A 100-Year Review: Microbiology and safety of milk handling¹

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ABSTRACT

Microbes that may be present in milk can include pathogens, spoilage organisms, organisms that may be conditionally beneficial (e.g., lactic acid bacteria), and those that have not been linked to either beneficial or detrimental effects on product quality or human health. Although milk can contain a full range of organisms classified as microbes (i.e., bacteria, viruses, fungi, and protozoans), with few exceptions (e.g., phages that affect fermentations, fungal spoilage organisms, and, to a lesser extent, the protozoan pathogens *Cryptosporidium* and *Giardia*) dairy microbiology to date has focused predominantly on bacteria. Between 1917 and 2017, our understanding of the microbes present in milk and the tools available for studying those microbes have changed dramatically. Improved microbiological tools have enabled enhanced detection of known microbes in milk and dairy products and have facilitated better identification of pathogens and spoilage organisms that were not known or well recognized in the early 20th century. Starting before 1917, gradual introduction and refinement of pasteurization methods throughout the United States and many other parts of the world have improved the safety and quality of milk and dairy products. In parallel to pasteurization, others strategies for reducing microbial contamination throughout the dairy chain (e.g., improved dairy herd health, raw milk tests, clean-in-place technologies) also played an important role in improving microbial milk quality and safety. Despite tremendous advances in reducing microbial food safety hazards and spoilage issues, the dairy industry still faces important challenges, including but not limited to the need for improved science-based strategies for safety of raw milk cheeses, control of postprocessing contamination, and control of sporeforming pathogens and spoilage organisms.

Key words: dairy food safety, cheese safety, pasteurization

INTRODUCTION

The first issue of the *Journal of Dairy Science* (JDS) in 1917 comprised 5 articles, including 1 article on the microbiology of milk authored by Cornell scientists R. S. Breed and W. A. Stocking (Breed and Stocking, 1917). The article reported both agar-based and direct microscopic bacterial counts for “market milk” collected in New York. This article ended with the noteworthy conclusion, “Research men, using technique which differs much in details, may be depended upon to secure much more consistent agar plate counts from ordinary samples of market milk than laboratory assistants working rapidly and using the routine methods of analysis recommended for the purpose.” Although we, the authors of the review presented here, are indeed located in Stocking Hall, which was named after the author of the first JDS microbiology paper, today research women and men are using different rapid detection methods and molecular biology tools, including whole-genome sequencing, to ensure the safety and quality of milk throughout the world.

Between 1917 and 2017, our understanding of the microbiology of raw milk and dairy products has undergone tremendous advances (see Appendix Table A1), in no small part because of the development and use of new microbiological techniques and methods, including development of improved selective and differential bacteriological media and development of molecular biology tools (e.g., PCR). In parallel, development and implementation of improved technologies and systems to control microbial food safety hazards and spoilage organisms have significantly improved dairy product quality and reduced public health hazards associated with dairy products to a level that would have been unimaginable in 1917, as illustrated by the modern availability of HTST pasteurized fluid milk with shelf lives of >21 d.

MICROBIOLOGY AND SAFETY OF MILK HANDLING FROM 1917 TO 2017

Transition to Pasteurization

The widespread implementation of pasteurization for raw milk has improved public health by preventing the

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spread of foodborne diseases across the United States. However, pasteurization was initially controversial and slow to be adopted as a common practice. In 1864, Louis Pasteur discovered that gradually heating wine, and then rapidly cooling it, prevented abnormal wine fermentation due to spoilage microorganisms; this process came to be known as pasteurization. Although Pasteur himself did not apply this principle to milk, implementation of milk pasteurization started considerably before 1917. As early as 1873, the American pediatrician Abraham Jacobi advocated boiling cow milk in bottles before feeding it to infants (Jacobi, 1873; Holsinger et al., 1997). Later, in 1886, the German chemist Franz von Soxhlet devised an apparatus for in-home, in-bottle milk sterilization for infants; this procedure involved boiling milk for 40 min (Andrews and Fuchs, 1944). By 1893, under the advice of Jacobi and others, philanthropist Nathan Straus opened milk depots in New York City, providing sterilized milk to infants of impoverished families (North, 1921; Steele, 2000). Despite an observed effect of sterilization on reducing infant mortality rates and growing support for in-home pasteurization of cow milk for infant consumption, implementation of widespread commercial pasteurization faced strong opposition (North, 1921; Andrews and Fuchs, 1944). Many public health officials and doctors in the United States opposed widespread commercial pasteurization of milk, fearing it would provide only a stopgap measure that might create a false sense of security for the processor while distracting farmers from the need to increase on-farm sanitary measures (North, 1921; Andrews and Fuchs, 1944). In addition, although some recognized pasteurization as a useful method for reducing milk spoilage, they remained concerned about its ability to effectively inactivate milkborne pathogenic microorganisms (Andrews and Fuchs, 1944). Nevertheless, in 1907, the first commercial-scale apparatus for pasteurizing milk by the holding method was installed in New York City (Andrews and Fuchs, 1944). A pivotal shift occurred in January 1908, when the US Public Health Service (USPHS) and Marine Hospital Service published *Milk and its Relation to the Public Health*, which revealed that the consumption of raw milk was dangerous and was often the cause of tuberculosis, typhoid fever, diphtheria, scarlet fever, and intestinal disorders of babies. In this document, US Surgeon General Walter Wyman famously wrote, "Pasteurization prevents much sickness and saves many lives" (Wyman, 1908). This report prompted states to respond to public health concerns surrounding diseases associated with raw milk. In July 1908, Chicago became the first American city to pass an ordinance requiring the pasteurization of all cow milk

entering the city, except for that from tuberculin-tested cows (Czaplicki, 2007). This ordinance was originally intended only as a temporary measure to control the spread of bovine tuberculosis; simultaneously, farmers were expected to bring their herds into compliance with tuberculin testing (Czaplicki, 2007). However, many cities followed suit, releasing similar ordinances regarding pasteurization (Andrews and Fuchs, 1944). As a result, commercial milk processing facilities were constructed throughout the United States to meet compliance needs, thus rapidly spreading the practice of pasteurization (Andrews and Fuchs, 1944). Once pasteurized milk was introduced to the public, pasteurized milk and milk products rapidly penetrated the market. According to Smith-Howard (2013), by 1916, 80 to 90% of the milk sold in Chicago, Boston, Philadelphia, and New York was pasteurized.

To assist in the prevention of milkborne diseases, in 1924 the USPHS published the *Standard Milk Ordinance for Alabama Municipalities*, later referred to as the first Standard Milk Ordinance; this document included standards for pasteurization (USPHS/FDA, 2016). Subsequently, Frank et al. (1927) reported on outcomes from the implementation of the Standard Milk Ordinance in 14 Alabama towns. Significant improvements were described in raw milk quality and USPHS milk sanitation ratings for both farms and processing facilities that were pasteurizing milk. Consequently, in 1926 a slightly modified version of the Standard Milk Ordinance was published (Frank et al., 1927). In 1927, the USPHS released an accompanying code to provide a uniform interpretation of the ordinance and to offer administrative and technical details regarding satisfactory compliance (USPHS/FDA, 2016). The ordinance and accompanying code were the precursors of the current US Grade A Pasteurized Milk Ordinance (PMO; USPHS/FDA, 2016).

As scientific research in the areas of milk production, processing, nutrition, and public health progressed and was shared with the public through publications such as JDS, the controversy surrounding pasteurization diminished to a point where it became more broadly accepted in the late 1930s (Steele, 2000). The practice of pasteurization achieved regulatory authority in 1947, when Michigan became the first state to implement a statewide milk pasteurization law, which compelled further expansion of pasteurization from cities to rural areas. Hence, 1947 represented a major landmark in dairy food safety. Since then, all other states have adopted similar requirements, signifying recognition of the importance of pasteurization in ensuring dairy food safety. Although some states still allow the intrastate sale of raw milk, interstate sale of raw milk and raw

milk products for human consumption is banned except for certain raw milk cheeses aged for >60 d (Steele, 2000; Weisbecker, 2007).

The public health impact of nationwide pasteurization requirements and improved dairy industry sanitation has been profound. In 1989, the US Food and Drug Administration (FDA) retroactively determined that about 25% of foodborne and waterborne illnesses in 1938 had been caused by consumption of contaminated milk and milk products; it is estimated that today less than 1% of foodborne and waterborne illnesses are caused by milk and milk products (USPHS/FDA, 2016). It is important to note, however, that consumption of raw milk and raw milk dairy products appears to be responsible for a disproportionate fraction of the human illnesses attributed to milk and milk products. Estimates by the Centers for Disease Control and Prevention based on data collected between 1993 and 2006 suggested that nonpasteurized milk and milk products carried 150 times greater risk of causing outbreaks and outbreak-associated illnesses per unit of product consumed relative to consumption of pasteurized products (Langer et al., 2012). A follow-up study on raw milk and cheese data for 2009 to 2014 estimated that consumption of these raw products carried an 840 times greater risk of illness compared with pasteurized products (Costard et al., 2017). Although these estimates illustrate the continued food safety challenges associated with consumption of raw milk (and to a lesser extent raw milk products), they were based on outbreaks linked to both legally produced and illegally sold raw milk and raw milk products. Therefore, one cannot necessarily conclude that consumption of a given raw milk product will carry an 840 times higher risk of foodborne illness compared with consumption of an equivalent pasteurized milk product. These estimates do highlight, though, the need for further research into the factors contributing to increased risk from consumption of raw milk products and novel intervention strategies to control the risk. For example, a joint assessment by the US FDA and Health Canada suggested that increased *Listeria* monitoring of finished product could greatly reduce the risk of contracting listeriosis from consumption of raw milk soft-ripened cheeses in the United States and Canada (FDA, 2015).

Refinement of Pasteurization Equipment, Requirements, and Other Processing Interventions to Control Microbial Contamination of Milk

In the early 1900s, recognized disease-causing microorganisms associated with the consumption of raw milk in the United States included *Mycobacterium tuberculosis*, *Salmonella* Typhi, *Corynebacterium diphtheriae*,

Vibrio cholera, *Shigella dysenteriae*, and *Brucella* spp. (Rosenau, 1908b); current bacterial nomenclature rather than that in use at earlier points is provided here and throughout the article. As the dairy industry slowly adopted pasteurization practices, numerous studies provided suggestions for pasteurization time-temperature combinations based on the determination of thermal death times for common pathogenic microorganisms found in raw milk (Rosenau, 1908a; North and Park, 1927). However, the scientific understanding of disease transmission and bacteriology was limited, as reflected by the studies performed. As late as 1927, there was no official agreement on effective time-temperature combinations, methods for determining combinations, or the specific organisms targeted for destruction by pasteurization (Rosenau, 1908b; North and Park, 1927).

For a period of time, *M. tuberculosis* cells were thought to be the most heat-resistant vegetative bacterial cells in milk. Therefore, many scientists between 1883 and 1906 developed heat treatment regimens targeting *M. tuberculosis* (Rosenau, 1908a). However, time-temperature combinations selected for these studies were somewhat arbitrary and methods were inconsistent (North and Park, 1927). In 1908, Milton J. Rosenau, then director of the Hygiene Laboratory of the US Public Health and Marine Hospital Service, published a comprehensive review that established 60°C for 20 min as the minimum time and temperature to heat milk to destroy *M. tuberculosis* (Rosenau, 1908b). This work inspired confidence in the use of relatively low temperatures to control milkborne pathogens and served as the foundation for general acceptance of the pasteurization process among health authorities (Andrews and Fuchs, 1944). In 1911, the National Committee on Milk Standards, a New York contingent of leading bacteriologists and public health officials, recommended a time-temperature combination of 62.8°C for 30 min (North and Park, 1927). In 1920, the Committee on Milk Supply officially recommended 62.8°C for 30 min (Westhoff, 1978). In the 1924 Standard Milk Ordinance, officials provided consensus and defined the pasteurization process as a heating process of no less than 61.1°C for 30 min using approved equipment (Frank, 1924). The suggested standard was later verified to provide a sufficient safety margin to destroy *M. tuberculosis* (North and Park, 1927).

Although the holding method was widely used through the 1930s, a new continuous method emerged as plate heat exchangers were developed, resulting in the HTST pasteurization method most commonly used today (Westhoff, 1978). However, HTST pasteurization requirements were difficult to establish from the existing literature, which did not report minimum treatment times for temperatures above 65.5°C. Additionally, pub-

lic health and regulatory officials were wary of accepting this method and associated equipment (Westhoff, 1978; Holsinger et al., 1997). As such, numerous studies were performed to determine time–temperature combinations capable of effectively controlling *M. tuberculosis* at higher temperatures (Westhoff, 1978). The year 1933 represents another landmark, as this was the year when HTST pasteurization equipment and methods were approved and when the first HTST time–temperature standards were included in the USPHS Milk Ordinance Code; these standards were a temperature of at least 160°F (71.1°C) for at least 15 s (Westhoff, 1978). As technology continued to evolve, continuous flash pasteurization methods gained popularity (e.g., higher heat, shorter time), and suitable standards were set accordingly to ensure the destruction of *M. tuberculosis* (Westhoff, 1978).

Although the time–temperature combinations for pasteurization were initially established for *M. tuberculosis*, the pasteurization target organism was redefined when Enright et al. (1957) determined, using animal experiments, that viable *Coxiella burnetii* survived in milk pasteurized at the existing standard conditions of 61.7°C for 30 min (Enright et al., 1957). *Coxiella burnetii* is the organism responsible for Q fever. In response, the USPHS immediately adjusted their recommendation for vat pasteurization to 145°F (62.8°C; typically rounded to 63°C) for 30 min, with the added recommendation that this limit be raised 3°C for products with more fat than that present in whole milk or in products with added sugar (Anderson, 1957). Consequently, parameters for HTST pasteurization were also adjusted to 161°F (71.7°C; often rounded to 72°C) for 15 s (Anderson, 1957; Holsinger et al., 1997). Today, batch (vat) pasteurization parameters of 63°C (145°F) for 30 min and continuous flow HTST pasteurization parameters of 72°C (161°F) for 15 s still represent the minimum pasteurization time–temperature combinations listed in the PMO, and *C. burnetii* remains the target organism for validation of pasteurization requirements for all US dairy products (USPHS/FDA, 2016); this approach also is considered an international standard (FAO/WHO, 2011). Another key date for dairy microbiology was August 10, 1987, when the FDA published a final regulation mandating the pasteurization of all milk and milk products in final package form for direct human consumption. This regulation, which banned the shipping of raw milk in interstate commerce, became effective September 9, 1987.

In parallel to advancement in pasteurization techniques (e.g., development of HTST) and refinement of minimum pasteurization time–temperature parameters, advancement in technologies that supported pasteurization implementation made essential contributions to

microbial dairy food safety and quality. For example, in 1935, Kay and Neave (1935) introduced the phosphatase test, a method used to determine relative alkaline phosphatase (ALP) activity. Alkaline phosphatase is found naturally in bovine milk and is inactivated once subjected to proper pasteurization conditions; hence, ALP measurement provides a simple test that can be used to verify proper pasteurization, which was particularly important in the early days of pasteurization (Burgwald, 1939). The Scharer method, which was developed in 1940, is an ALP colorimetric test that was applied until it was no longer accepted under the PMO in 2009 (Scharer, 1938; Rankin et al., 2010). Today, the industry commonly uses fluorometric and chemiluminescent tests, such as the Fluorophos ALP Test System (Advanced Instruments Inc., Norwood, MA) and Charm ALP/PasLite (Charm Sciences Inc., Lawrence, MA; Rankin et al., 2010). Currently, the PMO requires ALP testing using electronic methods for grade A pasteurized milk and milk products and bulk-shipped heat-treated milk products (USPHS/FDA, 2016).

One major challenge that has been described since the early days of JDS (Smith, 1919; Brew, 1922) and that remains in today's dairy industry is recontamination of pasteurized milk and dairy products with microbial pathogens and spoilage microbes after the pasteurization step—for example, during postprocessing filling or packaging. Major pathogens of current concern for postprocessing contamination are *Listeria monocytogenes* and to a lesser extent *Salmonella* as well as *Cronobacter*, particularly in infant formula. In the early days, postprocessing contamination was linked to both milk handlers and the processing environment. For example, according to the USPHS, 3 out of 11 disease outbreaks that were linked to pasteurized milk between 1929 and 1934 were traced to typhoid carriers who operated bottling machines or who handled bottles (Chilson et al., 1936). However, today, postprocessing contamination typically occurs from the built environment and equipment. Interestingly, the challenges of postprocessing contamination were recognized as early as 1917, when the International Dairy and Milk Inspectors' Committee published their report "Rules and Regulations Necessary for Securing a Clean and Safe Milk Supply," which suggested rules for sanitary milk control throughout production, transportation, handling, and delivery (Kelly et al., 1917). Among these were recommendations for implementation of strategies that today would be considered good manufacturing practices in addition to pasteurization. Consistent with this, and as a result of a series of comprehensive studies conducted between 1906 and 1921 (Rosenau, 1908a, b; North et al., 1925), the USPHS concluded that sanitary control throughout the dairy supply chain is essential for en-

suring the control of milkborne disease (Faulkner, 1957; USPHS/FDA, 2016). Thereafter, in 1923, the USPHS established an Office of Milk Investigations to assist the states in the development of effective milk-control programs (Faulkner, 1957). However, implementation of regulations was inconsistent across facilities and states for several decades (Dahlberg et al., 1953). As a consequence, in 1950 the US surgeon general requested that state milk sanitation regulatory agencies establish procedures for a voluntary Interstate Milk Shipper certification program, which resulted in the formation of the National Conference on Interstate Milk Shipments (NCIMS) and the Cooperative State Public Health Service Program for certification of interstate milk shippers. Responsibilities under this program were divided between state agencies and the Public Health Service Program. In 1969, the Public Health Service Program responsibilities were transferred to the FDA. Currently, all 50 states, the District of Columbia, and the US trust territories participate in the NCIMS. Biennial NCIMS meetings, which include representatives across the dairy spectrum, including producers, processors, and academics, are used to develop recommendations to modify the PMO. The NCIMS recommendations must be approved by the FDA before they are incorporated into the PMO. In addition to these government regulations, worldwide, the introduction of voluntary food safety management systems (e.g., hazard analysis and critical control point, International Organization for Standardization standards, the Global Food Safety Initiative) has contributed to the development of comprehensive systems to ensure dairy food safety. Simultaneously, starting in the 1930s, major advancements were made in sanitation practices. For example, clean-in-place methods were developed in 1950, and Tetra Pak (Pully, Switzerland) introduced UHT milk packaged in a multilayer carton in 1952.

1950: Introduction of the 60-d Holding Period for Raw Milk Cheese, with Subsequent Concerns Raised by Outbreaks and Laboratory Studies

The 60-d rule, which specifies that certain cheeses made from raw milk must be aged for more than 60 d, was introduced in the final years of World War II, when a lack of skilled cheesemakers in the United States coincided with 2 serious outbreaks of typhoid fever linked to cheese consumption (Johnson et al., 1990). In fact, during the decade encompassing the war (1935–1945), 40 foodborne disease outbreaks were attributed to cheese (Fabian, 1947). In response to this public health challenge, the surgeon general issued a letter on June 16, 1944, calling either for the produc-

tion of cheese from pasteurized milk or for raw milk cheeses to be adequately ripened for safety (Johnson et al., 1990). Many states, including California, Colorado, Indiana, Illinois, Missouri, Minnesota, New Jersey, and New York, responded to the letter by instituting regulations. It was in these local regulations that the first specific holding time requirements for raw milk cheeses (“cured for a minimum of 60 d”) can be found (Johnson et al., 1990). The FDA began standard of identity hearings for cheeses in 1947. These discussions included comments on the holding period, but it was not until August 24, 1950, that the final rule (15 FR 5653; US Federal Registrar, 1950) requiring a minimum 60-d holding period at 35°F or higher for specific cheeses was officially published as a national standard.

Much of the early scientific debate over the 60-d rule is lost to time, but the minimum holding time was at least in part based on research on the survival of *Brucella abortus* in Cheddar cheese (Gilman et al., 1946; Johnson et al., 1990). Although the study reported that cheeses intentionally inoculated with *B. abortus* were positive for up to 6 mo postmanufacture, it also reported that no *B. abortus* were found either in commercial Limburger cheeses produced with *B. abortus*-positive milk and held for 57 d or in cheeses made from milk collected from *B. abortus*-positive herds and stored for 41 d (Gilman et al., 1946). These observations, coupled with the absence of epidemiological data that linked Cheddar cheeses aged for more than 60 d to foodborne outbreaks, led the researchers to conclude that the 60-d holding period was a reasonable measure for control of foodborne pathogens (Gilman et al., 1946). While other studies at the time demonstrated long-term survival of *M. tuberculosis*, *Salmonella* Typhi, and hemolytic streptococci in various cheeses, the 60-d holding period was deemed to offer an adequate, though not absolute, protection from potential levels of pathogenic organisms in raw milk cheeses (Johnson et al., 1990; Boor, 2005).

Today the 60-d rule is captured in the US Code of Federal Regulations (CFR) under 7CFR§58.439 (US Code of Federal Regulations, 2016). The section states that if a “cheese is labeled as ‘heat treated,’ ‘unpasteurized,’ ‘raw milk,’ or ‘for manufacturing,’ the milk may be raw or heated at temperatures below pasteurization. Cheese made from unpasteurized milk shall be cured for a period of 60 days at a temperature not less than 35°F.” The standards of identity for cheeses can be found in 21CFR§133 (US Code of Federal Regulations, 2017a). These standards include requirements for permissible moisture content, minimum milk fat content, and the acceptability of pasteurized or raw milk in their production. Some standards of identity require holding periods longer than 60 d, but these aging requirements are aimed at ensuring proper development of charac-

teristics particular to the cheese variety and are not related to safety.

Over the past 70 yr, significant strides in cow health, milking hygiene, dairy processing, and disease surveillance across the farm, processor, and regulatory continuum have greatly improved the quality and safety of dairy products. Along with these improvements have come changes in the foodborne pathogens that are currently more frequently associated with dairy products. Nontyphoidal *Salmonella*, *Listeria monocytogenes*, Shiga toxin-producing *Escherichia coli* (STEC) and *Campylobacter* have replaced *B. abortus*, *M. tuberculosis*, *Salmonella* Typhi, and hemolytic streptococci as the primary bacterial pathogens of concern (Scallan et al., 2011). Recognition of the importance of these pathogens in dairy foods and their ability to survive in cheese has further challenged the efficacy of the 60-d rule for protecting public health. An early epidemiological example was an outbreak from 1980 to 1982 of *Salmonella* Muenster linked to raw milk Cheddar cheese in Canada (Wood et al., 1984). One of the implicated cheese lots remained positive for *Salmonella* Muenster for 125 d (Wood et al., 1984). A second Canadian outbreak in 1984, also linked to raw milk Cheddar cheese, determined that *Salmonella* Typhimurium persisted in the cheese for 8 mo (D'aoust et al., 1985). In the United States, 3 outbreaks associated with raw milk cheeses that had been aged for at least 60 d were reported from 1998 to 2011; 2 were caused by *E. coli* O157:H7 and the other was caused by *L. monocytogenes* (Gould et al., 2014). In March 2017, listeriosis was deemed responsible for 6 illnesses and 2 confirmed deaths linked to 60-d aged raw milk cheese from New York State (FDA, 2017a). Although environmental contamination sources after cheese making cannot always be excluded in outbreaks linked to raw milk cheeses, in several outbreaks pathogens present in raw milk were likely the root cause. For example, an investigation of a Dutch outbreak of *S. Typhimurium* phage type DT7 in a raw milk hard cheese aged for 9 mo found the rare *Salmonella* phage DT7 in both the farm's cattle and the dairy production room, highlighting the potential for raw milk contamination to carry through aging (Van Duynhoven et al., 2009). In the future, the use of whole-genome sequencing will enhance public health officials' ability to track specific strains and to identify sources of contamination for raw milk cheeses. Application of these tools will further help clarify the contributions of raw milk and the processing environment as pathogen sources.

Research over the past several decades has substantiated the ability of several foodborne pathogens to survive in cheese longer than the 60-d holding period. The ability of *L. monocytogenes* to persist for long periods of time in cheeses such as Cheddar (Ryser and Marth,

1987), raw milk semihard Swiss (Bachmann and Spahr, 1995), and soft cheeses (D'Amico et al., 2008) has been well documented. Similarly, several studies have demonstrated *E. coli* O157:H7 survival past the 60-d holding period for both Cheddar and Gouda (Reitsma and Henning, 1996; D'Amico et al., 2010). When the 60-d rule was originally adopted, it was understood that the ability of pathogens to survive the holding period varied among cheese types (Johnson et al., 1990) due to differences in attributes such as pH, salt, moisture, water activity, and temperature. More recently, researchers from various institutions have collaborated to provide more detail on the range of product parameters that exist in modern raw milk cheeses (Trmčić et al., 2017), but further study is needed to understand the influence of these parameters and ripening conditions on pathogen survival.

In 2016, required compliance to the Food Safety Modernization Act began for many cheese manufacturers in the United States. The act requires manufacturers to implement adequate controls to prevent or significantly reduce any hazards associated with the food being produced. Section 117.135 of 21CFR (US Code of Federal Regulations, 2017b) states that process controls "include procedures, practices, and processes to ensure the control of parameters during operations such as heat processing, acidifying, irradiating, and refrigerating foods. Process controls must include, as appropriate to the nature of the applicable control and its role in the facility's food safety system: (i) parameters associated with the control of the hazard; and (ii) the maximum or minimum value, or combination of values, to which any biological, chemical, or physical parameter must be controlled to significantly minimize or prevent a hazard requiring a process control." Based on epidemiological and challenge study data cited previously (e.g., Ryser and Marth, 1987; Bachmann and Spahr, 1995; Reitsma and Henning, 1996; D'Amico et al., 2008, 2010), the argument for the current 60-d rule as an adequate process control may be called into question. This notion is supported by a recent assessment of the risk of listeriosis from consumption of soft cheeses (using Camembert as an example), which was conducted by Health Canada and the FDA (FDA, 2015). The results from this study suggested that consumption of raw milk soft cheeses, even if aged for >60 d, presents an approximately 50 to 100 times higher risk for listeriosis compared with consumption of pasteurized milk cheeses. In fact, the assessment found that removal of the 60-d aging requirement for soft cheese would, in fact, reduce the risk of listeriosis from these products, albeit the reduction would be less than 2-fold. Specifically, sales of raw milk soft cheeses aged less than 60 d would be expected to reduce the risk of listeriosis because the shorter period

would permit less time for *L. monocytogenes* growth in these types of cheeses, which have high water activity and a high pH that increases during aging due to the metabolic activities of fungi or bacterial surface populations (e.g., *Brevibacterium*). More important, this risk assessment suggested that testing both raw milk and finished product could considerably reduce the risk associated with raw milk soft cheeses. This analysis underpins the decisions in some countries to remove the 60-d aging periods for certain cheeses; for example, in 2008, the provincial government in Québec (Canada) passed new regulations permitting the sale of soft and semisoft cheeses aged for less than 60 d. Further research into the efficacy of aging different cheese types for 60 d and the effect of additional measures, such as testing raw or finished product, will be important for determining the future of the 60-d rule.

1983: *Listeria* (Re-)Enters the Dairy Industry as a Pathogen of Concern—And Is Still Here in 2017

Listeria monocytogenes has a long history of being linked to milk and dairy products, including the fact that this pathogen causes severe disease (e.g., brain infections, abortions) in both cattle and humans. The first listeriosis outbreak linked to dairy was reported to have occurred in Halle, Germany, from 1949 to 1957; more than 100 stillbirths were suspected to have been caused by raw milk contaminated with *L. monocytogenes* (Seeliger, 1961). A comprehensive review on dairy-related listeriosis outbreaks was published in JDS in 2004 (Lundén et al., 2004). The second reported listeriosis outbreak linked to dairy occurred in Vaud, Switzerland, between 1983 and 1987; this outbreak was linked to Vaucherin Mont d'Or soft-ripened cheese and involved more than 120 cases with 32 associated deaths (Büla et al., 1995). This outbreak in Europe as well as a concurrent large listeriosis outbreak in 1985 in California, which was linked to Hispanic-style soft cheese, and a 1983 listeriosis outbreak in Massachusetts linked to contaminated pasteurized milk brought *L. monocytogenes* to the forefront of the dairy industry's attention. Since then, listeriosis has remained arguably the most important human foodborne pathogen associated with the dairy industry, and a considerable number of additional human listeriosis outbreaks around the world have been linked to dairy products. Different products (e.g., chocolate milk, fluid milk, butter, various cheeses, and, more recently, ice cream) have been implicated as sources of human listeriosis outbreaks (Lundén et al., 2004; Chen et al., 2016); however, Hispanic-style cheeses have been a particular concern with regard to human listeriosis as detailed in a recent review in JDS (Ibarra-Sánchez et al., 2017). In addition, surface

ripened and washed rind cheeses have been linked to several human listeriosis outbreaks and present a particular concern as potential sources of human listeriosis cases and outbreaks. For example, in 2008, a listeriosis outbreak in Chile with 165 reported cases and 14 deaths was associated with the consumption of Brie and Camembert (Montero et al., 2015). Raw milk consumption also is linked to human listeriosis cases and outbreaks; in 2016, a small listeriosis outbreak with 2 cases was linked to organic raw milk produced by a farm in Pennsylvania (CDC, 2016).

Our understanding of the sources of *L. monocytogenes* in dairy products also has evolved over the years. Although contaminated raw milk has been the likely source of some outbreaks (including outbreaks linked to raw milk cheeses and raw milk), studies conducted over the years suggest that *L. monocytogenes* contamination of dairy products typically occurs from the processing plant environment, where *Listeria* spp. and *L. monocytogenes* strains can survive for prolonged time periods and up to decades (for a review see Ferreira et al., 2014). For example, Kabuki et al. (2004) reported evidence for environmental *Listeria* persistence in 2 of 3 Latin-style cheese processing facilities enrolled in their study. In one facility, the persistent *L. monocytogenes* subtype was also identified in the finished products, supporting cross-contamination from the processing plant environment as a root cause of contamination of the finished product. Similarly, Beno et al. (2016) reported the persistence of *Listeria* spp. in 7 out of 9 small cheese making facilities included in a study on the development of pathogen environmental monitoring programs. The importance of environmental *L. monocytogenes* sources and cross-contamination is further illustrated by results from an investigation of a large listeriosis outbreak with 38 human cases in Québec, Canada; this outbreak was linked to a pasteurized soft-textured cheese, and postpasteurization contamination from the processing plant was the likely source of this outbreak. Importantly, investigation of this outbreak also suggested that extensive cross-contamination of different cheeses in retail establishments is a contributing factor (Gaulin et al., 2012). The recognition of the importance of postprocessing contamination with *L. monocytogenes* as a public health hazard has led to evaluation of different methods, including high-pressure processing, to inactivate *L. monocytogenes* in packaged products and in cheeses (e.g., Tomasula et al., 2014). Increased recognition has emerged regarding the essential nature of stringent pathogen environmental monitoring programs targeting *L. monocytogenes* as a means of reducing *L. monocytogenes* contamination of cheese and other dairy products, as illustrated by the FDA's recent publication of a draft guidance titled "Control of *Listeria monocy-*

togenes in Ready-To-Eat Foods: Guidance for Industry” (FDA, 2017b).

Since 1997, identification of human listeriosis outbreaks in general, including outbreaks linked to dairy products, has been facilitated by increasingly world-wide application of molecular subtyping (i.e., DNA fingerprinting) tools. Since 1999, routine subtyping of all *L. monocytogenes* isolates from human clinical cases in the United States has been performed using pulse field gel electrophoresis. This approach has been adopted for subtyping foodborne pathogens in many other countries through a system called PulseNet International, and this adoption will likely aid in the detection of more listeriosis outbreaks linked to dairy products in different parts of the world. Whereas large outbreaks may be detected without the use of subtyping methods, because pulse field gel electrophoresis and other subtyping methods have been increasingly used for routine surveillance of foodborne disease and, specifically, for listeriosis (Jackson et al., 2016), these methods have enabled the detection of a larger number of smaller outbreaks linked to cheese and dairy products. Improved detection of human listeriosis outbreaks will be further facilitated by routine implementation of whole-genome sequencing for surveillance and characterization of *L. monocytogenes* isolates from human clinical cases, foods, and food-associated environments. In the United States, routine characterization of *L. monocytogenes* through whole-genome sequencing was initiated in September 2013 (Jackson et al., 2016); this change in surveillance strategies enabled increased detection of listeriosis clusters and helped solve listeriosis outbreaks (5 and 9 in yr 1 and 2 of whole-genome sequencing implementation, respectively). Importantly, the outbreaks detected after implementation of whole-genome sequencing included 3 multistate outbreaks linked to different styles of soft cheeses as well as 2 outbreaks linked to ice cream (Jackson et al., 2016), illustrating the effect of this technology on the dairy industry. Whole-genome sequencing is also rapidly being implemented for foodborne disease surveillance in other countries (Deng et al., 2016).

1986 to 1992: *Enterobacter sakazakii* and STEC Are Recognized as Causes of Dairy-Associated Foodborne Disease Outbreaks—A Reminder that We Still Do Not Know All Dairy-Associated Pathogens

Several key pathogens were recognized as concerns to the dairy industry before 1917, including organisms such as *M. tuberculosis*, *Brucella* spp., and *Salmonella*. *Listeria monocytogenes* was added to the list of dairy-associated pathogens in the mid-1980s after it was linked to 2 large listeriosis outbreaks that were caused

by consumption of contaminated cheeses in both Europe and the United States. Two key pathogens of concern to the dairy industry (*Cronobacter* and STEC) were only recently recognized as dairy-related pathogens.

Cronobacter spp., which were originally designated as *Enterobacter sakazakii*, are a specific concern for the powdered infant formula industry (for a comprehensive review, including a summary of outbreaks, see Norberg et al., 2012). The first reported cases of *E. sakazakii* infections in neonates that were unambiguously linked to infant formula occurred in 1986 and 1987. An article published in 1989 (Biering et al., 1989) reported 3 cases of neonatal *E. sakazakii* infections that occurred in a hospital in Reykjavík, Iceland, over a 9-mo period in 1986 and 1987. As part of the outbreak investigation, *E. sakazakii* was isolated from several lots of the powdered milk formula used in the hospital, and *E. sakazakii* isolates obtained from the neonates were found to be indistinguishable (by biotypes, plasmid DNA profiles, and antibiograms) from 22 strains grown from the formula. Whereas Muytjens et al. (1983) had previously reported an association between *Cronobacter* spp. infections and consumption of infant milk formula, this group did not isolate *Cronobacter* spp. from the powdered infant formula despite its isolation from prepared formula and environmental samples. Since then, several *Cronobacter* outbreaks have been linked to contaminated infant formula, including some that have been linked to intrinsically contaminated powdered infant formula (i.e., formula contaminated in the processing facility) as well as several other outbreaks linked to infant formula that appears to have been contaminated from environmental sources during or after preparation. For example, one outbreak in Belgium in 1998 involved 12 neonates with necrotizing enterocolitis; in this outbreak, considerable evidence suggested that intrinsically contaminated powdered infant formula was a factor that contributed to this outbreak (van Acker et al., 2001). However, in this outbreak as well as in others, cross-contamination during formula preparation (e.g., either from intrinsically contaminated formula or from the environment in which preparation was conducted) may have been a contributing or sole factor responsible for transmission and infection. In addition, investigations of outbreaks and sporadic cases have also shown that problematic practices with regard to storage of prepared infant formula can be an important contributing factor to infection. For example, one study showed that some hospitals in France stored reconstituted infant formula for up to 24 h, which could allow for considerable growth of *Cronobacter* (Caubilla-Barron et al., 2007; Norberg et al., 2012). Consequently, important measures for prevention of *Cronobacter* infections include practices to prevent contamination of powdered

infant formula in processing facilities as well as compliance with guidelines for preparation and storage of reconstituted powdered infant formula (Norberg et al., 2012). Importantly, a paper published in JDS in 2011 detailed potential reservoirs and routes of transmission of *E. sakazakii* based on a study in a plant that produced milk powder (Jacobs et al., 2011), providing important guidance that can inform preventative strategies.

The *E. coli* strains causing enterohemorrhagic colitis (enterohemorrhagic *E. coli*; **EHEC**), including *E. coli* O157:H7, are another more recently recognized pathogen of concern for the dairy industry. Although EHEC are clinically characterized by their ability to cause hemolytic uremic syndrome (**HUS**), genetically these strains are characterized by the presence of *stx1* or *stx2* genes as well as the *eaeA* gene; *stx1* and *stx2* encode Shiga toxins (sometimes also referred to as verotoxins), whereas *eaeA* encodes intimin, which facilitates attachment of *E. coli* to intestinal epithelial cells. Because of the key importance of Shiga toxins in the pathogenesis of HUS, these *E. coli* are sometimes also referred to as STEC. Whereas EHEC were initially linked to cases and outbreaks caused by consumption of raw or undercooked beef products, it is now apparent that many different types of products that are produced without a bacterial control step (e.g., heat treatment) can transmit this pathogen, including produce, raw apple cider, raw milk, and certain raw milk dairy products (for a comprehensive review of STEC in dairy foods, see Farrokh et al., 2013). In 1986, 2 cases of *E. coli* O157:H7 infections were described in young children who consumed raw milk on farms where *E. coli* O157:H7 was subsequently also isolated from fecal samples from dairy cows, suggesting raw milk as a potential vehicle responsible for human O157:H7 infections (Martin et al., 1986). An early report of a linkage between EHEC and dairy products was also provided by Deschênes et al. (1996), who reported 4 HUS cases in a French village between March 1992 and May 1993 that were associated with consumption of a raw milk cheese. Since the first descriptions of EHEC infections linked to raw milk and dairy products, a considerable number of EHEC outbreaks around the world have been linked to consumption of raw milk. Although most of these outbreaks have been linked to isolates representing *E. coli* serotype O157:H7, some of them also have been caused by other serotypes (e.g., O22, O26; Farrokh et al., 2013). Enterohemorrhagic *E. coli* strains also have been responsible for some outbreaks linked to raw milk fermented dairy products and particularly raw milk cheeses. For example, between 1998 and 2011, 4 *E. coli* O157:H7 outbreaks in the United States were linked to consumption of raw milk cheeses (Gould et al., 2014). These findings are consistent with different

studies (Reitsma and Henning, 1996; D'Amico et al., 2010) that indicate that *E. coli* O157:H7 can survive in cheeses for more than 60 d. Interestingly, a few EHEC outbreaks linked to dairy products have been linked to pasteurized products, including one large outbreak in Scotland in 1994 linked to pasteurized fluid milk where *E. coli* O157:H7 was isolated from food contact surfaces located after the pasteurizer (Upton and Coia, 1994). These outbreaks further support that postpasteurization contamination with pathogens other than *L. monocytogenes* remains an issue even in the modern dairy industry.

1990: PCR Enters the Dairy Microbiology World and Changes it Forever

The advent of molecular methods for detection and characterization of dairy-associated microbes has had a major effect on dairy microbiology. Although some molecular methods, such as nucleic hybridization-based assays, were commercially available as early as 1985 (for a review, see Vasavada, 1993), the invention of PCR in 1985 opened the door to mainstream application of molecular methods. Application and use of PCR for detection of foodborne pathogens and other microorganisms started in the early 1990s. For example, the first PCR assay for *Listeria monocytogenes* was reported in 1990 (Border et al., 1990), and the first reports of using a PCR assay to detect *L. monocytogenes* in dairy products (soft cheese, milk) were published in 1991 (Furrer et al., 1991; Thomas et al., 1991; Wernars et al., 1991). Since then, PCR assays for virtually all foodborne pathogens of relevance to the dairy industry have been developed, are commercially available, and are increasingly routinely used for detecting pathogens in finished products as well as environmental samples. More slowly, PCR methods are also being developed for spoilage organisms of relevance to the dairy industry (e.g., Ranieri et al., 2012). However, developing molecular assays for spoilage organisms is more challenging than developing assays for pathogens because it is difficult to identify specific, well-defined molecular targets that yield high levels of sensitivity and specificity for spoilage organism detection. For pathogen detection, there is emerging consensus that molecular methods can provide more rapid, more specific, and more sensitive detection compared with many traditional or immunoassay-based methods (e.g., Abubakar et al., 2007). With continued improvements in ease of use and performance of molecular methods, including the development of easy-to-use isothermal methods for DNA amplification, molecular methods will likely continue to expand their penetration in the dairy pathogen testing industry. Increasing efforts to better characterize

spoilage organisms, including through whole-genome sequencing (e.g., Moreno Switt et al., 2014), may also provide new opportunities to develop better assays for detection of specific targeted spoilage organisms of relevance in the dairy industry.

1998: *Paenibacillus* spp. and *B. weihenstephanensis* Are Recognized as Psychrotolerant Spoilage Organisms of Concern in HTST-Pasteurized Fluid Milk

Similar to the changes in our understanding of food-borne pathogens associated with milk and dairy products, our understanding of spoilage organisms of concern has also changed considerably over time. Conceptually, spoilage organisms in products made from pasteurized milk may represent (1) organisms introduced from environmental sources or personnel after heat treatment or (2) organisms that survive pasteurization. In addition, thermosensitive bacteria that can grow in refrigerated milk and that produce thermostable spoilage enzymes (e.g., proteases) that are not inactivated by pasteurization are of concern (Murphy et al., 2016). For much of the 20th century, spoilage organisms introduced after pasteurization (e.g., *Pseudomonas* spp. and many types of coliforms, which can cause off flavors and defects) represented the major recognized spoilage concerns. For example, in the first volume of JDS in 1917, Harding et al. (1917) wrote, “The milk coolers and the bottling machines require special watching . . . not only because they frequently add large numbers of germs, but especially because they add them after the milk has been pasteurized.” Although spoilage organisms introduced after pasteurization still remain a major concern and challenge throughout the world, many dairy processors have effectively reduced postprocessing contamination. For example, data from Cornell’s Milk Quality Improvement program collected from 1991 to 2010 suggest that for many fluid milk processing plants in the northeastern United States, the ability to control postpasteurization contamination has significantly improved over time (Carey et al., 2005; Martin et al., 2012).

Bacterial spoilage organisms that survive commercial pasteurization include both thermotolerant non-sporeformers (e.g., *Microbacterium*, *Micrococcus*, *Streptococcus*, and *Lactobacillus*) and sporeforming bacteria (e.g., members of the order *Bacillales* and *Clostridiales*; Murphy et al., 2016). Sporeformers have long been recognized as spoilage organisms of concern in the dairy industry. For example, a 1981 review article in JDS (Collins, 1981) discussed that psychrotolerant bacteria in the genus *Bacillus* represent the most important heat-resistant psychrotolerant spoilage organisms in fluid

milk; common defects associated with these organisms were described as sweet curdling as well as bitter, fruity, rancid, and yeasty flavors. The frequent occurrence of these organisms in raw milk also was described more than 45 yr ago; for example, Chung and Cannon (1971) reported the detection of psychrotrophic sporeforming bacteria in 83% of the raw milk samples obtained from 18 individual producers. Early efforts to classify psychrotolerant *Bacillus* spp. isolates from milk (e.g., Shehata and Collins, 1971) indicated that these isolates had characteristics similar to the *Bacillus* species *brevis*, *circulans*, *cereus*, *coagulans*, *laterosporus*, *licheniformis*, *macerans*, *megaterium*, *polymyxa*, *pumilus*, and *subtilis*, although the isolates showed growth at temperatures lower than the growth temperature ranges reported for these species. In 1998, *Bacillus weihenstephanensis* was reported as a new species that belonged to the *B. cereus* group but was differentiated by its ability to grow at refrigeration temperatures (Lechner et al., 1998). Subsequent studies indicated that not all psychrotolerant strains in the *B. cereus* group necessarily represented *B. weihenstephanensis* (Stenfors and Granum, 2001), suggesting the existence of a distinct *Bacillus* species (*B. wiedmannii*) that is both psychrotolerant and able to cause disease (Miller et al., 2016). Further clarification of the classification of psychrotolerant sporeformers that cause milk spoilage started with the 1993 proposal to create a new genus *Paenibacillus* within the order *Bacillales* (Ash et al., 1993–1994); several psychrotolerant sporeformers obtained from pasteurized milk either were reclassified as *Paenibacillus* (e.g., *Bacillus polymyxa* became *Paenibacillus polymyxa*) or were identified and classified after description of the genus *Paenibacillus*. Improved definition and taxonomic classification of psychrotolerant *Bacillus* spp. following the description of *B. weihenstephanensis* in 1998 provided an improved ability to detect and define causes of HTST fluid milk spoilage due to the presence of psychrotolerant sporeformers (e.g., Huck et al., 2007a, b) and has led to the recognition that psychrotolerant sporeformers are the current biological limitation for HTST shelf-life extension of fluid milk past 24 d (Fromm and Boor, 2004; Ranieri and Boor, 2009).

1917 to 2017: Changing Microbiological Methods Affect Our Understanding of Dairy Microbiology

A key change that occurred between 1917 and 2017 is the evolution of methods for detection of microbes associated with milk and dairy products. We illustrate this with 3 examples, including (1) detection of the pathogen *L. monocytogenes*, (2) quantification of total bacterial numbers in raw milk, and (3) microbial indicator tests used for finished products.

A comprehensive review and summary of the development of different methods for detecting *L. monocytogenes* can be found in the book *Listeria, Listeriosis, and Food Safety*, particularly its 2 chapters dedicated to conventional and rapid methods for detection of *Listeria* (Brehm-Stecher and Johnson, 2007; Donnelly and Nyachuba, 2007). Direct plating was the initial method of choice for *L. monocytogenes* detection; however, often it was not successful in detecting the microbe. In 1948, the cold enrichment method was introduced by Gray et al. (1948), which essentially represented a nonchemical selective enrichment procedure. Starting in 1950, different selective chemical enrichment procedures that allowed for enrichment at 30 to 37°C were introduced and evaluated. Even following the introduction of selective enrichment and plating media, differentiation of the pathogenic *L. monocytogenes* from nonpathogenic *Listeria* spp. on plating media remained a challenge, particularly because product samples often can be contaminated with both *L. monocytogenes* and other nonpathogenic *Listeria* spp. Classical selective and differential plating media (e.g., Oxford agar, polymyxin-acriflavine-LiCl-ceftazidime-esculin-mannitol agar) do not differentiate between *L. monocytogenes* and nonpathogenic *Listeria* spp. Major breakthroughs that enabled improved detection of *L. monocytogenes* are represented by both molecular methods and chromogenic media that allowed differentiation between *L. monocytogenes* and nonpathogenic species. Today's standard methods for *L. monocytogenes* (or *Listeria* spp.) detection in dairy foods or processing plant environments typically involve a 24- to 48-h selective enrichment followed by a DNA amplification or antibody-based screening test or by plating on appropriate selective and differential media. Detection methods for other foodborne pathogens relevant to dairy (e.g., *Salmonella*, *Cronobacter*) have undergone similar improvements and evolution over the years.

Tests assessing total microbial loads in raw milk have played an important role in ensuring the safety and quality of raw milk for >100 yr. Even for pasteurized milk and dairy products, accurate methods for determining bacterial loads in raw milk are important for both quality and safety because the risk of pathogens and spoilage organisms surviving pasteurization increases with higher bacterial numbers in raw milk. Further, spoilage-associated enzymes that can be produced by bacteria present in raw milk may not be inactivated by pasteurization (Boor, 2001; Murphy et al., 2016). Consequently, federal and state regulatory agencies have developed standards for acceptable total bacteria counts in raw and pasteurized milk, whereas dairy industry organizations typically set more stringent standards. In 1924, the first version of the Standard

Milk Ordinance required that grade A raw milk have an average bacterial count of <50,000 cfu/mL (with standards of <200,000, <1 million, and <5 million cfu/mL for grade B, C, and D milk, respectively). The most recent version of the PMO (which covers only grade A milk) requires <100,000 cfu/mL for individual producer grade A milk in the United States (USPHS/FDA, 2016). Today, minimum standards for grade B milk ("milk for manufacturing purposes") are recommended by the Dairy Division of the USDA Agricultural Marketing Service. These were first published in 1972; the current minimum standard for grade B milk was published in 2011 and is 500,000 cfu/mL (USDA Agricultural Marketing Service, 2011). Historically, the PMO has required that laboratory procedures be compliant with the *Standard Methods for the Examination of Dairy Products* (SMEDP; Jezeski, 1956; USPHS/FDA, 2016). The SMEDP was first published in 1910 and since then has been updated 16 times to reflect changes to accepted methods of assessing the bacterial qualities of milk and milk products. In the 1910 SMEDP, the SPC was included as the accepted method for determining the total number of viable aerobic bacteria in raw milk; this method is still widely used. In 1916, the direct microscopic count was introduced as an alternative to SPC; this method is no longer deemed acceptable in the PMO because it is not sufficiently sensitive or accurate for regulatory or quality purposes (Jezeski, 1956; Laird et al., 2004). In 1929, the methylene blue test was introduced and was immediately controversial because results were not consistent with SPC data (Thornton and Hastings, 1930); this test was removed from acceptable methods in the PMO in 1961 (Luchterhand et al., 2009). In the 1980s, 3M Petrifilm aerobic count and plate loop count were introduced as alternatives to SPC (Thompson et al., 1960; Ginn et al., 1984). By the 1990s, 3M Petrifilm aerobic count was determined acceptable and is still considered equivalent to SPC (USPHS/FDA, 2016). In the 2000s, BactoScan (flow cytometry) and spiral plate count were introduced as alternative methods (Donnelly et al., 1976; Gunasekera et al., 2000; USPHS/FDA, 2001, 2005). For more detail on method development and procedures between 1905 and 1955, refer to Jezeski (1956); changes between 1956 and 2004 are detailed in previous editions of SMEDP, whereas current methods are detailed in the 17th edition of SMEDP (Laird et al., 2004).

In addition to specific tests for pathogens or spoilage organisms, the dairy industry has been using microbial hygiene indicator testing to detect lapses in sanitation and postprocessing contamination at the processing level for almost 100 yr. Hygiene indicator organisms are microbial markers whose presence relates to the hygienic quality of the food or environment (Chapin

et al., 2014). Advances both in our understanding of different bacteria associated with dairy products and in the development of specific methods for rapidly and reliably detecting specific bacteria associated with milk, dairy products, and dairy-associated environments have changed the types of indicator organisms used today compared with those used in the early 20th century. The use of coliforms as hygiene indicator organisms in milk was first suggested in 1919, although methods were not developed until 1927 (Finkelstein, 1919; Kessler and Swenarton, 1927). Although initially introduced for raw milk testing, coliform testing was later applied to evaluating pasteurized products (McCrary and Langevin, 1932; Chilson et al., 1936). Currently, the PMO limits coliforms to <10 cfu/mL in pasteurized milk and milk products (USPHS/FDA, 2016). Although coliforms historically have been used as hygiene indicator organisms that indicate fecal contamination, several studies have shown that the majority of coliforms originate from environmental sources and that coliform detection in milk and dairy products rarely indicates actual fecal contamination (for a review see Martin et al., 2016). Recent studies also show that coliforms represent only 7.6 to 26.6% of bacteria introduced into fluid milk by postpasteurization contamination (Martin et al., 2012). Most important, coliform tests do not detect *Pseudomonas* spp., which have been shown to represent the majority of postprocessing contaminants in fluid milk (Cousin, 1982; Sørhaug and Stepaniak, 1997). Because coliforms are imperfect indicators of postprocessing contamination, other hygiene indicators have been proposed in the dairy industry and are increasingly being used (Hervert et al., 2016; Martin et al., 2016). Specifically, testing for *Enterobacteriaceae* provides a better indicator for postprocessing contamination because it detects a wider set of organisms that represent a taxonomically consistent group, including *Salmonella* and *Yersinia*, 2 pathogens of concern in dairy products that are not detected with the coliform test (Hervert et al., 2016). Although coliform standards are still included in the PMO and hence coliform testing is still frequently used in the US dairy industry as well as in some other countries (e.g., Japan), *Enterobacteriaceae* testing is the microbial indicator test of choice for most dairy products in many countries in the world, particularly in most of Europe. However, *Enterobacteriaceae* tests do not detect *Pseudomonas* spp. As such, tests that provide for quantification of total gram-negatives are increasingly recommended as an alternative hygiene indicator, particularly for dairy products in which postprocessing contamination with *Pseudomonas* is a concern (e.g., fluid milk, fresh cheeses; Van Tassell et al., 2012; Hervert et al., 2016; Machado et al., 2017). Although total bacterial counts can also be used as a

hygiene indicator for some dairy products, the fact that both heat-resistant sporeformers and lactic acid bacteria (all of which represent gram-positive organisms) are also detected with these tests limits their value in many cases.

SUMMARY AND FUTURE DIRECTIONS

Despite the considerable advances that have been made in both our understanding of dairy microbiology and the application of this knowledge to improve dairy food safety (to a point where pasteurized milk and dairy products represent some of the safest foods available) and reduce food spoilage issues, considerable needs and opportunities remain for further advances in dairy microbiology. The emergence of new scientific technologies and tools such as whole-genome sequencing will provide new insight into currently unrecognized microbes in dairy products that can affect quality or that may present public health risks. On the other hand, changes in dairy production and processing, including new and improved processing technologies, will change the microbial ecology of dairy products, leading to recognition of different organisms not of previous concern to the dairy industry. For example, with increasing success of strategies for reducing raw milk contamination with vegetative bacterial cells as well as improved prevention of postpasteurization contamination, pathogenic and spoilage-associated sporeformers that survive pasteurization and heat treatments are likely to become an increasing concern for the dairy industry. In addition, parts of the dairy industry still rely on approaches and methods that have been used for >100 yr (e.g., coliform testing) and use knowledge that was created >60 yr ago (e.g., data on the time-temperature combinations used for HTST pasteurization; the 60-d holding period). Upgrades to both historical knowledge and procedures will thus also be essential for the dairy industry, particularly as requirements for science-based food safety practices are becoming more stringent around the world. Specific challenges and opportunities for different categories of dairy products are briefly discussed below.

Microbiological Challenges in Fluid Milk

Microbial food safety and quality issues will continue to evolve around (1) prevention of postpasteurization contamination and (2) control and reduction of thermotolerant and sporeforming organisms that can survive pasteurization, particularly HTST pasteurization. The specific challenges will depend on the type of pasteurization (HTST vs. UHT) used for production of fluid milk. Although UHT typically refers to a process that

includes aseptic packaging, microbiological challenges in this process still involve postpasteurization contamination, although mainly in facilities that may not have sophisticated quality management systems. There also are sporeformers that survive UHT treatment—in particular *Bacillus sporothermodurans*, which also has been identified in dairy farm environments (e.g., Scheldeman et al., 2006)—even though UHT spoilage by sporeformers occurs rarely. However, control of sporeformers that survive UHT treatment may be of some importance. Bacterial spoilage and food safety issues represent a much larger challenge for HTST milk. Postpasteurization contamination with psychrotolerant spoilage organisms (e.g., *Pseudomonas*) is not uncommon in many facilities around the world, and the development and consistent implementation of improved strategies for controlling these microbes will be important for this industry. It is likely that improved tools for source tracking (including whole-genome sequencing and metagenomics tools) will allow for an improved understanding of contamination sources and hence will facilitate improved control strategies for the prevention of postpasteurization contamination, which will also reduce the risk of pathogen introduction (in particular *L. monocytogenes*). In HTST processing facilities that effectively control postpasteurization contamination, sporeforming pathogens—for example, *B. cereus* and the cold-growing species *B. wiedmannii* (Miller et al., 2016)—and spoilage sporeformers that can grow at refrigeration temperatures (e.g., *Paenibacillus* spp.) will become major challenges for the industry. Control of sporeformers will require new systems approaches that will need to include efforts to minimize introduction of these organisms at the farm level as well as optimization and development of processing strategies that allow for spore removal (e.g., microfiltration).

Microbiological Challenges in Dairy Powders

Dairy powders (e.g., whey powders, milk powders) are becoming an increasingly important product for many countries (e.g., New Zealand, United States) and dairy processors. Because milk powders are an important ingredient in infant formula, control of pathogens (including but not limited to sporeformers such as *B. cereus* and *C. perfringens*) will be of particular importance for this group. For dairy powders used to produce foods for highly susceptible population subgroups, such as infants, control of opportunistic pathogens (including but potentially not limited to *Cronobacter* spp.) that may not be of concern in other products will also become increasingly important. In these products, nonpathogens that carry antimicrobial resistance genes in transferrable genetic elements, as well as the pres-

ence of antimicrobial resistance elements in general, may also emerge as a concern. With regard to spoilage organisms, control of a wide range of sporeformers, including thermophilic, mesophilic, and psychrotolerant sporeformers as well as *Clostridium* spp., will be of continued importance, particularly because dairy powders can be used as ingredients in a wide range of products, including reconstituted milk that then will be used to manufacture a range of dairy products (e.g., cheese, UHT milk). Hence, use of a systems approach to reduce sporeformers will be of particular importance for dairy powders, particularly because there is convincing evidence that for some thermophilic sporeformers contamination sources typically are located in the processing equipment (i.e., at locations that feature high temperatures that facilitate the growth of these organisms, such as regeneration sections of HTST units). Development and implementation of improved source tracking tools and better understanding of the ecology and diversity of powder-associated sporeformers will hence also be of particular importance for the control of sporeformers in the dairy powder production chain. Importantly, although specifications for sporeformer levels play an important role in dairy powders, methods for detection and enumeration of sporeformers in dairy powders show limited standardization and often involve different heat inactivation time–temperature combinations and media types. Standardization of spore detection methods for different sporeformer groups (e.g., thermophilic, mesophilic, and psychrotolerant sporeformers) thus will also be important.

Microbiological Challenges in Cheese

Cheeses represent a broad category, ranging from fresh cheeses with near-neutral pH to cheeses with low water activity and low pH that have been aged for 2 yr and beyond. Consequently, microbiological challenges associated with cheeses are equally diverse. With regard to food safety, rational, science-based approaches to ensuring the safety of raw milk cheeses, which are culturally and economically important products in many parts of the world, remain a major challenge that will need to be addressed. Improved control strategies for *L. monocytogenes* in cheese also remain a major challenge, particularly with several human listeriosis outbreaks associated with cheese worldwide linked to this high-fatality pathogen. Finally, there is a need for science-based information and data that support how sporeforming pathogens that can survive pasteurization (e.g., *B. cereus*) are controlled in different cheese types. With regard to spoilage, in certain types of hard cheeses, control of *Clostridium tyrobutyricum* is likely to continue to be a concern, particularly because the trend

toward “clean” labels makes addition of compounds that control growth of this organism less desirable. A systems approach that includes premium programs for raw milk that shows low numbers of this sporeformer may become more common in different countries of the world. Elimination and control of postpasteurization contamination with spoilage organisms will also remain an important area in cheese. Although bacterial spoilage organisms that lead to defects that are easily detected by consumers (e.g., blue color formation by *Pseudomonas*; Martin et al., 2011) are predominantly of concern in fresh and high pH cheeses, mold growth is a concern for a wide range of products. Development and implementation of improved tools for source tracking and for identification of spoilage organisms in cheese thus likely will be an important area of emphasis. Although cheese is the main dairy product in which production of mycotoxins may present a concern (Sengun et al., 2008), no foodborne illness cases due to mycotoxin contamination of cheese have been reported (Hymery et al., 2014). In addition to mycotoxin production due to mold growth on products, mycotoxins can be introduced into all dairy products through indirect contamination, which results when dairy cows ingest feed that contains mycotoxins that pass into the milk, such as aflatoxin M1 (Sengun et al., 2008).

Microbiological Challenges in Other Fermented Dairy Products

Microbial food safety and spoilage issues are generally of limited concern with fermented dairy products other than cheese because these products (e.g., yogurt) typically are characterized by low pH. However, fungal (i.e., yeast and mold) contamination is a related challenge; similar to cheese, we were not able to find records of mycotoxin-related illnesses attributable to mold-contaminated yogurt (mold growth is also less common in yogurt). Fungal related concerns in yogurt are increasing with the clean label trend, which is driving the need to eliminate antifungal compounds from these products. Thus, development and implementation of improved tools for source tracking and identification of fungal spoilage organisms will be important. In addition, development of alternative approaches to control yeast and mold (e.g., protective cultures that prevent or reduce growth of yeast and mold) will also be important for this sector of the dairy industry.

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APPENDIX

Table A1. Major milestones in milk and dairy food safety

Date	Milestone	Reference
1924	The US Public Health Service releases the Standard Milk Ordinance (precursor to today's Pasteurized Milk Ordinance), which defined pasteurization conditions targeting destruction of <i>Mycobacterium tuberculosis</i> .	Frank, 1924
1947	Michigan becomes the first US state to require pasteurization of milk.	Steele, 2000
1950	A 60-d holding period requirement for raw milk cheese is published in the United States.	Boor, 2005
1957	Minimum pasteurization time and temperature conditions are increased to ensure the destruction of <i>Coxiella burnetii</i> .	Enright et al., 1957
1980–1982	A series of <i>Salmonella</i> Muenster outbreaks in Canada is linked to survival of <i>Salmonella</i> in raw milk Cheddar that had been aged for at least 125 d.	Wood et al., 1984
1985	Jalisco cheese <i>Listeria monocytogenes</i> outbreak occurs in California.	Linnan et al., 1988

Continued

Table A1 (Continued). Major milestones in milk and dairy food safety

Date	Milestone	Reference
1986	Two cases of <i>Escherichia coli</i> O157:H7 infections are described in children who consumed raw milk on farms where <i>E. coli</i> O157:H7 was subsequently isolated from fecal samples from dairy cows, suggesting raw milk as a potential vehicle for human O157:H7 infections.	Martin et al., 1986
1986–1987	Three cases of neonatal infection caused by <i>Enterobacter sakazakii</i> (now named <i>Cronobacter</i>) are identified in a hospital in Reykjavik, Iceland; these appear to be the first <i>Cronobacter</i> cases definitively linked to infant formula.	Biering et al., 1989
1987	The US Food and Drug Administration (FDA) publishes a final regulation mandating the pasteurization of all milk and milk products in final package form for direct human consumption; this regulation banned the shipping of raw milk in interstate commerce.	US Federal Registrar, 1987
1990	After the invention of PCR in 1985, the first PCR assay for <i>Listeria monocytogenes</i> is reported.	Border et al., 1990
1996	PulseNet is launched by the Centers for Disease Control and Prevention (CDC).	Swaminathan et al., 2001
1998	<i>Bacillus weihenstephanensis</i> is described as a new psychrotolerant species of the <i>Bacillus cereus</i> group, which is found in heat-treated milk.	Lechner et al., 1998
2004	Psychrotolerant <i>Paenibacillus</i> is indicated as an important cause of HTST fluid-milk spoilage.	Fromm and Boor, 2004
2008	A large listeriosis outbreak with 38 human cases in Québec, Canada, is linked to pasteurized soft-textured cheese; cross-contamination of different cheeses in retail establishments is identified as a contributing factor.	Gaulin et al., 2012
2013	The FDA and CDC start routine use of whole-genome sequencing to characterize <i>Listeria monocytogenes</i> obtained from human cases and from foods.	Jackson et al., 2016
2015	Whole-genome sequencing links listeriosis outbreak to ice cream in the United States.	CDC, 2015