



## Bacterial counts on teat skin and in new sand, recycled sand, and recycled manure solids used as bedding in freestalls

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### ABSTRACT

On modern dairy farms, environmental mastitis pathogens are usually the predominant cause of mastitis, and bedding often serves as a point of exposure to these organisms. The objective of this longitudinal study was to determine bacterial populations of 4 different bedding types [deep-bedded new sand (NES), deep-bedded recycled sand (RS), deep-bedded manure solids (DBMS), and shallow-bedded manure solids over foam core mattresses (SBMS)] and of teat skin swabs of primarily primiparous cows housed in a single facility over all 4 seasons. Samples of bedding were collected weekly ( $n = 49$  wk) from pens that each contained 32 lactating dairy cows. Throughout the length of the same period, composite swabs of teat skin were collected weekly from all cows before and after premilking teat sanitation. Median numbers of streptococci and streptococci-like organisms (SSLO) were  $>8.6 \times 10^6$  cfu/g and  $>6.9 \times 10^3$  cfu/teat swab for all bedding types and teat swabs, respectively. Numbers of SSLO were greatest in samples of SBMS ( $2.1 \times 10^8$  cfu/g) and least in samples of NES ( $8.6 \times 10^6$  cfu/g), RS ( $1.3 \times 10^7$  cfu/g), and DBMS ( $1.7 \times 10^7$  cfu/g). Numbers of gram-negative bacteria in bedding ( $5.5 \times 10^4$  to  $1.2 \times 10^7$  cfu/g) were fewer than numbers of SSLO ( $8.6 \times 10^6$  to  $2.1 \times 10^8$  cfu/g). Numbers of coliform bacteria were greatest in samples of DBMS ( $2.2 \times 10^6$  cfu/g) and least in samples of NES ( $3.6 \times 10^3$  cfu/g). In general, the relative number of bacteria on teat skin corresponded to exposure in bedding. Numbers of gram-negative bacteria recovered from prepreparation teat swabs were greatest for cows bedded with DBMS ( $1.0 \times 10^4$  cfu/swab) and RS ( $2.5 \times 10^3$  cfu/swab) and least for cows bedded with NES ( $5.8 \times 10^2$  cfu/swab). Median numbers of coliform and *Klebsiella* spp. recovered from prepreparation teat swabs were below the limit of detection for all cows except those bedded with DBMS.

Numbers of SSLO recovered from prepreparation teat swabs were least for cows bedded with DBMS ( $6.9 \times 10^3$  cfu/swab) and greatest for cows bedded with RS ( $5.1 \times 10^4$  cfu/swab) or SBMS ( $1.6 \times 10^5$  cfu/swab). The numbers of all types of measured bacteria (total gram-negative, coliforms, *Klebsiella* spp., SSLO) on postpreparation teat swabs were reduced by up to 2.6 logs from numbers of bacteria on prepreparation swabs, verifying effective preparation procedures. Significant correlations between bacterial counts of bedding samples and teat skin swabs were observed for several types of bacteria. As compared with other bedding types, the least amount of gram-negative bacteria were recovered from NES and may indicate that cows on NES have a reduced risk of exposure to pathogens that are typically a cause of clinical mastitis. In contrast, exposure to large numbers of SSLO was consistent across all bedding types and may indicate that risk of subclinical mastitis typically associated with streptococci is not as influenced by bedding type; however, significantly greater numbers of SSLO were found in SBMS than in other bedding types. These findings indicate that use of different bedding types results in exposure to different distributions of mastitis pathogens that may alter the proportion of etiologies of clinical mastitis, although the incidence rate of clinical mastitis did not differ among bedding types.

**Key words:** mastitis, dairy cow, bacteria, milk quality

### INTRODUCTION

For decades, mastitis has been considered to be the most economically important and most commonly occurring disease of dairy cattle (Blackburn, 1958; Janzen, 1970; Hogeveen et al., 2011), and it continues to contribute to decreased profits for dairy farms throughout the world. Mastitis pathogens are frequently categorized as environmental or contagious based upon their primary reservoir and point of exposure (Smith and Hogan, 2001). Exposure to contagious mastitis often occurs during milking when teats of healthy cows are exposed to bacteria in milk that originated

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from infected quarters. The prevalence of contagious IMI has decreased due to adoption of recommended milking practices and selective culling of chronically infected cows. Exposure to environmental pathogens occurs when teats are exposed to large numbers of opportunistic organisms found in the animals' housing areas. As contagious pathogens have been controlled, environmental pathogens have come to account for the majority of IMI (Makovec and Ruegg, 2003). Possible sources of exposure include bacteria found in bedding, manure, and mud (Schreiner and Ruegg, 2003; DeVries et al., 2012; Hogan and Smith, 2012). Reducing bacterial exposure at the teat end is an important aspect of prevention of environmental mastitis. Dairy cattle spend 40 to 65% of their time lying down, and during these periods their teats may come in direct contact with bacteria found in bedding (Tucker and Weary, 2004; Cook et al., 2005; Hogan and Smith, 2012). The incidence of clinical mastitis has been shown to be associated with bacterial populations on teat ends (Neave et al., 1966), and teat-end bacterial populations have been correlated with bacterial populations found in bedding (Rendos et al., 1975; Hogan et al., 1999; Zdanowicz et al., 2004). Thus, to control environmental mastitis, dairy producers often focus on reducing exposure to pathogens found in bedding (Hillerton and Berry, 2003; Hogan and Smith, 2012).

Herds containing  $\geq 200$  milk cows currently produce 75% of all milk in the United States, and those containing  $\geq 500$  cows produce 63% of US milk (USDA-NASS, 2014). Bedding used on larger Wisconsin (WI) dairy farms includes fresh (58%) or recycled (10%) sand, organic materials (primarily wood products on top of mattresses; 22%), and manure products (10%; Rowbotham and Ruegg, 2015). Differences in the quality and quantity of milk produced per cow on larger WI dairy farms have been associated with the type of bedding used, and potential economic advantages exist for using inorganic bedding (Rowbotham and Ruegg, 2015). Whereas potential cost savings may be had for herds that use recycled bedding materials, those savings must be evaluated relative to potential differences in exposure to mastitis pathogens. The effect of different types of bedding on bacterial populations on teat skin is not well defined and is especially relevant for dairy farmers who must select bedding based on both cow management issues and environmental restrictions on manure management.

Numbers of streptococci on teats have been reported to be several log units greater than numbers of gram-negative or coliform bacteria (Rendos et al., 1975; Hogan et al., 1990). Correlations between bedding and teat-end bacterial populations differ between sand and sawdust (Zdanowicz et al., 2004), which emphasizes the

importance of studying both bedding and teat skin bacterial populations when comparing different bedding materials. Teat-end bacterial populations have also been associated with bedding DM (Proietto, et al., 2013). Most studies of bacterial populations in bedding have been of short duration (3 to 9 wk; Fairchild et al., 1982; Kristula et al., 2008; Sorter et al., 2014), with several focusing on bedding additives or treatments (Hogan et al., 1999, 2007, 2012). Few studies have compared bacterial populations in fresh and recycled sand (Kristula et al., 2005). In an observational study conducted on commercial dairy farms using clean or recycled sand (Kristula et al., 2005), seasonal differences in bacterial growth patterns were observed between summer and winter, but the study did not include organic bedding nor quantify teat skin bacteria. The objective of the current longitudinal study was to determine bacterial populations of 4 different bedding types [deep-bedded new sand (NES), deep-bedded recycled sand (RS), deep-bedded manure solids (DBMS), and shallow-bedded manure solids over foam core mattresses (SBMS)] and on teat skin swabs of primarily primiparous cows housed in a single facility over all 4 seasons.

## MATERIALS AND METHODS

### *Experimental Design and Quarter Enrollment Criteria*

The experiment was conducted at the University of Wisconsin–Madison, Marshfield Agricultural Research Station from January to December, 2013. Four bedding types were tested in a freestall barn 29.3 m wide by 59.4 m long with 4.3-m tall open side walls that contained identical pens ( $n = 4$ ), each 11.3 m wide by 26.1 m long. Each pen housed up to 32 lactating cows in 2 rows of 16 head-to-head freestalls. Freestall dimensions were: 1.65 m from rear curb to brisket locator, 1.78 m from rear curb to neck rail, 0.23 m curb height, and 1.28 m width (divider mounting on center). Alleys between freestalls and feed bunks were 4.04 m wide and alleys between freestalls and outside walls were 2.44 m wide. Throughout the period of the trial, each of the 4 pens contained a single type of bedding material: (1) NES, which was deep bedded, previously unused pit sand; (2) RS, which was deep bedded sand recycled on the farm using a screw-type sand separator designed to recover 80 to 90% of sand from manure for reuse as bedding (McLanahan, Hollidaysburg, PA); (3) DBMS, which was recycled on the farm using a screen press (PSS 1.2–520 FAN Separator, Bauer Group, Marktschorgast, Germany); and (4) SBMS, which was the same recycled manure solids as DBMS, shallow bedded over foam-core mattresses. Twice daily, as cows were milked, bedding was manually groomed and alleys were scraped. Fresh

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