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Review

Quantifying changes in spore-forming bacteria contamination along the milk production chain from farm to packaged pasteurized milk using systematic review and meta-analysis

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ABSTRACT

Approximately one-third of the fluid milk produced in the United States is lost annually. One important factor contributing to the loss is the contamination with spore-forming bacteria, which can not only survive the pasteurization process but also grow under refrigeration conditions, resulting in subsequent spoilage. The objective of this study was to describe the population dynamics of spore-forming bacteria in milk, from the farm to the packaged product, through a systematic review approach. A database search was conducted to identify, appraise, and summarize primary research studies that described the prevalence and/or concentration of spore-forming bacteria throughout the fluid milk supply chain. Due to variations in sampling points in reported studies, the pasteurized milk supply chain was standardized to include the following steps: “milking machine”, “raw milk”, “bulk tank”, “transportation”, “silo”, “pasteurized milk” and “packaged milk”. A literature search retrieved 9,778 citations, from which 46 relevant citations were selected for data extraction and subsequent meta-analysis. Concentration data were more abundant with 758 data points, as compared to prevalence data with 74 data points. In general, great heterogeneity was observed among studies on the contamination in milk samples with spore-forming bacteria. The findings showed that the concentration of spore-forming bacteria in milk samples increased within the range of 0.58–2.41 logs CFU/mL from raw milk to pasteurized milk according to the weighted mean estimates. Similarly, the prevalence of contaminated samples with spore-forming bacteria increased from 23% on farm, to up to 58% at the step of “pasteurized milk”. Meta-regression analysis indicates that the variables, season and location, of the study, accounted for 56.35% of the between-study heterogeneity. Although considerable primary research has been conducted on this topic, there are limited studies that comprehensively describe the dynamic changes of spore-forming bacteria under the current milk production system. In summary, the analyses based on comprehensively collected evidence show that the contamination of spore-forming bacteria originating from the farm remains stable with steady increases as the milk moves downstream. These findings indicate that in addition to on-farm interventions, special attention should be paid to introducing effective mitigation measures at the processing stage to further lower spore-forming bacteria levels introduced by the raw milk, and to prevent post-pasteurization contamination from raw ingredients and processing environments.

Contents

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1. Introduction

During milk processing, microbial contamination is the hurdle to overcome to provide safe products with a long shelf life. During production, contamination can be initially introduced into the milk supply chain, such as through milking and storage (Huck, Sonnen, & Boor, 2008). Some of these microorganisms produce a non-vegetative form called spore that provides resistance to heat, pressure, and detergents. Due to this elevated resistance, spores can survive pasteurization and thermal inactivation (Rodrigo B.A. Oliveira, Baptista, et al., 2018) and later germinate into their vegetative stages, which can cause milk spoilage. They can also form biofilms, which can become a continuing source of contamination in the processing environment (Rodrigo B.A. Oliveira, Baptista, et al., 2018). Spore-forming bacteria relevant to the dairy industry can be classified into two groups: i) aerobic spore-forming bacteria, such as *Bacillus cereus, Paenibacillus* spp. and *Geobacillus stearothermophilus*; and ii) anaerobic spore-forming bacteria, such as *Clostridium tyrobutyricum* (Doyle et al., 2015). Depending on their growth profile (i.e. psychrotrophic, mesophilic or thermophilic strains), aerobic spore-forming bacteria play an important role as indicators of the quality of milk powder (mesophilic and thermophilic) (Bienvenue, 2014; Watterson, Kent, Boor, Wiedmann, & Martin, 2014), and they may limit the shelf life of fluid milk (psychrotrophic) (Reid A Ivy et al., 2012; Ranieri & Boor, 2009). Anaerobic bacteria, like *Clostridium tyrobutyricum,* are characterized by the gas production and highlighted by their ability to cause defects in cheeses.

Research has described that the initial contamination of milk with spore-forming bacteria can occur on dairy farms. Contaminated teat skin is considered one of the major sources of spores in raw milk (McKinnon & Pettipher, 1983; Samaržija, Zamberlin, & Pogačić, 2012). It has also been documented that the number of spores present in raw milk is correlated to the degree of soil contamination on teats (Christiansson, Nilsson, Bertilsson, & Int Dairy, 1996). Raw milk is consequently transported to the processing facility where pasteurization occurs. The pasteurization procedure is able to effectively kill the vegetative cells, but is not capable of controlling the spores (Novak, Call, Tomasula, & Luchansky, 2005). In addition, research has suggested that the germination of these aerobic spores may be triggered by the pasteurization process (Hanson, Wendorff, & Houck, 2005).

During processing, bulk milk storage tanks, pipelines, and filling machines are also key contamination sources due to biofilm formation on food-contact surfaces of equipment (Marchand et al., 2012). Most of the spore-forming bacteria are able to create biofilms, which are very resistant to temperature and sanitation, and represent an additional hurdle for the dairy industry.

Certain spore-forming bacteria have the ability to grow under refrigeration conditions, leading to the production of different lipolytic and proteolytic enzymes that will break down the major constituents of milk, thus affecting its sensory quality (Samaržija et al., 2012). Even though this capability is only observed in certain genera/species, including *Paenibacillus* spp., *Viridibacillus* spp., and *B. weihenstephanesis* (R. A. Ivy et al., 2012; Ranieri, Huck, Sonnen, Barbano, & Boor, 2009), these strains seem to be the major cause of milk spoilage under refrigerated storage conditions. Defects in fluid milk, such as protein coagulation and rancidity, can be manifested due to the breakdown of proteins and fat lipolysis in low temperature conditions. In addition, for certain dairy products, such as milk powder, high levels (>500/mL) of spores in the final product limits their potential markets, leading to the loss of trade opportunities when quality standards of more profitable markets (e.g., global infant formula markets) cannot be met (Bienvenue, 2014). Finally, the persistence of spore-forming bacteria is especially relevant to cheese industry. A survey of spore-forming bacteria in Brazil reported that processed cheese had rates of occurrence up to 75.55% for some species, highlighting the importance of having low spore loads in raw ingredients (Rodrigo B.A. Oliveira, Lopes, et al., 2018).

Due to the unique problems posed by spore-forming bacteria for milk and milk products, the dairy industry needs to understand the dynamics of spore-forming bacterial populations across the milk supply chain, which can subsequently lead to potential intervention strategies to protect the safety and quality of their products. Therefore, there has been a marked increase in the number of publications documenting technical knowledge and scientific research on this topic. In general, the contamination of spore-forming bacteria at specific points along the milk supply chain have been reported. However, a limited number of studies have reported the cumulative impact of current practices over the entire system. In addition, research papers quantifying the contamination of spore-forming bacteria in milk are available, but with great heterogeneity that results from differences in study design and implementation, sampling schemes, and detection methods. A holistic and systematic understanding of the levels of spore-