J. Dairy Sci. 102:10213–10234
https://doi.org/10.3168/jds.2019-16692

Relationships among bedding materials, bedding bacteria counts, udder hygiene, milk quality, and udder health in US dairy herds

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ABSTRACT

Bedding is an important source of teat end exposure to environmental mastitis pathogens. To better control environmental mastitis, we need an improved understanding of the relationships among bedding selection and management, bedding bacteria counts (BBC), and udder health (UH). The objectives of this cross-sectional observational study were (1) to describe BBC, bedding characteristics, udder hygiene scores, bulk tank milk (BTM) quality, and UH in US dairy herds using 1 of 4 bedding materials; (2) describe the relationship between BBC and herd measures of UH; and (3) identify benchmarks for monitoring bedding hygiene. Local dairy veterinarians and university researchers enrolled and sampled 168 herds from 17 states. Herds were on a Dairy Herd Improvement Association (DHIA) testing program and used 1 of 4 bedding types for lactating cows: new sand, reclaimed sand, manure solids (MNS), or organic non-manure materials. Each herd was sampled twice (winter and summer) in 2016. Samples and data collected included unused and used bedding, BTM samples, udder hygiene scores, DHIA test data, and descriptions of facilities and herd management practices. Bedding was cultured to determine the total bacteria count and counts of *Bacillus* spp., coliforms, *Klebsiella* spp., non-coliform gram-negative organisms, streptococci or streptococci-like organisms (SSLO), and *Staphylococcus* spp. Bedding dry matter, organic matter, and pH were also measured. Bulk tank milk samples were cultured to determine counts of coliforms, NAS, SSLO, *Staphylococcus aureus*, and *Mycoplasma* spp. Udder health measures included DHIA test-day average linear score (LS); the proportion of cows with an intramammary infection (IMI), where infection was defined as LS $\geq$4.0; the proportion of cows with a new IMI, where new IMI was defined as LS changing from $<$4.0 to $\geq$4.0 in the last 2 tests; the proportion of cows with a chronic infection, where chronic was defined as LS $\geq$4.0 on the last 2 tests; and the cumulative incidence of clinical mastitis in the 30-d period preceding sample collection. Although much variation existed within and among bedding types, mixed linear regression showed the use of MNS bedding to be generally associated with higher BBC, dirtier udders, increased coliform and SSLO counts in BTM, and poorer UH measures compared with organic non-manure materials, reclaimed sand, or new sand bedding materials. While controlling for important farm traits and management practices, mixed linear regression showed that increased counts of coliforms, *Klebsiella* spp., SSLO, and *Staphylococcus* spp. in both unused and used bedding were associated with poorer values for 1 or more herd-level measures of UH. Achievable benchmarks identified for counts of coliforms (unused: $\leq$500 cfu/cm^3; used: $\leq$10,000 cfu/cm^3), *Klebsiella* spp. (0 cfu/cm^3 for unused and used), *Staphylococcus* spp. (0 cfu/cm^3 for unused and used), and SSLO (unused: 0 cfu/cm^3; used: $\leq$500,000 cfu/cm^3) can be used to monitor bedding hygiene in most bedding materials, with minor variations suggested for SSLO in unused MNS ($\leq$1,000 cfu/cm^3).
Key words: bedding, bacteria count, milk quality, mastitis

INTRODUCTION

With decades of progress in reducing the prevalence and effect of contagious mastitis pathogens such as *Staphylococcus aureus*, the control of infections with environmental pathogens has become a primary mastitis concern on most US dairy farms (Ruegg, 2017). Environmental mastitis is most frequently caused by environmental streptococci or streptococci-like organisms (**SSLO**; e.g., *Streptococcus uberis*, *Lactococcus lactis*), the coliform bacteria (e.g., *Escherichia coli*,

Received March 27, 2019.
Accepted June 29, 2019.
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Klebsiella spp.), and NAS (e.g., *Staphylococcus chromogenes*; Piepers et al., 2007; Oliveira et al., 2013). Environmental mastitis control strategies focus on 4 basic pillars, the first 3 of which were presented by Klaas and Zadoks (2018): (1) reduce bacterial load in the cow's environment, (2) remove bacterial load frequently from teats to prevent intrusion, (3) increase host resistance and resilience, and (4) increase or improve mastitis control practices (e.g., case detection and management, dry-off procedures).

Figure 1 offers a causal diagram depicting the possible relationships among these 4 pillars of mastitis control and udder health (**UH**). Specific strategies focused on pillars 2, 3, and 4 have been described elsewhere (Hogan and Smith, 2012; Ruegg, 2017). Although good progress has been made regarding pillar 1, there is still a great deal to learn regarding strategies to reduce teat end exposure to mastitis pathogens between milkings (Klaas and Zadoks, 2018). Cows spend 12 to 14 h/d lying down (Krawczel and Grant, 2009), so bedding is an important source of teat end exposure to environmental mastitis pathogens. Multiple studies have reported that bedding bacteria counts (**BBC**) are associated with bacterial load on the teat end (Bramley and Neave, 1975; Paduch et al., 2013; Rowbotham and Ruegg, 2016a). Furthermore, evidence is mounting to demonstrate a positive association between BBC and risk for IMI. In particular, high coliform counts in bedding have been associated with an increased risk for new coliform infections (Bramley and Neave, 1975; Carroll and Jasper, 1980; Hogan et al., 1989).

Closely related to this discussion are questions surrounding the importance of bedding material selection. Certain mastitis pathogens may be ubiquitous in some bedding materials, whereas others, such as *E. coli* or *Klebsiella* spp., may arrive due to contamination of bedding by fecal material, water, or feed (Klaas and Zadoks, 2018). Because bacteria require organic nutrients and moisture to survive, BBC are generally reported to be higher in organic bedding (**OB**) materials such as manure solids (**MNS**) or organic non-manure bedding (**ON**; e.g., sawdust, straw) compared with inorganic bedding (**IB**) such as new sand (**NS**; Rowbotham and Ruegg, 2016a; Bradley et al., 2018). Because reclaimed sand (**RS**) may have increased levels of OM, it may support higher BBC compared with NS (Kristula et al., 2005). Despite the differences in BBC among different bedding materials, studies report equivocal results regarding the relationship between bedding material selection and UH. In some studies, the use of IB (vs. OB) was associated with reduced clinical mastitis risk (Hogan et al., 1989) or lower SCC measures (Wenz et al., 2007; Rowbotham and Ruegg, 2015). However, a recent experimental study reported that bedding type

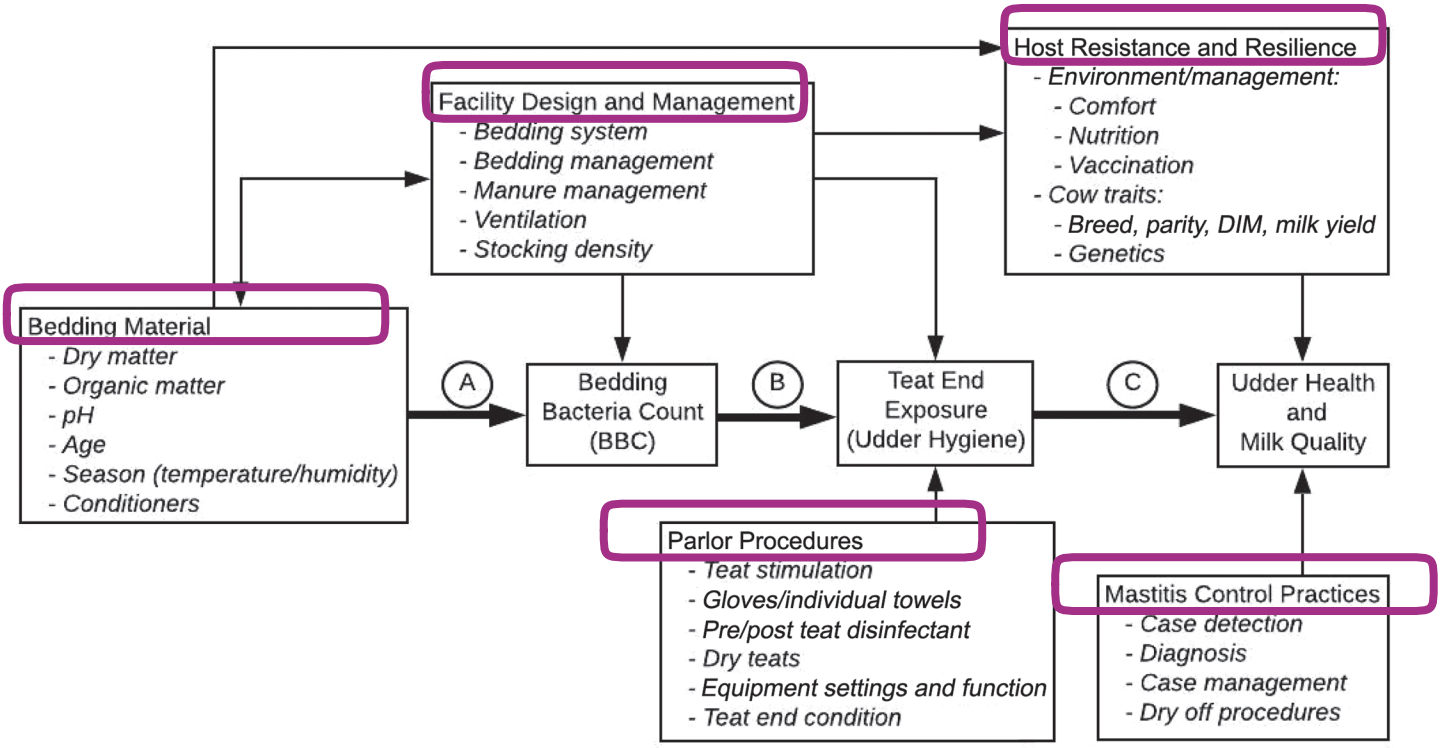

Figure 1. Suggested causal pathway describing major risk factors for environmental mastitis. Heavy arrows (lines A, B, and C) depict major relationships of interest in this study.

did not affect the incidence of clinical or subclinical mastitis in primiparous cows, although there was a tendency for greater delay in when clinical mastitis occurred for cows housed on deep-bedded NS compared with RS or deep-bedded MNS (Rowbotham and Ruegg, 2016b). Yet, the use of sawdust has been implicated in the epizootiology of coliform mastitis, particularly in *Klebsiella* mastitis (Carroll and Jasper, 1980). The relationship between bedding material and milk quality is also nebulous, with 2 recent observational studies, 1 in 325 Wisconsin herds and 1 in 125 UK herds, reporting no association between use of MNS (vs. IB or ON) and bacteria counts in bulk tank milk (**BTM**; Rowbotham and Ruegg, 2015; Bradley et al., 2018). An additional concern, though not a focus of this study, is that bedding type may influence mesophilic and thermophilic spore levels in bulk tank raw milk, which can cause spoilage of dairy products (Gleeson et al., 2013).

Assuming that BBC are associated with IMI risk, approaches to monitoring bedding hygiene are another area in need of study. Producers may submit bedding samples for laboratory culture to describe BBC. However, we need more evidence to establish benchmarks by which to interpret bedding culture reports. Previously suggested benchmarks have been limited to coliform bacteria and were most frequently derived for conventional OB materials such as shavings. For example, Bramley (1985) reported that *E. coli* mastitis was increased if cows were housed on sawdust with coliform counts exceeding 10^6 cfu/g of wet bedding.

To summarize, to improve environmental mastitis control, we need a better understanding of the relationships among bedding selection, BBC, and UH. Additionally, we need evidence-based benchmarks for monitoring bedding hygiene. As such, we set out to conduct a cross-sectional observational study to address the following 3 objectives: (1) describe BBC, bedding characteristics, udder hygiene scores, BTM quality, and UH in herds using 4 bedding types; (2) describe the relationship between BBC and herd measures of UH; and (3) identify benchmarks for monitoring bedding hygiene. For objective 1, we completed a simple analysis of factors associated with use of specific bedding materials, as identified on the main causal pathway proposed in Figure 1 (lines A, B, and C). For objective 2, we completed an analysis of the direct relationship between BBC and herd measures of UH while also controlling for, and describing the influence of, other herd factors related to housing, parlor procedures, routine mastitis control practices, and host resistance and resilience. When associations were detected between BBC and UH, we identified benchmarks (objective 3) to guide in the interpretation of bedding culture reports.

MATERIALS AND METHODS

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

Herd Enrollment and Sampling

A convenience sample of 189 herds was enrolled from 17 states with the assistance of 50 participating dairy veterinarians and 11 university researchers. Eligible herds had to use 1 of 4 bedding types for lactating cows: NS, RS, recycled MNS, or ON materials such as shavings or straw. Herds had to house all lactating cows on the same bedding type (no grazing allowed), be on a regular DHIA testing program, and keep records of clinical mastitis events. Sampling was conducted twice for each herd (winter and summer) in 2016 and was completed by the herd veterinarian or local university researcher using standardized protocols for collecting bedding and BTM samples and for udder hygiene scoring.

Bedding Sample Collection. Wearing clean disposable gloves, the sampler collected unused bedding from the bedding storage area by collecting grab samples from the top 5 cm of bedding from 15 random locations in the pile. After mixing in a clean bucket, a composite sample was transferred to two 1-quart (946.4 mL) Ziploc (SC Johnson, Racine, WI) bags. The age of the unused bedding (days that it had been in storage) was recorded. Used bedding was collected as a grab sample from the top 5 cm of bedding in the back one-third of 15 randomly selected stalls or locations in the yard, representing up to 5 lactating pens, and then mixed well in a clean bucket before being transferred into two 1-quart Ziploc bags. Samplers avoided manure pats. If more than 5 lactating pens existed, then samples were collected from 5 pens housing early- or peak-lactation cows and heifers. The age of the used bedding sample was recorded as the days since fresh bedding was most recently added to the stall or resting area. All bedding samples were placed on ice at the farm and then frozen at −20°C until being transported to the laboratory for analysis.

BTM Sample Collection. Producers collected duplicate BTM samples daily for 3 consecutive days within ±7 d of collecting bedding samples. Samples were collected either from the on-farm bulk tank or, for farms that directly loaded into tankers, from the tanker truck as the milk was unloaded at the milk processing facility. Samples were frozen immediately after collection and stored at −20°C until being transported to the laboratory for analysis.

Udder Hygiene Scores. Udder hygiene scoring was completed for 20 randomly selected cows housed in the same pens from which used bedding samples were collected using a 4-point scoring system described by Schreiner and Ruegg (2003), 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). If bedding was sampled from multiple pens, the 20 cows scored were selected in equal numbers from those same pens.

Herd Management Practices. At the first sampling event, the herd owner or manager completed a questionnaire describing herd characteristics, facilities, and practices surrounding bedding management, parlor routines, and mastitis control practices (Table 1). At the second sampling event, a shorter questionnaire was administered to identify any important management changes occurring in the interval between the first and second sampling events.

Herd Measures of UH. Herd-level DHIA test results for the test day immediately following each bedding sampling event were captured from the record processing center working with each herd (Dairy Records Management Systems, Raleigh, NC; AgSource, Verona, WI; Amelicor, Provo, UT; AgriTech, Visalia, CA). Information captured included herd ID, test date, number of lactating cows, average DIM, average test-day milk yield (kg/cow), average 305 ME (kg/cow), and the following UH measures: test-day average linear score (**AVLS**); the proportion of cows with an IMI, where infection was defined as linear score (**LS**) $\geq$4.0; the proportion of cows with a new IMI (**NIMI**), where an NIMI was defined as LS changing from <4.0 to $\geq$4.0 in the last 2 tests; and the proportion of cows with a chronic infection (**CRON**), where CRON was defined as LS $\geq$4.0 on the last 2 tests. Finally, we recorded the monthly cumulative incidence of clinical mastitis (**CLXM**) as the proportion of cows reported by the owner to experience a clinical mastitis event during the 30-d period preceding sample day. Because DHIA test intervals varied among herds, the NIMI variable was adjusted to reflect a standardized 30.5-d inter-test interval for each herd.

Laboratory Analysis of Bedding and Milk Samples

Bedding Culture. Frozen bedding and BTM samples were shipped on ice to the Laboratory for Udder Health (University of Minnesota Veterinary Diagnostic Laboratory, St. Paul) for analysis. After thawing at room temperature, 50 cm^3 of packed bedding material was weighed and measured into a new Whirl-Pak bag (Nasco, Fort Atkinson, WI), 250 mL of sterile water was added, and the contents were mixed and left to stand for 10 min. Serial 10-fold dilutions of the samples were made using sterile water (Becton Dickinson and Company, Franklin Lakes, NJ). Sample dilutions were plated onto MacConkey agar (gram-negative bacteria selection) and colistin naladixic acid agar (gram-positive bacteria selection, Becton Dickinson and Company) plates and incubated overnight at 37°C. For the MacConkey plates, lactose fermenting (pink) colonies were counted as coliform bacteria and all other colonies were counted as non-coliform gram-negative bacteria. Colonies with a confluent appearance on MacConkey agar were identified to the genus level using a MALDI Biotyper (Bruker Daltonics, Billerica, MA), and colonies identified as *Klebsiella* spp. were counted and reported as a percentage of total coliform count. For colistin naladixic acid plates, colony morphology in conjunction with catalase reaction and Gram stain were used to differentiate colonies of *Staphylococcus* spp., SSLO, and *Bacillus* spp. Total bacteria count (**TBC**) and counts of *Bacillus* spp., coliforms, *Klebsiella* spp., non-coliform gram-negatives, *Staphylococcus* spp., and SSLO were recorded as colony-forming units per cubic centimeter of wet bedding. The minimum limit of detection was 25 cfu/cm^3.

Bedding Characteristics (DM, OM, pH). Immediately after sampling for culture, bedding samples were refrozen (−20°C) and submitted for determination of OM percentage, DM percentage, and pH. After thawing to room temperature, each bedding sample was mixed in duplicate with 50 mL of deionized water and remixed at 10-min intervals (Godden et al., 2008). After 30 min at room temperature, pH of the mixture was determined using a Beckman Φ32 pH meter (Beckman Instruments, Fullerton, CA). Bedding (2 g) was dried in duplicate at 100°C for 24 h to determine DM (%) content, and dried bedding was ashed at 550°C overnight to determine ash content. Organic matter (%) was determined by subtracting the ash content from 100 percent.

BTM Culture. After thawing to room temperature, bulk milk and a 10-fold dilution of the bulk milk sample were plated onto MacConkey, Factor (gram-positive selective agar; University of Minnesota, 2016), and Focus (University of Minnesota, St. Paul, selective for SSLO bacteria) media plates and incubated for 2 d at 37°C. Lactose fermenting (pink) colonies on MacConkey medium were counted and reported as coliform bacteria. All β-hemolytic colonies on Focus medium were counted and identified to the species level using a MALDI Biotyper, as these colonies were suspect for *Streptococcus agalactiae.* All colonies on Focus medium that

Table 1. Farm traits, facilities, bedding management, parlor procedures and mastitis control practices collected in the herd management questionnaire

Management category	Factors described
Farm traits	State
	Region (Northeast, Midwest, West, South)
	Predominant breed
	Herd size (lactating/dry)
	Conventional (vs. organic)
Lactating cow housing	Lactating housing type
	Access to outside yard
	Ventilation system
	Subjective assessment of air quality
	If freestall or tiestall housing:
	Lying surface
	Presence of brisket locator
	Number of lactating cow pens
	Number of stalls per pen
	Average stocking density per pen (cows/stall)
	Stall length from brisket board to back of curb
	If loose housing (dry lot or bedded pack):
	Resting area (m^2/cow)
Lactating cow bedding	Bedding type (organic/inorganic)
	Bedding material (new sand, recycled sand, manure solids, organic non-manure)
	If new sand:
	Source of sand
	Washed or not
	If recycled sand:
	Method of recycling (passive sand lanes vs. mechanical)
	Average time to reuse
	Storage (open or under cover)
	If manure solids:
	Reclamation process (raw, digested, composted)
	Screw press (yes/no)
	Mechanical drying (yes/no)
	Average time to reuse (days)
Bedding management for lactating cows	Frequency of adding new bedding material to resting area and stalls
	Depth of bedding on lying surface
	Use (and frequency/type of product) of bedding conditioner
	Frequency of scraping alleyways
	If loose housing (dry lot or bedded pack):
	Frequency of raking or tilling bedding surface
	If deep bedding systems:
	Frequency of complete removal and replacement
Milking procedures	Milking frequency per day
	Milking system
	Frequency of spraying off milking units with water between cows
	Forestrip teats
	Use of predip
	Use of mechanical teat scrubber
	Use of individual towel to dry teats before milking unit attachment
	Use of postdip
	Clip or flame udders
	Dock tails or trim switches
Mastitis control practices	Clinical mastitis detection method (e.g., forestripping)
	Routine culture of clinical mastitis cases (yes/no)
	Routine culture of cows with elevated SCC (yes/no)
	Routine culture of fresh cows (yes/no)
	Mastitis records (yes/no)
	Protocols to treat mild, moderate, and severe clinical mastitis
	Number of clinical mastitis events in the 30 d preceding sampling
Dry-off routines	Use of long-acting intramammary antibiotic
	Use of teat sealant
	Vaccination for mastitis control
	Treatment of nulliparous heifers with antibiotics or teat sealants before first calving

were not identified as *Strep. agalactiae* were counted and recorded as SSLO. β-Hemolytic colonies on Factor medium were counted and identified to the species level using a MALDI Biotyper, and those with a confidence score ≥2.0 for *Staph. aureus* were counted and reported as such. Non-hemolytic colonies of *Staphylococcus* spp.

(based on colony morphology, catalase reaction, or Gram stain) were counted and reported as NAS. For *Mycoplasma* spp., 0.1 mL of BTM was swabbed across the entire surface of a *Mycoplasma* agar plate and incubated for 7 d in a 7% CO_2 incubator at 37°C. Plates were examined for *Mycoplasma* spp., and colonies were counted by a trained microbiology technician. For each BTM sample, total counts of coliforms, NAS, SSLO, *Staph. aureus*, *Strep. agalactiae*, and *Mycoplasma* spp. were recorded as colony-forming units per milliliter of milk. The minimum limit of detection for the BTM culture protocol was 5 cfu/mL.

Data Management and Analysis

Data were entered into an Excel database (Microsoft Corp., Redmond, WA). Statistical analysis was performed using SAS version 9.4 (SAS Institute Inc., Cary, NC), with the farm being the unit of analysis and 2 observations (summer and winter) available per farm. Information recorded for each observation included herd ID, season, sample collection date, information captured in the herd management questionnaire, udder hygiene scores, DHI test results, CLXM, BTM culture results, culture results for unused and used bedding, and bedding characteristics (DM, OM, pH). Udder hygiene scores were summarized both as the mean udder hygiene scores and as the proportion of cows with dirty udders (udder hygiene score $\geq$3). Descriptive statistics were calculated to evaluate the distribution of data and data integrity and to identify missing data. Because BBC (cfu/cm^3) and BTM culture results (cfu/mL) were not normally distributed, results were transformed ($\log_{10}$) before further analysis. Continuous variables underwent correlation analysis to identify variables that were highly associated ($R^2 \geq 0.60$). Simple associations among categorized variables were evaluated using a chi-squared test. Descriptive statistics were generated to describe herd characteristics, lactating cow housing, bedding management, milking procedures, and mastitis control practices. After stratifying by bedding type (NS, RS, MNS, and ON), descriptive statistics were also generated to describe BBC, bedding characteristics, BTM culture results, udder hygiene score measures, CLXM, and DHIA test-day results.

For objective 1, mixed linear regression (PROC MIXED) was used to investigate the direct relationship between bedding type (explanatory variable; NS, RS, MNS, or ON) and each of the following herd-level dependent variables: herd traits (herd size, average DIM, 305 ME), udder hygiene scores (mean udder hygiene score, proportion of cows with udder hygiene score $\geq$3), UH measures (AVLS, IMI, NIMI, CRON, CLXM), and BTM culture results. Because a moderate to high proportion of BTM samples were culture negative for *Staph. aureus* or *Mycoplasma* spp., respectively, a multivariable logistic model was used to evaluate the relationship between bedding material and risk for a positive (vs. negative) bulk tank culture result for *Staph. aureus* or *Mycoplasma* spp. In addition to including bedding type, variables describing season (summer and winter) and region (Northeast, Midwest, West, and South) were offered to each model but subject to removal using a backward stepwise elimination process if $P > 0.10$. Herd was included as a random effect. Overall statistical significance for main effects was declared at $P \leq 0.05$ and a trend at $0.05 < P \leq 0.10$. The Bonferroni correction factor was used to adjust the critical P-value to perform multiple contrasts among the 4 bedding types. A similar modeling approach was used to investigate the relationship between bedding type (explanatory variable) and BBC, DM, OM, and pH in both unused and used bedding samples (dependent variables), with different BBC groups analyzed, including TBC, *Bacillus* spp., coliforms, *Klebsiella* spp., non-coliform gram-negatives, SSLO, and *Staphylococcus* spp.

For objective 2, univariable linear regression (PROC MIXED) was initially used to describe unconditional relationships between each of the 5 UH dependent variables of interest (AVLS, IMI, NIMI, CRON, and CLXM) and each of the BBC explanatory variables in unused or used bedding (TBC, *Bacillus* spp., coliforms, *Klebsiella* spp., non-coliform gram-negatives, SSLO, and *Staphylococcus* spp.), bedding type (NS, RS, MNS, and ON), bedding age (d), and a variety of potential explanatory variables describing season, herd characteristics, housing, parlor procedures, and mastitis control practices (Table 1). Any explanatory variable that was unconditionally associated with 1 or more of the 5 UH outcomes of interest at $P < 0.20$ was offered into the final multivariable models investigating the relationship between BBC and UH. The only variable forced into these multivariable models was bedding type. Herd was included as a random effect. A backward stepwise variable selection process was used, with the least significant variables being removed one by one until all remaining predictors had $P \leq 0.10$. At each removal step, the potential for confounding was investigated by examining the effect of each explanatory variable on the estimate of the association between BBC and UH parameters. A variable was identified as a confounder and retained in the model if its inclusion resulted in >15% change in the estimate of the effect of BBC on the UH outcome. Upon reaching the final main effects model, we investigated whether nonlinear rela-

tionships existed between BBC measures and UH and explored all biologically plausible interactions between BBC measures and other covariates. Final models were selected based on lowest Akaike information criteria, and final model fit was assessed by plotting the deviance residuals. Statistical significance was declared at $P \leq 0.05$ and a trend at $0.05 < P \leq 0.10$.

For objective 3, we categorized BBC counts (e.g., low and high) for those bacteria groups that were determined to be associated with UH in objective 2. The cutpoints selected to create categories needed to be achievable by producers (i.e., ≥20% of samples achieved a "low" value), and the differences in UH performance for herds between low and high categories should be statistically and numerically different. Also, where possible, we aimed to identify cutpoints for categorization that could be used universally for any bedding material. The first step in this process was to examine BBC in unused and used samples by quartile to evaluate the variation present and how much overlap in distribution existed among the different bedding materials. For this analysis we further stratified the ON group to evaluate straw and shavings separately because preliminary analysis (not shown) suggested differences between these 2 materials when creating optimal benchmarks. The second step in this process was to categorize the BBC for each bacteria group into either 2 (low and high) or 3 (low, medium, and high) categories. For bacteria groups with ≥50% of samples achieving a value of 0 cfu/cm^3, only 2 categories (low and high) were created with an initial cutpoint of 0 (low) or >0 (high). However, for bacteria groups with ≤50% of samples achieving a value of 0 cfu/cm^3, 3 categories (low, medium, and high) were created, where the initial BBC cutpoints represented approximately the lower, middle, and upper third of samples. All categories initially created were tested for fit within a multivariable linear regression model to ensure that the BBC categorical variable (e.g., low or high; explanatory variable) was still associated with impaired UH (dependent variable). From there, we empirically lowered or raised the thresholds for BBC cutpoints by incremental degrees to create either low and high or low, medium, and high categories that produced the model of best fit, as indicated by the model Akaike information criterion value. The process of identifying the optimal cutpoints for BBC was first completed for all bedding materials combined in the same model while controlling for bedding material (forced) and including all other covariates that had been maintained in the initial models developed in objective 2 (e.g., parlor procedures, mastitis control practices, facilities). This modeling process was then repeated after stratifying the data by bedding material (NS, RS, MNS, straw, and shavings) to identify where similar cutpoints could be used among the 5 bedding materials of interest.

RESULTS

Description of Study Herds

Of 189 herds originally enrolled, 21 were omitted from analysis for the following reasons: use of limestone or using multiple bedding types for lactating cows (n = 3), use of multiple housing systems for lactating cows (n = 5), missing DHIA data (n = 10), or incomplete herd management questionnaire (n = 3). The final analysis included 324 records from 168 herds in 17 states: Wisconsin (74), Minnesota (69), California (27), Indiana (24), Ohio (22), New York (17), Iowa (16), Michigan (15), South Dakota (13), Maine (8), Missouri (8), Texas (8), Idaho (7), Georgia (6), Vermont (6), Florida (2), and Washington (2). Most herds were from the Midwest region (73.8%, n = 123), followed by the West (11.3%, n = 19), Northeast (10.1%, n = 17), and South (4.8%, n = 8). Of the 168 herds, 19.6% (n = 33), 34.5% (n = 58), 18.5% (n = 31), and 27.4% (n = 46) used MNS, ON, RS, and NS, respectively. All 4 bedding groups were represented in all regions, with the exception that no Southern herds used ON materials and no Western herds used RS.

The median (mean; range) number of lactating cows was 387 (897; 34–9,581), with a mean (±SD; range) DIM of 176.3 (±18.1; 92–278) and a mean 305 ME of 11,944 kg (±1,475; 7,132–14,795). The median DHIA testing frequency was 12 tests/yr; 160 herds had 8 or more tests/yr, 7 herds had 6 tests/yr, and 1 herd had 4 tests/yr. The median (mean; range) test-day average SCC was 196,000 cells/mL (218,765; 43,000–837,000). The overall (SD and range) AVLS, IMI, NIMI, CRON, and CLXM were 2.44 (±0.56; 1.0–4.1), 22.0% (±9.6; 1.0–55.0), 8.0% (±3.9%; 0–21%), 12.1% (±6.7%; 1.0–40.0), and 3.44% (±2.9; 0–19.6), respectively. The mean udder hygiene score and proportion of cows with dirty udders (udder hygiene score ≥3) was 1.85 (±0.40; 1.0–3.1) and 18.1% (±15.4; 0–85), respectively. Of 324 BTM samples cultured, 5.9% (19–324) and 45.7% (148–324) were positive for *Mycoplasma* spp. and *Staph. aureus*, respectively. No BTM samples were positive for *Strep. agalactiae*. The median (mean; range) age of unused and used bedding samples collected was 7 d (38.1; 0–730) and 3 d (4.6; 0–180), respectively. A description of processing and storage methods for unused bedding is provided in Supplemental Table S1 (https://doi.org/10.3168/jds.2019-16692). Freestall, tiestall, bedded pack, and dry lot facilities made up

85.7% (n = 144), 7.7% (n = 13), 4.2% (n = 7), and 2.4% (n = 4) of facilities, respectively. Detailed descriptions of housing characteristics (e.g., resting surface, ventilation, stocking density) and bedding and manure management practices (e.g., bedding depth, frequency of adding new bedding, manure removal) are provided in Supplemental Table S2 (https://doi.org/10.3168/jds.2019-16692). Detailed descriptions of routine milking practices and mastitis control practices are provided in Supplemental Tables S3 and S4 (https://doi.org/10.3168/jds.2019-16692), respectively.

BBC, Bedding Characteristics, Udder Hygiene Scores, BTM Quality, and UH in Herds Using 4 Bedding Types

The median herd size was larger for herds using MNS (1,478 cows) and RS (964 cows) compared with herds using NS (271 cows) or ON (212 cows; $P < 0.0001$; Table 2). Mean 305 ME was lowest for herds using ON bedding (11,481 kg) compared with NS (11,909 kg), MNS (12,072 kg), and RS (12,778 kg; $P = 0.0005$). The lowest and highest average udder hygiene scores were in herds using RS (1.73 ± 0.39) or MNS (1.94 ± 0.45), respectively, with intermediate scores for ON and NS ($P = 0.052$). The proportion of cows with dirty udders (score ≥3) tended to be lower in RS herds (14.2%) compared with MNS herds (22.1%; $P = 0.09$; Table 2). When evaluating milk quality, SSLO levels in BTM were lowest in herds using ON (2.78 ± 0.73) and NS (2.78 ± 0.90) and highest in herds using MNS (3.16 ± 0.74; $P = 0.017$; Table 2). Overall, 99.7, 86.4, and 85.8% of all BTM samples tested were positive for SSLO, NAS, and coliforms, respectively. The proportion of BTM samples positive for SSLO (MNS = 100%, ON = 100%, RS = 100%, NS = 98.9%; $P = 0.47$), NAS (MNS = 93.9%, ON = 83.8%, RS = 81.8%, NS = 87.0%; $P = 0.26$), or coliforms (MNS = 90.9%, ON = 87.4%, RS = 81.8%, NS = 82.6%; $P = 0.39$) was not different among the 4 bedding types. However, when evaluating counts of bacteria in BTM, coliform levels in BTM were lowest in herds using RS (1.43 ± 1.78) and NS (1.74 ± 1.36) and highest in herds using ON (2.02 ± 1.29) and MNS (2.07 ± 1.28; $P = 0.026$). A greater proportion of herds using ON (59.5%) or MNS (53.0%) was positive for *Staph. aureus* in BTM compared with RS (36.4%) or NS (29.4%; $P = 0.002$). When evaluating the relationship between bedding material and UH, UH measures (± SD) were generally worse (higher) in herds using MNS (LS = 2.66 ± 0.56; NIMI = 10.32 ± 3.95%; CLXM = 4.68 ± 3.68%) and generally the best in herds using NS (LS = 2.36 ± 0.52; NIMI = 7.22 ± 3.79%; CLXM = 3.28 ± 2.85%) or RS (LS = 2.32 ± 0.44; NIMI = 7.69 ± 3.50%; CLXM = 2.14 ± 1.48%; $P < 0.05$; Table 2).

When comparing BBC among the 4 bedding groups, BBC were always lower for unused (vs. used) bedding samples (Table 3). Although some exceptions existed, within unused samples BBC for MNS and RS were generally higher than those for ON or NS. For example, TBC, coliform, non-coliform gram-negatives, and SSLO counts were higher in unused samples of MNS and RS compared with ON or NS. When examining used bedding samples, there was no difference in SSLO counts among the 4 bedding groups. However, coliforms, *Klebsiella* spp., and *Staphylococcus* counts were highest in MNS and generally lowest in RS or NS. When comparing bedding characteristics, large variation existed both within and among the 4 bedding material groups (Table 3). When considering DM values in unused samples, there was a wide range in DM values within MNS (21.4–96.3%) and ON (34.9–94.3%) but less variation for RS (85.9–98.9%) and NS (83.6–100%). Mean DM (± SD) was lowest for MNS (57.0 ± 26.4%) and highest for RS (93.6 ± 2.7%) and NS (95.6 ± 2.4%; $P < 0.0001$). The same general relationships held true when evaluating DM values in used bedding samples. When considering OM values in unused samples, variation was extremely high for MNS (14.1–95.4%) and ON (30.4–100%) compared with RS (0.6–13.0%) or NS (0–15.8%). Mean OM values were highest for ON (93.1 ± 14.1%) and MNS (76.0 ± 19.4%) and lowest for RS (2.9 ± 2.3%) and NS (1.7 ± 2.4%; $P < 0.0001$). These same general relationships also held true for OM values in used bedding samples. When considering pH values in unused samples, the mean (±SD) values were lowest for ON materials (6.1 ± 1.9), with similar higher values for MNS (9.0 ± 0.6), RS (9.4 ± 0.3), and NS (8.8 ± 1.1; $P < 0.0001$). A similar pattern was observed in used bedding samples, with pH values being lowest in ON.

Relationship Between BBC and Herd Measures of UH

Results of the initial univariable analysis of factors unconditionally associated with UH outcomes at $P < 0.20$, including BBC, herd traits, facility and ventilation traits, parlor routines, and mastitis control practices, are reported in Supplemental Table S5 (https://doi.org/10.3168/jds.2019-16692). No BBC measure was associated with CLXM (results not reported), so this UH outcome was not further investigated. Similarly, *Bacillus* spp. and non-coliform gram-negative counts were not associated with any UH outcomes (results not reported). When evaluated independently in univariable models, increased TBC, coliform, *Klebsiella* spp.,

Table 2. Herd traits, udder hygiene scores, udder health measures, and bulk tank milk culture results for 168 US dairy herds using 1 of 4 bedding types[1]

Item	Bedding type				*P*-value
	Manure solids	Organic non-manure materials[2]	Reclaimed sand	New sand	
Samples (no.)	66	111	55	92	
Herd traits					
Milk cows[3] (no.)	1,478 (171–9,581)^{a}	212 (34–1,877)^{b}	964 (60–5,574)^{a}	271 (74–6,069)^{c}	<0.0001
DIM	176 ± 18 (92–278)^{a}	175 ± 18 (141–228)^{a}	173 ± 14 (146–221)^{a}	180 ± 20 (134–278)^{a}	0.26
305 ME (kg)	12,072 ± 1,461 (8,249–14,795)^{a}	11,481 ± 1,355 (8,235–14,617)^{b}	12,778 ± 1,026 (9,361–14,653)^{a,A}	11,909 ± 1,632 (7,132–14,792)^{ab,B}	0.0005
Udder hygiene score					
Average score	1.94 ± 0.45 (1.0–3.1)^{a}	1.88 ± 0.37 (1.1–2.8)^{ab}	1.73 ± 0.39 (1.0–2.6)^{b}	1.81 ± 0.37 (1.2–2.9)^{ab}	0.052
Score ≥3 (%)	22.1 ± 19.1 (0–85)^{a,A}	18.9 ± 15.1 (0–70)^{a}	14.2 ± 12.9 (0–45)^{a,A}	16.7 ± 13.6 (0–60)^{a}	0.091
Udder health measures					
Average SCC[4]	248.4 ± 122.5 (97–837)	224.6 ± 103.6 (82–579)	199.1 ± 80.7 (43–439)	202.3 ± 102.9 (55–621)	NA[5]
Average linear score	2.66 ± 0.56 (1.6–3.9)^{a,A}	2.43 ± 0.62 (1.0–3.8)^{ab}	2.32 ± 0.44 (1.3–3.5)^{b}	2.36 ± 0.52 (1.4–4.1)^{ab,B}	0.026
IMI[6] (%)	24.42 ± 9.39 (11.0–55.0)^{a}	22.17 ± 10.40 (8.0–49.0)^{a}	20.93 ± 7.78 (1.0–43.0)^{a}	20.71 ± 9.51 (5.0–52.0)^{a}	0.25
NIMI[7] (%)	10.32 ± 3.95 (2.0–19.0)^{a,A}	7.39 ± 3.82 (0–21.0)^{ab,B}	7.69 ± 3.50 (0–19.0)^{ab}	7.22 ± 3.79 (1.0–20.0)^{b}	0.031
CRON[8] (%)	13.35 ± 6.97 (4.0–40.0)^{a}	12.42 ± 7.20 (3.0–33.0)^{a}	11.25 ± 5.06 (1.0–24.0)^{a}	11.25 ± 6.52 (1.0–37.0)^{a}	0.25
CLXM[9] (%)	4.68 ± 3.68 (0–19.6)^{a}	3.47 ± 2.54 (0–12.5)^{ab}	2.14 ± 1.48 (0–8.78)^{b}	3.28 ± 2.85 (0–12.5)^{ab}	0.035
Bulk tank culture results (log_{10} cfu/mL)					
SSLO[10]	3.16 ± 0.74 (1.88–4.10)^{a}	2.78 ± 0.73 (1.18–4.10)^{bc}	2.84 ± 0.66 (1.60–4.10)^{ac}	2.78 ± 0.90 (0–4.10)^{bc}	0.017
NAS	1.79 ± 1.02 (0–4.10)^{a}	1.49 ± 1.01 (0–4.10)^{a}	1.25 ± 0.79 (0–4.10)^{a}	1.50 ± 0.91 (0–4.10)^{a}	0.28
Coliforms	2.07 ± 1.28 (0–4.24)^{a,A}	2.02 ± 1.29 (0–4.10)^{a}	1.43 ± 1.78 (0–4.10)^{b,B}	1.74 ± 1.36 (0–4.10)^{ab}	0.026
Staphylococcus aureus[11] (%)	53.0^{a}	59.5^{a}	36.4^{ab}	29.4^{b}	0.002
Mycoplasma[11] (%)	10.6^{a}	2.7^{a}	7.3^{a}	5.4^{a}	0.31

$^{a–c}$Means within a row with different lowercase superscripts are statistically different at $P \leq 0.0083$.

^{A,B}Means within a row with different uppercase superscripts have a tendency for difference at $P \leq 0.017$ (*P*-values adjusted for multiple contrasts).

[1]Values are crude mean ± SD (range in parentheses). Results (reported for both summer and winter sampling events) are the DHIA test-day results for the first test following bedding sample collection.

[2]Organic non-manure materials, including shavings (n = 68), straw (30), and other organic materials (13), such as cornstalks (n = 1), Casella organics (n = 3; Casella Organics, Rutland, VT), furniture waste (n = 1), fiber bed (n = 1), rice hulls (n = 1), or mixes (n = 7).

[3]Median (range).

[4]DHIA test-day average SCC (×1,000 cells/mL).

[5]NA = not available.

[6]Proportion of cows on test day with a linear score ≥4.0.

[7]NIMI = new IMI. Proportion of cows with a linear score <4.0 on the previous test day and a linear score ≥4.0 on the current test day.

[8]CRON = chronic infection. Proportion of cows with a linear score ≥4.0 on both the previous and current test days.

[9]CLXM = monthly cumulative incidence of clinical mastitis. Proportion of cows reported by the owner to experience a clinical mastitis event during the 30-d period preceding sample day.

[10]Streptococci or streptococci-like organisms.

[11]Proportion (%) of samples with positive bulk tank culture.

Table 3. Bedding bacteria counts (log_{10} cfu/cm^3) and bedding characteristics for 168 US dairy herds using 1 of 4 bedding types[1]

Parameter	Bedding type				*P*-value
	Manure solids	Organic non-manure materials[2]	Reclaimed sand	New sand	
Observations (no.)	66	111	55	92	
Unused bedding samples					
Total bacteria count	5.86 ± 0.93 (3.86–7.28)^{a}	3.41 ± 1.99 (0–6.94)^{b}	5.62 ± 0.75 (3.96–7.0)^{a}	3.16 ± 1.25 (0–5.74)^{b}	<0.0001
Bacillus spp.	5.73 ± 0.90 (3.86–6.80)^{a}	2.48 ± 2.02 (0–6.78)^{b}	5.47 ± 0.75 (0–6.76)^{a}	3.01 ± 1.36 (0–5.70)^{b}	<0.0001
Coliforms	2.44 ± 1.89 (0–6.80)^{a}	0.82 ± 1.41 (0–5.99)^{b}	1.58 ± 1.54 (0–5.40)^{c}	0.19 ± 0.66 (0–3.62)^{d}	<0.0001
Klebsiella spp.	0.69 ± 1.38 (0–6.46)^{a,A}	0.30 ± 0.95 (0–4.15)^{ac,B}	0.15 ± 0.65 (0–3.23)^{bc}	0.036 ± 0.34 (0–3.30)^{bc,C}	0.0002
Non-coliform GN[3]	4.01 ± 1.91 (0–6.80)^{a}	2.53 ± 2.18 (0–6.34)^{b}	3.60 ± 1.54 (0–6.56)^{a}	0.70 ± 1.33 (0–4.56)^{c}	<0.0001
SSLO[4]	3.89 ± 2.10 (0–6.80)^{a}	1.10 ± 1.45 (0–6.30)^{b}	3.77 ± 1.76 (0–6.40)^{a}	1.31 ± 1.25 (0–4.70)^{b}	<0.0001
Staphylococcus spp.	0.89 ± 1.78 (0–6.22)^{a}	0.56 ± 1.16 (0–4.51)^{ab}	0.39 ± 0.97 (0–4.70)^{ab}	0.17 ± 0.57 (0–3.11)^{b}	0.004
DM (%)	57.0 ± 26.4 (21.4–96.3)^{a}	83.5 ± 13.0 (34.9–94.3)^{b}	93.6 ± 2.7 (85.9–98.9)^{c}	95.6 ± 2.4 (83.6–100)^{c}	<0.0001
OM (%)	76.0 ± 19.4 (14.1–95.4)^{a}	93.1 ± 14.1 (30.4–100)^{b}	2.9 ± 2.3 (0.6–13.0)^{c}	1.7 ± 2.4 (0–15.8)^{c}	<0.0001
pH	9.0 ± 0.6 (7.0–10.0)^{a}	6.1 ± 1.9 (3.7–12.5)^{b}	9.4 ± 0.3 (8.3–10.0)^{a}	8.8 ± 1.1 (4.6–10.5)^{a}	<0.0001
Used bedding samples					
Total bacteria count	6.66 ± 0.56 (5.12–7.29)^{a}	6.58 ± 0.50 (4.93–7.28)^{b}	6.69 ± 0.40 (5.18–7.18)^{ab}	6.82 ± 0.34 (5.01–7.27)^{a}	0.003
Bacillus spp.	6.35 ± 0.69 (3.10–6.80)^{a}	5.15 ± 2.36 (0–6.80)^{b}	6.34 ± 0.99 (0–6.83)^{a}	6.64 ± 0.40 (4.10–7.11)^{a}	<0.0001
Coliforms	4.38 ± 1.30 (0–6.62)^{a}	3.30 ± 1.62 (0–6.80)^{b}	3.06 ± 1.07 (0–4.58)^{b,A}	3.67 ± 1.02 (0–6.41)^{b,B}	<0.0001
Klebsiella spp.	1.68 ± 2.03 (0–6.58)^{a,A}	1.20 ± 2.79 (0–6.49)^{ab,B}	0.61 ± 1.15 (0–3.47)^{b}	1.01 ± 1.50 (0–5.19)^{b}	0.004
Non-coliform GN	5.03 ± 1.60 (0–6.80)^{a}	4.55 ± 1.41 (0–6.80)^{bc}	4.78 ± 0.62 (3.28–6.45)^{ac}	4.55 ± 1.01 (0–6.03)^{bc}	0.0018
SSLO	5.71 ± 1.04 (0–6.80)^{a}	6.12 ± 0.87 (0–6.99)^{a}	5.82 ± 1.39 (0–6.80)^{a}	6.06 ± 0.73 (2.94–6.80)^{a}	0.42
Staphylococcus spp.	2.92 ± 2.42 (0–6.80)^{ab}	2.29 ± 2.34 (0–6.80)^{a,A}	1.38 ± 1.89 (0–6.64)^{ab,B}	1.36 ± 2.01 (0–6.34)^{b}	0.006
DM (%)	59.5 ± 19.3 (26.8–89.3)^{a}	72.1 ± 13.2 (29.3–88.2)^{b}	95.6 ± 2.1 (89.5–99.4)^{c}	95.7 ± 2.2 (84.0–98.9)^{c}	<0.0001
OM (%)	73.5 ± 18.4 (15.4–97.0)^{a}	89.4 ± 13.6 (33.2–99.0)^{b}	4.1 ± 3.0 (0.7–15.4)^{c,A}	2.8 ± 2.4 (0.5–14.9)^{c,B}	<0.0001
pH	9.3 ± 0.6 (7.0–10.0)^{a}	8.6 ± 1.0 (5.8–10.5)^{b}	9.7 ± 0.3 (9.0–10.4)^{c}	9.5 ± 0.4 (8.3–10.4)^{ac}	<0.0001

$^{a–d}$Means within a row with different lowercase superscripts are statistically different at $P \leq 0.0083$.

$^{A–C}$Means within a row with different uppercase superscripts have a tendency for difference at $P \leq 0.017$ (*P*-values adjusted for multiple contrasts).

[1]Values are crude mean ± SD (range in parentheses). Combined results reported for both summer and winter sampling events. Models adjusted for season and region.

[2]Organic non-manure materials including shavings (n = 68), straw (30), and other organic materials (13), such as cornstalks (n = 1), Casella organics (n = 3; Casella Organics, Rutland, VT), furniture waste (n = 1), fiber bed (n = 1), rice hulls (n = 1), or mixes (n = 6).

[3]Non-coliform gram-negatives.

[4]Streptococci or streptococci-like organisms.

SSLO, and *Staphylococcus* spp. counts in unused bedding were all associated with increased values for 2 or more of the 4 UH outcome measures, including AVLS, IMI, NIMI, and CRON. For used bedding, TBC and SSLO counts were not associated with UH outcomes, whereas positive associations were detected between coliform, *Klebsiella* spp., and *Staphylococcus* spp. counts and 2 or more UH measures.

Results of the final multivariable models describing the relationship between BBC in unused and used bedding and UH measures are shown in Tables 4 and 5, respectively. When competing against other BBC groups in the same model, TBC was not associated with any UH outcome (results not shown). In unused bedding, a 1-$\log_{10}$ increase in *Klebsiella* spp. tended to be associated with an estimated (SE) increase in AVLS and IMI of 0.035 (0.021) and 0.65 (0.038), respectively ($P < 0.10$). A 1-$\log_{10}$ increase in *Staphylococcus* spp. counts was associated with an estimated increase in IMI and CRON of 0.62 (0.33) and 0.66 (0.21), respectively ($P < 0.059$). Finally, a 1-$\log_{10}$ increase in SSLO was associated with an estimated increase in IMI of 0.50 (0.23; $P = 0.033$). For the model predicting IMI, either the *Klebsiella* spp. count or both the SSLO and *Staphylococcus* spp. counts could be retained in the same model but not all 3 could be retained together (Table 4). In the final multivariable models for used bedding, a 1-$\log_{10}$ increase in bedding coliform counts was associated with an estimated increase in IMI and CRON of 1.04 (0.30) and 0.48 (0.20), respectively ($P \leq 0.02$; Table 5). Alternately, a 1-$\log_{10}$ increase in *Klebsiella* spp. tended to be associated with an estimated increase in CRON of 0.27 (0.14; $P = 0.054$). A positive nonlinear relationship existed between *Staphylococcus* spp. and LS ≥4%, whereas a 1-$\log_{10}$ increase in *Staphylococcus* spp. counts was associated with an estimated increase in NIMI of 0.27 (0.088; $P = 0.003$). When considering all bedding materials together in the same model, there was no association between SSLO counts in used bedding and any of the UH measures. However, stratified analysis conducted later showed that within herds using shavings, a 1-$\log_{10}$ increase in SSLO counts in used shavings was associated with an estimated increase of 0.13 (0.04) in AVLS ($P = 0.0015$).

In addition to BBC, herd traits associated with poorer performance for 1 or more UH outcomes in the final multivariable models included use of MNS bedding, small herd size (<200 cows), lower herd-average milk yield (<10,000 kg/yr), increased test-day average DIM, herds in the Midwest or Western region, and the summer season (Tables 4 and 5). Facility traits associated with poorer UH outcomes included use of bedded pack facilities or tiestall barns (vs. freestalls) and fair or poor ventilation (vs. good) as subjectively assessed by the producer. The use of a disinfectant predip was associated with an increase in NIMI. Mastitis control practices associated with worse UH outcomes included failure to routinely culture milk from high-SCC cows and never or only occasionally using an intramammary antibiotic at dry off. A positive BTM culture for *Staph. aureus* and *Mycoplasma* spp. was associated with an increase in AVLS and IMI ($P < 0.05$). Additionally, a positive BTM culture for *Mycoplasma* spp. tended to be associated with an increase in CRON ($P = 0.09$).

Benchmarks for Monitoring Bedding Hygiene

Table 6 reports the results of the final multivariable models describing the relationship between BBC categories and UH outcomes when considering all bedding materials together. For each bacteria group, the table reports the optimal cutpoints identified for low and high or for low, medium, and high categories, the proportion of samples falling within each category, and the predicted adjusted mean (SE) value for each UH outcome at each category level. For example, when considering *Klebsiella* spp. counts in unused bedding, 91% of all samples fell in the low category, achieving a value of 0 cfu/cm^3 of wet bedding. The predicted mean (SE) AVLS for herds with *Klebsiella* spp. counts falling in the low or high category was 2.74 ± 0.10 and 2.87 ± 0.10, respectively ($P = 0.05$). In most cases, we were able to identify universally applicable cutpoints for benchmarks for coliforms, *Klebsiella* spp., and *Staphylococcus* spp. in unused and used bedding that were achievable and that were both statistically and numerically associated with improved UH outcomes. However, some minor exceptions were noted for specific bedding types. For example, for SSLO counts in used bedding, although UH measures were not significantly different across categories when considering all bedding materials together (Table 6; $P > 0.10$), these same categories for SSLO (low: ≤500,000; moderate: 500,001–2,000,000; high: >2,000,000 cfu/cm^3) were both numerically and statistically associated with UH for herds using shavings bedding (Table 7). Two other exceptions related to the selection of higher optimal cutpoints for categorizing SSLO and coliform levels in MNS bedding compared with cutpoints selected for the other bedding materials. For unused MNS, the optimal SSLO categories associated with UH were achieved when thresholds for the low, moderate, and high categories were set at ≤1,000, 1,001 to 750,000, and >750,000 cfu/cm^3, respectively. For used MNS, the optimal coliform categories associated with UH were achieved when thresholds for the low, moderate, and high categories were set at ≤10,000, 10,001 to 200,000, and >200,000 cfu/cm^3, respectively.

Table 4. Final multivariable models describing the relationship between bacteria counts in unused bedding and udder health outcomes[1]

	Udder health dependent variable							
	Average linear score		IMI[2] (%)		NIMI[3] (%)		CRON[4]	
Parameter	Estimate (SE)	*P*-value	Estimate (SE)	*P*-value	Estimate (SE)	*P*-value	Estimate (SE)	*P*-value
Intercept	2.09 (0.30)		23.08 (5.38)		1.80 (0.250)		12.07 (3.77)	
Bedding bacteria group								
Coliforms	NS		NS		NS		NS	
Klebsiella spp.	0.035 (0.021)	0.097	0.65 (0.38)	0.089[5]	NS		NS	
SSLO[6]	NS		0.62 (0.33)	0.059[5]	NS		0.66 (0.21)	0.002
Staphylococcus spp.	NS		0.50 (0.23)	0.033[5]	NS		NS	
Test-day average DIM	0.0043 (0.001)	0.0007	0.039 (0.023)	0.089	0.028 (0.01)	0.018	0.039 (0.015)	0.009
Bedding material								
Manure solids	0.29 (0.10)	0.007	2.58 (1.82)	0.33	2.02 (0.75)	0.06	2.07 (1.18)	0.094
Organic non-manure materials	−0.046 (0.093)		−0.62 (1.61)		0.60 (0.59)		−0.71 (1.10)	
Reclaimed sand	0.07 (0.10)		0.87 (1.86)		0.31 (0.73)		1.31 (1.19)	
New sand	Ref[7]		Ref		Ref		Ref	
Milk yield (kg/yr)								
>12,000	−0.41 (0.11)	<0.0005	−6.17 (1.96)	0.004	−2.05 (0.77)	0.01	−4.42 (1.35)	0.002
10,001–12,000	−0.41 (0.11)		−5.90 (1.85)		−2.16 (0.71)		−4.46 (1.27)	
≤10,000	Ref		Ref		Ref		Ref	
Herd size (no. of lactating cows)								
>500	NS		NS		2.04 (0.68)	0.01	NS	
201–500	—		—		0.75 (0.64)		—	
≤200	—		—		Ref		—	
Season								
Summer	NS		1.16 (0.50)	0.022	NS		NS	
Winter	—		Ref		—		—	
Region								
Midwest	0.059 (0.12)	0.035	−1.96 (2.05)	0.002	−1.35 (0.84)	0.02	−0.88 (1.39)	0.007
Northeast	−0.22 (0.15)		−8.03 (2.68)		−3.44 (1.10)		−4.70 (1.82)	
South	−0.25 (0.19)		−7.43 (3.30)		−1.15 (1.36)		−4.92 (2.38)	
West	Ref		Ref		Ref		Ref	
Housing								
Bedded pack	0.46 (0.17)	0.023	4.31 (3.0)	0.004	NS		5.60 (2.14)	0.008
Dry lot	0.02 (0.21)		−6.01 (3.57)		—		−1.40 (2.42)	
Tiestall	0.31 (0.14)		7.35 (2.37)		—		4.54 (1.64)	
Freestall	Ref		Ref		—		Ref	
Ventilation								
Poor or fair	0.27 (0.091)	0.004	4.62 (1.56)	0.004	1.10 (0.61)	0.07	4.32 (1.08)	0.0001
Good	Ref		Ref		Ref		Ref	
Predip use								
Yes	NS		NS		2.16 (1.00)	0.03	NS	
No	—		—		Ref		—	
Culture high-SCC cows								
Routinely	NS		−3.90 (2.06)	0.061	NS		NS	
Sometimes or never	—		Ref		—		—	
Dry-cow antibiotic therapy								
Always	NS		NS		NS		−3.97 (1.93)	0.042
Sometimes or never	—		—		—		Ref	
Bulk tank culture: *Mycoplasma*								
Positive	0.20 (0.087)	0.021	3.0 (1.57)	0.058	1.44 (0.85)	0.09	NS	
Negative	Ref		Ref		Ref		—	

Continued

Table 4 (Continued). Final multivariable models describing the relationship between bacteria counts in unused bedding and udder health outcomes[1]

Parameter	Udder health dependent variable							
	Average linear score		IMI[2] (%)		NIMI[3] (%)		CRON[4]	
	Estimate (SE)	*P*-value	Estimate (SE)	*P*-value	Estimate (SE)	*P*-value	Estimate (SE)	*P*-value
Bulk tank culture: *Staph. aureus*								
Positive	0.095 (0.043)	0.031	1.71 (0.78)	0.031	NS		NS	
Negative	Ref		Ref		—		—	

[1]Significant if $P \leq 0.05$; trend if $0.05 < P \leq 0.10$.
[2]Proportion of cows on test day with a linear score ≥4.0.
[3]NIMI = new IMI. Proportion of cows with a linear score <4.0 on the previous test day and a linear score ≥4.0 on the current test day.
[4]CRON = chronic infection. Proportion of cows with a linear score ≥4.0 on both the previous and current test days.
[5]This model accepts either *Klebsiella* spp. alone in the model or else SSLO and *Staphylococcus* together in the model. However, it will not accept all 3 bacteria groups together in the same model.
[6]Streptococci or streptococci-like organisms.
[7]Referent.

DISCUSSION

This is the first study of its scale designed to investigate the relationship among bedding materials, BBC, UH, and milk quality in US dairy herds and to establish evidence-based benchmarks for monitoring bedding hygiene in commonly used OB and IB materials. Samples were collected in summer and winter seasons, and participating herds represented several dairy regions, housing systems, parlor procedures, and mastitis control practices typical of modern conventional confinement dairies (USDA, 2015). However, this was a convenience sample of DHIA herds. In 2014, only 56.3% of US dairy operations participated in a DHIA testing program (USDA, 2014). The mean herd size and milk yield (897 cows and 11,944 kg/cow, respectively) were greater than that reported for all US dairy farms (234 cows and 10,328 kg/cow, respectively; Progressive Dairy, 2017). However, the mean SCC of 196,000 cells/mL was similar to the milk weighted bulk tank SCC of 193,000 cells/mL reported nationally (USDA, 2015). The proportion of BTM samples positive for *Staph. aureus* (45.7%) and *Mycoplasma* spp. (5.9%) was similar to the BTM prevalence of *Staph. aureus* (66.6%) and *Mycoplasma bovis* (8.7%) reported in a national study (USDA, 2015). Our findings that herds using MNS and RS were larger than herds using NS or ON and that herds using ON produced less milk per cow than herds using NS, RS, or MNS are consistent with findings from a survey of 286 Wisconsin dairy farms (Rowbotham and Ruegg, 2015).

BBC, Bedding Characteristics, Udder Hygiene Scores, BTM Quality, and UH in Herds Using 4 Bedding Types

Although objectives 2 and 3 were our major objectives, we first completed objective 1 to investigate whether direct associations existed between bedding type and each of the following 4 outcomes: BBC, udder hygiene scores, BTM quality, and UH. Our logic was that demonstrating these relationships would be a first step in supporting the biological plausibility of the causal pathway proposed in Figure 1. The finding that BBC were significantly higher in used versus unused bedding has been described in other studies and is attributed to the fact that once bedding is placed in stalls bacteria quickly multiply over the next 1 to 2 d, presumably as stalls are contaminated with bacteria, moisture, and nutrients to support bacteria growth (Sorter et al., 2014; Klaas and Zadoks, 2018). Interestingly, we did not find an association between bedding age and BBC. This may be due to the very wide variation in bedding age in our study, with the median values of unused and used bedding samples being 7 and 3 d, respectively.

Table 5. Final multivariable models describing the relationship between bacteria counts in used bedding and udder health outcomes[1]

	Udder health dependent variable							
	Average linear score		IMI[2] (%)		NIMI[3] (%)		CRON[4]	
Parameter	Estimate (SE)	*P*-value	Estimate (SE)	*P*-value	Estimate (SE)	*P*-value	Estimate (SE)	*P*-value
Intercept	2.06 (0.30)		21.07 (5.39)		1.09 (2.45)		11.55 (3.80)	
Bedding bacteria group								
Coliforms	NS		1.04 (0.30)	0.0006	NS		0.48 (0.20)	0.02[5]
Klebsiella spp.	NS		NS		NS		0.27 (0.14)	0.054[5]
SSLO[6]	NS		NS		NS		NS	
Staphylococcus spp.	NS		1.50 (0.53)	0.005	0.27 (0.088)	0.003	NS	
Quadratic term	NS		−0.25 (0.10)	0.016	NS		NS	
Test-day average DIM	0.0044 (0.001)	0.0005	0.037 (0.022)	0.10	0.026 (0.011)	0.03	0.044 (0.015)	0.004
Bedding material								
Manure solids	0.33 (0.099)	0.019	2.99 (1.73)	0.12	1.72 (0.74)	0.13	2.31 (1.19)	0.11
Organic non-manure materials	−0.028 (0.092)		−0.33 (1.60)		0.36 (0.57)		−0.27 (1.10)	
Reclaimed sand	0.082 (0.10)		2.56 (1.74)		0.29 (0.71)		1.57 (1.20)	
New sand	Ref[7]		Ref		Ref		Ref	
Milk yield (kg/yr)								
>12,000	−0.41 (0.11)	0.0004	−6.61 (1.96)	0.002	−1.99 (0.74)	0.008	−4.60 (1.36)	0.0008
10,001–12,000	−0.43 (0.11)		−6.39 (1.83)		−2.16 (0.69)		−4.81 (1.28)	
≤10,000	Ref		Ref		Ref		Ref	
Herd size (no. of lactating cows)								
>500	NS		NS		2.18 (0.66)	0.005	NS	
201–500	—		—		0.98 (0.62)		—	
≤200	—		—		Ref		—	
Region								
Midwest	0.085 (0.12)	0.026	−1.75 (2.06)	0.002	−0.94 (0.83)	0.03	−1.11 (1.41)	0.005
Northeast	−0.20 (0.15)		−8.05 (2.69)		−3.00 (1.08)		−5.16 (1.85)	
South	−0.24 (0.19)		−7.32 (3.30)		−1.04 (1.31)		−4.82 (2.40)	
West	Ref		Ref		Ref		Ref	
Housing								
Bedded pack	0.46 (0.17)	0.027	3.66 (2.98)	0.003	NS		5.20 (2.18)	0.012
Dry lot	−0.0012 (0.21)		−5.14 (3.57)		—		−1.37 (2.44)	
Tiestall	0.29 (0.14)		8.33 (2.40)		—		4.53 (1.65)	
Freestall	Ref		Ref		—		Ref	
Ventilation								
Poor or fair	0.27 (0.091)	0.0034	4.24 (1.56)	0.008	1.20 (0.59)	0.04	4.14 (1.09)	0.0002
Good	Ref		Ref		Ref		Ref	
Predip use								
Yes	NS		NS		2.06 (0.97)	0.04	NS	
No	—		—				—	
Culture high-SCC cows								
Routinely	NS		−4.16 (2.06)	0.046	NS		NS	
Sometimes or never	—		Ref		—		—	
Dry-cow antibiotic therapy								
Always	NS		NS		NS		−3.99 (1.96)	0.043
Sometimes or never	—		—		—		Ref	
Bulk tank culture: *Mycoplasma*								
Positive	0.22 (0.087)	0.014	3.38 (1.55)	0.031	1.45 (0.84)	0.09	NS	
Negative	Ref		Ref		Ref		—	

Continued

Table 5 (Continued). Final multivariable models describing the relationship between bacteria counts in used bedding and udder health outcomes[1]

	Udder health dependent variable							
	Average linear score		IMI[2] (%)		NIMI[3] (%)		CRON[4]	
Parameter	Estimate (SE)	*P*-value	Estimate (SE)	*P*-value	Estimate (SE)	*P*-value	Estimate (SE)	*P*-value
Bulk tank culture: *Staph. aureus*								
Positive	0.096 (0.044)	0.029	1.87 (0.78)	0.018	NS		NS	
Negative	Ref		Ref		—		—	

[1]Significant if $P \leq 0.05$; trend if $0.05 < P \leq 0.10$.
[2]Proportion of cows on test day with a linear score ≥4.0.
[3]NIMI = new IMI. Proportion of cows with a linear score <4.0 on the previous test day and a linear score ≥4.0 on the current test day.
[4]CRON = chronic infection. Proportion of cows with a linear score ≥4.0 on both the previous and current test days.
[5]This model accepts 1 of the 2 bacteria groups shown, but not both together in the same model.
[6]Streptococci or streptococci-like organisms.
[7]Referent.

Our finding that BBC were generally highest in MNS bedding agrees with several other studies, including one controlled study that reported higher coliform and *Klebsiella* spp. counts in used deep-bedded MNS compared with IB (Rowbotham and Ruegg, 2016a). Similarly, a recent observational study of 125 UK herds reported that coliform and *Staphylococcus* spp. counts in used bedding were higher in MNS compared with either sand or sawdust. The latter study also reported that SSLO counts were lowest in sand but equally high in MNS and sawdust (Bradley et al., 2018). One interesting finding in the current study was the large difference in BBC when comparing unused samples of RS and NS. Rowbotham and Ruegg (2016a) reported that coliform, *Klebsiella* spp., and SSLO counts were higher in used samples of RS compared with NS but did not report BBC values for unused samples.

Our finding that udder hygiene scores were generally higher in herds using MNS compared with RS differs from that of a national study reporting no association between bedding type and cow hygiene (Lombard et al., 2010). Increased cow hygiene scores and udder hygiene scores have both been associated with increased SCC and risk for IMI (Schreiner and Ruegg, 2003; Reneau et al., 2005). Apart from bedding type, it is certain that udder hygiene scores are also influenced by other factors, including facilities, bedding, and manure management and parlor procedures (Lombard et al., 2010).

In evaluating the association between bedding type and milk quality, we observed that SSLO counts in BTM were lower in herds using NS and ON compared MNS and that coliform counts in BTM tended to be lower in herds using RS compared with MNS or ON. These findings differ from those of 2 recent observational studies in Wisconsin and the United Kingdom that reported no association between use of MNS (vs. IB or ON bedding) and bacteria counts in BTM (Rowbotham and Ruegg, 2015; Bradley et al., 2018). Increased TBC and coliforms in BTM can be caused by bacteria from unsanitary milking equipment, inadequately chilled milk, contamination from soiled udders, and, occasionally, mastitic cows (Pantoja et al., 2009). Bradley et al. (2018) reported that udder hygiene and adequate udder preparation in the parlor were associated with reduced SSLO counts in BTM. Bacteria in BTM, regardless of source, are of concern to all segments of the dairy industry because they affect milk quality, ultimately resulting in milk with decreased manufacturing properties and dairy products with reduced shelf life. In this study, bacteria from bedding could be one source of bacteria in BTM, conceivably arriving from contaminated teat skin (if teats are not properly cleaned and disinfected before unit attachment) as well as from mastitic glands. One finding of interest was that herds

Table 6. Final multivariable models for all bedding materials describing the relationship between bacteria counts in unused and used bedding samples of all types (n = 324) and udder health outcomes when bedding bacteria count (BBC) is modeled as a categorical variable[1,2]

				Udder health outcome							
				Average linear score		IMI[5] (%)		NIMI[6] (%)		CRON[7]	
Group	Prevalence[3] (%)	Category	BBC cutpoint[4] (cfu/cm^3)	Adjusted mean (SE)	*P*-value	Adjusted mean (SE)	*P*-value	Adjusted mean (SE)	*P*-value	Adjusted mean (SE)	*P*-value
Unused bedding											
Coliforms	79	Low	≤500	2.72 (0.10)	0.34	23.35 (2.05)	0.08	7.96 (0.71)	0.09	16.29 (1.50)	0.13
	21	High	>500	2.78 (0.11)		25.16 (2.17)		8.87 (0.81)		17.35 (1.59)	
Klebsiella spp.	91	Low	0	2.74 (0.10)	0.05	23.84 (2.03)	0.12	8.14 (0.70)	0.09	16.54 (1.50)	0.51
	9	High	>0	2.87 (0.10)		25.72 (2.36)		9.28 (0.95)		17.05 (1.71)	
Staphylococcus spp.	83	Low	0	2.73 (0.10)	0.49	23.38 (2.03)	0.05	8.16 (0.71)	0.86	16.23 (1.49)	0.003
	17	High	>0	2.77 (0.10)		25.28 (2.15)		8.27 (0.82)		18.10 (1.57)	
SSLO[8,9]	36	Low	0	2.69 (0.11)		22.56 (2.14)	0.12	7.85 (0.79)	0.62	16.04 (1.55)	0.35
	32	Moderate	1–1,000	2.75 (0.10)		23.53 (2.07)		8.05 (0.77)		16.79 (1.53)	
	32	High	>1,000	2.75 (0.11)		24.79 (2.14)		8.45 (0.77)		16.45 (1.54)	
Used bedding											
Coliforms[9]	58	Low	≤10,000	2.74 (0.10)	0.84	22.89 (2.06)	0.047	8.16 (0.71)	0.84	16.24 (1.51)	0.26
	42	High	>10,000	2.74 (0.11)		24.50 (2.07)		8.25 (0.75)		16.83 (1.52)	
Klebsiella spp.	65	Low	0	2.74 (0.10)	0.60	23.27 (2.03)	0.04	8.22 (0.71)	0.84	16.28 (1.49)	0.006
	35	High	>0	2.76 (0.11)		24.74 (2.10)		8.14 (0.75)		17.55 (1.54)	
Staphylococcus spp.	52	Low	0	2.72 (0.10)	0.23	22.93 (2.04)	0.016	7.68 (0.71)	0.006	16.24 (1.51)	0.22
	48	High	>0	2.76 (0.10)		24.57 (2.06)		8.74 (0.71)		16.79 (1.51)	
SSLO[8,10]	27	Low	≤500,000	2.74 (0.10)	0.38	23.46 (2.05)	0.79	8.13 (0.74)	0.54	16.37 (1.51)	0.76
	29	Moderate	500,001–2,000,000	2.71 (0.11)		23.81 (2.15)		8.10 (0.76)		16.81 (1.55)	
	44	High	>2,000,000	2.77 (0.11)		24.06 (2.14)		8.54 (0.77)		16.57 (1.55)	

[1]The cutpoints identified for categorization can be universally applied to most bedding materials, with minor exceptions noted.

[2]All models offer to control for test-day average DIM, herd-average milk yield, herd size, season, region, housing, ventilation, predip use, culture of high-SCC cows, use of dry-cow antibiotic therapy, and bulk tank milk culture status for *Mycoplasma* spp. and *Staphylococcus aureus*. Herd was controlled for as a random effect.

[3]Proportion of all samples falling within the designated category.

[4]Minimum limit of detection for bedding culture is 25 cfu/cm^3 (reported as 0).

[5]Proportion of cows on test day with a linear score ≥4.0.

[6]NIMI = new IMI. Proportion of cows with a linear score <4.0 on the previous test day and a linear score ≥4.0 on the current test day.

[7]CRON = chronic infection. Proportion of cows with a linear score ≥4.0 on both the previous and current test days.

[8]Streptococci or streptococci-like organisms.

[9]Unique bacteria count cutpoints were used to categorize these bacteria groups for manure solids (see Table 8).

[10]Different strength of statistical association and estimated udder health differences exist for these categories for used shavings (see Table 8).

Table 7. Final multivariable models for manure solids and shavings describing the relationship between specific bacteria groups and udder health outcomes when bedding bacteria count (BBC) is modeled as a categorical variable[1,2]

				Udder health outcome							
				Average linear score		IMI[4] (%)		NIMI[5] (%)		CRON[6]	
Bacteria group	Prevalence[3] (%)	Category	BBC cutpoint (cfu/cm^3)	Adjusted mean (SE)	*P*-value	Adjusted mean (SE)	*P*-value	Adjusted mean (SE)	*P*-value	Adjusted mean (SE)	*P*-value
Unused manure solids (n = 66)											
SSLO	29	Low	≤1,000	3.07 (0.16)	0.03	21.91 (3.65)	0.03	8.62 (1.24)	0.43	17.51 (2.98)	0.12
	51	Moderate	1,001–750,000	3.11 (0.14)		24.56 (3.46)		9.25 (0.99)		17.38 (2.81)	
	20	High	>750,000	3.35 (0.15)		28.34 (3.62)		10.17 (1.20)		19.62 (2.88)	
Used manure solids (n = 66)											
Coliforms	27	Low	≤10,000	3.01 (0.14)	0.26	26.85 (2.32)	0.03	8.20 (1.20)	0.03	20.12 (2.13)	0.04
	55	Moderate	10,001–200,000	3.15 (0.13)		31.39 (2.09)		10.17 (1.02)		21.44 (1.97)	
	18	High	>200,000	3.02 (0.15)		28.13 (2.45)		7.79 (1.23)		24.06 (2.44)	
Used shavings (n = 68)											
SSLO	18	Low	≤500,000	2.47 (0.12)	0.01	23.89 (1.99)	0.19	8.19 (0.87)	0.71	13.66 (1.42)	0.04
	25	Moderate	500,001–2,000,000	2.65 (0.13)		23.42 (2.05)		9.19 (0.94)		13.51 (1.46)	
	57	High	>2,000,000	2.83 (0.11)		26.10 (1.77)		8.82 (0.76)		15.79 (1.32)	

[1]In contrast to Table 7, which presents cutpoints that may be applied to most bedding materials, this table presents unique bacteria count cutpoints used to categorize streptococci or streptococci-like organisms (SSLO) and coliforms for manure solids and presents the different strengths of statistical association and estimated udder health differences that exist for SSLO categories in used shavings.

[2]All models offer to control for test-day average DIM, herd-average milk yield, herd size, season, region, housing, ventilation, predip use, culture of high-SCC cows, use of dry cow antibiotic therapy, and bulk tank milk culture status for *Mycoplasma* spp. and *Staphylococcus aureus*. Herd was controlled for as a random effect.

[3]Proportion of all samples falling within the designated category.

[4]Proportion of cows on test day with a linear score ≥4.0.

[5]NIMI = new IMI. Proportion of cows with a linear score <4.0 on the previous test day and a linear score ≥4.0 on the current test day.

[6]CRON = chronic infection. Proportion of cows with a linear score ≥4.0 on both the previous and current test days.

Table 8. Summary of suggested benchmarks to interpret bedding culture results (cfu/cm^3 of wet bedding)

Bacteria group[1]	Bedding type[2]	Bedding bacteria count category[3]		
		Low	Moderate	High
Unused bedding				
Staphylococcus spp.	NWS, RS, ON, MNS	0	—	>0
Klebsiella spp.	NWS, RS, ON, MNS	0	—	>0
Coliforms	NWS, RS, ON, MNS	≤500	—	>500
SSLO	NWS, RS, ON	0	1–1,000	>1,000
	MNS	≤1,000	1,001–750,000	>750,000
Used bedding				
Staphylococcus spp.	NWS, RS, ON, MNS	0	—	>0
Klebsiella spp.	NWS, RS, ON, MNS	0	—	>0
Coliforms	NWS, RS, ON	≤10,000	—	>10,000
	MNS	≤10,000	10,001–200,000	>200,000
SSLO	NWS, RS, ON, MNS	≤500,000	500,001–2,000,000	>2,000,000

[1]Unused bedding was collected from the bedding storage area. Used bedding was collected from stalls or the cow resting area. SSLO = streptococci or streptococci-like organisms.

[2]NWS = new sand; RS = reclaimed sand; ON = organic non-manure materials (shavings or straw); MNS = manure solids.

[3]Minimum limit of detection for bedding culture is 25 cfu/cm^3 (reported as 0).

using MNS (53%) or ON (59.5%) were at higher risk for having a positive BTM culture for *Staph. aureus* compared with NS (29.4%). We hypothesize that this association may be indirect in nature, related to other herd characteristics or management practices surrounding the control of contagious pathogens. However, bedding should not be entirely overlooked as a potential source of exposure to contagious mastitis pathogens. Janzen et al. (1982) reported recovering higher counts of *Staph. aureus* from bedding samples, teat swabs, and milk samples of cows housed on MNS compared with limestone bedding. Furthermore, *Mycoplasma* spp. was discovered in high numbers in RS bedding from 3 Idaho dairies (Justice-Allen et al., 2010).

In evaluating the relationship between bedding type and UH, outcomes such as AVLS, NIMI, and CLXM were generally poorer (higher) in herds using MNS compared with herds using IB, with no difference in UH observed among herds using ON, RS, or NS. The latter finding is consistent with results of a 1-yr observational study that reported that although there were higher coliform, *Klebsiella* spp., and SSLO counts in OB (sawdust or straw) versus IB (sand or limestone), there was no association between bedding material and clinical mastitis risk (Hogan et al., 1989). Although relatively few studies have compared UH in herds using MNS versus other materials, most agree with our results in that they suggest at least a tendency for poorer UH in MNS herds (Wenz et al., 2007; Rowbotham and Ruegg, 2015; Rowbotham and Ruegg, 2016b). In a 1-yr experimental study, investigators reported no difference in incidence of clinical or subclinical mastitis when comparing primiparous cows housed on IB and either deep- or shallow-bedded MNS, although there was a tendency for greater delay in when clinical mastitis occurred for cows housed on NS compared with RS or deep-bedded MNS (Rowbotham and Ruegg, 2016b). An observational study of 325 Wisconsin herds reported a tendency for a higher proportion of cows with discarded milk and with nonfunctional quarters in herds using MNS versus IB or non-manure-based OB, which could be an indirect indicator of increased clinical mastitis incidence in MNS herds (Rowbotham and Ruegg, 2015). Finally, in a national survey of 1,013 US herds, the use of sand or newspaper bedding was associated with a lower bulk tank SCC, whereas use of composted manure tended to be associated with a higher bulk tank SCC (Wenz et al., 2007). A likely hypothesis is that MNS may be associated with poorer UH due to its ability to support higher levels of mastitis pathogens. However, our study showed a great deal of variation in BBC and UH within any of the 4 bedding groups evaluated, including MNS. This suggests that individual herds, including those using MNS, must have characteristics or management practices in place that help reduce BBC or mitigate IMI risk through other means. Such factors will be investigated in a separate analysis.

Relationship Between BBC and Herd Measures of UH

For this portion of the analysis, we identified that specific groups of bacteria, coliforms, *Klebsiella* spp., SSLO, and *Staphylococcus* spp. in both unused and used bedding were associated with 1 or more herd-level measures of UH. It was not surprising that coliform and *Klebsiella* spp. counts were mutually exclusive in the various models given that *Klebsiella* spp. is a subset of the coliform group. The TBC in bedding was not

associated with any UH measure, presumably because *Bacillus* spp., which is an uncommon cause of mastitis, represented the majority of TBC. These findings suggest that producers may get the most value from monitoring counts of coliforms, *Klebsiella* spp., SSLO, and *Staphylococcus* spp. in bedding culture reports but not necessarily TBC or *Bacillus* spp. For the most part, interactions did not exist between BBC and bedding type when examining the relationship between BBC and UH. However, there was 1 exception related to SSLO bacteria in used bedding. Although the multivariable model that included the 4 bedding types together (MNS, ON, RS, and NS) detected no overall association between SSLO counts and UH, when the ON group was later stratified to evaluate shavings and straw separately, increased SSLO counts in used shavings were associated with an increase in AVLS. Future analysis will investigate whether specific bedding characteristics or management practices unique to farms using shavings might explain this relationship. Our finding of no association between any BBC measure and the cumulative incidence of clinical mastitis in the 30-d period preceding sample day (CLXM) differs from some other studies. For example, in a 1-yr observational study of 9 commercial herds, Hogan et al. (1989) reported a positive linear relationship between clinical mastitis during lactation and counts of gram-negative bacteria and *Klebsiella* spp. in lactating cow bedding. We hypothesize that failure to detect an association in our study may be attributed to the tremendous variation that exists among the 168 participating herds in detecting and recording clinical mastitis events. Efforts to standardize these activities would benefit the industry.

Of some interest was that *Staphylococcus* spp. counts in both unused and used bedding were associated with UH. Once considered a minor pathogen, the NAS group is now recognized as the major cause of subclinical mastitis, can cause clinical mastitis, and is associated with elevated SCC (Piepers et al., 2007; Oliveira et al., 2013). Though long regarded as an opportunistic skin microbiota, the environment, including unused and used sawdust, has been reported as a reservoir of some species of NAS, including *Staph. haemolyticus*, *Staph. simulans*, and *Staph. xylosus* (Piessens et al., 2011). Although our bedding culture protocol did not allow for the identification and quantification of individual species within the *Staphylococcus* spp. group, our findings support the hypothesis that the environment may serve as a reservoir for at least some *Staphylococcus* species. There is much more to learn about the epidemiology of this group of staphylococci.

In addition to BBC, herd traits or management practices associated with poorer UH measures included a small herd size (<200 cows), lower average milk yield (<10,000 kg/cow per year), increased average DIM, herds in the West or Midwest regions, the summer season, and presence of *Staph. aureus* or *Mycoplasma* spp. in BTM. Additionally, UH measures were increased (poorer) in bedded-pack or tiestall versus freestall barns, in facilities with only fair or poor ventilation, in herds that did not routinely culture milk from high-SCC cows, and in herds that did not routinely infuse an intramammary antibiotic at dry off. Most, but not all, of these findings are consistent with risk factors described in other epidemiological studies. In a study of 292 dairy herds from New York, Oregon, and Wisconsin, investigators reported that an increased rate of clinical mastitis was associated with use of forestripping, presence of contagious pathogens in the bulk tank culture, proactive detection of mastitis in postpartum cows, and tiestall housing (Richert et al., 2013). A recent study of 325 Wisconsin dairies reported increased mean bulk milk SCC for summer versus winter months (Rowbotham and Ruegg, 2015). In a national survey of 1,013 dairy operations, the odds of an operation having a high bulk tank SCC (>200,000 cells/mL) were increased for herds in the Southeast region, in herds with a rolling herd-average milk production <9,090 kg/cow per year, and in herds not using a coliform mastitis vaccine. A systematic review of the literature reported that practices having the most consistent association with lower herd SCC were related to milking procedures. This included wearing gloves during milking, using automatic takeoffs, using postmilking teat dipping, milking problem cows last, conducting yearly inspections of the milking system, and using some technique to keep cows standing following milking (Dufour et al., 2011). Other practices associated with lower SCC were the use of a freestall system, use of sand bedding, cleaning the calving pen after each calving, surveillance of dry-cow udders for mastitis, use of blanket dry-cow antibiotic therapy, parenteral selenium supplementation, udder hair management, and frequent use of the California mastitis test (Dufour et al., 2011). Counterintuitively, we observed that use of a disinfectant predip was associated with an increase in NIMI. We hypothesize that this observation may represent an indirect association with other herd factors given that use of a disinfectant predip is a proven and widely accepted practice to aid in controlling environmental mastitis (Hogan and Smith, 2012).

Benchmarks for Monitoring Bedding Hygiene

We were able to develop benchmarks for coliform, *Klebsiella* spp., SSLO, and *Staphylococcus* counts in both unused and used bedding that were achievable and that were both statistically and numerically associ-

ated with improved UH performance (Tables 6 and 7). In most cases, the same benchmarks could be used in all bedding materials evaluated. However, it is worth noting that although the thresholds for categorizing SSLO counts in used shavings bedding were statistically associated with UH, these same thresholds were not associated with UH for other bedding materials. Additionally, the optimal thresholds identified for categorizing SSLO counts in unused MNS and coliform counts in used MNS were higher than for other bedding materials. This is primarily because the range for SSLO and coliform counts was higher in MNS than in other materials. The few studies that previously proposed BBC benchmarks limited their recommendations to the coliform or *Klebsiella* spp. groups and only specific bedding materials. For example, Bramley and Neave (1975) observed an increase in coliform infections when coliform bacteria counts in sawdust exceeded 10^7 cfu/g of wet bedding. In a later trial housing cows on sand or sawdust, Bramley (1985) stated that the incidence of clinical *E. coli* mastitis was increased if cows were housed on sawdust with coliform counts exceeding 10^6 cfu/g of wet bedding. Finally, in an observational study of 3 California dry lot dairies, Carroll and Jasper (1980) stated that an increase in clinical mastitis cases caused by *Klebsiella* spp. was observed when *Klebsiella* counts exceeded 10^6 cfu/g of wet bedding.

Readers should be cautious if trying to compare the BBC values and thresholds proposed in this study with those of previous studies because laboratories may report BBC in different units, including colony-forming units per cubic centimeter of wet bedding (Husfeldt et al., 2012), colony-forming units per gram of wet bedding (Rowbotham and Ruegg, 2016a), or colony-forming units per gram on a DM basis (Sorter et al., 2014). Although all 3 metrics are likely to be correlated with one another, we hypothesize that evaluating BBC on a volume basis (cfu/cm^3) may more closely reflect bacterial exposure to surface area on the teat skin and therefore IMI risk. However, this hypothesis requires investigation. Ultimately, the unit of measurement (cfu/cm^3 vs. cfu/g) might be most important if trying to make comparisons across different bedding materials because density values can vary dramatically between IB and OB materials. Producers monitoring bedding hygiene should be aware of the unit reported and be cautious comparing results from different labs if different units are reported. Additionally, it is important to establish a clear method of sampling, handling samples, and culturing and identifying bedding bacteria and to strictly adhere to these protocols so that the use of cutpoints can be made universally.

The estimated differences in UH outcomes, although statistically different, vary in magnitude for different BBC categories created. It would be ideal if we could estimate the economic implications associated with BBC. For example, what would the predicted cost savings be of achieving a low versus high *Klebsiella* count in unused bedding? Unfortunately, this question is problematic for several reasons, not the least of which is that this was an observational study, with associations described at the herd level potentially confounded by other herd effects. Researchers have attempted to estimate mastitis costs, with losses attributed to lost milk production, SCC program premiums and deductions, the economic effect of clinical mastitis, and losses due to culling or death (Fetrow et al., 2000). However, as Fetrow et al. (2000) pointed out, these costs will vary from herd to herd or over time within the same herd. Furthermore, bedding choices and bedding management may affect not only mastitis but also cow comfort, feed intake, milk production, or parlor throughput (Fetrow et al., 2000). Having established that a direct relationship exists between BBC and UH as well as benchmarks for monitoring bedding hygiene, our next step will be to investigate factors that affect BBC and UH (e.g., DM, OM, pH) and identify best practices for managing unused and used bedding materials. Future studies will need to investigate the economics associated with BBC as well as the cost–benefit of adopting specific management strategies designed to reduce BBC and improve UH.

One possible study limitation is that bedding samples were frozen before submission for culture. Although studies are needed to describe the effect of freezing on recovery of bacteria in bedding, studies have evaluated this in milk. For example, Schukken et al. (1989) reported that freezing and increased duration of storage resulted in a decrease in sample positivity for *E. coli* or *Actinomyces pyogenes* but an increase in sample positivity for NAS. As such, it is conceivable that freezing bedding samples for this study similarly altered recovery or concentration of bacteria recovered. That said, the methods used in this study are generalizable to real-world conditions because under most circumstances bedding samples would need to be frozen before submission to a reference laboratory, given that same-day culture is uncommon. Another potential limitation was the use of DHIA SCC data summarized at the herd level as an indirect measure of IMI and UH. Unfortunately, sampling and culturing milk from individual cows to describe the prevalence and etiology of infection was beyond the economic scope of this study. Although an SCC $\geq$200,000 cells/mL (LS $\geq$4.0) is the most commonly used threshold to predict the presence of IMI at the cow level, we recognize that predictive values from using this or other thresholds will vary by herd prevalence of IMI (Dohoo, 2001). Furthermore,

we acknowledge that herd measures such as AVLS and CRON could be influenced by mastitis management practices not assessed in this study, such as drying off chronic quarters or culling chronically infected cows. The use of NIMI as a key UH parameter is arguably a stronger indicator of recent new infections at the herd level.

CONCLUSIONS

Although variation existed among individual farms, the use of MNS bedding was generally associated with higher BBC, dirtier udders, increased coliform and SSLO counts in BTM, and poorer herd-level UH measures compared with herds using ON, RS, or NS bedding. There was no difference in UH outcomes among herds using NS, RS, or ON bedding. Increased counts of coliforms, *Klebsiella* spp., SSLO, and NAS in both unused and used bedding were directly associated with higher values for 1 or more herd-level measures of UH, including test-day AVLS, IMI, NIMI, and CRON. Achievable benchmarks identified for counts of coliforms (unused: $\leq$500; used: $\leq$10,000 cfu/cm^3), *Klebsiella* spp. (0 cfu/cm^3 for unused and used), *Staphylococcus* spp. (0 cfu/cm^3 for unused and used), and SSLO (unused: 0; used: $\leq$500,000 cfu/cm^3) can be used to monitor bedding hygiene in most bedding materials, with minor variations suggested for SSLO in unused MNS ($\leq$1,000 cfu/cm^3).

ACKNOWLEDGMENTS

Funding was provided by Boehringer Ingelheim Animal Health (St. Joseph, MO), Minnesota Dairy Herd Improvement Association (Buffalo, MN), Dairy Management Inc. (Rosemont, IL), and the University of Minnesota College of Veterinary Medicine (St. Paul, MN). Additionally, we give very special thanks to the participating herds and especially to each of the 50 American Association of Bovine Practitioners veterinarians and 11 participating university research and extension folks who assisted with herd recruitment and sampling. We also thank the 4 DHIA record processing centers for helping us retrieve the necessary test-day data: Dairy Records Management Systems (Raleigh, NC), AgSource (Verona, WI), Amelicor (Provo, UT), and AgriTech (Visalia, CA).

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