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Different management practices are associated with mesophilic and thermophilic spore levels in bulk tank raw milk

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ABSTRACT

Bacterial endospores (also referred to as spores) present in raw milk are capable of surviving pasteurization and other adverse conditions encountered during dairy powder production. Therefore, requiring low spore levels in raw ingredients (e.g., raw milk) may be necessary for producing dairy powders with low spore counts. To identify potential associations between management practices and spore levels in raw milk, we sampled bulk tank raw milk from 33 farms throughout New York State every other month for 1 yr. Following spore pasteurization (80°C for 12 min), samples were incubated at 3 different temperatures to enumerate psychrotolerant (6°C for 10 d), mesophilic (32°C for 48 h), and thermophilic (55°C for 48 h) spores. An additional enrichment procedure was used to detect spores present at low levels (<10 spores/mL). Overall, psychrotolerant, mesophilic, and thermophilic spores were detected (at levels ≥10 spores/mL) in 1, 74, and 58% of bulk tank raw milk samples, respectively. Although thermophilic spore levels could not be quantified (due to bacterial swarming), mesophilic spore levels ranged from below detection (<10 spores/mL) to 680 spores/mL. Data collected through surveys were used to identify management practices associated with either mesophilic or thermophilic spore levels. We found that different management practices are associated with mesophilic and thermophilic spore levels. Low mesophilic spore levels in bulk tank raw milk samples were associated with (1) large herd size, (2) use of sawdust or sand bedding, and (3) not fore stripping during the premilking routine. Management practices that were associated with lower odds of having a thermophilic spore level ≥10 spores/mL are (1) large herd size, (2) spray-based application of the postmilking disinfectant, (3) dry massaging the udder during the premilking routine, and (4) the use of straw bedding. Collectively, these results suggest that different management practices may influence mesophilic and thermophilic spore levels in raw milk.

Key words: spores, raw milk, management practices

INTRODUCTION

Sporeforming bacteria of the *Clostridiaceae* and *Bacillaceae* families represent important spoilage organisms for a variety of dairy foods (Scheldeman et al., 2005; De Jonghe et al., 2010; Ivy et al., 2012). In HTST pasteurized fluid milk, psychrotolerant *Bacillus* spp. and *Paenibacillus* spp. contribute off flavors and aromas which reduce product shelf life (Ranieri and Boor, 2009). In semi-hard cheeses, the anaerobic interior provides a favorable environment for *Clostridium tyrobutyricum*, which metabolizes lactate into hydrogen gas, resulting in "late blowing" defects (Klijn et al., 1995). In dairy powders, stringent customer specifications mandate low spore levels (A. Bienvenue, United States Dairy Export Council, Arlington, VA, personal communication). According to a recent survey by the US Dairy Export Council, dairy powders destined for use in baby formulas have aerobic mesophilic and thermophilic spore limits that range from <500 to 1,000 cfu/g (A. Bienvenue, United States Dairy Export Council, Arlington, VA, personal communication).

Raw milk has been implicated as an important source of endospores produced by mesophilic sporeforming bacteria (abbreviated here as mesophilic spores; **MS**), by thermophilic sporeforming bacteria (thermophilic spores; **TS**), and by psychrotolerant sporeforming bacteria (psychrotolerant spores; **PS**). However, several studies have shown that the spore populations of dairy powders are generally different from those of raw milk (Scott et al., 2007; Burgess et al., 2010; Hill and Smythe, 2012). For example, although TS of *Anoxybacillus* spp. and *Geobacillus* spp. are frequently isolated from dairy powders, they are rarely isolated from raw milk (McGuiggan et al., 2002; Rückert et al., 2004; Scott et al., 2007). It has been suggested that TS of *Anoxybacillus* spp. and *Geobacillus* spp. are either (1) present at very low levels in raw milk and are able to germinate and produce vegetative cells capable of growing in the product during processing, or (2) that the TS are able to attach to surfaces within the processing environment where they germinate and form biofilms

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that contaminate the product throughout the processing run (Burgess et al., 2010; Watterson et al., 2014).

A large proportion of MS and TS found in bulk tank (**BT**) raw milk are thought to originate from the farm environment, as high levels of MS and TS have been isolated from silage, bedding materials, and manure (McGuiggan et al., 2002; Vissers et al., 2007; Quiberoni et al., 2008). Furthermore, Julien et al. (2008) used DNA-based analyses to show that the same spore subtypes were isolated from both BT raw milk and the corn silage fed to milking cows, implicating feed as an important source of spore contamination.

Although numerous studies have examined management practices associated with SCC and SPC levels in BT raw milk (Barkema et al., 1998; Barnouin et al., 2005; Elmoslemany et al., 2010), our understanding of the influence of management practices on the transmission of spores from the farm environment into BT raw milk remains limited despite the significant economic importance of spore counts in the context of dairy powder production. Therefore the goals of this study were to (1) enumerate psychrotolerant, mesophilic, and thermophilic spores present in BT raw milk, and (2) determine management practices that are associated with spore levels in BT raw milk.

MATERIALS AND METHODS

Farm Enrollment

A total of 33 dairy farms located throughout New York State were included in the study. Participating farms were recruited based on their being enrolled in Quality Milk Production Services (**QMPS**) facilitated monitoring programs (College of Veterinary Medicine, Cornell University, Ithaca, NY). Farms were selected to represent 4 general geographic areas in upstate New York from the 4 QMPS laboratory locations (see Supplemental Table S1; http://dx.doi.org/10.3168/jds.2015-9406). At least 1 certified organic farm was included per region. Herds were also selected to represent a range of sizes and historical SCC levels (see Supplemental Table S1 for a detailed summary of farm characteristics; http://dx.doi.org/10.3168/jds.2015-9406).

Bulk Tank Raw Milk Sample Collection

Bulk tank raw milk samples were collected from each of the 33 dairy farms every other month between April 2012 and March 2013 (i.e., half of the farms had their first sample date in April 2012, whereas the remaining farms were first sampled in May 2012). Bulk tank raw milk was agitated before collection of approximately 400 mL of milk with a sterile sampling receptacle. Bulk tank raw milk samples were divided between two 300-mL polyethylene terephthalate vials and were held at ≤6°C during transport to the Milk Quality Improvement Program (**MQIP**) laboratory (Cornell University, Ithaca, NY). Samples were either brought directly to the laboratory by QMPS technicians, or were shipped via overnight delivery within 24 h of sampling; raw milk BT samples thus may have been tested between 24 and 72 h after milking. Because spores are unlikely to germinate at 6°C, time between milking and testing of BT milk was not presumed to affect the spore counts. Bulk tank raw milk sample temperatures were taken immediately upon receipt to the MQIP laboratory. If the sample temperature exceeded 6°C, a new sample was collected. Bulk tank raw milk samples were also submitted for SCC analysis (Dairy One Cooperative, Ithaca, NY).

Survey Administration

Surveys were administered verbally to dairy farm personnel at each sample collection by QMPS technicians. Questions focused on housing and bedding types used, premilking routine, and sanitation practices (see Table 1). Cow hygiene was assessed by technicians using a convenience sample of 10 cows per farm per sample date; a scale of 1 to 4 was used to score the cleanliness of the udder and hind legs of each cow, with lower scores indicating better hygiene (Barkema et al., 1998). Stall cleanliness was assessed using predefined terms (good, fair, and poor). All survey data were entered into an Access database (Microsoft Corporation, Seattle, WA).

Quantification of Psychrotolerant, Mesophilic, and Thermophilic Spores

Approximately 200 mL of each BT raw milk sample were aseptically transferred to a sterile glass Pyrex (Corning Inc., Corning, NY) bottle and agitated according to the *Standard Methods for the Evaluation of Dairy Products*, 17th ed. (**SMEDP**; Frank and Yousef, 2004). Each sample of BT raw milk was evaluated for total bacteria count on SPC agar (Difco, Franklin Lakes, NJ). Aliquots of each BT raw milk sample were spore pasteurized (**SP**) at 80°C for 12 min (Frank and Yousef, 2004), followed by rapid cooling to ≤6°C. Spore pasteurized samples were spiral plated (Autoplate 4000, Spiral Biotech, Norwood, MA) onto brain heart infusion agar (**BHI**; Difco, Franklin Lakes, NJ) in duplicate for (1) psychrotolerant spore count (**PSC**;