Furthermore, there are many variables determining milk production per cow (nutrition, breed, seasonality, DIM, etc.), 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, the “results in the literature indicate that high levels of milk production are possible in CBP,” and, as bedded packs potentially improve cow comfort, we may even expect greater milk production than in more traditional housing systems.

Udder health outcomes included in the current study, including % cows with elSCC, % cows with ceSCC, % cows with neSCC, BTSCC, and average LS did not differ significantly between facility types. Although the metrics studied have been varied, overall previous research has also found that udder health and milk quality measures on bedded pack farms are similar to that found on farms traditional using more traditional facility types (Eckelkamp, 2016a; Eckelkamp, 2016b; Black, 2013; Shane, 2010; Barberg, 2007; Lobeck, 2011; Heins, 2019). Specifically, subclinical mastitis prevalence levels did not differ between compost bedded packs and two types of freestall 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 compost bedded packs, cross-ventilated freestalls, and naturally-vented freestalls (Lobeck et. al, 2011). Additionally, Eckelkamp et. al 2016 (Sand bedded…) found no significant difference in subclinical mastitis prevalence in low-SCC CBP vs. sand-bedded freestalls in Kentucky (21.8 and 19.4%, respectively). Barberg et. al 2007b (Performance) found a subclinical mastitis prevalence of 27.7% for 12 CBP farms in Minnesota, which may be more representative of the general population of bedded pack farms in the state, as there were no inclusion criteria around maintaining a low SCC previous to the start of the study. Subclinical mastitis prevalence in the current study was comparable to this previous work, with a prevalence of 26% for farms using a bedded pack and 23.7% for freestall barns. In Brazil, Fávero et. al (2015) found much higher a prevalence of subclinical mastitis (43.8%) and percent new infections (20.9%) for three bedded pack farms than the current study (7 and 26% respectively for the three bedded packs with DHIA data).

With careful management of bedding material and excellent milking hygiene practices, the current study shows that both static and aerobically composting bedded pack barns are capable of achieving excellent milk quality. Three of the five bedded pack farms achieved a BTSCC of below 99,000 cells/mL, and the remaining two were below 200,000 cells/mL. Although historically static bedded packs made of organic material have been considered to be milk quality nightmares, the lowest BTSCC in the study (54,000 cells/mL) was achieved by a bedded pack farm using woodchips and straw that was not actively managing the pack to compost.

As udder health and hygiene for bedded packs compared to tiestalls and freestalls included in the current study, we feel that bedded pack systems can be considered a viable loose-housing option for the winter (in the Northeast? In Vermont?). These systems have a number of advantages for producers considering updating their facilities, including a smaller initial investment when compared to a new freestall or tiestall barn (Bewley et al., 2012), although the cost year-over-year for bedding is substantial. Bedded pack systems have considerable advantages from a welfare perspective, as it provides a housing option that doesn’t restrict animal movement, which is an issue of growing concern for both producers and the general public (Barkema et al., 2015). Additionally, bedded packs are designed for cow comfort (Barberg and Endres 2007, Bewley et al. 2017), and prevalence of lameness, foot, and leg injuries in bedded pack systems have been found to be significantly less than tiestalls and freestalls (Barberg and Endres, 2007, Lobeck et al. 2011, Burgstaller et al. 2016). The relationship between decreased milk production and lameness is well documented (Warnick et al. 2001, Green et al. 2002), so there is an additional financial incentive for dairy producers to decrease the prevalence of lameness in their herd. Lastly, manure management and environmental stewardship is a top concern for both dairy producers and the general public (Holly et al., 2018). Bedded systems may provide some advantages over other systems by decreasing the amount of liquid manure storage needed and making the manure drier before it is spread on fields, which may pose less of a risk for run-off into waterways. With no downsides due to hygiene or udder health concerns when properly managed on farms with excellent milking hygiene practices already in place, bedded packs may be an especially good option for small, pasture-based farms in the Northeast both now and in the future.

**Conclusion**

The current study is to the authors’ knowledge the first direct comparison of bedded packs to both tiestalls and freestall of similar size and management styles, for a population of entirely small to midsize organic dairy farms. It is the first publication describing udder health and hygiene on bedded pack systems in the Northeast, which is significant as the performance of a bedded pack system can be greatly influenced by climatic factors (Leso reference). Additionally, recent previous work has focused on describing bedded packs that are actively managed/cultivated for aerobic composting (Leso review). Leso et al. contrasted composting bedded packs (CBP) managed with daily cultivation, with conventional bedded packs, such as straw yards, noting the reduced cow cleanliness and increased risk of mastitis associated with the latter. While bedded pack systems are not common for housing lactating cows in Vermont, both composting and static systems are used (ref tucker’s paper). This infrequent use of bedded packs in our state created a challenge for enrolling 10 herds using this kind of system in this observational study. Despite this limitation, by including bedded pack farms managed in a variety of ways, the current work sheds light on a broader spectrum of options used within this loose-housing system utilizing organic bedding material.

Overall, farms with more deeply-bedded cows had improved bulk tank milk quality, as well as better udder health metrics and udder hygiene scores. Unsurprisingly, better udder hygiene was associated with improved udder health measures. Bedded pack systems did not differ significantly in their milk quality, udder health, or hygiene measures when compared to the more commonly used winter housing systems for organic cows in the state. Bedded packs can therefore be considered as a viable option for pasture-based herds looking for a more affordable loose-housing system.

**References**

**Figures**

**Tables**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table 1. Bulk tank milk aerobic culture outcomes by facility type for 21 Vermont organic dairy herds | | | | | | | | |
|  |  | Count |  |  | Mean | 95% CI | Range | Kruskal-Wallis χ2 |
| *Staph.* spp. (CFU/mL) | | 21 |  |  | 95.5 | 36-155 | 0-665 | 0.9 (df = 2, p = 0.62) |
|  | Bedded pack | 5 |  |  | 53 | 10-96 | 0-130 |  |
|  | Tiestall | 10 |  |  | 134.5 | 14-255 | 15-665 |  |
|  | Freestall | 6 |  |  | 65.9 | 28-104 | 5-125 |  |
|  |  |  |  |  |  |  |  |  |
| Non-*ag. Strep.* (CFU/mL) | | 21 |  |  | 156.4 | 42-271 | 10-1250 | 4.6 (df = 2, p = 0.10) |
|  | Bedded pack | 5 |  |  | 39 | 17-61 | 10-80 |  |
|  | Tiestall | 10 |  |  | 255.5 | 30-481 | 20-1250 |  |
|  | Freestall | 6 |  |  | 89.2 | 11-167 | 25-260 |  |
|  |  |  | Neg. for *S. aureus* (no. farms) | Pos. for *S. aureus* (no. farms) |  |  |  |  |
| *Staph. aureus* (CFU/mL) | | 21 | 8 | 13 | 43.6 | 14-73 | 0-320 | 3.4 (df = 2, p = 0.19) |
|  | Bedded pack | 5 | 3 | 2 | 9 | 0-21 | 0-30 |  |
|  | Tiestall | 10 | 3 | 7 | 63 | 5-121 | 0-320 |  |
|  | Freestall | 6 | 2 | 4 | 40 | 10-70 | 0-100 |  |
|  |  |  |  |  |  |  |  |  |
|  |  |  | Neg. for coliforms(no. farms) | 5 CFU/mL coliforms  (no. farms) | Mean | 95% CI | Range |  |
| Coliforms (CFU/mL) | | 21 | 16 | 5 | 1.2 | 0.3-2.1 | 0-5 | 0.4 (df = 2, p = 0.82) |
|  | Bedded pack | 5 | 4 | 1 | 1 | 0-3 | 0-5 |  |
|  | Tiestall | 10 | 7 | 3 | 1.5 | 0.003-3 | 0-5 |  |
|  | Freestall | 6 | 5 | 1 | 0.8 | 0-2.5 | 0-5 |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Table 2. Udder health and production outcomes by facility type for 21 Vermont organic dairy herds. | | | | | | |
|  |  |  | Continuous outcomes | | |  |
| Outcome |  | Sample size | Mean | 95% CI | Range |  |
| Bulk tank milk somatic cell count (cells/mL) | | 21 | 144,286 | 121,218-167,353 | 54,000-250,000 | One-way Fisher’s ANOVA: (F(2,18) = [2.137], p = 0.15) |
|  | Bedded pack | 5 | 107,600 | 70,275-144,925 | 54,000-160,000 |  |
|  | Tiestall | 10 | 146,400 | 114,136-178,664 | 97,000-250,000 |  |
|  | Freestall | 6 | 171,333 | 126,965-215,702 | 98,000-250,000 |  |
|  |  |  |  |  |  |  |
| Percent of cows with newly-elevated SCC (%) | | 19 | 5.7 | 4.2-7.3 | 0-12.3 | One-way Fisher’s ANOVA: (F(2,16) = [0.2128], p = 0.81) |
|  | Bedded pack1 | 3 | 7.0 | 2.8-11.2 | 2.9-12.3 |  |
|  | Tiestall | 10 | 5.4 | 3.0-7.8 | 0-10.5 |  |
|  | Freestall | 6 | 5.6 | 3.0-8.3 | 0-9.8 |  |
|  |  |  |  |  |  |  |
| Percent of cows with chronically-elevated SCC (%) | | 19 | 13.6 | 11.2-16.1 | 2.9-23.1 | One-way Fisher’s ANOVA: (F(2,16) = [0.3138], p = 0.74) |
|  | Bedded pack1 | 3 | 14.5 | 5.4-23.7 | 2.9-23.1 |  |
|  | Tiestall | 10 | 14.3 | 11.9-16.7 | 7.8-20.8 |  |
|  | Freestall | 6 | 12.0 | 6.7-17.3 | 5.7-23.1 |  |
|  |  |  |  |  |  |  |
| Percent of cows with SCC ≥ 200,000 cells/mL on current test date (%) | | 19 | 24.9 | 21.6-28.3 | 8.6-36.9 | One-way Fisher’s ANOVA: (F(2,16) = [0.1017], p = 0.90) |
|  | Bedded pack1 | 3 | 26.0 | 12.6-39.3 | 8.6-36.9 |  |
|  | Tiestall | 10 | 25.4 | 22.1-28.6 | 17.6-32.8 |  |
|  | Freestall | 6 | 23.7 | 16.9-30.5 | 11.6-36.5 |  |
|  |  |  |  |  |  |  |
| Standardized 150-day milk (pounds) | | 18 | 50.0 | 45.7-54.3 | 33.5-68.0 | One-way Fisher’s ANOVA: (F(2,15) = [0.4404], p = 0.65) |
|  | Bedded pack2 | 4 | 46.9 | 39.8-53.9 | 38.5-56.3 |  |
|  | Tiestall2 | 8 | 49.4 | 43.1-55.7 | 33.5-68.0 |  |
|  | Freestall | 6 | 53.0 | 43.5-62.5 | 38.7-67.7 |  |
|  |  |  |  |  |  |  |
| Avg. linear score of cows on farm | | 20 | 2.44 | 2.26-2.62 | 1.7-3.3 | One-way Welch’s ANOVA: (F(2,5.7) = [0.02891], p = 0.97) |
|  | Bedded pack2 | 4 | 2.38 | 1.84-2.91 | 1.7-3.1 |  |
|  | Tiestall | 10 | 2.45 | 2.31-2.59 | 2.2-2.8 |  |
|  | Freestall | 6 | 2.5 | 2.00-2.93 | 1.9-3.3 |  |
|  |  |  |  |  |  |  |
| 1 DHIA data not available for 2 farms | | | | | |  |
| 2 DHIA data not available for 1 farm | | | | | |  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Table 3. Selected results of univariate analysis identifying factors unconditionally associated with udder health and hygiene outcomes at p < 0.20 for 21 Vermont organic dairy herds | | | | |
| Parameter | | Group (sample size) | Coefficient estimate (SE) | *P-*value |
| Bulk tank milk somatic cell count (cells/mL) | |  |  |  |
|  | Lying surface | Mattress or concrete (n = 13) | 36,000 (23,454) | 0.14 |
|  |  | Deep bedding (n = 8) | Ref. | Ref. |
|  | Depth of bedding in stalls (cm)1 | Tiestalls and freestalls (n = 15) | -5,797 (3,970) | 0.17 |
| Percent cows with newly-elevated SCC (%)2 | |  |  |  |
|  | Glove use | Inconsistent glove use while milking (n = 9) | 2.83% (1.7) | 0.11 |
|  |  | All milkers consistently use gloves (n = 9) | Ref. | Ref. |
|  | Depth of bedding in stalls (cm)1 | Tiestalls and freestalls (n = 15) | -0.62% (0.24) | 0.02 |
| Percent cows with chronically-elevated SCC (%)2 | |  |  |  |
|  | Herds that clip or flame udders one or more times per lactation | Yes (n = 5) | -4.31% (2.9) | 0.16 |
|  |  | No (n = 14) | Ref. | Ref. |
|  | Proportion of cows with udder hygiene scores ≥ 3 | All herds with available test data (n = 19) | 12.7% (6) | 0.05 |
|  | Average hygiene score | All herds with available test data (n = 19) | 6.39% (3.1) | 0.05 |
| Percent cows with SCC ≥ 200,000 cells/mL on current test date (%)2 | |  |  |  |
|  | Depth of bedding in stalls (cm)1 | Tiestalls and freestalls (n = 15) | -1.2% (0.42) | 0.01 |
|  | Proportion of cows with udder hygiene scores ≥ 3 | All herds with available test data (n = 19) | 13.6% (8.5) | 0.13 |
|  | Average hygiene score | All herds with available test data (n = 19) | 7.7% (4.3) | 0.09 |
| Average linear score of cows on farm3 | |  |  |  |
|  | Herds that regularly do parenteral supplementation of dry cows with vit. E and selenium | Regular or occasional supplementation (n = 9) | -0.27 (0.18) | 0.15 |
|  |  | No parenteral supplementation (n = 11) | Ref. | Ref. |
|  | Herds that use any sort of approved organic intramammary product at dry-off | Yes (n = 5) | -0.29 (0.21) | 0.18 |
|  |  | No (n = 15) | Ref. | Ref. |
|  | Depth of bedding in stalls (cm)1 | Tiestalls and freestalls (n = 15) | -0.05 (0.03) | 0.1 |
|  | Proportion of cows with udder hygiene scores ≥ 3 | All herds with available test data (n = 20) | 0.75 (0.45) | 0.12 |
|  | Average hygiene score | All herds with available test data (n = 20) | 0.39 (0.23) | 0.11 |
| Proportion of cows with udder hygiene scores ≥ 3 | |  |  |  |
|  | Depth of bedded pack (m) | Bedded pack herds (n = 5) | -0.5 (0.06) | <0.01 |
|  | Lying surface | Mattress or concrete (n = 13) | 0.17 (0.08) | 0.06 |
|  |  | Deep bedding (n = 8) | Ref. | Ref. |
|  | Depth of bedding in stalls (cm)1 | Tiestalls and freestalls (n = 15) | -0.02 (0.02) | 0.13 |
| Average hygiene score | |  |  |  |
|  | Depth of bedded pack (m) | Bedded pack herds (n = 5) | -0.96 (0.15) | < 0.01 |
|  | Lying surface | Mattress or concrete (n = 13) | 0.33 (0.16) | 0.06 |
|  |  | Deep bedding (n = 8) | Ref. | Ref. |
|  | Depth of bedding in stalls (cm)1 | Tiestalls and freestalls (n = 15) | -0.06 (0.03) | 0.07 |
| 1 Stall bedding depth for freestalls and tiestalls bedded with wood shavings or sawdust | | | | |
| 2 DHIA data available for n = 19 herds. One herd included in average linear score analyses is seasonal and had no recent test data. | | | | |
| 3 DHIA data available for n = 20 herds. | | | | |

**Funding Sources**

This project was funded by the USDA Hatch Multistate Project VT-H02413MS.

**Acknowledgements**

We acknowledge the University of Vermont Genomics Core Facility for helping with MLST sequencing. We also thank all farms and farm workers who agreed to participate in this study. Finally, we thank all lab personnel who helped with sample collection and sample processing.

**Conflict of Interest**

The authors declare no conflicts of interest.

**Author Contributions**

Ashma: Lab work, data collection & analysis, manuscript writing

Chrsitine: Field sample collection

Ariela: Field sample collection, Lab work

Robert: Sample collection, lab work, data collection & analysis

Amanda: sample collection, lab work

John: Study design, field sample collection, lab work, data collection & analysis, manuscript preparation

***Comparison of bulk tank milk udder health measures, aerobic culture data, and hygiene scores by facility type***

****

**Figure 1.** Bulk tank milk somatic cell count by facility type. The central line of each box represents the median, while the upper and lower lines represent the upper (75th) and lower (25th) quartiles, respectively. The upper whisker represents the largest observation less than or equal to the 75th quartile plus 1.5 times the interquartile range, while the lower whisker represents the smallest observation greater than or equal to the 25th quartile minus 1.5 times the interquartile range. A one-way ANOVA revealed that there was not a statistically significant difference in bulk tank somatic cell count between any of the three facility types (F(2,18) = [2.137], p = 0.15).

**OR**

**Figure 1.** Bulk tank milk somatic cell count by facility type. Boxplot showing the 25th, 50th (median), and 75th percentiles of bulk tank raw somatic cell count data from 21 herds. The upper whisker represents the largest observation less than or equal to the 75th quartile plus 1.5 times the interquartile range, while the lower whisker represents the smallest observation greater than or equal to the 25th quartile minus 1.5 times the interquartile range. A one-way Fisher’s ANOVA revealed that there was not a statistically significant difference in bulk tank somatic cell count between any of the three facility types (F(2,18) = [2.137], p = 0.14).

****

**Figure 2.** Proportion of cows in a herd with a newly-elevated SCC by facility type, which was defined as the linear score changing from <4.0 to ≥4.0 in the last 2 tests. Boxplots showing the 25th, 50th (median), and 75th percentiles of DHIA data available from 19 herds. The upper whisker represents the largest observation less than or equal to the 75th quartile plus 1.5 times the interquartile range, while the lower whisker represents the smallest observation greater than or equal to the 25th quartile minus 1.5 times the interquartile range. Outliers are points located beyond the upper and lower whiskers. A one-way Fisher’s ANOVA revealed that there was not a statistically significant difference in proportion of cows with a newly-elevated SCC between any of the three facility types (F(2,16) = [0.2128], p = 0.81).



**Figure 3.** Proportion of cows in a herd with a chronically-elevated SCC by facility type, which was defined as a linear score ≥4.0 on the last 2 tests. Boxplots showing the 25th, 50th (median), and 75th percentiles of DHIA data available from 19 herds. The upper whisker represents the largest observation less than or equal to the 75th quartile plus 1.5 times the interquartile range, while the lower whisker represents the smallest observation greater than or equal to the 25th quartile minus 1.5 times the interquartile range. A one-way Fisher’s ANOVA revealed that there was not a statistically significant difference in proportion of cows with a chronically-elevated SCC between any of the three facility types (F(2,16) = [0.3138], p = 0.74).



**Figure 4.** Proportion of cows in a herd with elevated SCC by facility type, which was defined as having a linear score ≥4.0 on most recent test day. Boxplots showing the 25th, 50th (median), and 75th percentiles of DHIA data available from 19 herds. The upper whisker represents the largest observation less than or equal to the 75th quartile plus 1.5 times the interquartile range, while the lower whisker represents the smallest observation greater than or equal to the 25th quartile minus 1.5 times the interquartile range. Outliers are points located beyond the upper and lower whiskers. A one-way Fisher’s ANOVA revealed that there was not a statistically significant difference in proportion of cows with an elevated SCC between any of the three facility types (F(2,16) = [0.1017], p = 0.90).



**Figure 5.** Standardized 150 Day Milk by facility type. Boxplots showing the 25th, 50th (median), and 75th percentiles of DHIA data available from 18 herds. The upper whisker represents the largest observation less than or equal to the 75th quartile plus 1.5 times the interquartile range, while the lower whisker represents the smallest observation greater than or equal to the 25th quartile minus 1.5 times the interquartile range. Outliers are points located beyond the upper and lower whiskers. A one-way Fisher’s ANOVA revealed that there was not a statistically significant difference in Standardized 150 Day Milk between any of the three facility types (F(2,15) = [0.4404], p = 0.65).



*The variances are not homogenous for this analysis…. Violates an assumption of the regular ANOVA; can’t do Kruskal-Wallis with unequal variance either; so did Welch’s ANOVA (normally distributed, but unequal variances)*

**Figure 6.** Average linear score of cows in a herd by facility type. Boxplots showing the 25th, 50th (median), and 75th percentiles of DHIA data available from 20 herds. The upper whisker represents the largest observation less than or equal to the 75th quartile plus 1.5 times the interquartile range, while the lower whisker represents the smallest observation greater than or equal to the 25th quartile minus 1.5 times the interquartile range. A Welch’s ANOVA (unequal variances) revealed that there was not a statistically significant difference in average linear score of cows in a herd between any of the three facility types (F(2,5.7) = [0.02891], p = 0.97).



**Figure 7.** Mean hygiene score of cows in a herd by facility type. Boxplots showing the 25th, 50th (median), and 75th percentiles of data from 21 herds. The upper whisker represents the largest observation less than or equal to the 75th quartile plus 1.5 times the interquartile range, while the lower whisker represents the smallest observation greater than or equal to the 25th quartile minus 1.5 times the interquartile range. A one-way Fisher’s ANOVA revealed that there was not a statistically significant difference in mean hygiene score of cows in a herd between any of the three facility types (F(2,18) = [2.307], p = 0.13).



**Figure 9.** Proportion of cows with dirty udders (udder hygiene score ≥ 3) in a herd by facility type. Boxplots showing the 25th, 50th (median), and 75th percentiles of data from 21 herds. The upper whisker represents the largest observation less than or equal to the 75th quartile plus 1.5 times the interquartile range, while the lower whisker represents the smallest observation greater than or equal to the 25th quartile minus 1.5 times the interquartile range. A one-way Fisher’s ANOVA revealed that there was not a statistically significant difference proportion of cows with dirty udders in a herd between any of the three facility types (F(2,18) = [2.1031], p = 0.15).