

# Chipping away at the tough questions about bedding management and mastitis

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## Abstract

Over the past several decades, the dairy industry and mastitis researchers have, collectively, made great progress in improving our understanding of the role of bedding selection and management as a determinant of mastitis risk. Increased bedding bacteria counts (BBC) are associated with increased bacteria loads on teat ends and with increased risk for infection caused by environmental mastitis pathogens. Benchmarks for BBC have been established. The use of recycled manure solids is, on average, associated with higher BBC and poorer udder health, compared to inorganic materials or organic non-manure materials. However, BBC and udder health measures are highly variable among herds, regardless of the bedding material used. Several factors have been identified that are related to the processing and management of unused bedding and the management of bedding in stalls, which are associated with reduced BBC. Producers using recycled manure solids or sand bedding should strive to increase bedding dryness, with a view to reducing BBC. However, many questions remain, including the need to better understand the significance of organic matter levels in sand bedding, and to evaluate the impact of various methods of processing recycled manure solids on bedding characteristics, udder health, and economics.

**Key words:** dairy bedding, bacteria counts, mastitis

## Résumé

Au cours des dernières décennies, l'industrie laitière et les chercheurs de la mammite ont collectivement fait de grands progrès pour améliorer notre compréhension du rôle de la sélection et de la régie de la litière comme facteurs de risque de la mammite. Une augmentation du nombre de bactéries dans la litière (NBL) est associée à un accroissement de la charge bactérienne au bout des trayons et à un risque plus élevé d'infection causée par des pathogènes environnementaux de la mammite. Des normes pour les NBL ont été établies. L'utilisation de solides de fumier recyclé est généralement associée à une augmentation du NBL et à une moins bonne santé du pis par rapport aux matériaux inor-

ganiques ou aux matériaux organiques qui ne proviennent pas du fumier. Néanmoins, le NBL et les mesures reliées à la santé du pis sont très variables d'un troupeau à l'autre peu importe le matériel utilisé pour la litière. On a identifié plusieurs facteurs reliés au traitement et à la régie de la litière non-utilisée et à la régie de la litière dans les stalles qui sont associés à une réduction du NBL. Les producteurs qui utilisent des solides de fumier recyclé ou une litière à base de sable devraient s'efforcer de rendre la litière plus sèche dans le but de réduire le NBL. Toutefois, plusieurs questions demeurent incluant le besoin de mieux comprendre le rôle du niveau des matières organiques dans la litière de sable et l'évaluation de l'impact des différentes méthodes de traitement des solides de fumier recyclé sur les caractéristiques de la litière, la santé du pis et les retombées économiques.

## Introduction

With decades of progress in controlling contagious mastitis pathogens, improving the control of environmental mastitis has become the predominant concern on many North American dairies. Environmental mastitis is most frequently caused by coliform bacteria (e.g. *E. coli*, *Klebsiella* spp), environmental Streptococci and Strep-like organisms (SSLO) (e.g. *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Enterococcus* spp), and non-aureus Staphylococci (NAS). One very important environmental mastitis control strategy is to reduce teat-end exposure to bacteria in the cow's environment between milkings. Because cows spend 12 to 14 hours per day lying, bedding is an important source of teat-end exposure to environmental mastitis pathogens.<sup>16</sup> Multiple studies have reported that BBC are associated with bacterial load on the teat end.<sup>3,23,28</sup> Furthermore, evidence has been mounting to demonstrate a positive association between BBC and risk for intramammary infection (IMI). In particular, high coliform counts in bedding have been associated with an increased risk for new coliform infections.<sup>3,6,10</sup> However, a great many questions remain concerning bedding selection and management. This article will review current knowledge about the interrelationships between BBC, bedding materials, bedding management, and mastitis risk, addressing such questions as "Is there a relationship between BBC and udder health?"

“What should my BBC be?”, “Is there a best bedding material?” and, “What are the bedding characteristics and best management practices that we can manipulate to reduce BBC?”

### What is the Relationship between Bacteria Counts in Bedding and Udder Health?

Multiple studies have reported that bedding bacteria counts (BBC) are associated with bacterial load on the teat end.<sup>3,23,28</sup> Furthermore, evidence is mounting to demonstrate a positive association between BBC and risk for intramammary infection (IMI), although equivocal studies exist.<sup>8,21</sup> In particular, high coliform counts in bedding have been associated with an increased risk for new coliform infections.<sup>3,6,10</sup> In 1 year-long prospective observational study of 9 commercial herds using either inorganic bedding material (sand, crushed limestone), sawdust or chopped straw, Hogan et al reported a positive linear relationship between levels of coliform bacteria and *Klebsiella* spp in bedding and risk for clinical mastitis.<sup>10</sup> Unfortunately, that study did not include herds using recycled manure solids, which are increasing in use.

In one recent observational study of 168 herds from 17 states, herds were purposely selected to represent 1 of 4 bedding types; new sand (NS), reclaimed sand (RS), manure solids (MS) or organic non-manure materials (ON) such as straw or wood shavings.<sup>24</sup> Each herd was sampled twice (winter/summer) in 2016, collecting both unused (from the pile) and used (from stalls) bedding samples for aerobic culture. Results showed that increased counts of coliforms, *Klebsiella* spp, SSLO, and Staph bacteria in both unused and used bedding samples were associated with reduced udder health when evaluating 1 or more herd-level measures of udder health, including DHIA test day average linear score, the proportion of cows with an 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, and the proportion of cows with a chronic infection, where chronic was defined as a LS  $\geq$  4.0 on the last 2 tests. In a second more recent observational study, 80 herds from 10 major dairy states that used 1 of 4 common bedding materials (MS, ON, NS, RS) were recruited and visited in the summer and winter of 2017/2018.<sup>30</sup> At each visit, aseptic quarter-milk samples were collected from 20 randomly selected cows approaching dry-off ( $>$ 180 days pregnant), and samples of unused and used bedding were collected for aerobic culture. Results showed a positive association between the total bacteria count in unused bedding and odds (95% CL) for quarter-level infection caused by any pathogen (OR = 1.08 [1.00 – 1.07]), though this varied by bedding type. A positive association was also observed for counts of SSLO in unused bedding and odds for infection caused by SSLO (OR = 1.09 [1.00 – 1.09]). This study should be repeated with cows from all stages of lactation because infection dynamics will vary by stage of lactation. Taken as a whole, the body of research to date indicates that increased bacteria counts in bedding,

and specifically counts of coliforms, *Klebsiella* spp, SSLO, and Staph bacteria, are generally associated with increased mastitis risk.

### Is there a Best Bedding Type?

Closely related to the discussion about BBC are questions surrounding the importance of bedding material selection. Certain mastitis pathogens may be ubiquitous in some bedding materials, while others, such as *Escherichia coli* or *Klebsiella* spp, may arrive due to contamination of bedding by fecal material, water or feed.<sup>15</sup> Bacteria counts are generally reported to be higher in organic bedding materials, such as manure solids, straw or wood shavings, compared to inorganic bedding, such as new or recycled sand.<sup>2,28</sup> Because RS may have increased levels of organic matter, it may support higher BBC as compared to NS.<sup>17</sup> When comparing among organic bedding materials, Hogan et al reported that straw tended to have the highest streptococcal counts, while sawdust had the highest coliform counts.<sup>10</sup> However, the latter study did not include herds using MS. Patel et al observed that MS bedding samples generally had higher BBC compared to ON, RS, or NS bedding materials.<sup>24</sup>

Despite differences in BBC among different bedding materials, studies report equivocal results regarding the direct relationship between bedding material selection and udder health. In most studies, the use of inorganic (vs organic) bedding was associated with reduced clinical mastitis risk or lower SCC measures.<sup>10,27,33</sup> The use of recycled MS (vs other bedding materials) has been associated with increased environmental mastitis in many, but not all, studies.<sup>7,18,19,22,29</sup> One experimental study using survival analysis reported that bedding type did not affect the incidence of clinical or subclinical mastitis in primiparous cows, albeit there was a tendency for reduced risk of clinical mastitis for cows housed on deep-bedded NS as compared to RS or deep-bedded MS.<sup>29</sup> More recently, a 3-year-long clinical trial conducted with 734 cows on 1 research dairy reported that cows bedded with MS (deep bedded or on a mattress) exhibited a greater incidence of clinical mastitis than cows bedded with new or reclaimed sand (19.0 vs 8.4 cases/year).<sup>7</sup>

Patel et al observed that the use of MS bedding was associated with poorer herd level udder health measures, including elevated test day average LS and new IMI percentage, compared to ON, RS or NS, although considerable variation in udder health parameters existed among individual herds within any 1 of the 4 bedding groups studied.<sup>24</sup> Overall, herd-level udder health measures were not different among herds using NS, RS, and ON. Conversely, Rowe et al reported no significant difference in quarter-level prevalence of IMI in quarters of late-lactation cows exposed to MS (19.3%), NS (23.9%), ON (22.7%), and RS bedding (19.0%).<sup>30</sup> Similarly, in a survey study of 38 Midwest herds using MS in freestalls, authors reported that the average somatic cell count for study farms was comparable to the average in the region

and not excessively high.<sup>14</sup> Differences among studies could be attributed to a variety of factors including, but not limited to, differences in bedding materials, udder health measures, study herds and cows, or other unmeasured herd management factors associated with udder health.

The relationship between bedding material and bacteria levels in bulk-tank milk is nebulous, with 2 recent observational studies, 1 in 325 Wisconsin herds and 1 in 125 UK herds, reporting no association between use of MS (vs sand or other organic materials) and bacteria counts in bulk-tank milk.<sup>2,27</sup> However, Patel et al observed that SSLO counts in BTM were lower in herds using NS and ON, compared to MS, and that coliform counts in BTM tended to be lower in herds using RS as compared to MS or ON.<sup>24</sup> An additional concern, though not a focus of this review, is that bedding type may influence mesophilic and thermophilic spore levels in bulk tank raw milk, which can cause spoilage of dairy products.<sup>9</sup> Bacteria may arrive in bulk-tank milk from a variety of sources, including contaminated milking equipment/system, milk from an infected mammary gland, or contaminated teat skin. Patel et al reported that udder hygiene scores were generally higher in herds using MS compared to RS.<sup>24</sup> However, Lombard et al found no association between bedding type and cow hygiene.<sup>20</sup> Nonetheless, increased cow hygiene scores and udder hygiene scores have both been associated with increased SCC and risk for IMI.<sup>26,31</sup> Apart from bedding type, it is certain that udder hygiene score may be affected by other factors, including facilities, bedding and manure management, and udder preparation procedures in the parlor.<sup>20</sup>

Taken as a whole, studies suggest that, on average, the use of inorganic bedding is likely to result in better udder health than organic materials, and that, on average, herds using MS generally exhibit higher BBC and reduced udder health compared to herds using other bedding materials. However, equivocal results do exist among studies, and considerable herd-to-herd variation is evident. The fact that individual herds using MS can achieve lower BBC and good udder health indicates that management strategies or other mitigating factors must exist that can be employed to achieve good results, regardless of the bedding material in use.

### **What Should Producer Goals be for Bedding Bacteria Counts?**

It has been said: “You can’t manage what you can’t measure.” However, the utility of using bedding cultures as a monitoring tool was not previously well established, in part due to the historical absence of strong science-based cut points by which to interpret bedding culture results. Decades ago, Bramley and Neave observed an increase in coliform infections when coliform counts in sawdust bedding exceeded  $10^7$  cfu/g of wet bedding.<sup>3</sup> A separate study of 3 California dry-lot dairies reported an increase in clinical mastitis cases caused by *Klebsiella* spp when *Klebsiella* BBC exceeded  $10^6$  cfu/g of wet bedding.<sup>5</sup> Finally, Bramley stated that *E. coli* mas-

titis incidence was higher if cows were housed on sawdust with coliform counts exceeding  $10^6$  cfu/g of wet bedding.<sup>4</sup> However, these studies had several limitations, including that recommendations were limited to coliform bacteria and were usually derived from case studies or observational studies involving relatively few cows or herds. Moreover, these older cut points were often derived from studies of conventional bedding materials (e.g. shavings or straw) and may not have considered other materials in common use today (e.g. sand or manure solids). Given these limitations, more research is needed to establish thresholds for interpreting bedding culture results if this is to be a useful monitoring tool.

In a recent observational 168-herd study, Patel et al was able to identify benchmarks for monitoring bedding hygiene, whereby the cut points selected to create BBC categories (Low/High or Low/Moderate/High) were achievable by producers (i.e.  $\geq 20\%$  of samples were able to achieve a “Low” value) and differences in udder health for herds in the Low vs High BBC categories were both statistically and numerically different (Table 1).<sup>24</sup> In most cases, the same cut points can be used in all bedding materials evaluated, though some exceptions exist. Readers must be cautious when comparing bedding culture reports generated from different labs and among different studies, because laboratories may use different culture protocols and therefore may report BBC using different units, including cfu/cc of wet bedding, cfu/g of wet bedding or cfu/g on a dry-matter basis. It is important to establish a clear method of sampling, handling samples, and culturing/identifying bedding bacteria, so that the use of cut points can be made universally.

### **Management Strategies to Reduce Bedding Bacteria Counts**

Decades ago, Rendos et al, noted that the exact relationship between BBC and mastitis, and the degree to which their interaction is affected by management and individual cow factors, is unknown.<sup>25</sup> Since then, we have improved our understanding of a variety of important factors and management strategies affecting BBC and mastitis risk, including the characteristics of unused bedding and the management of bedding in stalls.

### **Characteristics of Unused Bedding**

Bacteria counts in unused bedding (i.e. in the pile/ready to use) are significantly associated with bacteria counts in used bedding (collected from stalls).<sup>24</sup> Therefore, a first step must be to identify and adopt strategies to reduce BBC in the original unused bedding. Bacteria require moisture and organic nutrients to multiply in bedding material.<sup>8</sup> As such, for some bedding materials, manipulating DM% or OM% may represent an important opportunity to reduce BBC in the unused bedding. Hogan and Smith suggested that a realistic goal for DM and OM for unused sand is  $>95\%$  and  $<5\%$ ,

**Table 1.** Suggested benchmarks to interpret bedding culture results (cfu/cc of wet bedding) (Source: Patel et al, 2019).<sup>24</sup>

Bacteria group	Bedding type	Bedding Bacteria Count Category (cfu/cc) <sup>1</sup>		
		Low	Moderate	High
Unused bedding (Collected from bedding storage area)				
Staph <sup>2</sup>	NS / RS / ON / MS	0	.	>0
<i>Klebsiella</i> spp	NS / RS / ON / MS	0	.	>0
Coliforms	NS / RS / ON / MS	≤500	.	>500
SSLO <sup>3</sup>	NS / RS / ON	0	1-1,000	>1,000
	MS	≤1,000	1,001-750,000	>750,000
Used bedding (Collected from stalls or cow resting area)				
Staph <sup>2</sup>	NS / RS / ON / MS	0	.	>0
<i>Klebsiella</i> spp	NS / RS / ON / MS	0	.	>0
Coliforms	NS / RS / ON	≤10,000	.	>10,000
	MS	≤10,000	10,001-200,000	>200,000
SSLO <sup>3</sup>	NS / RS / ON / MS	≤500,000	500,001-2,000,000	>2,000,000

NS: New sand, RS: Reclaimed sand, ON: Organic non-manure (Shavings/Straw), MS: Manure solids

<sup>1</sup> Minimum limit of detection for bedding culture is 25 cfu/cc (reported as 0)

<sup>2</sup> Staph: *Staphylococci* spp

<sup>3</sup> SSLO: Streptococci or streptococci-like organisms

respectively, and that a realistic goal for DM in MS is >35%.<sup>13</sup> However, research is needed to improve our understanding of the relative importance of these characteristics and to identify optimal DM and OM levels for specific bedding materials. In the aforementioned 168-herd observational study by Patel et al,<sup>24</sup> we were especially interested in pursuing these questions for unused MS and sand (discussed in sections a and b below), since these materials are widely used in US dairy herds and because a variety of processing techniques are available, which might be effective to manipulate DM or OM.

*a) Dry matter in unused recycled manure solids.* In the data collected by Patel et al, tremendous variation existed in DM values for the 56 MS samples within the data set, with the mean ± SD (range) value being 53.0 ± 26.1% (21.4 – 96.3).<sup>24</sup> Mixed multivariable logistic regression estimated that a 1 unit increase in DM (continuous explanatory variable) was associated with a reduced odds for the sample falling into a high BBC category for both SSLO [OR (95% CL): 0.961 (0.924, 0.998), P = 0.042] and total coliforms [OR (95% CL): 0.955 (0.922, 0.989), P = 0.012]. Using the same iterative process as previously described for creating categories for BBC, we were able to create the following recommended categories for DM% in unused MS: Poor: <35%; Moderate: 35 to 64.9%; and Excellent: ≥65%. When using these DM categories, 87.2%, 37.9%, and 24.3% of bedding samples in the poor, moderate and excellent DM categories, respectively, fell into the high BBC category for total coliforms. Achieving a higher DM category was also associated with reduced odds for a sample falling into a high SSLO category.

Studies investigating the impact of various processing techniques on characteristics of MS are extremely limited. One survey study evaluated bedding characteristics for 38 Midwest freestall herds with MS bedding, when processing methods included separation of raw (green) manure, sepa-

ration of anaerobic digested manure or separation of raw manure followed by mechanical drum composting for 18 to 24 h.<sup>14</sup> Authors reported that addition of a mechanical cool air blower post-separation and use of a shelter for bedding storage were associated with reduced moisture in fresh (unused) bedding samples but were not associated with BBC. In the observational study conducted by Patel et al,<sup>24</sup> of the 56 MS samples in the data set, 16 (28.6%) were composted, 5 (8.9%) were digested, 13 (23.2%) were passively dried (air/sun), and 22 (39.3%) were green/raw. Additionally, after initial processing, 9 of the 56 MS samples (16.4%) were mechanically dried using hot air. Mixed regression models estimated that mean (± SE) DM values in unused MS were higher in the West/Southern region (80.5 ± 2.5%) vs the Midwest/Northeast region (33.4 ± 2.2% DM), and increased in summer (57.1 ± 4.5%) vs winter months (51.1 ± 4.5%). However, a strong interaction existed between processing method and region. After stratifying the analysis, we observed that, in the Midwest/Northeast region, there was no association between initial processing methods, including digesting or composting (vs raw) and DM levels in MS. However, herds using mechanical hot air drying after initial processing exhibited significantly drier solids (44.0 ± 3.7%) compared to herds not using this processing technique (28.8 ± 2.7%) (P = 0.02). In a secondary analysis, after controlling for other initial processing techniques (i.e. digesting, composting), the use of hot air drying as a secondary processing technique for herds in the Midwest/Northeast region was associated with a significant reduction in monthly clinical mastitis incidence [est (SE): -9.19 % (2.52); P = 0.004] but not with other herd-level udder health outcomes. Readers should interpret these results with caution, given the observational nature of this study (may be confounded by other unmeasured factors) and given the relatively small sample size available for any

1 processing technique. Clearly, more research is needed to evaluate the potential benefits of various processing techniques for recycled MS.

*b) Dry matter and organic matter in unused sand bedding.* Patel et al observed that the unadjusted mean (SD) DM values were statistically higher for NS ( $95.6\% \pm 2.4$ ) ( $n=92$ ) vs RS ( $93.6\% \pm 2.7$ ) ( $n=55$ ) ( $P=0.0002$ ), though these differences were numerically small.<sup>24</sup> The crude mean (SD) OM values were lower for samples of NS ( $1.65\% \pm 2.45$ ) vs RS ( $2.92\% \pm 2.30$ ) ( $P = 0.016$ ), though, again, these differences were numerically small. These findings are in general agreement with an earlier study of herds using NS ( $n=13$ ) or RS ( $n=10$ ).<sup>17</sup> When the NS and RS data were combined, the median (range) DM and OM values for unused samples were 95.4% (83.6 to 100) and 1.5% (0 to 15.8), respectively.

After categorizing DM in unused sand (Low  $\leq 95\%$ ; High  $>95\%$ ), mixed multivariable logistic regression showed a consistent numeric reduction in risk for samples to fall into the high BBC category (dependent variable) when sand was in the high DM category (explanatory variable), even if this reduction in risk was not always statistically significant (unpublished). For example, the odds (95% CL) for a sample being classified as falling into the "High" category for SSLO, coliform or Staph bacteria in unused sand was 0.471 (0.215, 1.031) ( $P = 0.059$ ), 0.45 (0.137, 1.48) ( $P = 0.18$ ) and 0.402 (0.133, 1.211) ( $P=0.10$ ), respectively, if the sample fell into the high DM% ( $>95\%$ ) category. When including DM and OM in the same model, there was no association between OM and risk for samples being classified as falling into a high BBC category for the bacteria groups of interest, suggesting that OM may be of lesser importance as a determinant of BBC in sand. This deserves further study.

When investigating processing or management strategies that could increase DM or OM in unused sand on farms, it was observed that prior washing was associated with increased DM [est (SE) = 1.33 (0.59),  $P = 0.029$ ], but was not associated with OM levels, in new (virgin) sand. Increasing the days in storage prior to sampling also tended to be associated with higher DM levels in NS. For RS, the use of a shelter to store sand tended to be associated with increased DM [est ( $\pm$ SE) =  $1.31 \pm 0.87\%$ ,  $P = 0.14$ ], and samples were drier in summer (vs winter) months [est ( $\pm$ SE) =  $1.40 \pm 0.60\%$ ,  $P = 0.03$ ]. Somewhat surprising, DM and OM levels were not different in RS samples reclaimed using an active (mechanical) separation system (DM =  $93.69 \pm 0.69\%$ ; OM =  $3.53 \pm 0.74\%$ ) compared to using a passive (gravity) recovery system (DM =  $94.31 \pm 0.61\%$ ; OM =  $2.84 \pm 0.523\%$ ) ( $P \geq 0.45$ ).

### Managing Bedding in the Stalls

Regardless of the type of bedding material in use, studies repeatedly show that BBC increase rapidly once fresh bedding is placed into stalls.<sup>12,17</sup> Bedding material can quickly become contaminated with organic matter after the

introduction of feces in the stall. Furthermore, environmental conditions in the barn may promote bacterial replication in bedding. Hogan and Smith recommend removing wet, soiled material from the back third of stalls at least twice daily.<sup>13</sup> When organic bedding is used on mattresses or other shallow bedding systems, the complete replacement of material at least once daily will reduce bacterial load and is associated with reduced bulk milk SCC.<sup>27,32</sup> Multiple studies have reported that the application of some alkalizing or acidifying bedding conditioners may reduce BBC in some types of bedding.<sup>1,11,12,23</sup> However, the impact of bedding conditioners on udder health has not been described and the cost-benefit of this strategy requires investigation, since the duration of treatment effect is generally short lived (1 day). Pen management and facility design may also be important contributing factors: Hogan and Smith recommend that producers avoid overcrowding, as this increases manure contamination of alleys.<sup>13</sup> They also recommend that producers prevent or reduce the presence of standing water or mud, as either leads to increased splatter on hooves and legs, which will subsequently contaminate bedding in stalls. Finally, the correct sizing and design of stalls, relative to size of cows, can prevent cows from laying too far forward or at an angle in stalls; both of which increase defecation on the bedding surface.

### Conclusions

Increased bacteria counts in bedding are positively associated with bacteria loads on teat ends and are generally associated with increased risk for intramammary infection from environmental mastitis pathogens. The use of recycled MS is, on average, associated with higher BBC and poorer udder health measures, as compared to inorganic or other organic non-manure materials. However, BBC and udder health measures are highly variable among herds, regardless of the bedding material used. Several management strategies have been identified, related to bedding characteristics, the processing and management of unused bedding, and the management of bedding in stalls, which are associated with reduced BBC. Increased DM is associated with lower BBC in unused samples of MS and sand bedding. However, the association between OM and BBC in sand deserves further study. In the Midwest/Northeast region, herds using mechanical hot air drying after initial processing exhibited significantly dryer manure solids and a significant reduction in monthly clinical mastitis incidence. However, a great deal more research is needed to evaluate the impacts of various methods for processing manure solids on bedding characteristics, BBC, udder health and economics. In herds using new (virgin) sand, prior washing was associated with increased DM and increased days in storage tended to be associated with higher DM. Storing recycled sand in a shelter tended to be associated with increased sample DM.

## Acknowledgements

Financial support for studies conducted by the University of Minnesota was provided by Boehringer Ingelheim Animal Health (St. Joseph, MO),<sup>24</sup> Minnesota Dairy Herd Improvement Association (Buffalo, MN),<sup>24</sup> Dairy Management Inc.<sup>™</sup> (Rosemont, IL),<sup>24</sup> the University of Minnesota College of Veterinary Medicine (St. Paul, MN),<sup>24</sup> and Zoetis (Parsippany, NJ).<sup>30</sup> Special thanks go to the participating herds and to the many AABP veterinarians, university research and extension folks,<sup>24</sup> and Zoetis Quality Milk Specialist and Dairy Technical Service team members,<sup>30</sup> who assisted with herd recruitment and sampling. We also greatly appreciate the 4 DHIA record processing centers for helping us to retrieve necessary test day data: DRMS (Raleigh, NC), AgSource (Verona, WI), Amelcor (Provo, UT), and AgriTech (Visalia, CA).<sup>24</sup>

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