

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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Key Words:	Mastitis, organic dairy cattle, bedded pack, milk quality

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The STROBE-Vet statement checklist.

	Item	STROBE-Vet recommendation	Page #
Title and Abstract	1	(a) Indicate that the study was an observational study and, if applicable, use a common study design term	2
		(b) Indicate why the study was conducted, the design, the results, the limitations, and the relevance of the findings	2-3
Background / rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	(a) State specific objectives, including any primary or secondary prespecified hypotheses or their absence	6
		(b) Ensure that the level of organization is clear for each objective and hypothesis	6
Study design	4	Present key elements of study design early in the paper	2
Setting	5	(a) Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-8
		(b) If applicable, include information at each level of organization	NA
Participants ^b	6	(a) Describe the eligibility criteria for the owners/managers and for the animals, at each relevant level of organization	7-8
		(b) Describe the sources and methods of selection for the owners/managers and for the animals, at each relevant level of organization	7-8
		(c) Describe the method of follow-up	8
		(d) For matched studies, describe matching criteria and the number of matched individuals per subject (e.g., number of controls per case)	NA
Variables	7	(a) Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. If applicable, give diagnostic criteria	9-12
		(b) Describe the level of organization at which each variable was measured	9
		(c) For hypothesis-driven studies, the putative causal-structure among variables should be described (a diagram is strongly encouraged)	4-5

Data sources / measurement	8*	(a) For each variable of interest, give sources of data and details of methods of assessment (measurement). If applicable, describe comparability of assessment methods	9-12
		among groups and over time	
		(b) If a questionnaire was used to collect data, describe its development, validation, and administration	9-10
		(c) Describe whether or not individuals involved in data collection were blinded, when applicable	NA
		(d) Describe any efforts to assess the accuracy of the data (including methods used for "data cleaning" in primary research, or methods used for validating secondary data)	13
Bias	9	Describe any efforts to address potential sources of bias due to confounding, selection, or information bias	32
Study size	10	(a) Describe how the study size was arrived at for each relevant level of organization	8
		(b) Describe how non-independence of measurements was incorporated into sample-size considerations, if applicable	NA
		(c) If a formal sample-size calculation was used, describe the parameters, assumptions, and methods that were used, including a justification for the effect size selected	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	14
Statistical methods	12	(a) Describe all statistical methods for each objective, at a level of detail sufficient for a knowledgeable reader to replicate the methods. Include a description of the approaches to variable selection, control of confounding, and methods used to control for non-independence of observations	13-15
		(b) Describe the rationale for examining subgroups and interactions and the methods used	NA
		(c) Explain how missing data were addressed	17-18
		(d) If applicable, describe the analytical approach to loss to follow-up, matching, complex sampling, and multiplicity of analyses	NA
		(e) Describe any methods used to assess the robustness of the analyses (e.g., sensitivity analyses or quantitative bias assessment)	NA
Participants	13*	(a) Report the numbers of owners/managers and animals at each stage of study and at each relevant level of organization - e.g., numbers eligible, included in the study, completing follow-up, and analyzed	7-9

		(b) Give reasons for non-participation at each stage and at each relevant level of organization	7-9
		(c) Consider use of a flow diagram and/or a diagram of the organizational structure	NA
Descriptive data	14*	(a) Give characteristics of study participants (e.g., demographic, clinical, social) and	16, 42-43,
on exposures and potential		information on exposures and potential confounders by group and level of organization, if applicable	table S1-S5
confounders		(b) Indicate number of participants with missing data for each variable of interest and at all relevant levels of organization	17-18
		(c) Summarize follow-up time (e.g., average and total amount), if appropriate to the study design	NA
Outcome data	15*	(a) Report outcomes as appropriate for the study design and summarize at all relevant levels of organization	17-21, 42-48
		(b) For proportions and rates, report the numerator and denominator	NA
		(c) For continuous outcomes, report the number of observations and a measure of variability	17, 43
Main results	16	(a) Give unadjusted estimates and, if applicable, adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders and interactions were adjusted. Report all relevant parameters that were part of the model	43-48, 17-21
		(b) Report category boundaries when continuous variables were categorized	42
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done,-such as sensitivity/robustness analysis and analysis of subgroups	NA
Key results	18	Summarize key results with reference to study objectives	21-22
Strengths and Limitations	19	Discuss strengths and limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	21-22, 26, 32
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	21-34
Generalizability	21	Discuss the generalizability (external validity) of the study results	32
Funding	22	(a) Funding- Give the source of funding and the role of the funders for the present study	(a) 35
Transparency		and, if applicable, for the original study on which the present article is based (b) Conflicts of interest-Describe any conflicts of interest, or lack thereof, for each author	(b) 35

(c) Describe the authors' roles- Provision of an authors' declaration of transparency is recommended	(c) NA
(d) Ethical approval- Include information on ethical approval for use of animal and human	(d) 10 (IRB),
subjects	11 (IACUC)
(e) Quality standards-Describe any quality standards used in the conduct of the research	(e) 6

and implications and implications and, if applicable, for e. ^a Level of organization recognizes that observational studies in veterinary research often deal with repeated measures (within an animal or herd) or animals that are maintained in groups (such as pens and herds); thus, the observations are not statistically independent. This non-independence has profound implications for the design, analysis, and results of these studies.

b The word "participant" is used in the STROBE statement. However, for the veterinary version, it is understood that "participant" should be addressed for both the animal owner/manager and for the animals themselves. *Give such information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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Interpretive summary

10 Running head:

11 Milk quality and udder hygiene on VT organic dairies

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- 13 Relationship Between Facility Type and Bulk Tank Milk Bacteriology, Udder Health, Udder
- 14 Hygiene, and Milk Production on Vermont Organic Dairy Farms

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Abstract

Abstract
The primary objective of this cross-sectional observational study on organic dairies 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
collect bulk tank milk samples, udder hygiene scores, and complete a questionnaire on mastitis
risk and bedding management practices onenroll 40 farms, in order to compare herds using the
two most common housing systems (freestalls, tiestalls) with those using a bedded pack, for
organic dairy cattle in the state during the non-grazing season_(typically November-May).
Variation in bedded pack management practices was observed, with two general styles,
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 pack systems 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 SC	CS
(previous SCS <4.0 to current ≥4.0), and % cows with chronically elevated SCS (SCS ≥4.0 last	t
two tests). Multivariable linear regression models were performed to describe outcomes by	
facility type, but suffered from limited statistical power due to small group sample sizes. Final	
results from unconditional comparisons showed that farms grouped by facility type did not diff	er.
in metrics captured from Dairy Herd Improvement Association test data (cow-level udder healt	th
measures, milk production), bulk tank milk somatic cell count (BTSCC) and aerobic culture da	ıta,
or udder hygiene scores. Subsequently, a secondary analysis was conducted using univariate	
linear regression to identify associations between herd management factors and outcomes for a	11
21 farms combined. Although not all differences found were statistically significant in this	
secondary analysis which combined 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 elevated current SCS, lower average SCS, and better udder hygiene metric	cs.
Farms with lower mean udder hygiene scores had numerically lower chronically elevated SCS,	,
lower elevated current SCS, and lower average SCS. The current study provides insight on	
factors affecting bulk tank milk quality, udder health and hygiene measures on organic dairy	
farms in Vermont. We could not reject the null hypothesis that milk quality and udder health	
outcomes did not differ by facility type bedded packs were comparable to more frequently used	1
indoor housing systems (tiestalls and freestalls), we conclude that Bbedded pack facilities are	
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. More research including a larger number of herds is needed to test this hypothesis.

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Keywords: Mastitis, organic dairy cattle, housing, bedded pack, milk quality

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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 which 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).

91	The term "bedded pack" encompasses a variety of management styles (Bewley et al.,
92	2017), including compost bedded-packs (CBP), which utilize aerobic decomposition to break
93	down a bedding material of fine wood sawdust or shavings, as well as "conventional,"
94	"traditional," or "deep bedded packs" (Thurgood, 2009; Benson, 2012; Bewley et al., 2017; The
95	Dairyland Initiative, 2024). CBP can vary in depth, frequency and depth of aeration (tilling), type
96	of bedding material used, and in some regions the inclusion of forced air systems to dry the
97	bedding (Leso et al., 2020). In the Northeastern U.S., some producers are using deep bedded
98	pack systems where large volumes of straw or hay are added daily to an untilled surface in which
99	strata of bedding and waste accumulate throughout the period of time when cows are housed on
100	it (Benson, 2012). Oxygen is retained in the system by the selection of bedding material and the
101	timing of its application (Neher et al., 2022; Thurgood et al., 2009). A number of authors suggest
102	deep bedded -pack barns are synonymous with traditional straw yard housing systems (Bewley et
103	al., 2017; Leso et al., 2020; Ferraz et al., 2020). However, we find the deep bedded packs being
104	constructed on dairy farms in the Northeastern U.S. differ from traditional straw yards, in which
105	material is completely removed at approximately monthly intervals and the housing is used year-
106	round— (The Dairyland Initiative, 2024; Thurgood et al., 2009; Benson, 2012).
107	Bedded packs (BP) These loose-housing structures are perceived to integrate well into
108	Northeastern US pasture-based farm systems, and state and federal agencies in the U.S. are
109	providing financial incentives for dairies to build these structures as part of manure management
110	practices which improve water quality and contribute to soil conservation (USDA-NRCS;
111	Andrews et al., 2021; Thurgood et al., 2009).
112	As interest in bedded packsBP grows, it is important to better understand milk quality,
113	udder health and hygiene on farms using these housing alternatives. Understanding mastitis risk

for cattle housed on bedded packs <u>BP</u> 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 bedded packsBP could
pose a relatively higher risk for intramammary infections. Loose-housed cows continually add
manure to the bedded-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 bedded packsBP, 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 related to associated with a higher
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sand, which is an uncommon housing type for organic farms in Vermont (Andrews et al. 2021).
These include a study comparing actively managed composting bedded packs (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 two 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 on-for bedded
packBP farms and tiestall barns of similar size and management styleon 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 bedded pack barnBP 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 two most common indoor housing systems (freestalls, tiestalls) and farms using a bedded packBP for 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 bedded pack systemsBP 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 bedded packBP herds is inferior to that of more traditional housing types, as has been suggested by some previous research (Peeler et al., 2000; Fregonesi and Leaver, 2001; 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 bedded packs-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.

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).

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 in Winter 2018-2019 (all farms, n = 177). 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. During the non-grazing season (typically November-May in VermontWhen cows have no access to pasture in the winter non-grazing season are not on pasture), organic farms in Vermont house cows in a variety of indoor facility types. The Our previous Winter 2018-2019 industry survey aimed to quantifiedy 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

<u>current study farms were recruited from respondents to this survey</u> (Andrews et al., 2021). Daily
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 one of four
categories of bedding/housing combinations for their indoor housing system: 1) freestall-(FS)
barn bedded with sand, 2) freestall FS barn bedded with shavings or sawdust, 3) tiestall (TS) barn
bedded with shavings or sawdust, or 4) BP an enclosed loose housing facility deeply bedded with
organic material (hereafter, "bedded pack"). The first three 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 bedded packsBP 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 four 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
bedded pack systemBP as their primary indoor housing system. However, out of the 17 farms
from the 2018-2019 survey which indicated at least some use of a bedded pack systemBP, one
farm was not interested in any further participation, five did not use DHIA testing, and six only
used a bedded pack systemBP as a secondary housing system in conjunction with a tiestallTS
barn, or cows were only on the pack a few hours a day. Because the number of farms using
bedded packsBP was fewer than anticipated, the eligibility requirements were relaxed to include
one farm where cows spend the majority (two-thirds) of their time in a bedded packBP, with the
remaining time in a tiestall <u>TS</u> with wood shavings. Additionally, two bedded pack <u>BP</u> farms were
included that had limited DHIA information: one farm did not utilize cow-level testing, and cow-
level data for a second farm was limited due to their seasonal lactation schedule. This study was
intended to study cows while they were in their indoor housing system, so all herds visits were
completed before any grazing had begun for the season. 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,
two would be classified as "compost bedded-packs," utilizing 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 two 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; Bewley et al., 2017). The one remaining farm fell somewhere
between these two 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 freestallFS bedded with
sand, 5 freestallsFS bedded with wood shavings/sawdust, 10 tiestalls-TS bedded with wood
shavings/sawdust, 5 bedded packsBP) 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 for the
grazing season, 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 one farm
sampled using a freestallFS 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. The single sand freestallFS was combined with freestallsFS bedded with

wood shavings/sawdust (FS; n = 6), there were 10 <u>TS</u> tiestalls-bedded with wood shavings/sawdust (TS), and 5 bedded packs<u>BP</u> (BP).

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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 Data). 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 bedded pack systemsBP were asked additional questions to gather detailed information about bedded pack construction, management, monitoring practices, and perceptions comparing bedded packsBP 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

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(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 and bedding samples were collected. The bulk tank milk sample was collected directly from the top of the bulk tank using a 250-mL sterile single-use vial (Blue DippasTM, 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 Data). Additionally, measurements of the housing facilities were recorded for freestallsFS and tiestallsTS where appropriate (stall sizes, pen sizes, bedding depth, stocking density, trainer use), as well as observations about bedded packsBP when applicable (temperature, depth, pen size, and stocking density in m² per animal). If multiple pens were present (e.g., freestall barn), used bedding samples were collected from the pen containing the largest group of lactating cows, or from the highest producing group of animals if there were multiple pens of equal size. Bedding depth of freestallsFS and tiestallsTS was included as a producer reported value in the questionnaire. Bedding depth of bedded packBP 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 randomly selected cows on each farm (the first 30 able to be evaluated in a loose pen, or the first 30 encountered in a tiestall). Udder hygiene scores were taken from cows housed in the same pens from which used bedding samples were collected. A four-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).

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 \geq 200,000 cells/mL on most recent test day ("elevSCS"), where elevated SCS was defined as a somatic cell score of \geq 4.0; the proportion of cows with a newly elevated SCS ("newSCS"), which was defined as a SCS changing from <4.0 to \geq 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 \geq 4.0 on the last two tests (Schukken et al., 2003).

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).

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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 SSLO bacteria; University of Minnesota) media plates and incubated for two 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., streptococci and strep-like organisms (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.

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 two 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 $P \le 0.05$, visual assessment of distribution and
residuals, skewness, and comparison of the median and mean values. Raw bulk tank somatic cell
count (BTSCC) data was log ₁₀ 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.
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 three facility types. Statistical
significance for this test was declared at $P \le 0.05$. Multiple attempts were made using
multivariable analysis to compare the four aerobic culture outcomes for bulk tank milk, but all

modeling approaches suffered from over-parametrization even when data was 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 \geq 0.60), and unconditional associations among categorical variables were evaluated using a Pearson's chi-squared or Fischer's Exact test as appropriate ($P \leq$ 0.05). An ANOVA was used to check for correlation between numeric continuous variables and categorical variables ($P \leq$ 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 five observations. If any predictor had only one 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 "lme4" package to investigate the unconditional relationship between the six udder health and production outcomes (BTSCC, avg. SCS, newSCS, elevSCS, chronSCS, STD 150-day milk) and two hygiene outcomes (mean hygiene score, proportion of dirty udders) for each farm and the previously-described herd-level independent variables. The two 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. 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 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 two 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 until all remaining predictors had $P \le 0.10$. Final models were selected based on lowest Akaike information criteria, and an F-test to compare the final model to the model with facility type as the only predictor (significance declared at $P \le 0.05$). Overall statistical significance for facility type (the main predictor of interest) was declared at $P \le 0.05$. The multivariable modelling approach described above 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.

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 the six udder health and production outcomes (BTSCC, avg. SCS, newSCS, elevSCS, chronSCS, STD 150-day milk) and two hygiene outcomes (mean hygiene score, proportion of dirty udders). Unconditional relationships between the eight outcome variables and independent predictors are reported for a significance level of $P \le 0.20$ for an F-test, and only for predictor variables with group sizes of at least n = 5.

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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 bedded pack systemsBP in our region.

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Results

Description of study herds

Of the 21 herds enrolled, 5 used a bedded pack systemBP, 1 used a freestallFS bedded with sand, 5 used a freestallFS bedded with shavings/sawdust, and 10 used a freestallTS bedded with shavings/sawdust (Supplemental Table S1). Of the 5 BP farms, two bedded with shavings/sawdust and cultivated 2 times a day to promote aerobic composting, 1 bedded with straw and woodchips and cultivated 2 times/week, and 2 bedded mainly with straw, adding woodchips as needed, and did not cultivate the pack at all. 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 freestalls and tiestalls 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.

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

There was no difference in cfu count between the three facility types for any of the four bacterial groups measured using a nonparametric unconditional comparison (Table 2). The aerobic culture results for the four bacterial groups measured for bulk tank milk did not differ among facility types (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.

BTSCC, % cows with newly elevated SCS, % cows with chronically elevated SCS, % cows with elevated SCS, avg. SCS, and STD 150-day milk production did not differ by facility type (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 bedded packBP farms (n = 5), 2.5 (2.24-2.76) for tiestallTS farms (n = 10), and 2.15 (1.93-2.37) for treestallFS farms (n = 6). Mean udder hygiene score did not differ by facility type. The overall mean proportion of cows with

dirty udders in a herd (udder hygiene score \geq 3) was 40% (31-48). The mean proportion of cows with dirty udders (95% CI) was 32% (18-46) for bedded packBP farms, 49% (35-62) for tiestallTS farms, and 32% (20-44) for freestallFS farms. The proportion of cows with dirty udders did not differ by facility type.

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, two bedded packBP farms did not have available DHIA data (n = 19; group sizes: FS =6, TS = 10, BP = 3). One bedded packBP farm did not have average cow-level SCS data (n = 20; group sizes: FS = 6, TS = 10, BP = 4). For STD 150-day milk, one bedded packBP farm and two tiestallTS 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.

Bulk tank milk quality outcomes

There was no difference in cfu count between the three facility types for any of the four bacterial groups measured using a nonparametric unconditional comparison (Table 2). Multiple attempts were made using multivariable analysis to compare the four aerobic culture outcomes for bulk tank milk, but all modeling approaches suffered from over-parametrization even when data was log transformed and were not pursued further.

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 included facility type (forced) and herd size. Facility type was not associated with BTSCC in the final model (Table 4).

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), bedding amendment use, air quality, glove use, and mastitis record keeping practices. 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, and proportion of dirty udders. The final multivariable model included all four variables from univariate analysis, as well as 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).

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 three 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).

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).

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 $P \le 0.05$, possibly due to small sample size.

The depth of bedding in stalls for freestallFS and tiestallTS herds was unconditionally associated with multiple udder health outcomes. As the depth of bedding in freestallFS and tiestallTS herds increased, multiple udder health measures improved, including lower avg. SCS, BTSCC, elevSCS, and newSCS. Similarly, comparing farms where cows were on deep bedding (i.e., grouping all herds reporting deeply-bedded stalls plus bedded packBP 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 was 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 five herds using a bedded packBP, 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 three all-herds-freestalls and tiestalls reporting deeply-bedded stalls, plus the five bedded-packBP herds) had numerically lower average udder hygiene scores and proportion dirty udders

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compared to cows on stalls with bedding over a mattress or concrete surface. For the fifteen tiestalls and freestalls farms reporting bedding depth in stalls, increased bedding depth was associated with lower mean udder hygiene score and a numerically lower proportion of dirty udders.

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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 bedded packBP farms to both tiestallTS and freestallFS 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 bedded packBP systems are a viable option for housing in Vermont during the non-grazing season compared to the two most common indoor housing systems in the state (freestallsFS, tiestallsTS). This study is also the first to describe udder health and hygiene on bedded packsBP in the Northeastern US, which is significant as the performance of these systems can be greatly influenced by climatic factors. As BTM bacteriology, udder health and hygiene metrics, and milk yield did not differ for BP herds compared to TS and FS herds, there was insufficient evidence to reject our null hypothesis that these metrics would not vary by facility type. We conclude that bedded pack systems can be considered a viable loosehousing option for organic dairy cattle during the non-grazing season in the Northeastern US.

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

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Previous work describing bulk tank milk aerobic culture data for farms using a bedded pack system has primarily been limited to descriptive studies enrolling only composting bedded pack herds (Barberg et al., 2007b; Shane et al., 2010), with only one study directly comparing bacterial counts between composting bedded packs and freestall barns (Lobeck et al., 2012). 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 beddedpack 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). The current study is the first the authors are aware of directly comparing bacterial counts of bulk tank milk between bedded packs (both composting and static) and tiestall barns, and the first one to describe a population of exclusively organic dairies. The six farms included in Lobeck et al. (2012) used mainly wood sawdust as bedding material (with one using wheat straw by-product) as did the 12 farms in Barberg et al. (2007). This is similar to the current study, where three of five bedded packs used a combination of woodchips/shavings and straw/hay, and two used 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. 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 animals are primarily housed inside in Vermont. In contrast to
previous work, which evaluated milk culture results across the summer months (Barberg et al.,
2007b) and year-round (Lobeck et al., 2012), the current study focused solely on sampling
during the period when animals are primarily housed inside in Vermont. We were most
interested in studying bulk tank milk bacteriology for these organic herds during the non-grazing
season, as this is when these pastured based farms need to house their animals inside. All herds
included had excellent bulk tank milk quality; most (19/21) fell into the "low BTSCC" category
as defined by Jayarao et al. 2004 with the remaining 2 in the "medium BTSCC" category.
The <i>Staph</i> . spp. count for the five bedded packBP farms included in this study (median:
40 cfu/mL, range: 0-130) 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 <i>Staph</i> . spp. from
BTM collected just overin the winter months from six composting bedded pack CBP farms.
"Staph. spp." is comprised of a diverse group of different species, with 23 (Condas et al., 2017)
or 25 (De Visscher et al., 2017) different species isolated from intramammary infections in dairy
eattle. Within this highly heterogenous group of bacteria, some species are considered primarily
host-adapted (colonizing the skin or udder), while others are primarily found in the cow's

environment (reviewed in De Buck et al., 2021). Certain species have been associated with stall
surfaces, air, and unused sawdust bedding material (Piessens et al., 2011), some with different
facility types (Condas et al., 2017), and others with environmental contamination and poor teat
hygiene at milking time (De Visscher et al., 2016; De Visscher et al., 2017). Although the
specific source and routes of transmission for many Staph. spp. are still being elucidated, the
importance of post-milking teat-dip to control this group of bacteria has been established (Hogan
et al., 1987), while the efficacy of pre-dipping to control Staph. spp. other than S. aureus remains
controversial (Pankey, 1989). 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 (<u>Hogan et al., 1987</u> , Pankey et al., 1985; Pankey et al., 1987;
Quirk et al., 2012). All but one farm in the current study would fall into the "low" category for
Staph. spp. counts in the BTM (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 (SSLO) counts in BTM for bedded packsBP in the current study_were much lower than those from Minnesota composting bedded packsCBP in the winter (98-48,400 cfu/mL, -Shane et al. 2010; mean: 911 cfu/mL, 95% CI: 138-6,01, reported a range of SSLO 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 median SSLO counts for bedded pack farms included in the current study was 35 cfu/mL (range: 10-80). Work from Barberg et al. (2007) describing milk quality on composting bedded packsCBP in Minnesota noted that 6 of 12 farms sampled had "high" levels of SSLO. SSLO count did not differ between tiestallsTS, freestallsFS, and bedded packsBP in the current study. The overall SSLO count for all 21 farms included in the current study (median: 45 cfu/mL, range: 10-1250) was lower than that for the overall Strep.

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count for all-three facility types studied in Lobeck et al. 2012 (445 cfu/mL, 95% CI: 116-1704).
As the overall SSLO counts for all farm types included in the Minnesota studies are higher than
that found for all 21 farms in the current study, bMetter milking and bedding hygiene practices
amongst herds included in the current study may best explain this difference in BTM pathogen
profiles (Jayarao and Wolfgang, 2003).

All farms had low levels of coliforms in bulk tank milk (median: 0 cfu/mL, range: 0-5), indicating excellent hygiene practices at milking time (Jayarao and Wolfgang, 2003). Coliform counts did not differ between the three facility types. Bedded pack farms in the current study had The low very BTM low coliform counts in BTM for BP in the current study are (median: 0 efu/mL, range: 0-5), similar to those found for three compost bedded packCBP farms in a Brazilian studyBrazil (2.8 cfu/mL; Fávero et al. 2015). These low coliform counts are This is in contrast with previous work describing BTM quality for this kind of facility in the United States U.S.. Coliform counts for bedded packs in Minnesota (in the winter ranged from 15-1,128 cfu/mL, (Shane et al., 2010;), mean: 63.7 cfu/mL, 95% CI: 6-735, and the six bedded packs included in Lobeck et al. 2012. had a mean of 63.7 cfu/mL (95% CI: 6-735), although de 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 packsBP 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."

Prevalence of *Staph. aureus* was similar between the five VT bedded packBP farms in the current study (median: 0 cfu/mL, range: 0-30) 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 packsBP 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 bedded packsBP were certified organic, thise higher prevalence of *Staph. aureus* amongst 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 bedded packBP farms, where four or five 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).

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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.

Udder health outcomes included in the current study (percent cows with elevSCS, percent cows with chronSCS, percent cows with newSCS, BTSCC, and average avg. SCS) did not different significantly between facility types. For BTSCC, BP were numerically lower than the other two facility types; the difference in BTSCC for BP vs. to-FS and BP vs. TS equated to an increase of 34,628 and 28,105 cells/mL, respectively, which could eanamount to an important difference at the bulk tank level quality premiums under some systems. NewSCS was also numerically lowest for BP, at 2.3% lower than FS and more modestly at 0.43% lower than TS. Ruegg and Pantoja- (2013) propose a benchmark of having <8% of cows developing a new subclinical mastitis infections per month, whereas Schukken et al. (2003) suggest <10%. Using either of these figures, a difference of 2.3% between facility types would be substantial. BP had numerically lower chronSCS in comparison to FS (1.5%), but were equivalent to TS herds in this respect. As an industry benchmark is to have <10% of cows with chronic subclinical mastitis infections carrying over month to month (U. Minnesota Extension Dairy Team), 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 \geq 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 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 al
estimates, interpretation of the effect of facility type for these outcomes is challenging.

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Although some previous work has found BTSCC to be elevated for CBP farms (425,000 cells/mL over all four seasons, Black et. al 2013; 325,000 cells/mL during summer, Barberg et. al 2007b), other groups have also found udder health and milk quality measures on bedded packBP farms are similar to farms using more traditional facility types. Specifically, subclinical mastitis prevalence levels did not differ between compost bedded packs CBP and two types of freestallFS 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 packsCBP, cross-ventilated freestallsFS, and naturally-vented freestallsFS (Lobeck et al., 2011). Eckelkamp et. al 2016a found no significant difference in subclinical mastitis prevalence in CBP vs. sandbedded freestallsFS in Kentucky with a history of low BTSCC (21.8 and 19.4%, respectively), as well as no difference in BTSCC between the two 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 bedded packBP farms in that state as there were no inclusion criteria around maintaining a low SCC priorevious to the start of the study. The prevalence of subclinical mastitis for herds in the current study (26% for bedded packs) 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 three bedded packBP farms in Brazil than our study (26 and 7% respectively, for the three 3 bedded packsBP with available data).

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STD 150-day milk production did not differ significantly between facility type in the current study. 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. PThis aligns with previous research which has found no significant differences in various production metrics of cows housed on bedded packsBP vs. in freestallFS barns (Lobeck et al., 2011; Eckelkamp et al., 2016a; Costa et al., 2018). Varying production metrics for cows housed on bedded packsBP 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 bedded packsBP 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, the "results in the literature indicate that high levels of milk production are possible in CBP." As bedded packsBP potentially improve cow comfort, one may even expect greater milk production than in more traditional housing systems (Calamari et al., 2009; Ruud et al., 2010).

Our finding no difference in tThe two udder hygiene measures were not statistically different between the three facility types. TS farms had numerically higher proportion of dirty

752	udders and avg. udder hygiene score, while FS and BP systems were equivalent. However,
753	interpretation of these numerical differences is difficult, given that the standard errors for all four
754	estimates are large relative to the coefficient estimates. is in accordance with pPrevious work,
755	which found that cow hygiene on bedded packBP systems is was comparable to traditional
756	facility types in the Upper Midwestern U.S., Southeastern U.S., and Brazil (Barberg et al.,
757	2007b; Shane et al., 2010; Black et al., 2013; Eckelkamp et al., 2016b; a; Costa et al., 2018;
758	Adkins et al., 2022; Andrade et al., 2022). Black (2013) and Eckelkamp (2016a) reported that
759	increased pack moisture allows wet bedding material and manure to adhere more easily to
760	animals, meaning that cow hygiene is highly dependent on conditions of the bedded packBP.
761	This sentiment was echoed by the bedded packBP producers in the current study, who shared
762	that keeping their cows clean during periods of wet or humid weather could be a challenge.
763	However, all bedded packsBP in the current study had an average udder hygiene score of less
764	than 2.5, and the farm with the lowest mean average udder hygiene score overall was a bedded
765	pack farmBP. Although Cook (2002) as pointed out the challenges of comparing dairy cattle
766	hygiene between different facility types, we chose to focus on gathering observations of udder
767	hygiene. The relationship between udder hygiene and health is well-studied, and was a tractable
768	observation to make during non-grazing season farm visits where individual animals were often
769	roaming freely in a pen, or confined in a tiestall TS barn.
770	Objective 2: Analysis of farm management factors (non-facility) associated with bulk
771	tank milk quality, udder health, milk production, and udder hygiene scores for all
772	farms combined
773	As results from the multivariable models exploring the relationship between facility type
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and outcomes of interest suffered from limited statistical power due to small sample sizes, the

focus of the discussion will be on trends that emerged from the univariate analysis which combined all 21 farms.

One finding emerging from the univariate analysis combining all 21 farms from this work is that farms with deeper bedding had more favorable udder hygiene metrics (deeper bedding begets cleaner cows). When comparing farms that housed cows with a deep bedding system (deeply-bedded stalls or a bedded packBP) 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 freestallFS barns, including: Cook et al. 2016 (prevalence of dirty udders_was_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 tiestallsTS 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 bedded packBP, depth of bedding in freestallsFS and tiestallsTS) and hygiene score. As the measured height of bedding got deeper (height of bedded packBP, 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 three different

freestall FS bedding height groups (<0.56 cm, 0.56–1.75 cm, >1.75 cm). The our study, this
relationship between bedding depth and udder hygiene was especially strong for bedded packsBP
in particular, despite the although limited sample size was limited at of five herds. To the best of
our knowledge, this specific association has not previously been explored for bedded packBP
herds. There is clearly 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__(eleaner cows beget better milk). 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 three bedded packBP 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% for each one-unit increase in leg cleanliness score, respectively (Fávero et al., 2015). Curiously, although leg cleanliness score was associated with both mastitis outcomes on Brazilian bedded packBP, udder hygiene score was not.

A third interesting finding to emerge from the univariate regression results is that farms using deeper bedding had better milk quality outcomes (deeper bedding begets better milk).

Although there is an established recommendation of 15 cm for deep bedding of freestallsFS

(Bickert, 2000; Cook, 2002) and this depth appears to be based on optimizing cow comfort in
deep-bedded freestalls with no reference to udder hygiene or health. There is very-limited work
study exploring ideal bedding material depth for tiestall TS barns (Tucker and Weary, 2004;
Tucker et al., 2009), and this isthis work is again_solely-focused on the important concern of
cow comfort. As stated 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
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 Even still, the opportunity still exists for research exploring optimal stall bedding
depths of different organic materials in tiestall barns with a focus on mastitis and udder health
outcomes. It may be that recommending a particular depth of bedding to use for different types
of organic material would 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.).
to the goal was to Recent previous work has exclusively focused on describing bedded
packs that are actively managed for aerobic composting (Leso et al., 2020). Leso et al. contrasted
composting bedded packs managed with daily cultivation with conventional static 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 (Andrews et al., 2021). This infrequent

use of bedded packs in our state created a challenge for enrolling ten herds using this kind of system in our 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. Our current study shows that farms can achieve excellent milk quality using either a static or aerobically composting bedded pack system for indoor housing, e.g., three of the five bedded pack farms had a BTSCC ≤99,000 cells/mL, and the remaining two were ≤160,000 cells/mL. Furthermore, the lowest BTSCC in the study (54,000 cells/mL) was a static bedded pack farm using woodchips and straw. This low BTSCC was not just from selectively dumping milk from high-SCC cows; this farm also had the lowest overall % cows with elevated SCS (8.6%; data not shown).

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 may be interesting 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. The potential exists that producers who volunteered to participate in the current study are systematically more progressive or somehow different in their management practices than the general population of organic farms in Vermont. Additionally 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.

One Perhaps the biggest limitation of the current study is the small number of farms in each facility type. As state agencies had been promoting the use of bedded packBP 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 (i.e., teat-dipping, forestripping, using separate towels for individual cows) 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

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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 bedded packBP systems in the Northeastern US. Studies enrolling a larger
number of bedded pack farms bBy covering aenrolling 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 may have sufficient power to identify particular management factors which are
beneficial on bedded packsBP specifically.

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 ten 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 an aerobically composting bedded pack system for indoor housing;

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three of the five BP farms had a BTSCC ≤99,000 cells/mL, and the remaining two 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 just from selectively dumping milk from high-SCC cows; this farm also had the lowest overall % cows with elevated SCS (8.6%; data not shown).

Bedded packBP systems have a number of advantages for producers considering updating their facilities, including a smaller initial investment when compared to a new freestallFS or tiestallTS 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 tiestallTS and freestallFS 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 five 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 composted aged bedded 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). With no obvious disadvantages for udder health or hygiene when properly managed on farms with excellent milking hygiene practices already in place, bedded packs Bedded packs may be a an

especially 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 confirm that udder health, milk quality, udder hygiene and milk production compares favorably to more traditional housing systems.

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Conclusion

For five of the six studied udder health and production metrics, and both udder hygiene measures, numerically BP either performed slightly better or were 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 make it difficult to rule out biologically important effects of facility type for these outcomes. This is likely a result of 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. Bedded pack systems did not differ significantly in their milk quality, udder health, udder hygiene measures, or milk production, as compared to the more commonly used indoor housing systems (freestall or tiestall) for organic cows in Vermont. Bedded packs can therefore be considered as a viable option for pasture-based herds looking for a loose-housing system. Findings from the secondary analysis of results found evidence of the well-supported 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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1234 Tables

Table 1. Predictors offered to multivariable models for each of the eight 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

Deeply-bedded stalls or bedded pack; Stalls with bedding on a mattress or

Lying surface for cows¹ (deeply-bedded vs. not) 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

Routinely culture high somatic cell count cows

Always/Sometimes; Never

Always/Sometimes; Never

Ever perform culture of mastitic cows Yes; Never culture

Use intramammary product at dry-off (OMRI-listed)

Yes; No

All lactating cows regularly/

Parenteral supplementation with vit. E and selenium

Occasionally as needed; No All milkers consistently;

Glove use at milking Inconsistently/No

Check for clinical mastitis by noticing abnormal cow/abnormal udder and forestripping

Yes; No Parlor; Tiestall

Type of milking system used³ Farm-level udder hygiene metrics

Average udder hygiene score

Prop. dirty udders (%; udder hygiene score ≥3)

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Table 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

	Overall	Bedded	Tiestalls	Freestalls	
Bacteria group (cfu/mL)	(n = 21)	packs $(n = 5)$	(n = 10)	(n=6)	<i>P</i> -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 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 (log ₁₀ cells/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				
SCS ¹	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 \geq 4.0 current test ¹	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. SCS ²	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)

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

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

Table 4. Objective 1: Final multivariable models describing the relationship between facility type (forced) and milk quality, udder health, production, and udder hygiene outcomes

	Explanatory variable	Group (sample size)	Coefficient estimate (SE)	<i>P</i> -value
BTSCC (1	og ₁₀ cells/mL)			
	tercept		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 6	elevated SCS			
	Intercept		-1.6 (2.5)	
	Facility type (forced)	Freestall $(n = 6)$	2.3 (2.2)	0.33
		Tiestall $(n = 10)$	0.43 (1.9)	0.82
		Bedded pack $(n = 3)$	Ref.	Ref.
	Use bedding amendment	Yes (n = 5)	3.9 (1.8)	0.05
		No (n = 14)	Ref.	Ref.
	Subjective assessment air quality (researcher)	Good (n = 14)	3.6 (1.5)	0.04
		Fair $(n = 5)$	Ref.	Ref.
	Glove use at milking ¹	Never/Inconsistently $(n = 9)$	2.0 (1.3)	0.17
		Always $(n = 9)$	Ref.	Ref.
	Clinical mastitis events record keeping	Never kept records $(n = 6)$	4.4 (1.8)	0.03
		Sometimes/Temporarily kept records $(n = 6)$	1.1 (1.6)	0.52
		Always kept records $(n = 7)$	Ref.	Ref.
% chronic	cally elevated SCS			
	Intercept		5.3 (3.6)	
	Facility type (forced)	Freestall $(n = 6)$	1.5 (4.6)	0.75
		Tiestall $(n = 10)$	-0.08 (3.5)	0.98
		Bedded pack $(n = 3)$	Ref.	Ref.
	Feed supplemental vit. E and selenium ²	Yes (n = 11)	2.1 (2.8)	0.48
		No $(n = 7)$	Ref.	Ref.

	Use bedding amendment	Yes (n = 5)	5.7 (3.4)	0.12
		No $(n = 14)$	Ref.	Ref.
	Clip/flame udder hair	Yes (n = 5)	-6.3 (3.1)	0.07
		No $(n = 14)$	Ref.	Ref.
	% udder hygiene scores ≥3	Herds with available data $(n = 19)$	17.0 (6.1)	0.02
$\%$ SCS ≥ 4	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
Standardiz	zed 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			

²One farm unable to provide response

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Table 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)	<i>P</i> -value	Intercept
		1 (1 /	,		1
•	og ₁₀ cells/mL)	Matters 20 20 20 20 42 (n = 12)	0.12 (0.07)	0.12	<i>5</i> 1
Model 1	Lying surface	Mattress or concrete $(n = 13)$	0.12 (0.07)	0.12	5.1
36.110		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 milking ²	Never/Inconsistently $(n = 9)$	0.10 (0.07)	0.19	5.1
•	levated SCS ³	Always (n = 11)	Ref.	Ref.	
Model 4	Glove use at milking	Never/Inconsistently $(n = 9)$	2.83 (1.7)	0.11	4.3
		Always $(n = 9)$	Ref.	Ref.	
Model 5	Depth of bedding in stalls (cm) ¹	Tiestalls and freestalls $(n = 15)$	-0.62 (0.24)	0.02	8.3
% chronica	ally elevated SCS ³				
Model 6	Clip/flame udder hair	Yes (n = 5)	-4.31 (2.9)	0.16	14.8
		No $(n = 14)$	Ref.	Ref.	
Model 7	% udder hygiene scores ≥3	Herds with available data $(n = 19)$	12.7 (6.1)	0.05	8.6
Model 8	Avg. udder hygiene score	Herds with available data $(n = 19)$	6.39 (3.1)	0.05	-1.2
$\%$ SCS ≥ 4	.0 current test ³				
Model 9	Depth of bedding in stalls (cm) ¹	Tiestalls and freestalls $(n = 15)$	-1.2 (0.42)	0.01	30
Model 10	% udder hygiene scores ≥3	Herds with available data $(n = 19)$	13.6 (8.5)	0.13	19.6
Model 11	Avg. udder hygiene score	Herds with available data $(n = 19)$	7.7 (4.3)	0.09	7.1
Average So					
Model 12	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 13	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 14	Depth of bedding in stalls (cm) ¹	Tiestalls and freestalls $(n = 15)$	-0.05 (0.03)	0.10	2.6

Model 15	% udder hygiene scores ≥3	Herds with available data $(n = 20)$	0.75 (0.45)	0.12	2.1
Model 16	Avg. udder hygiene score	Herds with available data $(n = 20)$	0.39 (0.23)	0.11	1.5
Standardiz	ed 150-day milk (pounds) ⁵				
Model 17	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 18	Herd size	Herds with available data $(n = 18)$	0.26 (0.14)	0.07	33.1
% udder hy	ygiene scores ≥3				
Model 19	Depth of bedded pack (m)	Bedded pack herds $(n = 5)$	-0.5 (0.06)	0.004	0.97
Model 20	Lying surface	Mattress or concrete $(n = 13)$	0.17 (0.08)	0.06	0.30
		Deep bedding $(n = 8)$	Ref.	Ref.	
Model 21	Depth of bedding in stalls (cm) ¹	Tiestalls and freestalls $(n = 15)$	-0.02 (0.02)	0.13	0.54
Avg. udde	r hygiene score				
Model 22	Depth of bedded pack (m)	Bedded pack herds (n = 5)	-0.96 (0.15)	0.008	3.4
Model 23	Lying surface	Mattress or concrete $(n = 13)$	0.33 (0.16)	0.06	2.1
		Deep bedding $(n = 8)$	Ref.	Ref.	
Model 24	Depth of bedding in stalls (cm) ¹	Tiestalls and freestalls $(n = 15)$	-0.06 (0.03)	0.07	2.6
¹ Stall bedo	ling depth for freestalls and tiestalls bedded with wood sl	havings or sawdust			
² One farm	used automatic milking system				
³ DHIA da	ta available for $n = 19$ herds.				
⁴ DHIA da	ta available for $n = 20$ herds.				
⁵ DHIA da	ta available for $n = 18$ herds.				

⁵ DHIA data available for n = 18 herds.

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Interpretive summary

Previous studies reported bedded packs improve cow welfare and comfort and have
advantages for manure management, soil health, and water quality. Consensus is lacking on
whether bulk tank milk quality, udder health, udder hygiene and milk production are compromised
on bedded packs. In an observational study of 21 organic dairies in Vermont during the non-
grazing season (November-May), bedded packs were similar to tiestalls and freestalls in their bulk
tank milk quality, udder health, udder hygiene and milk production. For producers considering a
transition from tiestalls, bedded packs may be a viable option for dairy cattle housing in the
Northeastern US.

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11 Milk quality and udder hygiene on VT organic dairies

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- Relationship Between Facility Type and Bulk Tank Milk Bacteriology, Udder Health, Udder
- 14 Hygiene, and Milk Production on Vermont Organic Dairy Farms

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Abstract

The primary objective of this cross-sectional observational study on 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 two most common housing systems (freestalls, tiestalls) with those using a bedded pack, for organic dairy cattle in the state during the non-grazing season (typically November-May). Variation in bedded pack management practices was observed, with two general styles, 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 pack systems 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

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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 \geq 4.0), and % cows with chronically elevated SCS (SCS \geq 4.0 last two tests). Multivariable linear regression models were performed to describe outcomes by facility type, but suffered from limited statistical power due to small group sample sizes. Unconditional comparisons showed that farms grouped by facility type did not differ 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 which combined 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 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. The current study provides insight on factors affecting bulk tank milk quality, udder health and hygiene measures on organic dairy farms in Vermont. We could not reject the null hypothesis that milk quality and udder health outcomes did not differ by facility type. Bedded packs were comparable to more frequently used indoor housing systems (tiestalls and freestalls). Bedded pack facilities are may be a viable option for confinement housing during the winter nongrazing season for pasture-based herds interested in a loose-housing system in the Northeastern US. More research including a larger number of herds is needed to test this hypothesis.

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Keywords: Mastitis, organic dairy cattle, housing, bedded pack, milk quality

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 (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 in which strata of bedding and waste accumulate throughout the period of time when cows are housed on it (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). A number of 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 on dairy farms in the Northeastern U.S. differ from traditional straw yards, in which 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 two 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.

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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 two most common indoor housing systems (freestalls, tiestalls) and farms using a BP for 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 is 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.

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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).

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Herd enrollment and selection

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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 in Winter 2018-2019 (all farms, n = 177). 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 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 one of four 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 three 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 four 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 which indicated at least some use of a BP, one farm was not interested in any further participation, five did not use DHIA testing, and six 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 one 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, two BP farms were included that had limited DHIA information: one 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, two would be classified as "compost bedded-packs," utilizing 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 two 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; Bewley et al., 2017). The one remaining farm fell somewhere between these two 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

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to not resume the study, and the final analysis included the 21 herds sampled in 2019. As there was only one farm sampled using a FS 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. 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.

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 Data). 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

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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 collected directly from the top of the tank using a 250-mL sterile single-use vial (Blue DippasTM, 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 Data). 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 tiestall). A four-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).

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 \geq 200,000 cells/mL on most recent test day ("elevSCS"), where elevated SCS was defined as a somatic cell score of \geq 4.0; the proportion of cows with a newly elevated SCS ("newSCS"), which was defined as a SCS changing from <4.0 to \geq 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 \geq 4.0 on the last two tests (Schukken et al., 2003).

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 SSLO bacteria; University of Minnesota) media plates and incubated for two 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., streptococci and strep-like organisms (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.

Data management and analysis

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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 two farm-level udder hygiene measures: 1) mean udder hygiene score, and 2) proportion of cows with dirty udders (udder hygiene score \geq 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 $P \leq$ 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 \log_{10} 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.

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 three facility types. Statistical significance for this test was declared at $P \le 0.05$. Multiple attempts were made using multivariable analysis to compare the four aerobic culture outcomes for bulk tank milk, but all modeling approaches suffered from over-parametrization even when data was 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 \geq 0.60), and unconditional associations among categorical variables were evaluated using a Pearson's chi-squared or Fischer's Exact test as appropriate ($P \leq$ 0.05). An ANOVA was used to check for correlation between numeric continuous variables and categorical variables ($P \leq$ 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 five observations. If any predictor had only one 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 "lme4" package to investigate the unconditional relationship between the six udder health and production outcomes (BTSCC, avg. SCS, newSCS, elevSCS, chronSCS, STD 150-day milk) and two hygiene outcomes (mean hygiene score, proportion of dirty udders) for each farm and the previously-described herd-level independent variables. The two 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. 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 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 two 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 until all remaining predictors had $P \le 0.10$. Final models were selected based on lowest Akaike information criteria, and an F-test to compare the final model to the model with facility type as the only predictor (significance declared at $P \le 0.05$). Overall statistical significance for facility type (the main predictor of interest) was declared at $P \le 0.05$. The multivariable modelling approach described above 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.

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 the six udder health and production outcomes (BTSCC, avg. SCS, newSCS, elevSCS, chronSCS, STD 150-day milk) and two hygiene outcomes (mean hygiene score, proportion of dirty udders). Unconditional relationships between the eight outcome variables and independent predictors are reported for a significance level of $P \le 0.20$ for an F-test, and only for predictor variables with group sizes of at least n = 5.

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.

Results

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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 freestalls and tiestalls 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.

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

There was no difference in cfu count between the three facility types for any of the four
bacterial groups measured using a nonparametric unconditional comparison (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.
BTSCC, % cows with newly elevated SCS, % cows with chronically elevated SCS, %
cows with elevated SCS, avg. SCS, and STD 150-day milk production did not differ by facility
type (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 farms (n = 5), 2.5 (2.24-2.76)
for TS farms ($n = 10$), and 2.15 (1.93-2.37) for FS farms ($n = 6$). Mean udder hygiene score did
not differ by facility type. The overall mean proportion of cows with dirty udders in a herd
(udder hygiene score ≥3) was 40% (31-48). The mean proportion of cows with dirty udders (95%)
CI) was 32% (18-46) for BP farms, 49% (35-62) for TS farms, and 32% (20-44) for FS farms.
The proportion of cows with dirty udders did not differ by facility type.
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

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, two 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, one

BP farm and two TS farms were missing DHIA data ($n = 18$; group sizes: $FS = 6$, $TS = 8$, $BP = 18$
4). Farms with missing data for a particular outcome were excluded for the analyses of that
outcome.

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 included facility type (forced) and herd size. Facility type was not associated with BTSCC in the final model (Table 4).

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), bedding amendment use, air quality, glove use, and mastitis record keeping practices. 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, and proportion of dirty udders. The final multivariable model included all four variables from univariate analysis, as well as 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).

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 three 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).

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).

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 $P \le 0.05$, possibly due to 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, 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 was 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 five 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 three freestalls and tiestalls reporting deeply-bedded stalls, plus the five 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 tiestalls and freestalls reporting bedding depth in stalls increased bedding depth was associated with lower mean udder hygiene score and a numerically lower proportion of dirty udders.

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 two most common indoor housing systems in the state (FS, TS). This study is also the first to describe udder health and hygiene on BP in the Northeastern US, which is significant as the performance of these systems can be greatly influenced by climatic factors. As BTM bacteriology, udder health and hygiene metrics, and milk yield did not differ for BP

herds compared to TS and FS herds, there was insufficient evidence to reject our null hypothesis that these metrics would not vary by facility type.

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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 byproduct, 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 five 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 <i>Staph</i> . spp. from BTM in the winter months from six 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 one farm in the current study would fall into the "low" category for
Staph. spp. counts in the BTM (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 (SSLO) 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. SSLO count did not differ between TS, FS, and BP in the current study. 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). Milking and bedding hygiene practices amongst herds included in the current study may best explain this difference in BTM pathogen profiles (Jayarao and Wolfgang, 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 three 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."

Prevalence of *Staph. aureus* was similar between the five VT BP farms in the current study and the six 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 four or five 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.

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. For BTSCC, BP were numerically lower than the other two 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 at the bulk tank level quality premiums under some systems. NewSCS was also numerically lowest for BP, at 2.3% lower than FS and more modestly at 0.43% lower than TS. Ruegg and Pantoja (2013) propose a benchmark of having <8% of cows developing a new subclinical mastitis infections per month, whereas Schukken et al. (2003) suggest <10%. Using either of these figures, a difference of 2.3% between facility types would be substantial. BP had numerically lower chronSCS in comparison to FS (1.5%), but were equivalent to TS herds in this respect. As

an industry benchmark is to have <10% of cows with chronic subclinical mastitis infections carrying over month to month (U. Minnesota Extension Dairy Team), 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 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.

Although some previous work has found BTSCC to be elevated for CBP farms (425,000 cells/mL over all four 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 BP farms are similar to farms using more traditional facility types. Specifically, subclinical mastitis prevalence levels did not differ between CBP and two 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 (Lobeck et al., 2011). Eckelkamp et. al 2016a found no significant difference in subclinical mastitis prevalence in CBP vs. sand-bedded FS in Kentucky with a history of low BTSCC (21.8 and 19.4%, respectively), as

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well as no difference in BTSCC between the two 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 the 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 three BP farms in Brazil than our study (26 and 7% respectively, for 3 BP with available data).

STD 150-day milk production did not differ significantly between facility type in the current study. 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 may even expect greater milk production than in more traditional housing systems (Calamari et al., 2009; Ruud et al., 2010).

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The two udder hygiene measures were not statistically different between the three facility types. TS farms had numerically higher proportion of dirty udders and avg. udder hygiene score, while FS and BP systems were equivalent. However, interpretation of these numerical differences is difficult, given that the standard errors for all four 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 highly 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) as pointed out the 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.

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 three 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 sample size was limited at five 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 three 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% for each one-unit increase in leg cleanliness score, respectively (Fávero et al., 2015). Curiously, although leg cleanliness score was associated with both mastitis outcomes on Brazilian BP, udder hygiene score was not.

A third interesting finding to emerge from the univariate regression results is that 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 study exploring ideal bedding material depth for TS barns (Tucker and Weary, 2004; Tucker et al., 2009), this work is focused on the important concern of cow comfort. 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. It is likely that the effect seen in the current work of deeper bedding and better udder health outcomes is mediated through the

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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 may be interesting 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.

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Perhaps the biggest limitation of the current study is the small number of farms in each facility type. 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 on BP specifically.

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

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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 ten 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 an aerobically composting bedded pack system for indoor housing; three of the five BP farms had a BTSCC ≤99,000 cells/mL, and the remaining two 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 just from 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). 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 confirm that udder health, milk quality, udder hygiene and milk production compares favorably to more traditional housing systems.

Conclusion

For five of the six studied udder health and production metrics, and both udder hygiene measures, numerically BP either performed slightly better or were 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 make it difficult to rule out biologically important effects of facility type for these outcomes. This is likely a result of 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

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number of farms using each type of housing are needed to more definitively explore these relationships. Findings from the secondary analysis of results found evidence of the well-supported 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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1107 Tables

Table 1. Predictors offered to multivariable models for each of the eight 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

Deeply-bedded stalls or bedded pack; Stalls with bedding on a mattress or

Lying surface for cows¹ (deeply-bedded vs. not) 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

Always; Sometimes/Temp.; Never

Always/Sometimes; Never

Always/Sometimes; Never

All lactating cows regularly/ Occasionally as needed; No

All milkers consistently; Inconsistently/No

Yes; Never culture

Yes: No

Yes: No

Parlor; Tiestall

Keep record of clinical mastitis events

Routinely culture mastitic milk

Routinely culture high somatic cell count cows

Ever perform culture of mastitic cows

Use intramammary product at dry-off (OMRI-listed)

Parenteral supplementation with vit. E and selenium

Glove use at milking

Check for clinical mastitis by noticing abnormal cow/abnormal udder and forestripping

Type of milking system used³

Farm-level udder hygiene metrics

Average udder hygiene score

Prop. dirty udders (%; udder hygiene score ≥3)

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Table 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

	Overall	Bedded	Tiestalls	Freestalls	
Bacteria group (cfu/mL)	(n = 21)	packs $(n = 5)$	(n = 10)	(n=6)	<i>P</i> -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 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 (log ₁₀ cells/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)

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

¹¹⁰⁸

% chronically elevated				
SCS ¹	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 \geq 4.0 current test ¹	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. SCS ²	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

Table 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)	<i>P</i> -value
BTSCC (le	og ₁₀ cells/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 e	elevated SCS			
	Intercept		-1.6 (2.5)	
	Facility type (forced)	Freestall $(n = 6)$	2.3 (2.2)	0.33
		Tiestall $(n = 10)$	0.43 (1.9)	0.82
		Bedded pack $(n = 3)$	Ref.	Ref.
	Use bedding amendment	Yes (n = 5)	3.9 (1.8)	0.05
		No (n = 14)	Ref.	Ref.
	Subjective assessment air quality (researcher)	Good $(n = 14)$	3.6 (1.5)	0.04
		Fair $(n = 5)$	Ref.	Ref.
	Glove use at milking ¹	Never/Inconsistently $(n = 9)$	2.0 (1.3)	0.17
		Always $(n = 9)$	Ref.	Ref.
	Clinical mastitis events record keeping	Never kept records $(n = 6)$	4.4 (1.8)	0.03
		Sometimes/Temporarily kept records $(n = 6)$	1.1 (1.6)	0.52
		Always kept records $(n = 7)$	Ref.	Ref.
% chronic	ally elevated SCS			
	Intercept		5.3 (3.6)	
	Facility type (forced)	Freestall $(n = 6)$	1.5 (4.6)	0.75
		Tiestall $(n = 10)$	-0.08 (3.5)	0.98
		Bedded pack $(n = 3)$	Ref.	Ref.
	Feed supplemental vit. E and selenium ²	Yes (n = 11)	2.1 (2.8)	0.48
		No $(n = 7)$	Ref.	Ref.

	Use bedding amendment	Yes (n = 5)	5.7 (3.4)	0.12
		No $(n = 14)$	Ref.	Ref.
	Clip/flame udder hair	Yes (n = 5)	-6.3 (3.1)	0.07
		No $(n = 14)$	Ref.	Ref.
	% udder hygiene scores ≥3	Herds with available data $(n = 19)$	17.0 (6.1)	0.02
$\%$ SCS ≥ 4	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
Č	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	Yes (n = 5) No (n = 15) Yes (n = 5) No (n = 15)		
	(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
Standardiz	zed 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			

²One farm unable to provide response

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Table 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

O de la Falla de Maria II		Coefficient estimate	D 1	T
Outcome Explanatory Variable	Group (sample size)	(SE)	<i>P</i> -value	Intercept
BTSCC (log ₁₀ cells/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 milking ²	Never/Inconsistently $(n = 9)$	0.10 (0.07)	0.19	5.1
	Always $(n = 11)$	Ref.	Ref.	
% newly elevated SCS ³				
Model 4 Glove use at milking	Never/Inconsistently $(n = 9)$	2.83 (1.7)	0.11	4.3
	Always $(n = 9)$	Ref.	Ref.	
Model 5 Depth of bedding in stalls (cm) ¹	Tiestalls and freestalls $(n = 15)$	-0.62 (0.24)	0.02	8.3
% chronically elevated SCS ³				
Model 6 Clip/flame udder hair	Yes (n = 5)	-4.31 (2.9)	0.16	14.8
	No $(n = 14)$	Ref.	Ref.	
Model 7 % udder hygiene scores ≥3	Herds with available data $(n = 19)$	12.7 (6.1)	0.05	8.6
Model 8 Avg. udder hygiene score	Herds with available data $(n = 19)$	6.39 (3.1)	0.05	-1.2
% SCS \geq 4.0 current test ³				
Model 9 Depth of bedding in stalls (cm) ¹	Tiestalls and freestalls $(n = 15)$	-1.2 (0.42)	0.01	30
Model 10 % udder hygiene scores ≥3	Herds with available data $(n = 19)$	13.6 (8.5)	0.13	19.6
Model 11 Avg. udder hygiene score	Herds with available data $(n = 19)$	7.7 (4.3)	0.09	7.1
Average SCS ⁴				
Model 12 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 13 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 14 Depth of bedding in stalls (cm) ¹	Tiestalls and freestalls $(n = 15)$	-0.05 (0.03)	0.10	2.6

Model 15 % udder hygiene scores ≥3 Model 16 Avg. udder hygiene score	Herds with available data ($n = 20$) Herds with available data ($n = 20$)	0.75 (0.45) 0.39 (0.23)	0.12 0.11	2.1 1.5
c 76	Tierds with available data (ii 20)	0.57 (0.25)	0.11	1.3
Standardized 150-day milk (pounds) ⁵				
Model 17 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 18 Herd size	Herds with available data $(n = 18)$	0.26 (0.14)	0.07	33.1
% udder hygiene scores ≥3				
Model 19 Depth of bedded pack (m)	Bedded pack herds $(n = 5)$	-0.5 (0.06)	0.004	0.97
Model 20 Lying surface	Mattress or concrete $(n = 13)$	0.17 (0.08)	0.06	0.30
	Deep bedding $(n = 8)$	Ref.	Ref.	
Model 21 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 22 Depth of bedded pack (m)	Bedded pack herds (n = 5)	-0.96 (0.15)	0.008	3.4
Model 23 Lying surface	Mattress or concrete $(n = 13)$	0.33 (0.16)	0.06	2.1
	Deep bedding $(n = 8)$	Ref.	Ref.	
Model 24 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 wo	ood 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.				
DITIA data available for it – 10 licius.				

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⁵ DHIA data available for n = 18 herds.