ABSTRACT

Raw milk typically has little bacterial contamination as it leaves the udder of the animal; however, through a variety of pathways, it can become contaminated with bacteria originating from environmental sources, the cow herself, and contact with contaminated equipment. Although the types of bacteria found in raw milk are very diverse, select groups are particularly important from the perspective of finished product quality. In particular, psychrophilic and psychrotolerant bacteria that grow quickly at low temperatures (e.g., species in the genus *Pseudomonas* and the family *Enterobacteriaceae*) and produce heat-stable enzymes, and sporeforming bacteria that survive processing hurdles in spore form, are the 2 primary groups of bacteria related to effects on processed dairy products. Understanding factors leading to the presence of these important bacterial groups in raw milk is key to reducing their influence on processed dairy product quality. Here we examine the raw milk microbiological parameters used in the contemporary dairy industry for their utility in identifying raw milk supplies that will perform well in processed dairy products. We further recommend the use of a single microbiological indicator of raw milk quality, namely the total bacteria count, and call for the development of a whole-farm approach to raw milk quality that will use data-driven, risk-based tools integrated across the continuum from production to processing and shelf-life to ensure continuous improvement in dairy product quality. 

Key words: raw milk quality, microbiological testing, bacterial contamination, farm practices

INTRODUCTION

The microbiological composition of raw bovine milk has been extensively studied for over a century. Although it is expected that raw milk will contain low levels of microbial contamination (here the focus will be on bacterial contamination), contemporary and historical attention has emphasized the production of high-quality raw milk as an indicator of overall hygienic production practices at the farm. The term “high-quality,” from a microbiological perspective, is often used to refer to raw milk with low total bacteria counts; however, the term may also be used to describe raw milk with low levels of certain groups of bacteria (e.g., coliforms). Indeed, tests for various groups of raw milk bacteria have been used as indicators of raw milk quality beyond the total bacteria count (TBC), most notably, preliminary incubation (PI) count for psychrophilic bacteria, coliform count (CC), laboratory pasteurization count (LPC) for thermoduric bacteria, and, less frequently, spore count testing. Results of these tests may be used to indicate specific deficiencies in hygienic practices; for example, the presence of high levels of coliforms (e.g., >100 cfu/mL) may indicate that cows are not adequately cleaned before milking unit attachment (Murphy and Boor, 2000).

Despite the common use of these tests to assess raw milk quality, they are primarily used as indicators of conditions on the farm and not as an indicator of performance in finished products, an important consideration when defining raw milk quality. An extensive discussion of the effects of raw milk microbial contaminants on processed dairy product quality will not be included here, as a recent review thoroughly examined this topic (Murphy et al., 2016). However, it is noteworthy to our review that Murphy et al. (2016) identified 2 primary groups of raw milk microbial contaminants that influence processed dairy product quality, including (1) psychrophilic and psychrotolerant bacteria (e.g., *Pseudomonas*) and (2) sporeforming bacteria (e.g., *Paenibacillus* and *Clostridium*). Briefly, high initial concentrations of psychrophilic and psychrotolerant bacteria, or growth due to improper cooling or excessive storage times, may lead to heat-stable enzyme development by *Pseudomonas* and other related bacteria. Even though the bacterial cells themselves do not survive processing hurdles, the heat-stable enzymes produced when cell concentrations reach 5 to 8 log cfu/mL continue
breaking down milk components even after processing, leading to flavor, odor, and body defects in finished products (Sørhaug and Stepaniak, 1997). For example, cheese made from raw milk with elevated bacterial numbers before processing (e.g., >1,000,000 cfu/mL) leads to reduced yields and flavor defects during aging (Banks et al., 1988). In UHT milk products, which are stored at ambient temperatures, residual heat-stable proteases and lipases produced by elevated raw milk microbial loads cause gelation of the product over the extended shelf-life, a defect termed “age-gelation,” along with other textural defects such as sedimentation, and off-odors and flavors (Murphy et al., 2016).

The second key group of bacteria in raw milk that are relevant to finished product quality are sporeforming bacteria, a diverse group belonging to the orders Bacillales and Clostridiales. These bacteria produce endospores, or spores, that originate from natural environments on dairy farms, are transferred into raw milk, survive processing hurdles, and subsequently grow, causing spoilage in finished dairy products (Gopal et al., 2015). For example, psychrotolerant sporeforming bacteria grow at refrigeration temperatures, causing fluid milk spoilage (Ranieri and Boor, 2009). Studies have demonstrated that psychrotolerant sporeforming bacteria are responsible for approximately 40 to 50% of fluid milk in the United States reaching the Pasteurized Milk Ordinance bacterial limit of 20,000 cfu/mL during shelf-life (Ranieri and Boor, 2009; Alles et al., 2018; Reichler et al., 2018) and, importantly, are the primary bacterial agents limiting the extension of fluid milk shelf-life. Another important group of sporeforming bacteria, anaerobic butyric acid bacterial spores (i.e., Clostridium spp.), cause late-blowing defect in cheese (Klijn et al., 1995). Late blowing is of particular concern in hard and semi-hard aged cheeses such as Gouda and does not typically develop until 60 to 90 d into aging, resulting in economic losses for cheesemakers (Martin et al., 2021). A focus on these raw milk microbial drivers of finished product quality is necessary to prioritize efforts that will result in improved finished product quality. This is critical to the entire dairy continuum, as consumer dissatisfaction with product quality influences their willingness and intent to continue purchasing and consuming that product (Quech and Ash, 1980).

In this review we discuss common contemporary microbiological parameters used to evaluate raw milk quality, types of bacterial contaminants targeted by these tests, sources of contaminants, and farm factors associated with these parameters, as well as potential shortcomings of these parameters for evaluating raw milk quality from the perspective of finished product quality. Further, we propose a new definition of raw milk quality that utilizes (1) an overall raw milk quality indicator as a process control test to assess consistency and compliance with hygienic farm practices, (2) a set of tests that will aid in troubleshooting when the overall raw milk quality indicator is at high levels, and (3) tests that specifically assess the presence and levels of groups of bacteria that influence finished product quality. Finally, we discuss the potential for development of predictive decision support tools that integrate knowledge of raw milk microbial populations, as well as farm management practices that influence microbial presence and levels in raw milk and their effects on finished product quality. Moving from the current reactive, test-based system to a proactive and predictive approach is a critical step in ensuring high-quality dairy products across the continuum from production to consumption.

### RAW MILK MICROBIOLOGICAL PARAMETERS

**Total Bacteria Count: An Overall Indicator of Raw Milk Microbiological Quality and Hygienic Production Practices**

The TBC is the most widely used measure of microbial quality of raw milk and is measured in the United States using several approved methods including the standard plate count (SPC), plate loop count (PLC), Petrifilm (3M) aerobic count, flow cytometry methodologies (e.g., Bactoscan, Foss Analytical), and others (USPHS/FDA, 2019). In the US, the Pasteurized Milk Ordinance limits the bacteria count in grade “A” raw milk to 100,000 cfu/mL for individual producers or 300,000 cfu/mL for commingled raw milk (USPHS/FDA, 2019). Outside of the US, similar limits on TBC are required. For example, the limit for total plate count of raw milk intended for processing is 100,000 cfu/mL in Europe (European Commission, 2021), whereas in New Zealand the limit is 300,000 cfu/mL (Ministry for Primary Industries, 2022), and Canadian standards limit total aerobe mesophilic bacteria to 50,000 cfu/mL (National Dairy Code, 1997). However, reported mean bulk tank TBC typically fall well below these regulatory limits. For instance, Elmoslemany et al. (2009a) measured TBC and other microbiological quality parameters over a 2-year period from approximately 11,100 bulk tank raw milk samples collected from 235 dairy farms in Prince Edward Island, Canada. The authors report a TBC geometric mean of 5,300 cfu/mL with only 6% of samples above 50,000 cfu/mL. Similarly, Pantoja et al. (2009) measured TBC from 7,241 bulk tank raw milk samples from 16 farms in Wisconsin (USA) during a 1-year period, reporting a mean TBC of 3.1 log cfu/mL (1,259 cfu/mL), with just 1.6% (n = 142) above the
regulatory limit of 100,000 cfu/mL. In a more recent study, Rodrigues et al. (2017) evaluated 472 raw bulk tank milk samples from 19 farms in New York State and reported mean TBC values by farm ranging from 3.01 log cfu/mL (1,023 cfu/mL) to 4.11 log cfu/mL (12,882 cfu/mL). Although these reports indicate that TBC values well below common regulatory limits in the US and in other countries are commonplace, producers are encouraged to pursue continuous improvement in reducing bulk tank raw milk TBC, primarily through the lever of premium payments from processors and cooperative. Guidelines suggest that TBC targets for good raw milk are in the range of <5,000 to <10,000 cfu/mL (Murphy and Boor, 2000; Jayarao and Wolf-gang, 2003), yet these recommendations are somewhat dated for contemporary producers. It should also be noted that the regulatory limit for TBC (i.e., 100,000 cfu/mL) is not an appropriate cutoff for defining raw milk quality, as raw milk with this level of microbial contamination is at high risk of resulting in finished product quality deterioration.

Farm sources and management practices associated with elevated TBC in bulk tank raw milk have been widely studied. Many possible pathways of bacterial contamination throughout the production continuum may lead to increased TBC in bulk tank milk, so comprehensive management is essential to limit contamination and subsequent growth of bacteria during storage. The first potential source of bacterial contamination is, of course, the udder itself, especially when an active bacterial infection is present. For example, Hayes et al. (2001) examined a variety of raw milk microbiological quality parameters, including SPC, Petrifilm aerobic count, presumptive gram-negative bacteria on Mac-Conkey agar, and presumptive Streptococcus spp. on Edwards medium, from bulk tank milk from 13 farms over a 2-week period to characterize the causes of spikes (i.e., sudden elevations above normal values) in TBC. The authors found that 11 of the 20 observed spikes were driven by increases in the mastitis pathogen Streptococcus uberis (Hayes et al., 2001). Similarly, Zadoks et al. (2004) found that streptococcal, staphylococcal, and gram-negative counts were all significantly and positively associated with bulk tank raw milk TBC, with streptococcal counts accounting for 69% of TBC variability in their study of mastitis-causing bacteria in bulk tank raw milk on 48 New York farms. The relationship between mastitis and TBC is further illustrated by the relationship between SCC and TBC. For example, in a study of the associations between raw milk quality indicators in bulk tank raw milk on 16 farms, Pantoja et al. (2009) reported that the odds of increased TBC increased by 2.4% for every 10,000 cell/mL increase in SCC in the same milk load. A study of SCC and PLC, a measure of TBC, from monthly producer data collected from 5 large milk processing facilities in New York from March 1999 to December 2000, indicated that farms with SCC below 200,000 cells/mL had lower PLC levels compared with milk with higher SCC (van Schaik et al., 2002). Additionally, Borneman and Ingham (2014) reported a highly significant correlation (P-value <2 × 10⁻16) between SPC and SCC in 2012 bulk tank raw milk samples; however, the authors also report that the R² value for this association was quite small (0.02–0.03), suggesting that many other factors also drive SPC outcomes. Indeed, the effects of mastitis pathogens on bulk tank raw milk TBC depend on multiple factors, including the causative bacterial agent, the proportion of the herd infected, and the size of the herd (Hayes et al., 2001).

In addition to the contribution of mastitis to bulk tank TBC, other major factors that influence this parameter include milking time hygiene (for example, sufficient removal of soil from teat surfaces before milking) and equipment and raw milk handling factors (e.g., proper cleaning and sanitation of equipment and adequate cooling of raw milk after harvest). Elmoslemany et al. (2010) identified risk factors for elevated total aerobic count, including the amount of dirt on teats before milking, use of water to wash teats during milking preparation without subsequent drying, use of the same towel for drying multiple cows, and manual cleaning of the bulk tank. The authors also noted that the lowest bulk tank bacterial counts were associated with predipping of teats during milking preparation followed by drying with single-use towels, compared with other methods of teat preparation. Similarly, Jayarao et al. (2004) studied microbial quality parameters in bulk tank raw milk from 126 dairy farms in Pennsylvania and found that when cows were subjected to pre- and post-dipping, SPC values were significantly lower, whereas SPC values were significantly higher when cows were sprayed with teat dip instead of using a cup for application. According to a study by Doyle et al. (2016), the microbial population of the teat surface was the most significant source of contamination for raw milk, which highlights the importance of pre-milking hygiene to management of TBC in bulk tank raw milk. Further, these studies also provide additional insight into the associations described earlier between TBC and SCC, as effective milking preparation and hygiene are essential to prevent mastitis from contagious and environmental mastitis pathogens (Hogan, 2017; Middleton and Fox, 2017), which ultimately cause elevated SCC in bulk tank milk. Finally, equipment hygiene and water quality factors also play an important role in management of TBC in bulk tank raw milk. For example, Elmoslemany et al. (2009b) reported that high slug scores,
indicating sufficient physical cleaning of the pipeline, along with use of water softener, were associated with lower TBC in bulk tank raw milk and related. Hard water and infrequent evaluation of milk house water for bacterial contamination were associated with elevated TBC in bulk tank raw milk. In a study specifically focused on the effects of cleaning and hygiene of milking equipment on bacterial counts in raw milk, Bava et al. (2011) categorized farms into a high SPC group (n = 14 farms, mean SPC = 4.25 log cfu/mL) and a low SPC group (n = 8, mean SPC = 3.68 log cfu/mL) and examined equipment cleaning and sanitation factors and equipment contamination between the groups. The authors report that the high and low groups differed significantly between bacterial contamination of liners and milk receivers, as well as in water temperature reached during the detergent phase of cleaning milking equipment. Unfortunately, many of these studies report associations, and few studies have assessed whether the identified associations represent true “cause and effect” relationships.

Importantly, raw milk handling, with particular attention paid to achieving and maintaining proper cooling (i.e., maintaining milk temperature below 40°F, or 4.4°C), is also critical to preventing increases in TBC during storage, especially for raw milk supplies that will be stored for longer than 24 h before processing. O’Connell et al. (2016) showed that, when stored at 6°C, the bulk tank raw milk TBC increased significantly from 3.43 log cfu/mL to 4.87 log cfu/mL after 96 h of storage, but storing raw milk at 2°C resulted in no significant increase between 0 and 96 h of storage. Driving increases in raw milk TBC during prolonged storage, or storage at abusive temperatures (e.g., ≥6°C), is the growth of Pseudomonas. De Jonghe et al. (2011) demonstrated that under suboptimal simulated storage conditions (i.e., 6°C storage with 7 brief spikes to 10°C over 4 d, to simulate the addition of warm milk to the bulk tank) not only was there a significant increase in raw milk TBC compared with optimal storage conditions (i.e., 3.5°C storage with 7 brief spikes to 6°C over 4 d, to simulate the addition of warm milk to the bulk tank), but Pseudomonas was the primary bacterial agent causing the increase in TBC. This is of particular importance, as noted earlier in this review, because a variety of Pseudomonas species are known to produce heat-stable enzymes that ultimately affect the quality of processed dairy products.

As the name suggests, the TBC approximates the total levels of bacterial contaminants in raw milk, yet the types of microbial contaminants in bulk tank raw milk are diverse, and, importantly, not all of these contaminants are relevant to finished product quality, as will be discussed. Advances in culture-independent molecular biological methodologies over the last 20 years have allowed for more comprehensive evaluation of microbial populations in raw milk using methods such as targeted metagenomics. Although study design plays a large role in the microbiome identified in any particular study due to variations between farms and over time on the same farm (Skeie et al., 2019), by stage of lactation (McHugh et al., 2020), by geographic region (Skeie et al., 2019), and under different meteorological conditions (Kable et al., 2016), there are commonalities to be drawn with regard to the raw milk microbiome from these studies. In a recent thorough review of the raw milk microbiome by Parente et al. (2020), the authors evaluated results from 5 studies on the microbiome of bulk tank raw milk from different geographic regions to determine the most prevalent and abundant taxa in raw bulk tank milk. These taxa included members of the phyla Proteobacteria, Firmicutes, Bacteroidota (formerly Bacteroidetes), Actinobacteria, and Mycoplasmatota (formerly Tenericutes), with the most prevalent and abundant genera including Pseudomonas, Streptococcus, Lactococcus, Acinetobacter, and Staphylococcus (Parente et al., 2020). In addition to the commonalities in raw milk microbial content, it should be noted that, overall, the microbiome of raw milk is tremendously diverse, with many relevant bacterial taxa that are important from the perspective of milk quality, human safety, and animal health often present at very low levels. For example, sporeforming bacteria typically are found in raw milk at very low levels (Martin et al., 2019). Psychrotolerant sporeforming bacteria, which are the primary biological limiting factor for HTST fluid milk shelf-life (Ranieri and Boor, 2009), have been found to have a mean concentration of less than 1 spore per milliliter of raw bulk tank milk [mean of −0.72 log_{10} most probable number (MPN)/mL; Buehler et al., 2018a] in a study of 99 farms across New York State. Further supporting that psychrotolerant sporeforming bacteria are typically found at low levels, a recent study of spores in bulk tank raw milk from 17 New York State dairy farms reported that only 42% of the 34 bulk tank raw milk samples tested had detectable levels of psychrotolerant spores in the 1 mL of raw milk that had been heat-treated and plated for spore enumeration (Martin et al., 2019). In the aforementioned review by Parente et al. (2020), Paenibacillus, the predominant genus of psychrotolerant sporeforming bacteria causing HTST fluid milk spoilage (Ranieri and Boor, 2009), was not identified in the top 25 most prevalent and abundant bacterial genera, yet it is a major contributor to fluid milk spoilage and waste in the US (Martin et al., 2021), highlighting the challenges associated with detecting low-level bacterial contaminants of relevance to raw milk quality.
A high TBC, whether gauged by the regulatory limit (i.e., 100,000 cfu/mL) or by current examples of limits defining high-quality raw milk (e.g., 5,000 cfu/mL; Jayarao and Wolfgang, 2003) in and of itself is not a definitive indication that dairy products manufactured from that milk will have reduced quality. Indeed, as discussed previously, the primary raw milk microbial drivers of finished product quality include groups of bacteria capable of producing heat-stable enzymes and spore-forming bacteria. Other bacterial contaminants, even when present at elevated levels, may have no discernable effect on finished product quality, although they may indicate a farm-level deficiency in cleaning, sanitation, or other management factors. However, evidence suggests that, when TBC counts are high, the bacteria responsible for the overall increases are of the groups that have been shown to produce heat-stable enzymes. For example, a study of the predominant microflora of low-quality bulk tank milk (as defined by TBC >3.0 × 10^5) in Denmark revealed that gram-negative, oxidase-positive bacteria, including *Pseudomonas*, *Chryseobacterium*, *Alcaligenes*, *Comamonas*, and *Pasteurella*, were present in 72% of samples (Holm et al., 2004). Similarly, Rodrigues et al. (2017), in a study of bulk tank raw milk microbiome in high-SPC (>3.6 log cfu/mL) and low-SPC (≤3.6 log cfu/mL) samples identified that high-SPC samples had significantly higher abundances of *Acinetobacter*, *Enterobacteriaceae*, *Corynebacterium*, and *Streptococcus* (Rodrigues et al., 2017).

It is demonstrable that, as an indicator of raw milk quality, the TBC has important utility from both farm hygiene and management practice perspectives, as well as potentially from a finished product perspective, as the types of bacteria that typically drive high TBC levels are those that are likely to produce heat-stable enzymes; however, this parameter is not without limitations. Studies using culture-independent methods to evaluate microbial populations in raw milk (Doyle et al., 2016; Rodrigues et al., 2017; Skeie et al., 2019; McHugh et al., 2020) have provided a great deal of insight into types and sources of bacterial contamination; however, these insights have also raised questions about the limitations of our current industry test standards to truly assess the quality of raw milk. To illustrate this point, many bacterial taxa found using culture-independent methods in bulk tank raw milk are obligately anaerobic, including various members of the classes *Clostridia* (e.g., *Romboutsia*, *Ruminococcus*, *Mogibacterium*) and *Bacteroidia* (e.g., *Bacteroides*, *Alistipes*; Parente et al., 2020). This is no surprise, as these obligate anaerobes are associated with the gut microflora and are present in high levels in manure (McGarvey et al., 2004). However, culture-dependent methods currently used to evaluate the TBC (e.g., SPC, Petrifilm aerobic count) are performed under aerobic conditions, a potential limitation for the interpretation of these tests as they relate to raw milk quality. Further limitations of these standard culture-dependent TBC methods include the use of mesophilic incubation temperatures (typically 30–35°C; Laird et al., 2004), which do not allow for the growth of obligate psychrophiles or thermophiles, and the common presence of bacteria that grow in clumps or chains (e.g., gram-positive cocci such as *Streptococcus* and *Staphylococcus*), resulting in multiple cells presenting as single colonies on agar plates (Sutton, 2012). Fortunately, the increased use of culture-independent flow cytometric methods (e.g., Bactoscan) for estimation of TBC in bulk tank raw milk has addressed many of the major limitations associated with traditional culture-dependent methods discussed here, although, to ensure equivalency to the standard method (i.e., SPC), the output of individual bacteria count from the flow cytometric methods is often converted to colony-forming units, as the individual bacteria count is often considerably higher due to the ability to enumerate cells not detected by culture-based methods. This conversion uses a linear regression equation that varies by system and eliminates some of the benefits of this method. Stakeholders may consider using individual bacteria count values instead of converting to colony-forming units, to maintain the benefits of this method over culture-based methods.

**Preliminary Incubation Count: Enrichment of Psychrophilic Bacteria**

The PI count has a long history of use in the dairy industry, with Johns (1958) first suggesting the use of this parameter in North America. As Johns describes it, the adoption of bulk tank use on farms with proper cooling may mask the sanitary conditions used to produce the milk, and therefore a novel test should be used to identify milk produced under poor sanitary conditions. The PI test conditions include incubation of raw milk for 18 h at 12.8°C, thereby enriching for psychrophilic bacteria, followed by enumeration using the SPC method (Frank and Yousef, 2004). The US currently has no regulatory requirements for PI count, but guidelines typically suggest that PI count should be either below a specific target (e.g., 50,000 cfu/mL) or below a certain increase (e.g., 3- to 4-fold) above the SPC value (Murphy and Boor, 2000a). Jayarao and Wolfgang (2003) suggest that a good PI count should fall below 10,000 cfu/mL. Reported PI counts vary based on study, although few studies on contemporary raw milk supplies have been published. Boor et al. (1998) examined the microbial quality of raw bulk tank
milk from New York producers between 1993 and 1996, reporting a mean PI count of 81,000 cfu/mL, with farm means ranging from 13,000 cfu/mL to 216,000 cfu/mL (Boor et al., 1998). Jayarao et al. (2004) reported a mean PI count of 8,740 cfu/mL for 126 producers in Pennsylvania over an 8-wk time period, with a range of 500 to 139,750 cfu/mL. In a study of approximately 11,100 raw bulk tank milk samples collected from 235 dairy producers in Prince Edward Island, Elmoslemany et al. (2009a) reported a geometric mean PI count of 12,000 cfu/mL.

Elevated PI counts have been tied to inadequate cleaning and sanitation at the farm, in particular in milking and milk storage equipment, but also potentially of the cows themselves. Jayarao et al. (2004) suggest that, when PI counts are elevated, investigation should focus on equipment factors such as inadequate water temperature used to clean equipment and water quality issues (e.g., water hardness, pH), temperature of the bulk tank 2 h after milking, and pre- and post-milking hygiene procedures (e.g., use of pre- and post-dip, use of forestripping). Similarly, Murphy and Boor (2000) indicate that elevated PI counts are most likely the result of dirty equipment, poor cooling, or residual soil on teat surfaces at the time of milking. In a more recent publication, Elmoslemany et al. (2009b) reported that farm management practice risk factors for elevated PI count included environment and cow hygiene factors, including cow stall hygiene, teat cleanliness, teat wash, and pre-dip, as well as equipment hygiene factors, including temperature of bulk tank alkaline wash, pipeline alkaline wash alkalinity, and milk house water quality factors (Elmoslemany et al., 2009b).

The groups of bacteria detected by the PI method are typically dominated by psychrophilic or psychrotolerant taxa, as the procedure itself enriches the populations that can grow at 12.8°C. The predominant populations reportedly identified from PI counts include streptococci, coliforms, *Pseudomonas*, and at lower rates, *Staphylococcus* and gram-positive rods (Gillespie et al., 2012). In a dated study, Johns and Landerkin (1969) reported that the primary organisms responsible for increases in PI counts were gram-negative rods. Similarly, a more recent report by Martin et al. (2011) indicated a moderate correlation ($R^2 = 0.68$) between PI counts on SPC agar versus PI count on gram-negative selective agar (i.e., crystal violet tetrazolium agar), indicating that the predominant population were gram-negative bacteria. However, it should be noted that the raw milk samples tested in Martin et al. (2011) were collected from storage tanks at processing facilities, not from producer bulk tanks, which likely influenced the overall bacterial populations. These limited studies indicate that, although *Pseudomonas* and other gram-negative bacteria seem to predominate the population of bacteria enriched during the PI method, the microbial drivers of this parameter are more diverse than is typically assumed.

The limitations of the PI count are driven by the fact that this method is meant to predict the keeping quality of raw milk under certain abusive conditions, namely (1) storage at the farm or processing facility for long periods of time (e.g., >2–3 d total), (2) storage at higher-than-optimum temperature (i.e., >4°C) for shorter periods of time, or (3) both conditions. Given that these conditions do not necessarily occur for any given load of raw milk, the PI count has no significant correlation with finished product quality (Martin et al., 2011) despite selecting for psychrophilic bacteria that are often capable of producing heat-stable enzymes. If storage and handling conditions at the farm and the processing facility are adequately managed, most bacteria detected by the PI count will not reach levels where they produce heat-stable enzymes, and therefore will not affect finished product quality. Importantly, total bacteria levels were considerably higher in the 1950s and 1960s when the PI count was recommended by Johns (1958), with the high-quality group in that study having SPC values ranging from 5,000 to 29,000 cfu/mL (mean of ~14,600 cfu/mL, calculated here from raw data presented in Johns), which would not be considered high quality under contemporary definitions. Even so, some argue that, even in the contemporary raw milk continuum, some milk supplies are held for 2 to 3 d at the farm and potentially another 2 to 3 d at the processing facility before pasteurization, and therefore using the PI count is still an appropriate assessment of the risk of reduced keeping quality. A more direct and quantitative approach to consider when raw milk with initial low TBC (i.e., <5,000 cfu/mL) is held under abusive conditions, as defined here, is to evaluate for TBC immediately before processing, to identify total bacteria levels reaching concentrations that would allow for production of heat-stable enzymes.

Coliform Count: Indicators of Fecal and Environmental Contamination

Coliforms are a method-defined group of bacteria that are aerobic or facultatively anaerobic gram-negative, non-sporeforming rods capable of fermenting lactose to produce gas and acid within 48h at 32 to 35°C (Davidson et al., 2004). Coliforms have been used for over a century as indicators of fecal contamination in water, dairy, and other food products (Martin et al., 2016), although it is noteworthy that the coliform group in general is not strictly associated with fecal contamination, and, indeed, fecal coliforms are a small subset of
the larger coliform group, many of which are associated with environmental sources (e.g., water, vegetation). The standard method for enumeration of CC is the use of violet red bile agar, with confirmation testing of typical colonies for gas and acid production in brilliant green bile medium; however, the use of dehydrated film media (e.g., 3M Coliform Petrifilm) is pervasive due to the simplicity of the method (Davidson et al., 2004). The Pasteurized Milk Ordinance does not set forth a regulatory limit in the US for coliforms in raw milk; however, California has established a regulatory limit of 750 cfu/mL for raw milk (CDFA, 2022). Coliforms are commonly evaluated in raw milk as indicators of hygienic milking practices, specifically as indicators of fecal contamination, and guidelines suggest that good-quality raw milk should contain no more than 10 cfu/mL (Jayarao et al., 2001), with levels between 100 and 1,000 cfu/mL indicating poor milking hygiene (Ruegg and Reinemann, 2002). Pantoja et al. (2009) reported a mean CC of 1.7 log cfu/mL (50 cfu/mL) from over 7,200 raw milk samples from 16 dairy farms in Wisconsin, and Jayarao et al. (2004) reported a mean of 70 cfu/mL from 126 dairy farms in Pennsylvania, both at or above the guidelines for good- and acceptable-quality raw milk, respectively. Jayarao and Wang (1999) reported a considerably higher CC mean from 130 bulk tank raw milk samples from South Dakota and Minnesota, of 3.4 log cfu/mL (2,500 cfu/mL).

Farm sources and management practices associated with high CC may include poor milking hygiene (e.g., not adequately removing residual soil from teats before unit attachment), or environmental contamination through insufficiently cleaned equipment or, occasionally, from presence of coliform mastitis in the milking herd (Murphy and Boor, 2000). Jayarao and Wolfgang (2003) suggest that, when bulk tank raw milk CC levels are high, pre-milking hygiene should be evaluated, including whether udders are wet during milking, whether the milking unit falls into manure during milking, whether rubber hoses and gaskets show wear, and whether clinical coliform mastitis occurs in the herd. A study by Elmoslemany et al. (2009b) indicated that cow hygiene factors, including leg hygiene score and teat cleanliness score, are significantly associated with bulk tank raw milk CC; in addition, several equipment factors, including temperature of multiple equipment wash steps, cleaning and sanitation chemistry factors, and milk house water quality factors are also significantly associated with bulk tank raw milk CC. Pantoja et al. (2011) also report that handling of milking unit clusters, specifically reducing rate of unit fall-offs and increasing cluster washes, are associated with in-line CC and that milking machine wash failures (e.g., lower-than-normal wash temperature reached, failure to dispense preset amount of detergent) are strongly associated with in-line raw milk CC. The authors further found a positive association between SCC and CC, indicating that milking cows with coliform mastitis may have contributed to CC levels in their study (Pantoja et al., 2011).

Coliforms are a diverse group of bacteria, falling primarily within the Enterobacteriaceae family but also including members of the Aeromonadaceae family (Abbott et al., 2003). Genera commonly isolated from raw milk include Citrobacter, Enterobacter, Escherichia, and Klebsiella (Jayarao and Wang, 1999). Kagkli et al. (2007) further report the presence of Hafnia and Serratia in bulk tank raw milk. Of these genera, only Escherichia coli is predominantly associated with fecal contamination, whereas the remaining coliform contaminants are often associated with, and persist in, environmental contamination, as from soil, water, and vegetation (Martin et al., 2016), which is consistent with the reports that bulk tank raw milk CC is significantly associated with equipment cleaning and sanitation factors. Indeed, even members of the coliform group traditionally thought to be superior indicators of fecal contamination, Klebsiella and Escherichia coli, have been shown to persist and grow in natural environments such as water and soil (Ferguson and Siguroretto, 2011). Many genera of environmental coliforms, including Enterobacter, Citrobacter, and Serratia, have also been shown to grow substantially at low temperatures (Masiello et al., 2016), meaning that, over prolonged cold storage of raw milk, these organisms may contribute to high overall TBC in bulk tank raw milk. Finally, coliforms that cause environmental mastitis include Escherichia, Klebsiella, Enterobacter, and Serratia (Hogan and Smith, 2003), which may also contribute to CC in bulk tank raw milk. Hayes et al. (2001) found that, of 20 bulk tank raw milk TBC spikes evaluated over a 2-wk period from 13 farms, 4 were associated with high levels of Escherichia coli, potentially due to milking of mastitic cows; however, the authors suggested that these spikes were more likely due to insufficient cleaning and sanitation of equipment.

As discussed previously, elevated raw milk CC (>100–1,000 cfu/mL) is an indicator of inadequate cow hygiene, milking time hygiene, or equipment hygiene. However, similarly to the other hygiene indicators discussed here, the ability of CC to influence finished product quality is almost entirely dependent on the conditions allowing these organisms to reach levels where they may begin to produce heat-stable enzymes. Coliforms, as detailed for other gram-negative bacteria (e.g., Pseudomonas), have been shown to produce a variety of enzymes when cell concentrations reach high enough levels (Trmič et al., 2015), some of which are heat stable (Vithanage et al., 2016). Due to the
limited ability of CC to indicate fecal contamination, this parameter does not provide considerable additional information beyond that of other indicator organisms from the perspective of finished product quality.

**Laboratory Pasteurization Count: A Measure of Thermoduric Bacteria**

The use of the LPC method dates back to the early 20th century, when early dairy bacteriologists studied the presence of thermoduric bacteria in raw milk and their ability to survive pasteurization treatments, in particular low-temperature, long-time pasteurization (Hileman, 1940). This method consists of heating raw milk to 62.8°C for 30 min to eliminate heat-sensitive bacteria, followed by enumeration using the SPC method (Frank and Yousef, 2004), although variations on this method will be discussed further herein. Similar to the PI and CC parameters, there are no regulatory limits for LPC in raw milk in the US, but this parameter is often used as a component of producer quality programs. It is generally recommended that LPC be below 100 cfu/mL as an indication of high-quality milk and that levels above 200 cfu/mL are an indication of poor equipment hygiene (Murphy and Boor, 2000; Jayarao and Wolfgang, 2003). Several publications have reported LPC values for bulk tank raw milk, including a mean of 1.9 log cfu/mL (79 cfu/mL) from a study of 7,220 bulk tank raw milk samples collected from Wisconsin producers (Pantoja et al., 2009), a mean LPC of 129 cfu/mL from 855 bulk tank raw milk samples in New York (Boor et al., 1998), and a mean of 43 cfu/mL from 1,141 bulk tank samples collected from Tennessee dairy farms (Gillespie et al., 2012).

It is widely accepted that LPC values exceeding guidelines as described above (i.e., >200 cfu/mL) are associated with poor equipment cleaning and sanitation. These associations date back to the earliest publications on the use of LPC to identify populations of bacteria capable of surviving pasteurization. A review of the thermoduric bacteria in pasteurized milk, published in the *Journal of Dairy Science* by Hileman in 1940, describes improperly sterilized milk contact surfaces, dirty milk cans, and improper caring for equipment and utensils as sources of thermoduric bacteria in raw milk (Hileman, 1940). It should be noted that, in this 1940 review, a low LPC is designated as 5,000 cfu/mL, nearly 2 orders of magnitude higher than current recommendations for LPC limits for good-quality milk. Certainly, in the last century, cleaning and sanitation practices of milking and milk handling equipment have advanced dramatically. Current research, however, corroborates these early reports that equipment hygiene is of particular importance to LPC levels in bulk tank raw milk. Elmoslemany et al. (2009b) evaluated the association between LPC in bulk tank raw milk and farm risk factors, reporting that higher temperatures used during equipment cleaning and sanitation, high chlorine concentration, high slug score (indicating sufficient physical cleaning of the pipeline), and the use of water softener (to eliminate hard water) are all protective against high LPC (Elmoslemany et al., 2009b). Meanwhile, Bava et al. (2011) found that LPC of liner swabs before milking (indicating residual thermoduric bacteria in cleaned and sanitized milking liners) is significantly and positively associated with bulk tank raw milk SPC levels, further supporting the importance of equipment cleaning and sanitation on bacterial contamination of the bulk tank milk.

Early studies indicated that predominant populations detected by the LPC consisted primarily of streptococci, micrococci, coryneform bacteria, aerobic sporeforming rods, and occasionally gram-negative rods (Thomas et al., 1967). More modern evaluations of thermoduric populations in raw milk revealed similar outcomes, with Kikuchi et al. (1996) reporting the presence of *Bacillus* (30.7% of total isolates), *Microbacterium* (26.9% of total isolates), *Micrococcaceae* (23.4% of isolates), coryneform bacteria (7.9% of isolates), *Streptococcus* (6.7% of isolates), and others at a lower rate in 400 laboratory pasteurized raw milk samples from Japan. Delgado et al. (2013) reported that nearly 60% of isolates from 22 raw milk samples from Spain subjected to laboratory pasteurization were identified as *Streptococcus thermophilus* (56%), with other major contributors identified as *Lactobacillus* (18%), *Enterococcus* (13%), and others at below 1% of the isolates identified, including aerobic bacilli (Delgado et al., 2013). Finally, Ribeiro Júnior et al. (2018) reported 8 genera of thermoduric bacteria isolated from 20 Brazilian raw milk samples, including *Bacillus, Brachybacterium, Enterococcus, Streptococcus, Micrococcus, Kocuria, Paenibacillus,* and *Macrococcus*, with *Bacillus* representing approximately 50% of the isolates identified. Although the overall types of thermoduric bacteria found in raw milk over time appear to be consistent across studies, the proportions vary considerably by study and presumably by raw milk supply, although this cannot be confirmed through current literature. It should also be noted that only one (Delgado et al., 2013) of the above-cited studies indicates what criteria were used to select bacterial isolates (e.g., all colonies selected, selected based on colony morphology, other), so appropriate caution must be used when interpreting the specific bacterial population proportions reported here.

Limitations of the LPC method for identifying high-quality raw milk include methodological issues and interpretation issues. First, regarding methodological
limitations of the LPC test, the recommendation is to pour plate using SPC agar, as many of the expected thermoduric bacteria selected by the laboratory pasteurization treatment are micro-aerophilic and therefore do not form robust colonies on the surface of agar, making enumeration difficult. Many contemporary laboratories have moved away from using the pour plating method, as it is more time consuming and requires the use of an autoclave. In its place, dehydrated film plates (e.g., 3M Petrifilm) are increasingly used, because they require no specialized equipment beyond a pipette and an incubator. A study by Byrne and Bishop (1991) evaluated 3M Aerobic Count Petrifilm as a suitable alternative for LPC enumeration compared with SPC pour plates. The authors reported that, for 45 samples of naturally contaminated raw milk, there was no significant difference between LPC conducted using SPC pour plates and AC Petrifilm. However, when evaluating raw milk experimentally contaminated with various thermoduric bacteria, the authors found that recovery of 6 of the 7 Micrococcus species tested was significantly lower on AC Petrifilm than on SPC pour plates. The authors further noted that, when raw milk samples were allowed to sit at room temperature for 2 to 4 h before plating, this difference diminished. It should therefore be noted that, although overall differences between these 2 plating methods for naturally contaminated milk appear to be small based on this one study, high proportions of Micrococcus in the thermoduric population of any given raw milk supply would likely result in far lower LPC with the use of AC Petrifilm.

Beyond the LPC methodological issues discussed, the issue of interpretation represents a much larger limitation to the contemporary dairy industry. The first misinterpretation of the LPC test is that it represents populations of thermoduric bacteria capable of surviving pasteurization. Although that may be true for low-temperature, long-time pasteurization, also called batch or vat pasteurization, which is conducted at 63°C for 30 min, it is not true for HTST pasteurization (72°C for 15 s), which is the heat treatment used for the vast majority of fluid milk in the US. Previous studies of HTST fluid milk populations indicate that the predominant bacteria recovered are Bacillus spp., whereas, at the end of shelf-life, the predominant bacteria are the psychrotolerant sporeforming bacteria Paenibacillus (Huck et al., 2008; Ranieri and Boor, 2009). Of course, Bacillus and other aerobic sporeforming bacteria are among the bacterial taxa selected for by the LPC method, as previously seen. However, given the large variation in the proportions of these organisms within the overall thermoduric population found in raw milk supplies, LPC is a poor predictor of the microbiological performance of pasteurized fluid milk, as demonstrated by Martin et al. (2011). Another frequent misconception is that the LPC is a proxy for spore populations in raw milk. As described earlier, aerobic sporeformers may represent a range of proportions of the thermoduric populations in raw milk and are typically present in very low levels. For these reasons, if a raw milk spore count is desired, the method used should be specific for spores, as will be described in the next section. Finally, it should be acknowledged that, although non-sporeforming thermoduric bacteria are not drivers of processed dairy product quality in most cases, these organisms can represent important non-starter lactic acid bacteria, especially in raw milk cheeses.

**Sporeforming Bacteria: Low-Level Contaminants Contributing to Dairy Product Quality**

The final raw milk microbiological parameter that will be discussed in this review is the spore count. Overall, this parameter is used less frequently to monitor raw milk quality than the others discussed here, and, indeed, to call it one parameter is a misnomer; as sporeforming bacteria are diverse and have varied effects on finished product quality, several different methods are used to quantify different types of sporeformers. The primary groups of sporeforming bacteria monitored in raw milk supplies are (1) psychrotolerant sporeformers (e.g., Paenibacillus spp.), which cause spoilage of fluid milk; (2) mesophilic and thermophilic sporeformers (e.g., Bacillus licheniformis), which contribute to levels of spores in dairy powders; and (3) anaerobic butyric acid bacteria (e.g., Clostridium tyrobutyricum), which cause late-blowing defect in certain styles of cheese. The methods used to detect each of these groups include application of an initial heat treatment to eliminate all vegetative cells in the sample, including other thermodurics (e.g., Micrococcus) and sporeforming bacteria that are not in spore form, followed by selective enumeration methods that allow for growth of the group of interest. For psychrotolerant, mesophilic, and thermophilic spore count, raw milk is heat treated to 80°C for 12 min, followed by plating on standard methods agar, which is then incubated at 6°C for 10 d, 32°C for 48 h, and 55°C for 48 h before enumeration, respectively (Martin et al., 2019). Anaerobic butyric acid bacteria (BAB) are enumerated using a MPN method due to their presence in low levels in raw milk, which is performed by distributing raw milk into tubes of Bryant and Burkey medium in successive dilution series (e.g., 5 tubes with 9 mL of medium and 1 mL of raw milk; 5 tubes with 9.9 mL of medium and 0.1 mL of raw milk), which are then capped with melted paraffin wax to maintain an anaerobic environment and heat treated at 75°C for 15 min, followed by incubation...
at 35°C for 6 d. Tubes are scored for gas production (as indicated by the movement of the wax plug upward in the tube), and final MPN is calculated (Martin et al., 2019).

The US currently has no regulatory spore count limits for raw milk, and spore count monitoring is primarily driven by processors that want to control finished product quality or spoilage originating from spores in raw milk. Unlike other bacterial parameters described herein, there are no generally accepted guidelines for limits of spores in bulk tank milk, with the exception of a limit of 3.0 log spores/L (1,000 spores/L or 1 spore/mL) for BAB spores suggested by Vissers et al. (2006) to prevent late-blowing defect in cheese. Spore levels in raw milk are reportedly very low, which contributes to the limitations of this method, as we will discuss. Martin et al. (2019) reported mean psychrotolerant spore count of $-0.24 \text{ log cfu/mL}$ (0.57 cfu/mL), mesophilic spore count (MSC) of 0.50 log cfu/mL (3 cfu/mL), and thermophilic spore count (TSC) of 0.36 log cfu/mL (2.3 cfu/mL) in 34 samples of bulk tank raw milk collected from 17 New York dairy farms. Similarly, Buehler et al. (2018a) reported a mean psychrotolerant spore count of $-0.72 \text{ log MPN/mL}$ (0.19 MPN/mL) based on data collected by Masiello et al. (2014), also demonstrating low-level psychrotolerant spore contamination in raw milk. In a study of bulk tank raw milk from farms in 18 US states, Murphy et al. (2019) reported mean MSC and TSC of 0.26 log cfu/mL (1.8 cfu/mL). Finally, BAB spores are also present in raw milk at low levels. For example, Vissers et al. (2007) reported a mean BAB from 24 farms in the Netherlands of 2.70 log spores/L (501 spores/L or 0.5 spores/mL; Vissers et al., 2007). Despite these low levels of contamination, spores present in raw milk survive processing hurdles and go on to cause quality deterioration of finished dairy products, making them especially important parameters of raw milk quality. With no generally accepted limits on aerobic spore levels in raw milk to prevent finished product quality issues, an alternative approach for quantifying and reducing the effects of these organisms is to use predictive modeling tools such as those discussed later in this review. Briefly, stakeholders use established distributions of target sporeforming populations (e.g., psychrotolerant spores) in raw milk, or develop distributions based on their own supply chain, to predict the impact on finished product outcomes. These models can then be used to determine the incremental improvement on finished product outcomes (e.g., spoilage) when spore concentrations are reduced in the supply.

Numerous publications have demonstrated the role that farm management practices and factors play in the levels of sporeforming bacteria found in bulk tank raw milk. These can broadly be categorized as (1) housing area and bedding practices, (2) milking routine practices, (3) cow-level factors, and (4) feed factors. For example, Murphy et al. (2019) demonstrated that bedding material is associated with spores in bulk tank raw milk, with level of spores in the bedding material directly associated with MSC and TSC in raw milk. Magnusson et al. (2007) reported the same finding when specifically investigating the levels of raw milk contamination with *Bacillus cereus* spores. Miller et al. (2015a) also reported that the use of sand or sawdust bedding for lactating cows was associated with lower MSC in bulk tank raw milk, and the use of straw bedding was associated with lower TSC in bulk tank raw milk. Murphy et al. (2019) and Martin et al. (2019) both reported that the increased frequency of topping up or changing bedding was associated with reduced bulk tank raw milk spore levels. Studies have also suggested that milking routine practices may affect spore levels in bulk tank raw milk; this may be driven by the effectiveness of different milking practices with regard to removal of residual soil (e.g., manure, bedding material) from teat ends. Relatedly, the amount of soil on teat and udder surfaces has also been shown to be significantly associated with bulk tank spore levels (Vissers et al., 2006; Masiello et al., 2014; Martin et al., 2019; Murphy et al., 2019). Indeed, Evanowski et al. (2020) reported that significant reductions in bulk tank MSC and TSC were observed after milking employees were trained on enhanced teat end cleaning procedures and laundered towels used for milking preparation were prepared by washing with detergent, sanitizing with bleach, and thoroughly drying, demonstrating the importance of milking preparation procedures on transmission of spores into bulk tank raw milk. Finally, feed factors influence BAB spore levels in bulk tank raw milk. For example, Vissers et al. (2007) found that the driving factor for BAB spore levels in bulk tank raw milk was the concentration of BAB spores in mixed silage fed to the lactating herd. The pathway of contamination from feed to milk includes concentration of spores in the manure and subsequent contamination of the udder and teat surfaces. These studies all demonstrate the importance of comprehensive management in control of the transmission of spores from environmental sources into bulk tank raw milk.

Concerning the characterization of sporeforming bacteria found in bulk tank raw milk, major genera reported include *Bacillus*, *Paenibacillus*, *Clostridium*, *Brevibacillus*, *Lysinibacillus*, *Sporosarcina*, *Ureibacillus*, *Viridibacillus*, *Oceanobacillus*, and others (Coorevits et al., 2008; Miller et al., 2015a). Multiple reports suggest that the most prevalent sporeforming bacterium present in raw milk is *Bacillus licheniformis* (Crielly et al.,...
Bacillus species identified in raw milk include Bacillus clausii, Bacillus species identified in stream dairy products. Other spoilage and potential foodborne disease risks in downstream dairy products. Other Bacillus species identified in raw milk include Bacillus clausii, Bacillus pumilus, Bacillus subtilis, and others (Miller et al., 2015b). Paenibacillus dominates the psychrotolerant sporeforming taxa found in raw milk (Ivy et al., 2012), but other genera found in raw milk have also been reported to grow at low temperatures, including Bacillus weihenstephanensis and Viridibacillus spp. (Trmčić et al., 2015). Similarly, strictly anaerobic sporeforming bacteria are predominantly within the genus Clostridium (Doyle et al., 2015), with the notable member being Clostridium tyrobutyricum as the primary causative agent of late-blowing defect in certain styles of cheese (Klijn et al., 1995). Finally, raw milk is occasionally demonstrated to be a source of sporeforming extremophiles that survive very harsh conditions that are destructive to most other sporeforming organisms. For example, Schellingman et al. (2005) found a surprising diversity of highly heat-resistant spores (recovered after heat treatment of 100°C for 30 min) in raw milk supplies from 17 farms in Belgium, including Bacillus sporothermodurans and Geobacillus spp. These extremophiles have particular importance to the quality of dairy powders and UHT fluid milks (Miller et al., 2015b; Alonso et al., 2021). Overall, the diversity of sporeforming bacteria in raw milk supplies contributes to their ability to cause quality deterioration in finished dairy products and represents a challenge for detecting and enumerating specific subgroups, as we will describe subsequently.

The primary limitations of monitoring raw milk supplies for sporeforming bacteria are related to their presence in very low concentrations, often below 1 cfu/mL, which is the detection limit of most traditional microbiological methods. In response, methods that estimate low-level concentrations of spores (i.e., MPN) are used for BAB and have been described for psychrotolerant spores (Masiello et al., 2014); however, these methods are time consuming and tedious, and require a great deal of space and laboratory ware (e.g., test tubes), which are barriers to routine use. Due to the low levels of contamination and the associated methodological challenges with enumerating low-level contaminants—as the number of recovered colonies are often at such low levels (i.e., <20–25 cfu/mL) that reliable assessment of spore loads is not assured—the association of raw milk spore count with finished product quality is low. For example, Martin et al. (2011) found no significant association between spore count and fluid milk SPC at 17 d of refrigerated storage, despite Paenibacillus being the predominant genus detected at that point in shelf-life. The authors acknowledged that the limitation of the enumeration method, specifically that many samples tested had spore counts below the limit of detection, likely influenced the outcome of their study. It is further noteworthy that, in addition to recommendations for BAB limits in raw milk intended for use in cheesemaking, as described earlier, no well-established guidelines for aerobic spore limits in raw milk currently exist, limiting the ease of interpretation of this parameter to dairy industry stakeholders.

Defining Raw Milk Microbiological Quality from the Perspective of Finished Product Quality: A 3-Tiered Approach

Each of the microbiological parameters discussed herein has benefits and limitations for identifying and defining raw milk quality, but, importantly, it has been demonstrated that these various parameters are often significantly associated with each other and with other non-microbiological measures of quality, specifically bulk tank SCC. For example, Jayarao et al. (2004) reported that bulk tank raw milk with low SPC (<5,000 cfu/mL) also had a significantly lower SCC (<200,000 cells/mL), PI (<10,000 cfu/mL), LPC (<100 cfu/mL), and other bacterial measures of quality. Similarly, Pantoya et al. (2009) found that an increased TBC was 6.3 times more likely for bulk tank raw milk with elevated CC, and 1.3 times more likely for bulk tank milk with elevated LPC. The authors further note that the odds of increased TBC increased by 2.4% for every 10,000 cell/mL increase in bulk tank raw milk SCC (Pantoya et al., 2009). Schukken et al. (1992) reported that herds that produced milk with higher PLC, a measure of TBC, had higher SCC, and Gillespie et al. (2012) identified a significant association between PLC and PI. Finally, Masiello et al. (2017) reports a significant association between milk SCC and TBC, PI, and psychrotolerant spore count. A key takeaway from the associations between raw milk microbiological parameters for dairy industry stakeholders is that monitoring raw milk quality, especially as it pertains to identifying raw milk supplies likely to perform well throughout the continuum from production to processing and shelf-life, should be streamlined to focus on an overall microbiological quality indicator, which we propose should be TBC. As noted by Hutchison et al. (2005), although TBC is not without its own shortcomings, no better bacterial marker for overall raw milk hygiene currently exists, especially given its ease and rapidity of measure-
Table 1. Suggested 3-tiered approach to using microbiological testing to define raw milk quality

<table>
<thead>
<tr>
<th>Goal of testing</th>
<th>Recommended microbiological test(s)</th>
<th>Testing frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Process control</td>
<td>Total bacteria count (TBC)</td>
<td>High frequency, every load of milk</td>
</tr>
<tr>
<td>Monitoring parameters that</td>
<td>Spore counts including butyric acid bacteria</td>
<td>High/moderate frequency initially to develop a baseline</td>
</tr>
<tr>
<td>directly influence finished</td>
<td>count and psychrotolerant spore count,</td>
<td>distribution in the supply, then subsequently lower</td>
</tr>
<tr>
<td>product quality</td>
<td>lactic acid bacteria</td>
<td>frequency to identify deviations from the baseline</td>
</tr>
<tr>
<td>Troubleshooting</td>
<td>Preliminary incubation count, coliform count,</td>
<td>Low frequency, only when needed to troubleshoot high</td>
</tr>
<tr>
<td></td>
<td>and laboratory pasteurization count</td>
<td>TBC, paired with on-farm risk assessment</td>
</tr>
</tbody>
</table>

Beyond the use of TBC as an overall process control test, we suggest that other microbiological tests discussed in this review should be utilized in 1 of 2 ways: (1) monitoring parameters that directly influence the finished product, or (2) troubleshooting causes of high TBC (Table 1). The first, monitoring tests, should be used periodically to assess parameters that directly affect finished product quality, including various types of spore tests, especially BAB count and psychrotolerant spore count. Additional microbial monitoring parameters should be considered based on the specific dairy product manufactured from the raw milk supply. For example, although not covered in detail here because their presence in raw milk is not routinely tested for, certain raw milk lactic acid bacteria (lactobacilli, streptococci, and others) may affect the quality of artisanal raw milk cheeses, in the development of both desirable and undesirable attributes, making this group an appropriate target for raw milk microbial monitoring in some cases. Initially, stakeholders should monitor these parameters frequently (e.g., weekly) to develop a baseline distribution of the spore concentrations (or alternative monitoring target) in a particular supply and to ensure that this baseline falls within expected ranges based on research such as that discussed herein. Once a baseline is established, monitoring tests may be performed less frequently (e.g., monthly) unless a deviation from the baseline is detected. Outcomes of monitoring tests may also be used to reward producers who consistently produce raw milk with low spore concentrations through premium payments. The second method, troubleshooting tests, should be used only when TBC exceeds the acceptable quality specification and includes the PI, CC, and LPC tests. In conjunction with evaluation of on-farm risk factors (e.g., pre-milking routine, equipment cleaning, and sanitation), troubleshooting tests should be used on a case-by-case basis to investigate causes of increased TBC.

Overall, this 3-tiered approach to identifying high-quality raw milk through the strategic use of raw milk microbiological test parameters will facilitate a more efficient system with better outcomes for stakeholders across the dairy industry and can be used as the basis of establishing a quality control program for raw milk.

Applying Data-Driven, Risk-Based Tools to Ensuring Raw Milk Microbiological Quality for Optimum Finished Product Performance

In addition to the raw milk microbiological testing approach outlined here, future dairy industry stakeholders should adopt predictive tools that allow for the integration of data across the dairy continuum to support risk-based decision making at the farm. The research discussed in this review reveals considerable overlap in on-farm risk factors for different raw milk microbiological parameters. For example, adequate equipment hygiene factors are associated with elevated levels of TBC, CC, PI, and LPC (Elmoslemany et al., 2009b; Bava et al., 2011). Leveraging the research on these common factors will be essential to transition from a solely reactive, test-based system that is predominantly used in the dairy industry today to a whole-farm, risk-based system where farm management practices and testing data are paired to proactively reduce the likelihood of adverse events occurring, as relating to raw milk microbiological quality and resulting finished product quality.
The development of decision support tools at the production and processing levels has demonstrated how relevant data can be used to predict outcomes of interest to dairy industry stakeholders. For example, Cabrera (2018) outlines the development and application of over 20 computerized dairy farm decision support tools to assist producers with farm management related to nutrition, reproduction, calf and heifer management, replacement, price risk, and environment (Cabrera, 2018). Other similar tools have also been described for dairy production (Rose et al., 2016; Balhara et al., 2021), and numerous commercial products exist on the marketplace (e.g., iDDEN.org, MyDairyDashboard.com). Similarly, decision support tools have been developed to assist processors with dairy product quality improvements. For example, Monte Carlo simulations have been used to predict the shelf-life of fluid milk based on the presence of psychrotolerant spores in raw milk supplies (Buehler et al., 2018a), to predict consumer exposure to spoiled yogurt (Buehler et al., 2018b), and to predict the occurrence of late-blowing defect in cheese (Qian et al., 2022).

Despite the demonstrated utility of data-driven, risk-based decision support tools, they are not without their limitations. In particular, dairy producers face several barriers to the adoption of these tools. A recent commentary on improving the adoption rates of integrated decision support systems identified major barriers including (1) perceived limited value by producers, (2) issues of ease of use or interpretation, (3) lack of practical and direct application, (4) data collection, quality, and standardization challenges, (5) lack of data integration, (6) limited data sharing and farm infrastructure, and (7) limited multidisciplinary collaboration between scientists developing new knowledge and those developing decision support tools (Baldin et al., 2021). Similarly, Rose et al. (2016) outlined a similar set of factors affecting adoption of decision support tools, including what the authors describe as core factors (e.g., ease of use, peer recommendation, cost), enabling factor (i.e., facilitating conditions such as internet and phone connection), driving factors (e.g., level of marketing and legislative compliance), and modifying factors (e.g., producer age, scale of business; Rose et al., 2016). Importantly, these barriers are likely to have the largest influence on dairy producers who are most in need from the perspective of quality improvements (e.g., those with limited resources, remote farm locations). However, despite these limitations, development of data-driven, risk-based decision support tools applied to the microbiological quality of raw milk, integrated throughout the production to processing chain, has the potential to revolutionize how dairy industry stakeholders define raw milk quality. Further cross-disciplinary efforts should be made, to ensure broad adoption and implementation of these tools in the future. For example, co-ops and processors could use these models to set target levels for measures that are directly linked to finished product quality and conversion efficiency (e.g., BAB counts, psychrotolerant spore counts), with associated premiums. This approach would allow for strategic targeting of specific on-farm practices to ensure consistent production of raw milk that meets specifications, and subsequent verification of this approach through periodic, low-frequency monitoring of relevant parameters (e.g., BAB counts) paired with frequent process control monitoring (i.e., TBC testing) on every load of milk.

CONCLUSIONS

Bacterial populations in raw milk play an important role in the quality of processed dairy products. However, as discussed in this review, parameters currently used to assess microbiological raw milk quality have varying degrees of capacity to inform stakeholders on their potential implications for finished product quality. The future of raw milk quality determination should focus on a whole-farm approach that acknowledges the microbiological risk factors at various stages of the production process and transitions from the reactive test-based system of our current dairy industry to a predictive, risk-based system. This requires standard use of an appropriate microbiological indicator, namely TBC, periodic monitoring of microbial parameters that directly affect finished product quality, use of troubleshooting tests when the process control test is out of compliance, and implementation of integrated decision support tools that will provide producers with the necessary guidance to produce high-quality raw milk for processing into high-quality finished dairy products. This approach will require stakeholder collaboration throughout the dairy continuum from production, processing, regulatory agencies, and academic institutions.

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