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Effect of post-pasteurization contamination on fluid milk quality[1]

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ABSTRACT

Fluid milk quality in the United States has improved steadily over the last 2 decades, in large part due to the reduction in post-pasteurization contamination (PPC). Despite these improvements, some studies suggest that almost 50% of fluid milk still shows evidence of PPC with organisms that are able to grow at 6°C, even though PPC may be much less frequent in some facilities. Several gram-negative bacteria, when introduced as PPC, can grow rapidly at refrigeration temperatures around 6°C and can lead to bacterial levels above 20,000 cfu/mL (the regulatory limit for bacterial numbers in fluid milk in the United States) and spoilage that can be detected sensorially within 7 to 10 d of processing. Importantly, however, storage temperature can have a considerable effect on microbial growth, and fluid milk stored at 4°C and below may show considerably delayed onset of microbial growth and spoilage compared with samples stored at what may be considered mild abuse (6°C and above). Notable organisms that cause PPC and grow at refrigeration temperatures include psychrotolerant *Enterobacteriaceae* and coliforms, as well as *Pseudomonas*. These organisms are known to produce a variety of enzymes that lead to flavor, odor, and body defects that can ultimately affect consumer perception and willingness to buy. Detecting PPC in high temperature, short time, freshly pasteurized fluid milk can be challenging because PPC often occurs sporadically and at low levels. Additionally, indicator organisms typically used in fluid milk (i.e., coliforms) have been shown to represent only a fraction of the total PPC. Recent studies indicate that coliforms account for less than 20% of the total gram-negative organisms introduced into fluid milk after pasteurization. In contrast, *Pseudomonas*, which is not a coliform and therefore is not detected using coliform media, is the most commonly isolated genus in PPC fluid milk. To reduce PPC, processors must (1) use testing methods that can detect both coliforms and non-coliform gram-negatives (i.e., *Pseudomonas*) to understand true contamination rates and patterns, and (2) establish cleaning and sanitation protocols and employee and management behaviors that target persistent and transient PPC organisms.

Key words: fluid milk, post-pasteurization contamination, *Pseudomonas*, sanitation, *Enterobacteriaceae*

INTRODUCTION

Raw milk, even when produced under ideal circumstances, has a diverse bacterial ecology that reflects the lifestyle of the animal and the environment in which the commodity is produced. A wide range of gram-positive and gram-negative bacteria, pathogens, spoilage bacteria, and organisms that are commensal with the animal or cause animal disease are found in raw milk. Fortunately, pasteurization, which was widely adopted in the United States in the 1940s, reduces the levels of many of these organisms by up to 6 orders of magnitude (Villamiel and de Jong, 2000). Certain heat-resistant or thermoduric bacteria (e.g., *Micrococcus*) are capable of surviving pasteurization conditions (e.g., 72°C/15s) in vegetative form, but these organisms are typically not able to grow under refrigeration (Gleeson et al., 2013). Additionally, spore-forming bacteria can survive pasteurization in spore form; importantly, several aerobic sporeformers that can grow under refrigeration conditions have been identified in both raw milk and HTST-pasteurized fluid milk (Ivy et al., 2012). When post-pasteurization contamination (**PPC**) occurs with organisms that can grow at refrigeration temperatures, the gram-negative organisms introduced typically cause spoilage and reach levels above the Pasteurized Milk Ordinance (**PMO**) limit of 20,000 cfu/mL before growth of psychrotolerant sporeformers occurs and appear to outcompete these sporeformers. In the absence of PPC with gram-negative, psychrotolerant organisms, aerobic psychrotolerant sporeformers present in raw milk typi-

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cally grow to spoilage levels after 14 d at 6°C (Ranieri and Boor, 2009). The predominant spore-forming bacteria capable of growing at refrigeration temperatures are certain strains of *Paenibacillus* and *Viridibacillus*, along with *Bacillus weihenstephanensis* (Ivy et al., 2012). We might expect that much of the fluid milk supply would be spoiled by these aerobic spore-forming bacteria that originate in raw milk and survive pasteurization, yet almost 50% of the fluid milk supply shows evidence of contamination with heat-labile gram-negative bacteria that originate from the processing facility environment and recontaminate fluid milk after pasteurization.

Post-pasteurization contamination of fluid milk with psychrotolerant spoilage bacteria plays a significant role in limiting the quality and shelf life of conventionally pasteurized fluid milk. From the earliest days of pasteurization, recontamination of fluid milk after pasteurization has been identified as a problem. In 1920, milk inspectors were urged by Russel S. Smith of the Department of Agriculture to "not rest assured of a safe product because of the mere presence of a milk pasteurizing plant in their city. Special attention must be given to the operation of such a plant in view of the fact that unless it is properly operated it may become a chance source of infection" (Smith, 1920). Smith goes on to outline locations within the processing facility that should receive particular attention to prevent recontamination; namely, pumps, bottling machines, bottles, and milk cans (Smith, 1920). Despite the passage of nearly a century since the above advice to the dairy industry, PPC remains an important cause of fluid milk spoilage. This review focuses on the effects of PPC on fluid milk quality with special attention paid to the organisms commonly responsible for PPC, where they are typically introduced into fluid milk, and diagnostic tools for detecting and tracking them in product and processing environments.

PSEUDOMONAS IS THE PRIMARY CAUSATIVE AGENT OF PPC IN FLUID MILK

Pasteurization is designed to reduce the populations of the most heat-resistant vegetative pathogen found in milk, *Coxiella burnetii*, to levels that would not pose a public health risk (Holsinger et al., 1997). The resultant pasteurization parameters, a minimum of 72°C for 15 s for HTST processing, are reported to deliver a considerable reduction in psychrotolerant gram-negative bacteria (Champagne et al., 1994), and at least a 6-log reduction in some species of *Pseudomonas* (Villamiel and de Jong, 2000). Therefore, the presence of *Pseudomonas* and other gram-negative bacteria in pasteurized fluid milk is typically an indication that a contamination event has occurred post-processing. However, pasteurization failures and presence of high levels of gram-negative bacteria (with subsequent survival of pasteurization of some bacterial cells) in raw milk may also be responsible for presence of gram-negative bacteria in finished HTST products. The PMO limits total bacterial counts in grade 'A' commingled raw milk to 300,000 cfu/mL (FDA, 2015). However, if this raw product is held for an extended period of time or at an elevated temperature before pasteurization, bacterial numbers may reach concentrations where even a 6-log reduction would result in residual bacterial cells in pasteurized finished product. Although these deviations (i.e., pasteurization failure and very high pre-pasteurization bacterial levels) are less likely to be an issue in countries with well-developed and sophisticated dairy industries, high levels of gram-negative bacteria in raw milk are not unusual in countries that lack effective on-farm cooling practices and an effective farm-to-processing plant refrigeration chain.

Four primary groups of psychrotolerant bacteria are important in PPC of fluid milk (Table 1): (1) *Pseudomonas*; (2) coliforms; (3) non-*Pseudomonas*, noncoliform gram-negative bacteria; and (4) gram-positive spore-forming bacteria. *Pseudomonas* is, by far, the most commonly reported organism responsible for PPC of HTST fluid milk in the United States (Ranieri and Boor, 2009; Martin et al., 2011b) and globally, including Sweden (Ternström et al., 1993; Eneroth et al., 1998), Australia (Juffs, 1973; Deeth et al., 2002), the UK (Schröder, 1984; Stevenson et al., 2003), and others. Several factors contribute to the success of *Pseudomonas* as an agent of PPC, the first being its ability to grow rapidly at low temperatures (Ternström et al., 1993). Ranieri and Boor (2009) reported that samples of HTST fluid milk contaminated with *Pseudomonas* had significantly higher bacterial numbers by 7 d after pasteurization than samples lacking PPC, with those samples contaminated with *Pseudomonas* reaching the PMO limit of 20,000 cfu/mL at d 8 after pasteurization, on average, compared with d 15 for samples with no PPC but with presence of psychrotolerant sporeformers. Trmčić et al. (2015) demonstrated that various *Pseudomonas* strains were capable of growing more than 4 log cfu/mL over 21 d at a slightly stressed refrigeration temperature (i.e., 6°C). Additionally, *Pseudomonas* are known to be particularly adept at outcompeting other spoilage microorganisms due in part to the ability of many strains to produce antibacterial and antifungal agents and siderophores (Gram et al., 2002), which are excreted into the growth medium where they bind and solubilize iron. *Pseudomonas* produce a variety of siderophores, notably pyoverdin (Brown and Luke,