* Description of considerations for enrolling a herd
  + Dairy farms who responded to the survey (cite previous paper), indicated they met enrollment criteria AND were interested in further participation
    - Responded to survey in Winter 2018-2019
    - Responded at least "sorta" or "very" interested (no "not really"/"nope")
    - Testing with DHIA at least monthly
    - Herd size 35-120 lactating cows
  + Contacted if they were interested, met criteria, and in 1 of 4 categories of bedding/housing combination we were interested in
    - What we WANTED
      * 10 FREESTALL with SAND
      * 10 FREESTALL with WOOD
      * 10 BEDDED PACKS
      * 10 TIESTALL with WOOD
    - What we GOT
      * 1 FREESTALL with SAND
      * 5 FREESTALL with WOOD
      * 5 BEDDED PACKS
      * 10 TIESTALL with WOOD
* Description of administration of survey, sample collection
  + Collection of BTM and bedding
    - Agitate, dipper, etc.
    - Description of collecting used, unused bedding- grabs from different spots? Mixed them? Subsampled?
  + Survey- used KoboCollect, tablet
* Description of culture procedures at Minnesota for BTM and bedding, St. Albans for culture data from them?

***Bedding and BTM M and M from patel’s paper:***

Frozen bedding and BTM samples were shipped on ice to the Laboratory for Udder Health (University of Minnesota Veterinary Diagnostic Laboratory, St. Paul) for analysis. After thawing at room temperature, 50 cm3 of packed bedding material was weighed and measured into a new Whirl-Pak bag Nasco, Fort Atkinson, WI), 250 mL of sterile water was added, and the contents were mixed and left to stand for 10 min. Serial 10-fold dilutions of the samples were made using sterile water (Becton Dickinson and Company, Franklin Lakes, NJ). Sample dilutions were plated onto MacConkey agar (gram-negative bacteria selection) and colistin naladixic acid agar (gram-positive bacteria selection, Becton Dickinson and Company) plates and incubated overnight at 37°C. For the MacConkey plates, lactose fermenting (pink) colonies were counted as coliform bacteria and all other colonies were counted as non-coliform gram-negative bacteria. Colonies with a confluent appearance on MacConkey agar were identified to the genus level using a MALDI Biotyper (Bruker Daltonics, Billerica, MA), and colonies identified as Klebsiella spp. were counted and reported as a percentage of total coliform count. For colistin naladixic acid plates, colony morphology in conjunction with catalase reaction and Gram stain were used to differentiate colonies of Staphylococcus spp., SSLO, and Bacillus spp. Total bacteria count (TBC) and counts of Bacillus spp., coliforms, Klebsiella spp., non-coliform gram-negatives, Staphylococcus spp., and SSLO were recorded as colony-forming units per cubic centimeter of wet bedding. The minimum limit of detection was 25 cfu/cm3 (max count of 6,250,000 cfu/mL).

After thawing to room temperature, bulk tank milk and a 10-fold dilution of the 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 d at 37°C. Lactose fermenting (pink) colonies on MacConkey medium were counted and reported as coliform bacteria. All β-hemolytic colonies on Focus medium were counted and identified to the species level using a MALDI Biotyper, as these colonies were suspect for *Streptococcus agalactiae*. All colonies on Focus medium that were not identified as *Strep. agalactiae* were counted and recorded as SSLO. β-Hemolytic colonies on Factor medium were counted and identified to the species level using a MALDI Biotyper, and those with a confidence score ≥2.0 for *Staph. aureus* were counted and reported as such. Non-hemolytic colonies of Staphylococcus spp. (based on colony morphology, catalase reaction, or Gram stain) were counted and reported as NAS. For Mycoplasma spp., 0.1 mL of BTM was swabbed across the entire surface of a Mycoplasma agar plate and incubated for 7 d in a 7% CO2 incubator at 37°C. Plates were examined for Mycoplasma spp., and colonies were counted by a trained microbiology technician. For each BTM sample, total counts of coliforms, NAS, SSLO, *Staph. aureus*, *Strep. agalactiae*, and Mycoplasma spp. were recorded as colony-forming units per milliliter of milk. The minimum limit of detection for the BTM culture protocol was 5 cfu/mL (max count of 62,500 cfu/mL).

For the beddings, we use MacConkey and CNA agar.

Bulk Tanks use Factor (gram positive selective), Focus (Strep selective; FKA MKTK) and MacConkey

* Considerations for dealing with 40 herd bedding data
  + Cut points from culture data (e.g., less than 10 CFU; max count of 6,250,000)
    - Make them categorical?
  + Zeroes in bedding culture data
    - Make difficult to just log transform bedding bacteria counts

***Random notes from meeting with John re: what this study could cover/purpose of manuscript***

* A descriptive study can serve for other researchers to help with their study design- this study can describe variability present in various predictors (info gathered in survey) for other researchers to use in power calculations for studies of farms like this (small, organic dairy farms in the NE)
  + A descriptive pilot study
  + How much are **static** bedded pack systems described in the literature? Here we show farms like this **CAN** successfully manage a system like this (still pretty “good” hygiene- define “good”- good milk quality; BW, SW, CF kindof)
* Notes on boxplots for manuscript
  + ~~Tighten up jitter on points~~
  + ~~Get rid of background grid (vertical lines, anyhow)~~
* Since sample size so limited, merged all 21 herds to make 6 models of udder health outcomes (facility type forced into models, check effect of including it in model vs. not to try and gauge how much this influences outcomes)
  + This work can still describe milk quality on 21 organic dairy herds in VT
  + Or, predictors of milk quality on organic dairies in VT

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***Data analysis notes***

For Patel’s Objective 1, linear regression model:



***Description of steps taken in data management and statistical methods***

* Udder hygiene scores were summarized both as the mean udder hygiene scores and as the proportion of cows with dirty udders (udder hygiene score ≥3)
* Descriptive statistics were calculated to evaluate the distribution of data and data integrity and to identify missing data (means, variances, percentiles for numeric continuous variables, frequencies tabulations and percentages for categorical variables). Descriptive statistics (using predictors) were generated to describe general/overall herd characteristics (farm traits), lactating cow housing/facilities, lactating cow bedding management, milking/hygiene procedures/practices, mastitis control practices for all 21 herds included in the study. Also did descriptive statistics of **outcome measures** (overall for 21 herds, then by facility or bedding type too)
* All udder health outcome measures checked to make sure they were roughly normally distributed; visually assessed. Distribution of raw SCC, log2 transformed SCC, and log10 transformed SCC was assessed to check if it was normally distributed, but all were fairly normally distributed/log transformed data not “better”/raw SCC data not that “bad” so raw SCC was chosen for ease of interpretation
* 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, were discluded from further analysis (but listed in descriptive statistic tables)
* univariate analysis used to screen predictors, if unconditionally associated at a level of p<0.2 (using linear regression, single predictor for 6 UH outcomes which were numeric, continuous) were candidates for inclusion in multivariable model
* predictors that were completely correlated with one facility type (predictor of interest that will be forced into the model) were discluded from further analysis (but listed in descriptive statistic tables); binary categorical predictors with a category of less than 5 were unable to be combined and discluded from further analysis (but listed in descriptive statistic tables)
* pearson’s correlation coefficient was calculated for all numeric continuous predictors to check for high levels of correlation between predictor variables, and if found to be greater than 0.6 the predictor with a more highly significant relationship found in univariate analysis was eligible for inclusion in the multivariable model; chi-square, fisher’s exact tests (where appropriate) used to check for correlation between categorical variables (cut-off?); ANOVA used to check for correlation between numeric continuous variables and categorical variables (cut-off?)

***Comparison of Bulk Tank Udder Health Measures, Aerobic Culture Data, and Hygiene Scores by Facility Type***

* .. imported into R Statistical Programming Environment (R Core Team, 2022); R version 4.1.3 (One Push-Up) was released on 2022-03-10.
* R Core Team. 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
* notes re: ANOVA/boxplots for 8 outcomes (SCC, wLS, unLS, %New, %chron, %any, avgHyg, 34Hyg)… Checking the assumptions for ANOVA: were checked for (1) outliers, (2) normality using a Shapiro-Wilk test of normality for each group being compared (with significance at p = 0.05) and (3) homogeneity of variances (using Levene’s test and also looking at the residuals vs. fitted values plot). The Tukey method was used for adjusting p-values for multiple comparisons using the “TukeyHSD” function of the “stats” package in R (R Core Team, 2022).