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Longitudinal assessment of dairy farm management practices associated with the presence of psychrotolerant Bacillales spores in bulk tank milk on 10 New York State dairy farms

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ABSTRACT

The ability of certain spore-forming bacteria in the order Bacillales (e.g., *Bacillus* spp., *Paenibacillus* spp.) to survive pasteurization in spore form and grow at refrigeration temperatures results in product spoilage and limits the shelf life of high temperature, short time (HTST)-pasteurized fluid milk. To facilitate development of strategies to minimize contamination of raw milk with psychrotolerant Bacillales spores, we conducted a longitudinal study of 10 New York State dairy farms, which included yearlong monthly assessments of the frequency and levels of bulk tank raw milk psychrotolerant spore contamination, along with administration of questionnaires to identify farm management practices associated with psychrotolerant spore presence over time. Milk samples were first spore pasteurized (80°C for 12 min) and then analyzed for sporeformer counts on the initial day of spore pasteurization (SP), and after refrigerated storage (6°C) for 7, 14, and 21 d after SP. Overall, 41% of samples showed sporeformer counts of >20,000 cfu/mL at d 21, with *Bacillus* and *Paenibacillus* spp. being predominant causes of high sporeformer counts. Statistical analyses identified 3 management factors (more frequent cleaning of the bulk tank area, the use of a skid steer to scrape the housing area, and segregating problem cows during milking) that were all associated with lower probabilities of d-21 Bacillales spore detection in SP-treated bulk tank raw milk. Our data emphasize that appropriate on-farm measures to improve overall cleanliness and cow hygiene will reduce the probability of psychrotolerant Bacillales spore contamination of bulk tank raw milk, allowing for consistent production of raw milk with reduced psychrotolerant spore counts, which will facilitate production of HTST-pasteurized milk with extended refrigerated shelf life.
Key words: *Bacillus* spp., *Paenibacillus* spp., spoilage, management practice

INTRODUCTION

Bacterial spoilage is the predominant limiting factor in the shelf life of pasteurized fluid milk (Boor, 2001; Durak et al., 2006). In the absence of postpasteurization contamination of the product, spore-forming bacteria are the predominant residual organisms in pasteurized fluid milk. Gram-positive *Bacillus* spp. and *Paenibacillus* spp. form heat-resistant spores that are able to withstand the HTST [72°C (161°F), 15 s] pasteurization commonly used for fluid milk processing (Collins, 1981; Fromm and Boor, 2004; Ranieri et al., 2009), with certain strains able to grow at refrigerated storage temperatures, resulting in milk spoilage (Washam et al., 1977; Huck et al., 2008). Previous work identified *Bacillus* spp. as predominant sporeformers early in the refrigerated shelf life of fluid milk, whereas the prevalence of *Paenibacillus* increased over shelf life (Fromm and Boor, 2004; Ranieri et al., 2009). Characterization of bacterial isolates showed that only a few specific *Bacillus* species (e.g., *Bacillus weihenstephanensis*) but many *Paenibacillus* species are able to grow during refrigerated storage of milk. These spore-forming spoilage bacteria, via metabolic activities, can lead to loss of product quality (e.g., curdling, off-odors or off-flavors; Ageitos et al., 2007; Dutt et al., 2009). Controlling these psychrotolerant Bacillales spores in raw milk is critical to the extension of fluid milk shelf life.

Elimination of psychrotolerant spore-forming bacteria is challenging as they are found throughout the general environment (e.g., soil, decaying matter, plant surfaces, mammalian digestive tracts; Gilliam et al., 1984; Gilliam, 1985; Sarkar, 1991; Fredrickson and Onstott, 1996; Nicholson, 2002) and, more specifically, the dairy farm environment. For example, *Bacillus* spp. were frequently isolated on Scottish dairy farms, from

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both the dairy farm environment and raw bulk tank milk (Crielly et al., 1994). *Paenibacillus* spp. have also been isolated from silage, dairy cow feed concentrate, and raw milk (Vaerewijck et al., 2001; te Giffel et al., 2002; Scheldeman et al., 2004). In a recent study in the United States, spore-forming psychrotolerant bacteria were isolated from samples taken along the dairy processing continuum from milk trucks to packaged final products; identification of the same bacterial subtypes in both raw and pasteurized milk samples suggests that pasteurized fluid milk spoilage can result from spores that originate from raw milk on the farm (Huck et al., 2007b).

A previous cross-sectional study on the relationship between on-farm management practices and psychrotolerant Bacillales spore levels in bulk tank milk from 99 dairy farms in New York State (**NYS**) found that practices related to milking time hygiene may influence the levels of spores present in bulk tank raw milk (Masiello et al., 2014). Specifically, improved udder cleanliness was linked to reduced psychrotolerant Bacillales spore counts (Masiello et al., 2014). Due to the cross-sectional nature of that study, it did not evaluate how spore levels or management practices may change over time on a dairy farm. Exploring the factor of time is important, because previous work has identified associations between the isolation of psychrotolerant *Bacillus* spp. from fluid milk and specific seasons (Phillips and Griffiths, 1986; Griffiths and Phillips, 1990; Sutherland and Murdoch, 1994). For example, studies on both bulk tank and pasteurized milk in Scotland found that psychrotolerant spore-forming bacteria were more commonly isolated in the summer and autumn months (Phillips and Griffiths, 1986; Griffiths and Phillips, 1990). Another Scottish study exploring seasonal occurrence found that psychrotolerant *Bacillus* spp. were most prevalent during the summer and autumn months whereas mesophilic (growth at 30°C for 72 h) *Bacillus* spp. often dominated in the winter months (Sutherland and Murdoch, 1994).

We hypothesized that specific farm-associated factors, including on-farm management practices and seasonal factors, may be associated with psychrotolerant spore contamination of bulk tank raw milk. With the goal of future development of specific recommendations for management of spore numbers in bulk tank milk, the objectives of this study were to (1) assess and characterize psychrotolerant spore contamination patterns in bulk tank milk across and within dairy farms over time; and (2) evaluate the associations of on-farm management practices and seasonal factors with psychrotolerant spore contamination in bulk tank milk over the course of 1 yr.

MATERIALS AND METHODS

Farm Selection

A longitudinal study with monthly sampling repetitions was conducted on 10 NYS dairy farms from February 2011 to February 2012 (excluding December 2011). Herds were selected from the Quality Milk Production Services (**QMPS**; College of Veterinary Medicine, Cornell University, Ithaca, NY) program clientele at 4 QMPS locations, representing 4 regions in NYS, including regions surrounding Ithaca (3 farms), Canton (2 farms), Cobleskill (3 farms), and Geneseo (2 farms). Farms were selected based on willingness to participate and previous classification of psychrotolerant Bacillales spore levels, as described by Masiello et al. (2014). Specifically, 5 farms previously identified as having a "high" psychrotolerant Bacillales spore level [≥3 log cfu/mL after 21 d of 6°C storage after spore pasteurization (80°C, 12 min)] and 5 farms previously identified as having a "low" psychrotolerant Bacillales spore level (<3 log cfu/mL over all 21 d of 6°C storage after spore pasteurization) were included in the study. All participants were fully informed of the design of the study, the nature of the data being collected, and their future use, and all were aware that their participation in the study was voluntary. Each participant signed an informed consent document acknowledging the above items.

Farm Characteristics

Herd sizes ranged from 32 to 1,368 cows, with a mean herd size of 535 ± 480 cows. Cows were housed in freestalls (65%), tiestalls (25%), or on pasture (10%). Cow breeds included Holstein (60%) and multiple or mixed breeds (40%). The number of lactating cows per farm ranged from 26 to 909 (mean of 371 ± 326 lactating cows), with farms milking between 2 and 4 times daily (30% at 2×, 60% at 3×, and 10% at 4×). Average milk production across farms was 10,821± 2,714 kg (23,857 ± 5,984 lb) per cow per year and ranged from 4,468 to 14,250 kg (9,850 to 31,415 lb).

Survey Design

The survey used in this study was modified from an existing QMPS survey that included questions on herd health, housing cleanliness, equipment maintenance, milking time procedures, and medication usage (Table 1). The modified survey included 4 pages previously used by QMPS and 1 page that focused on specific potential risk factors deemed relevant for spore contamination (Table 1); the supplemental 1-page survey