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Title: Characterization of Milk Hauling Practices and Their Impact on Raw Milk Microbiological Quality

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The dairy industry has indicated that milk hauling sporadically compromises milk quality, but often the reason is unknown. Milk hauling practices are an underexplored area of research, and are in need of attention because during hauling milk is most exposed to the external environment in comparison with any other step of modern dairy processing. Milk hauling is defined as the activities associated with the transfer of raw milk from producer to tanker truck, which is then transported and unloaded into storage silos at a processing facility. Tanker are often used to haul several loads within a 24-h period without cleaning and sanitizing in between; a practice that is mandated by the Grade "A" Pasteurized Milk Ordinance (PMO). Repeated tanker usage between cleans is necessary in the modern dairy industry; less cleaning reduces chemical and water usage, and time. There is no specification on maximum loads hauled or idle time (empty and dirty) between loads. Additionally, many routine practices outlined in the PMO use vague wording (as needed) to describe frequency; this is to provide flexibility to industry since each facility is unique. However, this vagueness does not inform industry on what best

practices entail; potentially leading to unexplained sources of contamination due to weaknesses in practices.

The overarching hypothesis of our research is that milk hauling sanitation and operation practices have the potential to negatively contribute to the microbiological quality of raw milk and impact finished product quality. In the scope of our study, negative impact from hauling is defined as an increase in microbiological counts or microflora proteolytic and lipolytic enzyme activity. The aim of our research was to explore a vast range of hauling situations to see if they had potential to compromise raw milk quality; this was achieved by i) characterizing variability in industry hauling sanitation and operational practices, ii) identifying circumstances in which hauling contributes to a degradation in the microbiological quality of raw milk by analyzing two years of historic raw milk microbiological data from producers and tanker trucks of a Northwest co-op. and iii) investigating impact of worst-case hauling conditions (e.g. extended idle time between loads) by measuring raw milk microbiological counts and enzyme activity for two scenarios: a) a small-scale using stainless steel milk cans, b) commercial study using tanker trucks and a pre-selected route.

As anticipated, variability in industry practices exists, especially for equipment that required manual cleaning, and preventative maintenance programs such as replacement of aged equipment and parts. Analysis of historic raw milk microbial counts indicated that microbiological counts were not likely to be influenced by hauling, but rather are influenced by on-farm milk quality. Low counts from the on-farm bulk tank will be maintained if best sanitation and operating practices implemented at every step of the process, including milk hauling. Our small-scale milk can study demonstrated that extended idle time (> 6 hours) between loads has potential to negatively impacts milk quality, and provided a proof-of-concept for scaling up to a commercial study. Negative impact on milk quality was demonstrated to not be measurable for commercial tankers remaining dirty and idle for periods of <6 h between loads.

Current PMO regulations (clean per 24 hours) appears to be adequate as long best sanitation and operation practices are implement. Future advances in rapid microbiological testing may facilitate better methods for measuring raw milk quality, especially as we better understand influences of raw milk microflora on downstream quality of dairy products.

Characterization of Milk Hauling Practices and Their Impact on Raw Milk Microbiological Quality

By
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Chapter 1- Introduction

Scoping the Problem

Milk quality is susceptible to deterioration the moment it leaves the cow, and continues to be influenced through handling, transporting, and processing; milk quality can only be negatively influenced due to opportunistic microbial contamination in the dairy supply chain (Doyle et al., 2016; Marchand et al., 2012). Milk hauling is defined as the activities associated with the transfer of raw milk from producer to tanker truck, which is then transported and unloaded into storage silos at a processing facility. The Grade "A" Pasteurized Milk Ordinance (PMO) mandates milk hauling operation and sanitation practice; we are focused on the regulation in regards to repeated tanker usage in Appendix B (Milk Sampling, Hauling and Transportation), stating that "it is allowable to pick up multiple loads continuously within a 24 h period, provided the milk tanker truck is washed after daily use" (Food and Drug Administration, 2015). Tanker usage frequency greatly varies depending on producer and processor demands, and can be categorized as either i) short distance, high frequency hauling, where several loads will be collected within 24 hours, and ii) long distance, low frequency hauling where a tanker remains empty and dirty (e.g. idle) for an extended period of time between loads (Darchuk et al., 2015a,b).

Importance of best hauling practices seems to be minimized due to the perceived simplicity of the process. Milk hauling is more than transporting milk from producer to processor. In fact, milk is most exposed to the external environment during hauling than in any other step of modern dairy processing; on-farm bulk tanks are opened to sample, measure, and grade milk before loading into tankers, and tankers are opened upon reception to check temperature and test for antibiotics. Vague language (i.e. as needed) is used in the PMO to describe frequencies of sanitation and operational practices, which leads to gaps in practices and leaves industry to question if milk hauling is an unexplained source of negative impact on dairy product quality. The most recent hauling research has demonstrated that milk hauling practices in a standard industry setting in both short distance, high frequency and long distance, low frequency hauling situations had no measurable impact on subsequent loads for standard plate count, preliminary incubation count, and thermophilic spore count (Darchuk et al., 2015 a,b). However, another set of studies by Teh et al., (2011, 2012, 2013) demonstrated that several genera of microflora obtained from internal surfaces of tankers can produce either or both proteolytic and lipolytic enzymes, which may be thermo-tolerant and have potential to compromise downstream dairy product quality. Additionally, raw milk microflora populations can rapidly shift during transfer from bulk tank to tanker and tanker to silo (Kable et al., 2016; Huck et al., 2008).

We scoped our research to highlight hauling situations that are both standard and suboptimal to increase the likelihood of capturing scenarios that have a negative impact on raw milk quality. In the scope of our research we have defined negative impact from milk hauling as a significant increase in i) microbiological counts, or ii) culture-based proteolytic and lipolytic activity, when comparing milk samples obtained from producer on-farm bulk tanks and from tankers prior to unloading at processing facilities (tanker unloading > on-farm bulk tank).

Milk Hauling Overview

Historical Practices

The earliest milk hauling operations consisted of the use of flat bed, horse-drawn wagon to haul 10-gallon milk cans. Transition from milk cans to bulk milk haulers occurred in the early 1960s (Erba, et al., year unknown). In early years, tankers were cleaned after every load, but the consolidation of the industry has led to longer routes and more frequent use of tankers between cleans (Darchuk et al., 2015; Dommett et al., 1980). A single tanker load or "route" is defined as the collection, transport, and unloading of one or more producers' raw milk to a processing facility (Eba, et al. year unknown). Routes are designed to maximize tanker usage; if several producers are on a given route that typically indicates that they produce smaller volumes of milk and require consolidation for pickup of more producers for a given route. Pickup frequency depends on farm bulk tank capacity and regulatory factors, e.g. bulk tanks must be cleaned and sanitized every 72 h (Food and Drug Administration, 2015). In modern dairy practice, where the number of farms is on the decline and herd sizes are on the rise, most produce enough milk to be collected from at least once a day. Additionally, the number of processing plants is on the decline; this results in haulers having to travel farther between producers and processors (Erba, et al., year unknown). Repeated tanker use is necessary to improve efficiency of milk collection and reduce usage of resources such as water, chemicals, and personnel (USDA, 2014).

Function of milk haulers

A milk hauler's job includes more than transporting raw milk from farm to processor. In modern dairy processing, milk is rarely exposed to the external environment, with the exception being during hauling. Haulers inspect equipment to ensure integrity and cleanliness, to prevent contamination, and participate in recordkeeping practices. Upon arrival at a dairy farm, the hauler must check the milk temperature, collect a representative milk sample from the bulk tank(s), and accurately measure the milk to ensure the transaction between the producer and processer is fair and shrinkage is minimal. Shrinkage is the difference between what a hauler reports as the amount picked up and the actual amount delivered, typically most agreements allow the shrinkage rate to be between 0.25% and 0.50%; values exceeding the acceptable tolerance indicate that the processor is paying for air (Erba, et al., year unknown). Darchuk reported that the typical shrinkage rate for tankers is ~0.02% (e.g. two gallons per tanker). Factors that contribute to exceeding tolerance shrinkage limits includes incorrect reading of the farm bulk tank dipstick, errors in converting dipstick unit (e.g. inches) to pounds or gallons, errors in recordkeeping, spillage in transfer, and improperly calibrated bulk tanks. Milk hauler positions tend to have a high turnover rate due to long hours, weekend shifts, and having several driving and non-driving responsibilities, making it difficult to retain experienced and skilled haulers (Erba et al., unknown).

Tankers and tanker accessories: design, function, and challenges

Tankers are designed to maintain cold milk temperature during transport using a design similar to that of a Thermos®. Tankers are equipped with stainless end caps, an interior tank, an exterior shell, and between the tank and shell is a ~3.8 cm polystyrene core for support and insulation (Darchuk, 2015). Theoretically, in hot weather conditions (35°C) a fully loaded tanker would only increase by ~1°C in 24 h. (Darchuk et al., 2015). Pantoja et al. (2009) reported that tankers are more susceptible to seasonal temperature fluctuation in comparison to on-farm bulk tanks; however, this observation was of a small magnitude (<0.5°C) and unlikely to contribute any negative impact. Interior surfaces of tankers must be constructed of smooth, non-absorbent, corrosion-resistant, non-toxic material; 300 series AISI stainless steel is typically used to facilitate optimum cleanability (Food and Drug Administration, 2015). Tankers are cleaned and sanitized by an automated process called clean-in-place (CIP), where chemicals are circulated through a piping with tubular flow and are dispersed into the tanker via spray ball or other mechanism of dispersal (Memisi, et al., 2015). CIP systems needs to be validated at least annually. Validation activities include testing i) temperature, ii) flow and mechanical action, iii) conductivity, iv) time, and v) equipment inspection (Food and Drug Administration, 2015; DeLaval, 2013).

Tanker accessories are components necessary to transfer milk in a sanitary fashion, including transfer hoses, caps, pumps, valves, and gaskets. These components fall under the same sanitation regulations as tankers (repeated use per 24 h); however, tanker accessories are non-insulated and typically exposed to the external elements, and for these reasons are likely more susceptible to microbiological contamination. Two types of transfer hoses are used in milk hauling: i) farm hose, used to transfer milk from bulk tank to tanker, and ii) receiving hose, used to transfer milk from tanker to storage silo at processing facility. Farm hoses are usually stored on the tanker, but it should be noted that farm hoses are not typically used in mega dairy farm operation, where instead milk is passed through a plate cooler and collected directly in the tanker, rather than in a bulk tank. Receiving pumps are sometimes designed with a purge feature to remove residual milk from the receiving hose and pump between loads.

Inadequate tanker sanitation provides opportunities for increased microbiological counts and biofilm formation, which can compromise raw milk quality. Biofilm formation occurs when a planktonic bacterial cell attaches to a nutrient rich substrate (e.g. milk) and reproduces to form a resilient extracellular polysaccharide layer which mitigates the efficacy of cleaning and sanitizing agents (Marchand, et al., 2012). Raw milk microflora isolated from internal surfaces of tankers has been demonstrated to form biofilms in vitro on stainless steel coupons which resulted in 2.7 to 7.6 log cfu/cm^2 (Teh et al., 2012). Darchuk et al., (2015) reported that internal surfaces of tankers average 3.4 log cfu/900 cm^2. Raw milk storage sanitation can influence psychrotrophic (cold-thriving) microflora; one study reported that inadequate raw milk storage sanitation can result in elevated psychrotrophic populations (>75%). Conversely, adequate sanitation mitigates psychrotrophic populations (<10%) (Hantsis-Zacharvo and Halpern, 2007).

Several raw milk psychrotrophic bacteria are known to produce proteolytic and lipolytic enzymes, some of which are thermo-tolerant and can remain active post-pasteurization (Teh et al., 2011, 2012). General consensus is that the cell concentration needs to be above 6 log cfu/mL for sufficient enzyme activity to compromise downstream product quality (SMEDP 17th edition). However, certain products and processes may be more susceptible to residual microbial enzymes at lower concentrations. For example, fluid milk shelf-life can be compromised by the presence of proteases, which degrade casein and result in bitter flavors and gelation of milk, and lipases degrade milk fats and form free fatty acids that give rancid, soapy off flavors (Marchad et al., 2012).

Milk Receiving Operations

Standard procedures

Milk receiving is the process of unloading tanker trucks at processing facilities. Receiving operations are permitted to be either partially or fully enclosed from the external environment, and, depending on the facility capacity, they can unload anywhere from one to four tankers simultaneously. Prior to unloading, each load of milk must be tested for antibiotic residues, temperature checked (≤7°C). Milk samples are obtained by either using a sanitized stainless steel dipper and are collected from the top hatch or using a syringe to collect from an in-line sample port (Food and Drug Administration, 2015). Loads positive for antibiotic residues (beta-lactams) are infrequent. One study reported that in New York State only 0.4% of loads (163 of 41,351 loads) tested positive for antibiotic residues (Schaik et al., 2002); however, tetracycline testing is currently being phased in and may impact these rates. Aside from out of specification temperature and presence of antibiotic residues, tanker loads may be rejected several other reasons, including i) incomplete, incorrect, or missing paperwork, ii) past due for CIP, iii) negative organoleptic properties of milk (e.g. off-odor or appearance), and iv) tanker displays signs of improper cleaning or cross-contamination from prior use (Food and Drug Administration, 2015). The party responsible for tanker load rejection is required to cover the cost of loss. For example, the producer is responsible if the milk tests positive for antibiotic residues, but the hauler is responsible for compromised or missing paperwork, past due for cleaning, and elevated temperatures. It may seem counterintuitive to blame haulers for elevated milk temperatures, but haulers are responsible for checking on-farm bulk tank temperature prior to transferring milk to the tanker. As mentioned previously, temperature should not significantly increase during hauling.

Once a tanker load is approved for reception, a receiving hose is connected to the tanker outlet valve and milk is pumped into raw milk storage silos to await further processing. Receiving hoses are constructed out of thick rubber and range from 2.5” to 6” in diameter. Receiving pumps are often designed with a purge mechanism that removes residual milk between loads. Receiving hoses can be used repeatedly for 24 h between CIP. When not in use, receiving hoses need to be capped and hung up to mitigate contamination (Food and Drug Administration, 2015).

Milk reception is a bottleneck in the modern dairy industry. Receiving operations are often delayed due to silos being at maximum capacity or unavailable during cleaning and sanitizing operations. Long waiting periods can add many hours to a hauler's day, and it ties up hauling equipment so that it cannot be used to pick up and transport additional milk. In addition to reducing efficiency, haulers are often not paid for idle time at processing plants (Erba, et al., year unknown; industry contact 2016). Unloading time is contingent on the receiving hose diameter and pump speed, and can range from under 6 minutes to over 40 minutes. Some processors select smaller hose diameters and slower pump speeds to mitigate risk of physically damaging milk quality, while mega facilities utilize larger hose diameters for efficiency (industry contacts, 2016).

Raw Milk Microbiological Quality

Overview

High quality raw milk can be defined by several parameters: complete nutritional composition, free from undesirable organoleptic qualities (e.g. off-flavor and odors), free of adulterants (e.g. detectable antibiotic residues, added water, etc.), low microbial counts, and low somatic cell counts (SCC) (Murphy et al., 2016). Microbiological quality of raw milk is influenced on-farm by the health and hygiene of cows, milking equipment sanitation and design, personnel, and the external environment (Gargouri et al., 2013). Elevated SPC that exceeds the PMO limit (>1,000,000 cfu/mL) have a direct negative impact on product quality (SMEDP 17th edition). Elevated microbiological counts and SCC are associated with increased enzyme activity that compromises milk components and can result in product defects (Murphy et al., 2016). One challenge with current industry raw milk microbiological tests is that when counts are below the regulatory limit

(<100,000 cfu/mL) the results do not predict shelf-life in dairy products (Martin et al., 2011). However, lower counts may be linked to specific organisms and issues, for example presence of a prolific producer of thermo-stable enzymes or thermoduric bacteria below this threshold could compromise downstream quality (Murphy et al., 2016). There is necessity for rapid, readily available testing methods that better characterize raw milk quality.

Baseline milk quality is established at the farm, therefore it is critical that the microbiological activity is minimal to maintain low counts throughout the dairy supply chain; maintaining low counts provides flexibility and extended use of raw milk storage and transportation. Historically, dairy processors perform microbiological tests monthly for individual producers to meet requirements for regulatory agencies, but with increasing herd size and improved testing technologies, processors are routinely testing for the maintenance of low microbial counts (Jayarao et al., 2004, Pantoja et al.,2009). On-farm bulk tank counts can vary greatly. One study analyzed a single producer's bulk tank counts for two years (n = 7,241 samples) and reported the mean SPC to be 3.1 log (12,500 cfu/mL); however, individual samples ranged from 0 to 2 x 10^6 cfu/mL (Pantoja et al., 2009). Monitoring milk quality daily allows processors and producers to identify on-farm sanitation and equipment issues early on and reduce long-term negative impact on the milk supply (Pantoja et al.,2009). Tier levels of premium payment programs vary. Murphy et al. (2016) states that a monthly average SCC < 100,000 cells/mL and microbial counts < 15,000 cells/mL are considered "premium" quality, while Gillespie et al. (2012) reported <10,000 cells/mL to be "premium" quality.

Seasonality

Intuitively, the elevated temperature of summer months are thought to correspond with elevated microbiological counts; however, the findings in literature vary. The diversity of raw milk microflora in tankers and storage silos at a large-scale dairy manufacturing facility was seasonally characterized, and while microflora was highly diverse and varied with season, spring had the highest species diversity, but cell density was only marginally higher than fall and summer (Kable et al., 2015). Gillespie et al., 2012 reported that winter had the highest PIC (4.1 log cfu/mL), while there was no difference between summer and winter SPC. Costello et al. (2003) found that SPC was greatest in the winter for a single farm for 11 years, Shaik et al. (2002) found counts to be highest in summer for several New York farms (2 years), and Pantoja et al. (2009) observed farm to farm variability, but no dominant season for elevated counts (22 months). Farm geological location and climate may play a role in individual farm counts. For instance, farms located in the Pacific Northwest may have poorer milk quality in winter months due to high rainfall levels. Seasonality may impact producers at variable degrees, suggesting that time of year could be a contributing factor to raw milk quality, but it is more likely that on-farm practices will be the dominant factor (Pantoja et al., 2009).

Standard industry microbiological test methods

Common industry microbiological methods used to determine raw milk quality are standard plate count (SPC) or aerobic plate count (APC), coliform count (CC), lab pasteurization count (LPC), preliminary incubation count (PIC), and somatic cell count (SCC) (SMEDP 17th edition). Acceptable raw milk quality is quantified as SCC > 750,000 cells/mL, and SPC > 100,000 cfu/mL for a single farm and > 300,000 cfu/mL for commingled milk (Food and Drug Administration, 2015). Grade "B", or manufacturing grade, milk has the same SCC standards as grade "A", but less strict microbial limits (<500,000 cells/mL) (USDA, 2011). It should be noted that grade "B" milk only accounts for ~1% of US milk supply and is not typically included in incentive programs (USDA, 2015; Murphy et al. 2016).

Traditional cultural enumeration methods include standard plate count (SPC) and aerobic plate count (APC) and are reported as colony forming units (CFU) per weight or volume of sample. SMEDP 17th edition recommends incubation at 32°C for 48 hours prior to enumeration. SPC is often interpreted as an estimate of total bacteria, but does not represent the entire population due differences in required growth conditions and nutrients for various bacterial subpopulations. Flow cytometry is a modern enumeration method that can rapidly (<10 minutes) estimate individual bacteria counts (IBC) in raw milk samples. Each individual cell is stained with ethidium bromide and is injected into a capillary, where each cell passes through a light beam and viable cells fluoresces and are detected by the optical system and reported as IBC/mL (Cassoli et al., 2016). Compared to traditional culture-based enumeration where only colonies that grow under incubation conditions are accounted for, flow cytometry accounts for every viable cell.

Preliminary incubation count (PIC) is used by the dairy industry to estimate sanitation and hygiene. Elevated PIC can be an indicator of poor sanitation and hygiene practices (Gillespie et al., 2012). The PIC procedure is similar to SPC, with the additional step that milk samples are incubated at 21°C for 18 h prior to plating or other analysis. This is to encourage growth of psychrotrophic spoilage organisms that may be present in undetectable levels when using SPC (SMDPE 17th edition). Microflora associated with healthy cows (LAB and Staphylococcus) are not expected to rapidly reproduce under PI conditions, but common spoilage organisms associated with poor sanitation (e.g. Pseudomonas) will thrive under PI conditions (Gillespie et al., 2012; Murphy et al., 2012). Pseudomonas are psychrotrophic, meaning that they are capable of growing at refrigeration temperatures (≤ 7°C), and are known be prolific producers of thermo-tolerant extracellular protease and lipase enzymes (Vithanage, et al., 2016).

Lactic acid bacteria (LAB) are Gram-positive, non-sporeforming, lactose-fermenting organisms that enter the dairy supply chain by i) natural microflora; non-starter lactic acid bacteria (NSLABs), and ii) intentionally for cultured dairy products. LAB tend to be weak producers of proteolytic and lipolytic enzymes in raw milk (Hantsis-Zacharvo and Halpern, 2007). Some NSLABs are thermo-tolerant and can result in negative defects in cheese production. For example, *Lactobacillus wasatchensis* is a recently identified NSLAB associated with late gas blowing in aged Cheddar cheese (Oberg, et al. 2016). *Lactobacillus curvatus* is another NSLAB that produces carbon dioxide gas that creates cracks in Cheddar cheese (Porcellato et al., 2015).

Coliforms are Gram-negative, non-sporeforming, lactose-fermenting bacteria that are found in aquatic, fecal, vegetative, and soil environments (Hogan and Smith, 2003; Pantoja et al.,2011). Common genera include Escherichia (*E. coli*), *Klebsiella*, and

Enterobacter. Coliform counts are often used to quantify hygiene. Coliforms are heat labile and are easily destroyed by pasteurization. The PMO does not regulate coliform counts in unpasteurized milk; however, coliform counts must be < 10 cells/mL or g in pasteurized dairy products (Food and Drug Administration, 2015). Elevated coliform counts in raw milk are associated with weaknesses in on-farm management practices. For example, inadequate sanitation conditions in milk equipment and CIP systems, such as not meeting the wash temperature (>7°C) or not adding sufficient detergent (Pantoja et al.,2011).

Moving Forward: investigating hauling practices from different angles

Milk hauling needs more attention to better understand implications that current practices have on raw milk quality. While Darchuk et al. (2015 a,b) was unable to demonstrate negative impact in a standard industry setting, we believe that negative impact due to hauling is sporadic and is more likely to occur as the result of weaknesses in sanitation and operating practices. As with any industry project, it can be challenging to representatively capture true practices because personnel may perform at higher levels when being observed (McCambringe et al, 2013). We approached this challenge from several angles; i) we surveyed several dairy processing facilities in the Pacific Northwest to capture variability in practices, ii) we designed a hauling route using historical raw milk quality data to identify producers with both historically good and poor quality milk, and iii) we collaborated with a mega dairy operation that intakes ~280 tanker trucks per day. Another challenge in hauling research is attributed to dilution factor and scale, making it difficult to capture a measurable negative impact. Upon unloading a tanker, it will have a residual milk volume of ~0.02% (i.e. about two gallons), theoretically a highly contaminated milk load (150,000 cfu/mL) would only contribute less than 0.003 log cfu/mL to the next load of milk (Darchuk, 2015). To address this challenge we included a small-scale worst-case hauling scenario experiment to demonstrate that hauling does have the potential to negatively impact subsequent loads of milk.

Research Objectives

i) Characterize variability in industry hauling sanitation and operational practices.

ii) Identify circumstances in which hauling contributes to a degradation in the microbiological quality of raw milk by analyzing historic raw milk microbiological data from producers and tanker trucks of a Northwest co-op.

iii) Investigate impact of worst-case hauling conditions (e.g. extended idle time between loads) by measuring raw milk microbiological counts and enzyme activity for two scenarios: a) a small-scale using stainless steel milk cans, b) commercial study using tanker trucks and a pre-selected route.

Chapter 2. Analysis of raw milk historical data to identify trends in milk hauling

Interpretative Summary

Trends in the microbial quality of raw milk at the time of farm collection and receipt at processing facility: a case study in the Pacific Northwest.

Shifts in raw milk microbiological counts from on-farm bulk tank to tanker trucks at milk receiving are not well characterized. Frequent monitoring of raw milk microbial counts enables processors and producers to diagnosis and troubleshoot sanitation equipment in a timely manner, rather than going unnoticed for extended periods of time. Baseline raw milk quality is established by on-farm management practices and is very rarely influenced by hauling. Maintaining low microbiological counts at each step of the process strengthens dairy product safety and quality.

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Abstract

Shifts in raw milk microbiological counts from on-farm bulk tanks to tanker trucks at milk receiving are not well understood. Baseline milk microbiological quality is established by on-farm management practices, but it is influenced by subsequent handling and processing steps in the dairy supply chain. Tanker trucks are often used to haul several loads within a 24-h period without cleaning and sanitizing in between; a practice that is mandated by the Pasteurized Milk Ordinance (PMO). The aim of this study was to identify circumstances in which hauling contributes to a degradation in the microbiological quality of raw milk. We investigated two years (2014-2015) of historical microbiological counts for individual producers (n = 106 producers, 59,855 samples) and tanker trucks (n = 23,270 loads) at a Pacific Northwest Dairy co-op. Raw milk samples had been previously enumerated using flow cytometry (BactoScan) and reported as individual bacterial count (IBC) and preliminary incubation individual bacterial count (PI-IBC). Analysis focused on PI-IBC due to greater magnitude of variability and its use as an indicator of sanitation or equipment failure. The top 1% individual producer PI-IBC were classified as outliers (>305 PI-IBC, n=599 loads). Milk hauling is unlikely to measurably impact raw milk quality (i.e. increased microbiological counts during transportation). Instead, producer microbiological quality was influential on the tanker load. Single producer tanker loads were significantly higher than having one or more producers on each load due to a dilution effect. Emphasis on daily monitoring raw milk microbiological counts allows for spikes to be identified and the necessary corrective action taken before

the problem persists for extended periods of time, and potentially impacted commingled milk in processing facilities.

Keywords: hauling, preliminary incubation

Introduction

Dairy product quality is influenced by harvesting, storage, handling, and processing steps from initial collection of raw milk on the farm to consumption by the end user. The microbiota of raw milk begins to be affected as soon as it comes in contact with the teat surface (Doyle, et al, 2016). As raw milk is further manipulated, its microbial quality can only decrease; however, excessive contamination can be mitigated through good sanitation and operation practices.

The Pasteurized Milk Ordinance (PMO) dictates practices that are designed to maintain optimum milk quality, including specific requirements for milk transportation from the farm's bulk tank to the processor's bulk silo (Food and Drug Administration, 2015). Milk is transported from the farm by tanker trucks, which can be single or double trailers that haul between 18,000 and 36,000 kg (~40,000 and 80,000 pounds) of milk per load (not including tanker weight). Prior to transferring raw milk from a bulk tank to a tanker truck, the hauler must collect a raw milk sample (producer sample) that will be used for analyses such as somatic cell count (SCC), fat, protein, water content, microbial analysis, and for traceback to farm in the case of a positive antibiotic test of a tanker. Processors use data from these samples to identify trends and to incentivize producers by paying increased prices for higher quality raw milk. Tankers are filled by collecting milk from one to five (or more) producers along predetermined routes typically assigned by the processor. Milk from multiple farms is commingled into a tanker and then further commingled in storage silos (2,500 to 150,000 liters per silo) at the dairy processing facility. Raw milk quality is defined by several factors: composition of macro components, free from undesirable organoleptic qualities (e.g. off-flavor and odors), free of adulterants (e.g. detectable antibiotic residues, added water, etc.), legal level microbial counts (raw milk Standard Plate Count (SPC) ≤100,000 cfu/mL for a single producer or ≤300,000 cfu/mL for commingled milk (PMO, Food and Drug Administration, 2015), and low somatic cell counts (SCC) (Murphy et al., 2016). Raw milk microflora is highly diverse and microbial composition changes rapidly when transferring milk from tankers to storage silos. While highly diverse, raw milk contains a core microbiota of 29 taxonomic groups (Kable, et al., 2016). While the majority of bacteria are destroyed during pasteurization it should be recognized that several genera of raw milk microflora produce thermo-resistant proteases and lipases that can result in off-flavors and odors (Teh et al., 2011, 2012; Murphy et al., 2016). Therefore, a single farm or tanker has the potential to significantly influence the quality of a large volume of raw milk and negatively impact further processed dairy products. It is of critical importance to identify and prioritize best practices at every step from milking through production to maximize milk quality (Kurt and Ozilgen, 2013).

The dairy industry has communicated their belief that milk hauling can have a negative impact on the microbial of raw milk between on-farm collection and receiving at the processor; however, the evidence to support this claim is anecdotal. Previous research was unable to demonstrate a negative impact on raw milk microbiological quality from repeated tanker use on either short- or long-haul routes (Darchuk et al., 2015a, b). Based on further discussions with the dairy industry, it was communicated that the occurrence of hauling having a negative impact on raw milk quality was quite rare (<1%). We hypothesize that historic raw milk quality data is useful for identifying circumstances in which hauling contributes to a degradation in the microbiological quality of raw milk. This was accomplished by analyzing two years of raw milk microbiological data from producers and tanker trucks of a Northwest dairy co-operative.

Materials and Methods

Collection of microbiological data from industry partner

Microbiological data from the analysis of raw milk samples during the 2015 and 2016 calendar years were provided through a partnership with a Northwest Dairy Cooperative. In addition to mandatory PMO receiving requirements (i.e. antibiotic residue and temperature), this facility conducts microbial testing on raw milk samples collected from the producers' bulk tanks (n = 59,855) as well as on raw milk samples obtained from tankers upon arrival at the processing facility (n = 23,270). Microbiological counts were enumerated by the facility's quality lab using a FOSS Bactoscan (Bactoscan FC, Foss, Hillerød, Denmark) and were reported as individual bacterial count per milliliter (IBC) and preliminary incubation individual bacteria count per mL (PI-IBC). For PI-IBC enumeration, samples were incubated at 13°C for 18 h prior to enumeration. Flow cytometry is designed to counts every viable cell, rather than just the ones that grow under incubation conditions with traditional enumeration methods, such as SPC, where methodologies differ based on protocol guidelines (e.g. AOAC versus IDF/ISSO) which contribute to the variability of results. Conversion between CFU and IBC is non-linear and requires frequent calibration to establish standard curves for individual instruments (Cassoli et al., 2016). Standard curves were not readily available for the entire data set, so units were kept as IBC.

Data processing

Raw IBC and PI-IBC data were extracted from MADCAP milk quality program (MADCAP Software, Inc., La Jolla, CA) as .csv files that were converted to spreadsheets using Microsoft Excel (Microsoft, Redmond, WA). Data from each month were extracted separately with producer and tanker counts in unlinked .csv files. Each month of data included IBC, PI-IBC, tanker manifest number, producer number, and number of pickups for each day. Manifest numbers were used to link producer counts to their corresponding tanker counts. Each month of data was checked for errors or gaps. The majority of tanker loads included milk from more than one producer; therefore, commingled producer counts were estimated by calculating an average of the IBC and IPC of producer samples by tanker load. For each load of milk from January 2014 through December 2015, the producer load average was compared to the corresponding tanker unloading sample and the potential impact from hauling was calculated for IBC and PI-IBC:

Hauling impact = tanker count – commingled producer count

Data analysis

Processed data were imported to JMP Pro 12 Statistical software (SAS Institute, Cary, NC). A box-and-whisker plot was constructed to compare annual and monthly data. Outliers were defined as the top 1% highest microbial counts in the data set (n=599). Outliers were further examined to determine commonalities that existed. Frequency of milk collection, producer, and seasonality were evaluated for significance using a Chi-square with Yates' correction using GraphPad QuickCalc (www.graphpad.com).

Results and Discussion

Characterizing Overall Producer and Tanker Milk Quality

In the 2015-2016 calendar years, the mean ± SE of individual producer raw milk samples (59,855 total bulk tank samples) for IBC and PI-IBC were 16.2 ± 0.17 and 36.3 ± 1.6, respectively (Figure 2.1). Mean ± SE for IBC and PI-IBC of corresponding tanker unloading samples (n = 23,270 loads) were 16.4 ± 0.16 and 47.6 ± 3.3, respectively. We investigated if seasonal differences and rainfall could account for variability. Year 2015 had significantly more outliers than 2014. Bacterial counts for five of the twenty-four months were significantly different; counts in January and February 2015 were significantly lower than January and February 2014, and October, November, and December 2015 were significantly higher than for the same months in 2014 (data not shown). While there was not a clear trend of seasonality, it should be noted that outlier microbiological counts were much greater in winter months and seemed to impact some producers more than others (data not shown). Seasonal impact on raw milk quality conflicts in the literature; some have reported elevated counts in summer (van Shaik et al. 2002), while others have reported elevated counts in winter (Gillespie et al., 2012; Costello et al. 2003). Kable et al., (2016) investigated the diversity of raw milk microflora in tankers (n=899) and storage silos (n=5) at a large-scale dairy manufacturing facility. Microflora was highly diverse and varied with season, spring had the highest species diversity, but cell density was only marginally higher than fall and summer. Additionally, it was found that 29 taxonomic genera make up the core raw milk microflora, regardless of season. Pantoja et al., 2009 tracked temperature variability in milk stored in on-farm bulk tanks and tanker trucks1 over the course of one year, There was no significant difference in bulk tank milk temperature due to seasonality. Tanker milk was warmer in the summer, but only by a marginal difference (~0.5°C) and unlikely to contribute increased microflora growth and well within the parameters of the PMO (<7.2°C). Seasonality impacted producers at variable degrees, suggesting that time of year could be a contributing factor to raw milk quality, but it is more likely that on-farm practices will be the dominant factor (Pantoja et al., 2009).

Producer Variability: PI-IBC

Individual producer (n = 106) PI-IBC data was further investigated to identify producer to producer variability. Producers' discussed within this study are identified as

[1] Large-scale dairies often milk directly into tankers, rather than store in on-farm bulk tanks. Milk is chilled in a plate-cooler before reaching the tanker.

producer A through O. Thirteen of the 106 producers were significantly different (one-way ANOVA); 10 producers (H, M, O, K, D, C, B, A, G, N) were significantly higher and 3 were significantly lower (I, E, F) (Figure 2.2). PI-IBC was selected as the primary focus due to the greater variation and range in counts for individual producers as well as its better correlation with finished product defects. Preliminary incubation enumeration is used in the dairy industry as an indicator for sanitation and hygiene (Gillespie et al., 2012). The underlying theory of PI-IBC is that growth of psychrotrophic spoilage organisms (capable of growing at refrigeration temperatures ≤ 7°C), that may be at undetectable levels under SPC incubation conditions are encouraged by incubating milk samples at 12.8°C for 18 h prior to plating or before enumeration via flow cytometry (SMDPE 17th edition). Pseudomonas are the dominant psychrotrophs in raw milk and are prolific producers of heat stable extracellular enzymes which could influence downstream product quality (Vithanage, et al., 2016).

Individual producer outliers

Outliers were classified as the top 1% of individual producer counts (Table 2.1; PI-IBC > 305). Individual producer outlier frequencies were determined and the majority of producers had less than 2% of on-farm bulk tank loads exceeding 305 PI-IBC (Figure 2.3). Sixteen of 106 producers had significantly higher percentage of samples with PI-IBC >305 than the co-op as a whole. The six producers with the most instances of bulk tank outliers (number of outliers/ total number of bulk tank loads) are: producer C (16/320), producer D with (19/364), H with (6/24), producer K with (19/364), producer O with (62/470), and producer A with (50/3049). Producers A and O accounted for 20.2% of outliers (112/600). Interestingly, most of these “problematic” producers are small producers that have less frequent milk collection (every other day) (Figure 2.3; small producers < 500 bulk tank loads for 2014-2015). Smaller producers are more likely to lack well defined or documented sanitation procedures and are more likely to have manual operations and use older equipment which is more difficult to clean and sanitize (Opivo et al, 2013).

Small producer case studies

The differing patterns of PI-IBC counts from two small producers (J and O) demonstrates the value of monitoring producer milk quality. Both producers have a single bulk tank that is collected from every other day (n=446 and 470, respectively). In the first case study, producer J historically has good milk quality (IBC: 14.9 ± 0.32; PI-IBC: 54.0 ± 9.8); however, 3.4% (15/446) of this producer’s bulk tank loads were PI-IBC outliers (>305). Of these outliers, 13 occurred over the course of 17 days in October 2015, which accounted for 41.9% (13/31) of their bulk tank loads for that month (Figure 2.4). The causation of the count spike in this case is unknown, but elevated PI-IBC can be indicative of on-farm sanitation or equipment failure (Murphy et al., 2012). This example demonstrates a major benefit of monitoring on-farm milk microbial counts daily. If the producer is rapidly notified and troubleshooting processes can be expedited, the risk of milk contamination can be mitigated quickly protecting the farmer from losses due to poor quality and protect the processor from downstream product spoilage or defects. In contract, producer O has frequently elevated PI-IBC counts (200 ± 30.6) with 13.2% of samples (62/470) being in the outlier category (PI-IBC >305) (Figure 2.5). These outliers occurred in 15 of the 24 months in the study period and contributed approximately 10% of the outliers for the entire co-op. It is clear that this producer needs assistance to consistently improve PI-IBC count.

Large producer case study

Producer A has two bulk tanks and has milk picked up on average two to four times per day (n = 3049 bulk tank loads total). Overall, this producer has good quality milk (IBC: 27.1 ± 1.0; PI-IBC: 66.4 ± 7.8); however, this farm contributed approximately 10% (n = 59) of the outlier samples for the entire co-op. The months with the highest mean PI-IBC were July 2015 (498.6 ± 34.1) and November 2015 (374.3 ± 57.2) (Figure 2.6a). Both months were further investigated to see if there was an explanation for elevated counts. July 2015 had a couple of days were all loads had elevated counts (data not shown). In November 2015 PI-IBC counts gradually increased from November 11th through the 20th and then returned to normal (Figure 2.6b). All loads collected during these dates had elevated counts, indicating that both bulk tanks were impacted, which could potentially be the result of a sanitation or equipment failure. While all producers should strive to produce high quality milk, it is especially important for producers who supply a significant portion of a processor's milk supply since a larger volume of milk with high microbiological counts will be much more impactful on commingled milk than a very small farm.

Improving efficacy of monitoring raw milk quality

Even in the best operations, sanitation and equipment failures will occur sporadically. Identification of sporadic spikes in counts should not be a punishment to the producer, but instead producers and processors need to collaborate to identify issues rapidly and mitigate the potential for negatively influencing the milk supply. Milk supply microbiota populations can rapidly shift in transfer from bulk tank, tanker, to silo (Kable et al., 2016; Huck et al., 2008). Traditional culture enumeration methods are not ideal for monitoring daily raw milk quality due to slow turnaround time (48 h for SPC) (SMDPE 17^{th} edition). Flow cytometry is gaining popularity as an effective tool for rapidly assessing raw milk microbiological quality. Daily analysis enables processors to capture spikes in on-farm and tanker microbiological counts which allows for corrective action before the issue persists for extended periods of time and impacts large volumes of milk.

Premium payment incentives are often used by co-ops to encourage producers to strive for low microbiological and somatic cell counts. The PMO allows for raw milk microbiological counts to be up to 100,000 cfu/mL for individual producers, where premium incentive programs consider <10,000 cfu/mL to be of good quality (Gillespie et al., 2012). Historically, the industry standard for dairy processors was to perform microbiological tests every two to four weeks for individual producers to comply with regulatory requirements (Flores-Miyamoto et al., 2014.; Pantoja et al., 2009). More frequent testing of raw milk quality is becoming more common as herd sizes have increased and testing technologies become more efficient (Jayarao et al., 2004; Pantoja et al., 2009). More frequent monitoring of on-farm bulk tank quality is necessary for capturing issues that may impact milk quality, such as sanitation and equipment failure. For perspective, the scenarios described in this paper would not have been captured if bulk tank counts were only being monitored twice a month and would have likely persisted for a longer period of time prior to being noticed.

Tanker Variability: PI-IBC

This co-op receives 20 to 30 tanker loads per day, equating to 23,270 loads in the 2014-2015 calendar year. Mean tanker unloading PI-IBC was 47.6 ± 3.3, with the top 1% outliers classified as PI-IBC > 405 (Table 2.1). In the vast majority of samples, there was minimal or no difference in actual tanker PI-IBC and predicted tanker PI-IBC (using producer data; data not shown). This outcome was anticipated because when standard operating and sanitation practices are implemented as intended, microbial counts are not expected to significantly increase as a result of hauling (Darchuk, et al. 2015). Analyzing an expansive data set of tanker microbiological counts has not been reported in the literature to our knowledge; our findings support the current PMO regulations of repeated tanker usage between cleans.

Producer bulk tanks are unloaded by tankers every other day to up to two times per day. A given tanker load contains milk collected from one to eight bulk tanks. Loads that contain milk from more than one producer are considered “commingled”. To evaluate the impact of commingling to mitigate poor quality producer loads, the top 1% of producer PI-IBC counts were compared with their respective tanker PI-IBC counts. Tanker loads containing milk from more than one producer were significantly lower than their highest single producer count when more than one producer was in each load (Figure 2.7). Tankers are reported to retain ~0.02% residual milk from previous loads (Darchuk et al., 2015 a, b), therefore, poor microbiological quality from a single bulk tank is significantly mitigated by the addition of milk from at least one other farm due to dilution. While sporadic, we identified a couple situations where hauling impact was measurable. For example, figure 2.8 displays a snapshot in January 2014 where producer PI-IBC was low for each load for a given tanker, but the tanker unloading milk counts increased over four loads, and then dropped back, presumably after CIP of the tanker. A similar situation occurred in November 2015 during the instance with producer A (Figure 2.6b.; hauling impact data not shown).

Conclusion

Baseline milk microbiological quality is established by on-farm management practices, and has potential to be compromised in subsequent processing steps. Adequate sanitation throughout transport and processing is essential mitigating microbial counts. Based on analyzing trends and outliers in microbial counts of raw milk for producers and tankers during receiving, milk hauling does not appear to significantly impact raw milk microbiological quality within the parameters of this analysis. Negative impact due to hauling is sporadic and highly infrequent; we observed when tanker loads consist of more than one producer and there is a high count producer included in that load, it will likely be diluted out by the producers on load and be unmeasurable. Interestingly, smaller producers (those collected every other day) are more likely to have elevated microbiological counts. While negative impact from hauling is often unmeasurable, routine monitoring of microbiological counts of on-farm bulk tanks and tankers at unloading strengthens overall dairy product quality by allowing industry to identify spikes microbial counts and troubleshoot, rather than having the issue persist unknowingly for extended periods of time.

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Chapter 3. Characterization of milk hauling practices through industry survey

INTERPRETATIVE SUMMARY

Short Communication: Characterization of Industry Milk Hauling Practices

Milk hauling sanitation and operation practices are mandated by the Pasteurized Milk Ordinance (PMO). Several of the regulatory practices are described using vague terminology (e.g. as needed) and need further clarification to help industry determine best practices. Our aim was to characterize current industry milk hauling practices and provide recommendations to enhance current practices. We characterized industry hauling sanitation and operational practices by i) surveying several Northwest dairy processors, and ii) enumerating the microbiological load of internal surfaces of raw milk transfer hoses to evaluate their potential as an unexplained source of contamination in dairy processing.

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Abstract

The Pasteurized Milk Ordinance (PMO) mandates milk hauling sanitation and operational practices; however, the use of vague language (i.e., as needed) and gaps in processes lead to variability in industry practices. Our aim was to characterize industry milk hauling practices and identify areas that may be an unexplained source of contamination in the dairy processing continuum, and communicate this information with industry to cultivate best practices. The objectives of this study were to i) survey industry hauling sanitation and operation practices in the Pacific Northwest region of the United States, and ii) quantify microbial populations (APC, LAB, coliforms) on the internal surfaces of transfer hoses (tanker and receiving bay) at two facilities to determine their potential contribution to the microbiological quality of raw milk. Eleven facilities (78% response rate) participated in our survey. All facilities surveyed were compliant with the PMO; however, overall milk reception layout, sanitation practices, and routine maintenance greatly varied between facilities. Farm hoses (n=115) greatly varied in microbiological counts (0.0 to 7.0 log cfu/100 cm^2), while receiving hoses (n=7 from two facilities; A and B) contained consistently low levels of contaminants (0.0 to 5.6 log cfu/100 cm^2). Increasing microbial populations were not correlated with time since last cleaning for either tanker or receiving bay hoses. Microbial content of farm hoses is likely to reflect the microbial quality of the previous milk transferred through the hose, making on-farm management practices the primary consideration to maintain low microbiological counts downstream. Upon arrival at the processor, 10% of farm hoses were missing caps. While this did not correlate with elevated microbiological counts, uncapped farm hoses are exposed to the farm environment, provide opportunity for contamination, and are in violation of the PMO. Through observations made during our studies, manual cleaning procedures appear to be a major weakness in hauling practices and need more attention. Recognizing and communicating variability and areas of weakness allows industry to elevate their hauling sanitation and operational practices to maintain optimum milk quality.

Keywords: milk hauling, milk transportation, raw milk quality, transfer hoses

Introduction

Optimum milk quality is established as soon at the milk is collected from the teat of the cow (Doyle et al., 2017). As milk is further manipulated by handling, transportation, and processing, milk quality can only be negatively influenced due to opportunistic microbial contamination (Marchand et al., 2012). For this reason, it is critical that best sanitation and operation practices are implemented at every step of the process to produce the highest quality dairy products. The dairy industry has expressed concerns that hauling sporadically contributes to reduced milk quality. Milk hauling can be defined as the period when raw milk is transferred from the farm bulk tank to tanker, transported, and then unloaded from tanker to silo at a processing facility. Repeated tanker usage (without cleaning between loads) reduces costs in energy, water and chemical usage, and sanitation labor and is a necessary efficiency with the consolidation of the dairy industry (USDA, 2014). The Grade "A" Pasteurized Milk Ordinance (PMO) defines and mandates standard hauling operation and sanitation practices, such as repeated use of tankers for up to 24 hours prior to a mandatory clean-in-place (CIP) (Food and Drug Administration, 2015). Previous research has verified that when standard practices are followed, hauling is unlikely to compromise bulk raw milk quality (Darchuk et al, 2015a,b).

While the PMO clearly defines a necessary sanitation schedule for tankers, maintenance and sanitation of other accessory components are vaguely defined in the PMO with a recommended schedule of "as needed" (Food and Drug Administration, 2015). Knowledge gaps exist in understanding how hauling practices can negatively impact the dairy processing continuum, but it is speculated that negative impact typically occurs when a collection of practices are not implemented as intended (Darchuk et al., 2015a,b ; Teh et al., 2011 and 2012). For example, lacking a dynamic CIP system validation and verification program increases the risk of inadequate sanitation. Tanker components and accessories such as gaskets, pumps, and caps that require manual cleaning (clean-out of-place; COP) are another area of concern due to variability in cleaning schedules as well as cleaning efficacy due to personnel (Memisi et al., 2015).

The overarching hypothesis of our research is that milk hauling sanitation and operation practices have the potential to negatively contribute to the microbiological quality of raw milk and impact finished product quality. However, if this occurs, it is likely infrequent and difficult to measure due to the scale of production. To identify potential contributing factors, it is essential to accurately characterize various aspects of hauling practices. We hypothesized that various milk transportation accessories (hoses, pumps, and associated parts) contribute negatively to raw milk quality. The objectives of this study was i) to characterize sanitation and operation practices associated with collection of milk on-farm and delivery of milk at the processing facility, and ii) to quantify the microbial load of accessory hauling components (i.e., transport hoses) in a commercial setting to evaluate potential impact on raw milk quality.

Materials and Methods

Surveying Industry to Identify Trends in Milk Hauling Practices

Fourteen dairy processing facilities of various sizes in the northwestern United States were asked to participate in the survey (78% response rate). Quality assurance and milk receiving personnel of various positions (operators, supervisors, managers) were contacted via email to request their participation in an industry survey about milk hauling and receiving practices in their facilities. Following initial contact, industry representatives were provided with an electronic copy of the survey via follow-up email and requested to return the survey via postal mail, email, or retrieved during an on-site visit to the facility. The survey consisted of 12 questions to characterize type and frequency of sanitation and operating procedures related to milk hauling and receiving practices. Observations and responses were compiled and evaluated to determine commonalities and gaps in practices with reference to the PMO regulations.

Sampling Internal Surfaces of Transfer Hoses

Transfer hoses were sampled at two milk processing facilities (A and B) using 3M sponge swabs with buffered peptone water (St. Louis, MO). For facility A, the internal surfaces (100 cm^2) of receiving bay hoses (n = 3) were swabbed once after a day of standard use (approximately 10 loads in 24 hours) for three consecutive days in September 2016. At facility B, interior surfaces (100 cm^2) of receiving bay hoses and tanker farm hoses were sampled during two consecutive days of operation in March 2017. Receiving bay hoses (n = 4) were sampled once every hour over the course of 6 hours. Farm hoses located on tanker trucks (n = 115) were sampled upon arrival at the receiving bay and time of last CIP was documented.

Samples were immediately cooled (< 4.4°C), transported to the laboratory, and processed within 24 hours of collection. Samples were serially diluted in 0.1% peptone water and plated on Aerobic Plate Count (APC) Petrifilm (3M), coliform Petrifilm (3M), and deMan, Rogosa and Sharpe (MRS) Agar (MRS; Difco, Sparks, MD). Petrifilms were incubated at 30°C for 24-48 h, and MRS plates were incubated for 30°C for 72 hours under anaerobic conditions. Counts were reported as log cfu/cm^2.

Data analysis

Survey results were compiled and qualitatively observed for trends in practices. Transfer hose microbiological counts were analyzed by one-way ANOVA in JMP Pro 13 (SAS Institute, Cary, NC).

Results and Discussion

Survey Results

Our survey highlights milk reception layout, operational and sanitation practices, and routine maintenance practices (Table 3.1). Eleven of the fourteen facilities (78%) completed and returned the milk hauling survey. Regulatory practices are outlined in PMO section 7 (12p.) Cleaning and Sanitizing of Containers and Equipment and Appendix B. Milk Sampling, Hauling and Transportation (Food and Drug Administration, 2015).

Facility layout. A majority (55%) of receiving bays in the Pacific Northwest are partially enclosed: facilities with an overhead cover, but lack complete sides/wall). The remainder of receiving facilities (45%) are fully enclosed. Facilities ranged in the number of receiving bays ranges from one to three, where the majority of facilities have two receiving bays (64%), indicating that they can unload multiple tankers at a time and/or continue receiving if one bay is unavailable due to sanitation schedule or maintenance. The number of receiving hoses per bay range from one to five with two hoses being the most common response (45%). Most facilities have more receiving hoses than bays. This allows for flexibility during operation to support cleaning activities and divert milk to alternative storage sites (surplus milk). All facilities reported that their receiving hoses are hung during storage and between receiving loads. These practices are in compliance with the PMO mandate that receiving hoses be capped and hung up when not in use (Food and Drug Administration, 2015). The most common cap styles used in the region were threaded cap (45%) and tri-clamp with cap (37%); however, two facilities reported an alternative style (i.e., mounted cap housing).

Milk reception practices. The frequency of milk delivery to processing facilities in the Pacific Northwest varied substantially throughout the industry. A majority of these facilities (82%) receive >6 tanker loads of raw milk per day. The largest number of tankers received was 30 per day. The smallest processing facility received 2-3 tankers every other day. Prior to unloading, raw milk from each tanker load must be tested for the presence of antibiotics (beta-lactams) and milk temperature must be checked to verify it is below the cutoff (<7.2°C) (Food and Drug Administration, 2015). The majority of facilities collect milk receiving samples from the top hatch of the tanker using a sanitized stainless steel dipper (91%). One facility aseptically collects samples via an in-line where a syringe is punctured into a rubber septum covered sample port.

Small parts: replacement and sanitation frequency. The PMO mandates that replacement of small parts such as hoses, caps, gaskets, and cleaning tools are replaced as needed. Cleaning and sanitation of small parts is completed a minimum of every 24 h and additionally as needed throughout daily use (Food and Drug Administration, 2015). Most facilities (82%) replace gaskets and farm hoses as needed; however, 18% schedule routine replacement every 3-6 months. Similarly, nearly all facilities (91%) replace cleaning equipment such as buckets and brushes as needed. A single facility reported having a routine replacement schedule for their cleaning tools of 2-4 weeks. In the Pacific Northwest, most facilities clean and sanitize small parts (e.g., hoses, caps, gaskets) multiple times per day (82%); however, some facilities (18%) clean these parts once per day.

Tanker sanitation frequency. Tankers are permitted for continuous use within a 24-h period before required CIP treatment (Food and Drug Administration, 2015). Processors have the ability to mandate a more frequent sanitation schedule, if desired. The majority of facilities (63%) follows the PMO requirements allowing for repeated tanker use between CIPs. Four facilities in this study implement a CIP treatment after each load of milk. Facilities that clean after every load tended to be ones that receive less than 10 loads per day.

CIP validation frequency. The PMO requires validation of the CIP system at least annually (Food and Drug Administration, 2015). CIP system parameters that are checked in the validation process are 1) temperature, 2) flow and mechanical action, 3) conductivity, 4) time, and 5) equipment inspection (DeLaval, 2013). Overall tanker sanitation is only as good as the efficacy of any given facility's regular practices (Darchuk, 2015). To ensure adequate tanker sanitation an effective validation and verification program needs to be established. Validating CIP systems more frequently could mitigate risks of inadequate sanitation. In our survey, 55% of facilities validate their CIP system on an annual basis. Several facilities indicated that CIP validation is performed more frequently: quarterly (27%), monthly (18%), and daily (9%). It should be noted that daily validation is unlikely and is rather a daily verification activity, such as ATP, microbiological, and residual protein swabs, and rinse samples. Lunining et al. (2009) reported that industry personnel often confuse the concepts of validation and verification. It is essential that industry is comprehensive in sanitation validation and verification activities to ensure adequate tanker sanitation.

Transfer Hoses: From Farm to Receiving

Transfer hoses are the connecting components used to transfer raw milk from producer on-farm bulk tank to processor storage silos. Farm hoses are used to transfer milk from farm bulk tanks into the tanker. They are constructed of transparent flexible plastic that meets the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, 20##", and are typically 6.4 to 7.6 cm (2.5 in to 3 in) diameter and may be up to 46 m (150 ft) long (Food and Drug Administration, 2015). These hoses are accessed through the pump box and are stored either coiled or housed within a slanted pipe along the side of the tanker. Receiving hoses are used to unload milk from tankers and are constructed out of thick rubber material. The inside of these hoses are cleaned and sanitized as part of the tanker CIP at the frequency determined by the processor (after every load to once per 24 h). While the tanker body is well insulated to maintain the bulk fluid temperature, these hoses and the residual milk within them quickly reach ambient temperature in between loads.

Farm hoses. Figure 3.1 displays tanker farm hoses (n = 115) microbiological counts. Mean APC was 4.7 log cfu/100 cm^2 and ranged from 0.0 to 7.0 log cfu/100 cm^2. Mean LAB count was 3.5 log cfu/100 cm^2 and ranged from 0.0 to 5.4 log cfu/100 cm^2. Mean coliform count was 2.2 log cfu/100 cm^2 and ranged from 0.0 to 6.3 log cfu/100 cm^2. Time (h) since last CIP was categorized into five hour increments; microbiological counts did not increase with time since last CIP (one-way ANOVA; p-value$_{APC}$ = 0.3, p-value$_{LAB}$ = 0.3, and p-value$_{coliforms}$ = 0.8). The vast majority of tankers (93%; 107/115) were under 24 h since last CIP. Of the eight tankers (7.0%) that were past 24 h since last cleaning, three (2.7%) of these tankers collected the last load prior to 24 h since first use (permitted by the PMO). The remaining five loads (4.3%) were past 24 h since first use: three loads were from unique tankers and may have been single-use, long-haul loads where 72 h use is permitted for grade B milk, and two loads were from the same tanker and collected ~6 h apart (42 h and 48 h since last cleaning) in clear violation of the PMO. With the exception of the one tanker that was >40 h overdue for cleaning, all tankers were in compliance with the PMO.

High variability in farm hose microbial counts was observed; however, there was no correlation with time since last CIP or number of loads hauled. For example, we sampled the farm hoses from the same tanker up to three times per day that did not increase in microbial load (data not shown). In comparison to microbiological counts of raw milk surfaces reported in other literature, farm hoses seem to have higher counts than other raw milk surfaces. Darchuk et al., (2015) reported that internal surfaces of tankers averaged 3.4 log cfu/900 cm^2. Teh et al., (2012) demonstrated biofilm formation using microflora obtained from tankers in vitro using stainless steel coupons, which resulted in 2.7 to 7.6 log cfu/cm^2, it should be noted that temperature conditions for this study were extreme (25°C for 24 h), this type of use would be unlikely in the United States (Darchuk et al., 2015).

Receiving hoses. Facility A receiving hoses had average APC and coliform counts (3.7 and 1.4 log cfu/100 cm^2) after standard use (unloading ~10 tankers in 24 h) for all three sample days (Table 3.2). Facility B average APC, LAB, and coliform counts were comparable to facility A (2.1, 0.8, and 0.6 log cfu/100 cm^2, respectively). There were no trends in microbial growth with hourly sampling and there was a lack of variability between hoses (one-way ANOVA; p-value <0.05). Low counts in facility B[2] receiving hoses were expected due to high volume intake (~280 tankers per day), where receiving hoses are almost always in constant use which mitigates the opportunity for microbial growth on internal surfaces. Repeated use of receiving hoses does not appear to be a source of contamination in milk hauling.

Characterizing weaknesses in hauling practices

One challenge to consider with industry studies is the observation effect (e.g. Hawthorne Effect), where there is the possibility of personnel performing at a higher level when being observed in comparison to unobserved “normal” operation (McCambringe et al, 2013). Despite this challenge, we did observe several weaknesses in hauling sanitation and operations practices:

A) Manual cleaning procedures is an area of hauling that needs more attention. We observed personnel neglecting to disassemble and use detergent to clean pump boxes, dome lids and air vents, and gaskets. In fact, one tanker had so

[2] Facility B is not included in the survey due to geographical location; all facilities surveyed are located in the Pacific Northwest region of the United States.

much fouling on the air vent that the cap could barely be removed. While sampling at facility B we witnessed several dirty pump boxes and hose caps with visible fouling on the internal surface. In contrast to CIP systems, manual cleaning requires more human involvement. During tanker sanitation, the farm pump needs to be disassembled and manually cleaned with detergent; however, the tanker and farm hose can be connected to the CIP system without ever dissembling the pump.

B) Most facilities do not have an established routine for replacing small parts (e.g. gaskets, buckets, brushes) that are susceptible to deterioration. Deteriorated gaskets were observed on one of facility B's receiving hoses (age was unknown). The cleanablity of deteriorated gaskets is reduced, providing an optimal environment to support biofilm formation (Storgards et al., 1999). Longevity of parts susceptible to deterioration depends on material and usage, but replacement is recommended as a preventative measure rather than replacement when functionality is lost. Gasket longevity can be extended by not overtightening clamps.

C) Several farm hose caps had visible fouling and 10% of farm hoses were missing caps (data not shown). According to the PMO (section 7, item 15p.A), "hoses must be capped or otherwise properly protected when not in use, and should only be exposed to external elements for the brief moment of uncapping and connecting to a pump" (Food and Drug Administration, 2015). Though we did not find a relationship between missing hose caps and elevated microbial counts (data not shown), hoses are dragged on the farm ground when being unloaded from the tanker, providing ample opportunity for contamination.

Conclusion

Variability exists in industry milk hauling sanitation and operation practices. Negative impact from hauling appears to be sporadic and difficult to measure due to the scale of production; however, weaknesses in practices can compound and potentially compromise microbiological quality of raw milk and impact finished product quality. Characterizing variability of hauling practices aids in identifying and communicating best practices to elevate industry practices and contribute to better dairy product quality. In the modern dairy industry, repeated tanker and accessory usage is essential for economic reasons; it reduces chemical and water usage, time, and money. Therefore, hauling sanitation and operating procedures must be efficient and effective as part of the production of high quality dairy products. Current hauling practices are sufficient when practices are implemented as intended; however, we have demonstrated that variability in industry hauling practices exist, even though the survey responses were in alignment with the PMO. The most effective way to mitigate poor practices is to educate and monitor personnel on proper use of cleaning and sanitizing agents and procedures (Kurt and Ozilgen, 2013). Additionally, when educating personnel, it is important to emphasize food safety and quality aspects to clarify why procedures need to be followed. It is also recommended that industry implement thorough preventative maintenance programs, including routine replacement of hauling accessories susceptible to deterioration, such as gaskets, farm hoses, and cleaning buckets and brushes.

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Chapter 4. Impact of leaving milk trucks empty and idle for 6 hr between raw milk loads

Interpretative Summary

The Pasteurized Milk Ordinance (PMO) allows for repeated tanker usage between cleanings per 24 h. Raw milk microbiological quality was investigated in two worst-case milk hauling scenarios: small-scale with milk cans and commercial-scale with tankers. A worst-case hauling scenario is defined as a hauling vessel left empty and dirty (idle) for extended periods of time between loads. A small-scale experiment was conducted first for proof of concept of negative impacts from hauling. Negative impact on milk quality was demonstrated to not be measurable for commercial tankers remaining dirty and idle for periods of <6 h between loads.

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Abstract

The Pasteurized Milk Ordinance (PMO) allows for milk tanker trucks to be used repeatedly for 24 hours before mandatory clean-in-place (CIP) cleaning, but there are no specifications for the length of time a tanker can be empty between loads. We defined a worst-case hauling scenario as a hauling vessel left empty and dirty (idle) for extended periods of time between loads, especially in warm weather. Initial studies were conducted at a small-scale using milk cans as a proof-of-concept and to determine timeframes that could contribute negatively to raw milk quality from simulated hauling scenarios. Based on small-scale results, a commercial hauling study was conducted through partnership with a Pacific Northwest dairy co-op to investigate if extended idle time (6 h) between loads influences microbiological populations and enzyme activity in subsequent loads of milk. Milk cans were used to haul raw milk (load 1), emptied, incubated at 30°C for 3, 6, 10, and 20 h, and refilled with commercial HTST whole milk to measure cross-contamination. For the commercial study, we selected two producers (Farm A and Farm B) from a co-op based historical microbiological data, with farm A having substantially higher microbiological counts than farm B. For both experiments, milk samples were obtained each farm's bulk tank and from the milk can or tanker prior to unloading. Each sample was microbiologically assessed in for standard plate count (SPC), lactic acid bacteria (LAB), coliforms counts. Colony isolates were assessed for lipolytic and proteolytic activity using spirit blue agar (SBA) and skim milk agar (SMA), respectively. Our milk can study demonstrated the potential for negative impact from hauling, while our commercial study demonstrated that maximum idle time (6 h) would

mitigate negative impact. We have demonstrated that current milk hauling practices are adequate and it is recommended that industry emphasizes on sanitation efficacy and preventative maintenance of tankers, rather than increased cleaning frequency.

Keywords-milk hauling, milk receiving, milk transportation, raw milk microbiology

Introduction

Milk hauling practices encompass the handling and transportation of raw milk from producer to processor. Sanitation and operation practices have drastically changed since the earliest milk hauling operations which utilized flatbed horse-drawn wagons to transport 10-gallon milk cans. Clean-in-place systems (CIP) were developed in the 1950s with the dairy industry being the first to utilize this automated cleaning method for pipelines and tanks. By the early 1960s, hauling transitioned from milk cans to bulk milk tanker trucks (Erba, et al., year unknown). Historically, tankers were cleaned between every load, but as the industry continued to consolidate (e.g. decrease in number of producers and processors), demand increased for more frequent use of tankers (Darchuk et al., 2015a, b ; Dommett et al., 1980). Repeated tanker usage between cleans is practiced by the majority of the modern day industry. The Pasteurized Milk Ordinance (PMO) allows tankers to be used repeatedly for up to 24 hours before a mandatory clean-in-place (CIP) (Food and Drug Administration, 2015). Repeated tanker usage maximizes milk collection efficiency and reduces water, chemical, and energy usage. Tanker usage frequency greatly varies and can be categorized as i) short-distance high frequency hauling, where several loads will be collected within 24 hours, or ii) low frequency hauling where a tanker may remain empty and dirty (parked/idle or en route) for an extended period of time between loads (Darchuk et al., 2015a,b; Kuhn et al., 2017 in review). Industry is concerned that extended periods of time between loads at elevated temperatures could promote microbiological growth that could potentially impact subsequent loads of milk.

PMO hauling regulations have been demonstrated to be sufficient when standard practices are followed. Darchuk et al., (2015a, b) studied milk hauling practices in a standard industry setting and investigated both short-distance high frequency and long-distance low frequency hauling. These studies did not identify a measurable impact from subsequent loads for standard plate count, preliminary incubation count, and thermophilic spore count. However, demonstrating measurable negative impact from hauling on a commercial scale can be challenging due to microbial load of raw milk coupled with the volume: surface area dilution factor when the tank in refilled. There is necessity for a cost-effective strategy to demonstrate that microbial growth of raw milk microflora can grow on raw milk contact surfaces (e.g. inside the tanker and tanker accessories).

Milk cans are the ideal hauling vessel to emulate worst-case practices hauling practices because like commercial tanker trucks, they are constructed out of stainless steel. In contrast, milk cans lack of insulation and have increased surface area to volume ratio, this creates a situation where residual milk from previous loads on a hot day will likely encourage higher rates of microbiological growth, that can inoculate subsequent loads and potentially result in a measurable negative impact. We hypothesized that if negative impact could be demonstrated on a small-scale under worst-case conditions, this information could be used to provide a liberal estimate of the growth that would happen in a commercial tanker truck.

Materials and Methods

Experimental design

Cross-contamination in milk cans. Five-gallon stainless steel milk cans (Hamby Dairy Supply, Maysville, MO) were used as hauling vessels to demonstrate worst-case scenario impact on milk quality. Raw milk was collected from the Oregon State University (OSU) dairy farm (Corvallis, Oregon). Prior to filling the milk cans, a sample of raw milk (90 ml) was aseptically collected from the farm's bulk milk tank for baseline microbial analysis. The milk cans were filled and loaded into the back of a standard cab truck and hauled for 30 minutes prior to unloading at OSU's Arbuthnot creamery. The raw milk was held in the milk cans at ambient temperature (~27°C) for 1.5 hours before sampling. Milk from each can (90 ml) was aseptically sampled in sterile snap vials (Nelson and Jameson, Marshfield, WI). Following sampling, milk cans were emptied After emptying, milk cans were either manually cleaned (Ecolab Liquid 90, St. Paul, Minnesota) and sanitized (Ecolab Mikroklene, St. Paul, Minnesota) or were left uncleaned. The empty milk cans (cleaned/sanitized and uncleaned) were then held at 30℃ for up to 20 h. At pre-determined time points (3, 6, 10, and 20 h), milk cans were filled with five gallons of commercially pasteurized whole milk purchased from a local grocery store. The pasteurized milk was from the same lot code and samples (90 ml) were collected from representative containers and composited prior to determining baseline microbial quality. The milk cans were loaded into the back of a standard cab truck, hauled for an additional 30 min, and returned to the campus creamery. Milk cans were unloaded and milk samples (90 ml) were collected and analyzed for microbial loads. All milk samples were stored at 4°C between the time of collection and analysis (<24 hours).

Milk cans were numbered and randomized for each sample day Each time point was completed in triplicate, with three cans per replicate, totaling in nine cans per time point. For each day two of the eight cans served as a control (appendix) Microbiological analyses for this portion of the study were conducted in the Food Safety Systems laboratory on campus.

Cross-contamination in milk tankers. A single trailer milk tanker was used to haul milk (~5,440 kg) from a single farm (Farm A) and deliver it to a Northwest dairy processing company. The truck would then either i) immediately (within 30 min) collect milk from a second farm (Farm B) or ii) stand idle and uncleaned for 6 hours before collecting milk from the second farm (Farm B). This experiment was repeated over the course of six consecutive days with the two scenarios (i and ii) being randomized across the six days. Farms were selected based on previous trends in the microbiological quality of their raw milk (Table 2) with Farm A being a historically high count farm and Farm B being a historically low count farm (Kuhn et al 2017).

Milk samples were collected using 90 mL sterile snap vials from the farm bulk tanks and tankers (PMO, Appendix B).All samples were stored at <4.4°C for up to 24 hours prior to conducting microbiological testing. Microbiological testing took place in the Northwest dairy processing facility’s quality lab.

Microbiological testing

Milk samples were removed from 4°C storage and shaken prior to sampling. Serial dilutions were prepared in 0.1% peptone water (Acumedia, Lansing, MI) and spread plated in duplicate on a variety of media to enumerate different microbial populations. These media included Coliform Petrifilm (3M, St. Paul, Minnesota), Tryptic Soy Agar (TSA; Acumedia), de Man, Rogosa, and Sharpe Agar (MRS; Difco, Sparks, MD), Spirit Blue Agar (SBA; HiMedia, Mumbai, India) with lipase reagent (Difco, Sparks, MD), and Skim Milk Agar (SMA; HiMedia). Media were incubated and enumerated as shown in Table 2.

A relative proportion of representative colonies (morphological and enzymatic) on from SBA (n = 12/sample) and SMA (n = 12/sample) plates were further assessed for lipolytic and proteolytic activity by transferring to fresh SBA and SMA plates. These plates were incubated as described above with isolates being categorically classified as proteolytic (P+) or non-proteolytic (P-) based on SMA growth and also classified based on a gradation of lipolytic activity on SBA from non-lipolytic (L-) to highly lipolytic (L+++). Following characterization, isolates were transferred to Tryptic Soy Broth (TSB; Acumedia) and incubated at 32℃ for 24 hrs. Broth cultures were mixed with an equal volume of 30% (v/v) glycerol in cryogenic tubes and stored at -80℃.

Data analysis

Microbiological counts were calculated as CFU/mL or CFU/cm^2 and log transformed prior to statistical analyses. The significance of treatments was determined by comparing their microbiological counts using one-way ANOVA. Negative impact on milk quality was defined as a significant increase in microbiological counts in pasteurized milk samples. Statistical tests to enumeration data and treatment differences were performed using JMP Pro 12 (SAS Institute, Cary, NC). Relative isolate characterization data was analyzed for significance using Fisher's exact test (GraphPad, La Jolla, CA).

Results

Proof-of-Concept: Impact of Hauling Practices Using Milk Cans

Raw milk collected from the farm bulk tank and used as the initial load (Load 1) for the milk can study had baseline bacterial levels of 3.9 ± 0.2 log cfu/mL SPC, 2.9 ± 0.3 log cfu/mL LAB, and 2.4 ± 0.2 log cfu/mL coliforms (Figure 1). At the time of unloading following the emulated hauling scenario, the microbial populations of SPC, LAB, and coliforms were similar to those at the time of loading (bulk tank). Microbiological counts did not increase during transportation of load 1, this was expected due to the short timeframe of hauling coupled with the maintenance of low milk temperature from bulk tank collection to unloading (data not shown).

Commercial HTST whole milk was selected for load 2 due to having reduce background microflora and increasing the likelihood of measuring negative impact from cross-contamination. HTST milk used for load 2 met the quality standards for pasteurized milk for each replicate; <20,000 cfu/mL and <10 coliform cells/mL (Food and Drug Administration, 2015). The SPC of the HTST milk used in this study was 1.5 ± 0.1 log CFU/mL; LAB and coliform counts were negligible (0.1 and 0.0 log cfu/mL, respectively). As anticipated, emulated hauling had no impact on the microbiological contents of the

HTST milk (Load 2) in cans that had been cleaned and sanitized between loads, regardless of time since cleaning (3-20 h) (Figure 1).

Emulating hauling had a significant negative impact on the microbiological contents of the HTST milk (Load 2) when milk cans were not cleaned and sanitized between loads (Figure 1). Load 2 microbiological quality increased significantly with extended incubation times of dirty milk cans prior to loading (Figure 1). The SPC of load 2 with following dirty can incubation times of 3, 6, 10, and 20 h were 2.9, 3.0, 4.3, and 6.0 log cfu/mL, respectively. Load 2 milk SPC and LAB levels were significantly influenced by the dirty milk cans at all incubation times, demonstrating that microbial contamination from load to load occurs. Load 2 SPC following 3 and 6 h incubations were not significantly different (p-value >0.05), inferring that residual milk microflora had not yet reached measurable growth. Load 2 SPC for 6, 10, and 20 h significantly increase with each incubation time point. LAB counts followed a similar trend. Interestingly, coliform counts were undetectable (<1 cfu/mL) for HTST milk after 3 h incubation of empty fouled cans, but steadily increased between 6 h (1.5 ± 0.3 log) and 20 h (5.1 ± 0.4 log). Measurable contamination between loads was demonstrated as was that increasing the time between milk collections can lead to microbial growth in the hauling vessel leading to negative impacts on subsequent loads.

Figure 2a and 2b displays relative total percentage of microflora proteolytic and lipolytic activities for each milk can sampling point (n = 1152 total isolates). Isolate data is not shown for HTST whole milk (Load 2) samples due to very low levels of microbial contaminants on skim milk agar and spirit blue agar (<1 cfu/mL). The majority of isolates at all times points were non-proteolytic and non- to weakly lipolytic. As incubation time of dirty cans increased, the relative percentage of non-proteolytic and non- to weakly lipolytic isolates tended to increase.

Commercial Hauling Study

For the commercial hauling study, the initial load of raw milk upon collection from the farm (Farm A bulk tank) had SPC, LAB, and coliform counts of 3.9 ± 0.1, 3.3 ± 0.2, and 2.6 ± 0.3 log CFU/mL, respectively (Figure 3). Microbial counts were unchanged after hauling (Load 1). The microbiological quality of raw milk from Farm B for SPC, LAB, and coliform counts were 3.2 ± 0.1, 3.0 ± 0.1, and 1.4 ± 0.1 log cfu/mL, respectively. As expected, based on historical data (Table 2), Farm A bulk tank raw milk quality was significantly poorer than Farm B bulk tank raw milk quality throughout this study (SPC, LAB, and coliform count p-values: 0.0003, 0.03, and <0.0001). On days when Load 2 from Farm B was collected immediately after Load 1 was delivered, SPC, LAB, and coliform counts were 3.4 ± 0.1, 2.9 ± 0.1, and 1.4 ± 0.3, respectively. These counts were similar on days when Load 2 from Farm B was collected 6 h after Load 1 was delivered. Overall, the microbial milk quality of Load 1 (Farm A) did not measurably influence the microbial milk quality of the subsequent load (Farm B), regardless of immediate tanker usage or 6 h idle time. However, coliform counts were significantly higher in the subsequent load (Farm B) compared to bulk tank samples when looking at immediate use and 6 h idle time prior to farm B collection (0.8 to 1.4 = 0.7 log increase; p-value=0.03).

Farm A and Farm B bulk tank and tanker unloading milk proteolytic and lipolytic colony enzyme activities are displayed in figure 4a and 4b. Interestingly, Farm A and Farm

B had distinctly different enzyme activity distributions (Fisher's exact test two-sized p-value < 0.001). Farm A bulk tank isolates were predominantly non-proteolytic (70.6%) and non- to weakly lipolytic (non-lipolytic = 27.8% and weakly lipolytic = 43.8%). The relative distribution of enzyme activity of isolates from Farm A and Load 1 were nearly identical, as expected. The majority of Farm B isolates were proteolytic (56.3%) and highly lipolytic (68.8%). This distribution in was not significantly different in Load 2, regardless of time between loads, indicating that Load 1 did not have a significant influence on the distribution of microbial populations with these phenotypes. Due to the dilution factor and the surface area-to-volume ratio, this data confirms that a 6 h idle time for tankers will not produce a measurable impact on the microbial quality of raw milk of subsequent loads.

Discussion

Our results from the small-scale study demonstrate that hauling practices could contribute negatively to raw milk quality and is a plausible concern for the milk industry. Load 1 residual milk measurably contaminated Load 2, regardless of incubation time between loads (Figure 1). Increased incubation time increased the severity of contamination, with the exception of SPC between 3 and 6 h. Dilution effect creates a challenge for hauling research because tankers are reported to retain ~0.02% residual milk volume (~8 liters) post-unloading, which is diluted with subsequent milk collection (Darchuk et al., 2015a, b). This challenge was overcome in this experiment due to milk cans having an increased surface area-to-volume ratio that is 10-fold larger than a commercial tanker (milk can = 0.27 SA:V; commercial tanker = 0.027 SA:V). The milk cans' internal temperature rapidly equilibrated to ambient temperature (data not shown), this is due to being constructed of a single layer of stainless steel with of lack insulation. In contrast, commercial tankers are designed to insulate and maintain milk temperature, and are constructive of an internal stainless steel tank, layered with thick polystyrene, and shelled with stainless steel. However, internal temperature of tankers have potential to rapidly increase upon unloading and idle time, especially since tankers require air flow to prevent implosion during unloading. While tankers design is adequate for maintaining milk temperature when full, Teh et al.(2011, 2012, 2013) reported that internal surfaces of empty milk tankers reached 20°C in New Zealand summer months and demonstrated that tanker contaminants are capable of producing thermostable lipolytic enzymes at these temperatures. Our collaborating industry partner allows tankers that stand idle (empty and dirty) for a maximum of 6 h or they must be cleaned and sanitized prior to next use. The data from our milk can and commercial-scale study demonstrates this practice as an effective strategy to minimize the potential losses in milk quality due to hauling. Load 1 milk quality did not measurably influence Load 2 milk quality, regardless of immediate pick up or 6 h idle time, nor did Load 1 measurably impact the distribution of enzyme-producing bacteria in Load 2. In the scope of this experiment, proteolytic and lipolytic enzyme producing isolates did not appear to be the dominant population; however, this is dependent on milk source microflora, which are known to be highly diverse and readily shift during pre-processing storage (Kable et al., 2016).

Our study also characterized the relative distributions of bacterial populations capable of producing enzymes on two different farms. Interestingly, Farm A had significantly higher microbiological counts, but Farm B's microbial population is far more enzymatically active. Presence of residual microbial enzymes could potentially compromise finished dairy product quality and may contribute to reduced shelf-life and undesirable sensory attributes. In order to capture psychrotrophic activity we incubated isolates at 21℃; many psychrotrophs will grow above cold temperatures (> 7℃) (SMEDP 17th edition). Raw milk storage sanitation conditions influence microflora populations; poor sanitation yields elevated counts of psychotropic bacteria (Hantsis-Zacharvo and Halpern, 2007). In order to capture psychrotrophic activity we incubated SMA and SBA at 21℃ (SMEDP 17th edition). Several psychotropic genera producer proteolytic and lipolytic enzymes, which remain active post-pasteurization, so increased levels in raw milk are more likely to compromise raw milk quality. While we did not identify these isolates, previous research has classified raw milk microflora by enzyme activity. Isolates that produce lipolytic enzymes only are often Pseudomonas or Acinetobacter and isolates that produce both lipolytic and proteolytic enzymes are typically Microbacterium. Isolates with proteolytic activity alone are less as common. Lactic acid bacteria (Lactococcus and Leuconostoc) do not typically have significant enzyme activity (Hantsis-Zacharvo and Halpern, 2007).

Utilization of microbiological media that demonstrates enzyme activity may be an important and meaningful tool for the dairy industry to characterize raw milk quality. Interpretation of proteolytic activity on skim milk agar (SMA) was straightforward. Freshly prepared SMA is opaque milky white in appearance, non-proteolytic colonies will not change the appearance of the media, and proteolytic colonies will create transparent halo clearings under and around each colony. Interpretation of lipolytic activity on sprit blue agar (SBA) is more challenging and morphological interpretations vary in the literature. Prepared SBA appearance is slightly transparent, light blue, and has sheen due to the presence of trybutirin (milk glyceride). The manufacture of SBA (HiMedia) describes sprit blue dye as an inert and ideal indicator of lipolysis by yielding clear halos around lipolytic colonies (HiMedia, 2011). Teh et al., (2011, 2012) and Hantsis-Zacharvo and Halpern (2007) characterized lipolytic activity as a royal blue zone around colonies, whereas the SMEDP 17th edition (2004) describes lipolytic activity as clear zones with or without a deep blue color around or under each colony. Trmcic et al., (2015) used a 3-point measurement scale; (-) was no visible zone of cleaning, (+) <2mm clearing, and (++) <2mm clearing; however, color change of agar was not mentioned. In our preliminary work, we found that colonies produced four types of reactions on sprit blue agar that we described in this paper. It is speculated that L+ is likely weak/or false indicator of lipolysis because the media becomes transparent around colonies, but no defined halo or clearing. In contrast, L- were colonies that did not change the appearance of the media. L++ were well defined halos around each colony that is described as matte in contrast to the sheen of the media's original appearance. L+++ indicated a royal blue reaction, as described by the literature mentioned; however, only some L+++ colonies produced a well-defined matte halo. Characterization of culture-based lipolytic activity methods needs to be improved to produce consistent results and correct interpretation.

Conclusion

Raw milk was originally transported in 10-gallon milk cans, and as we demonstrated, they are susceptible to compromising raw milk quality through poor insulation and potentially sanitation, and not to mention inefficient for transporting mass quantities. Tankers have revolutionized dairy processing capabilities and are an essential component in modern industry. The PMO does not specify maximum time that tankers can be empty and dirty between loads, only that they must be cleaned every 24 h. In our commercial study we were able to demonstrate that 6 h idle time between loads is unlikely to have measurable impact on subsequent load quality; however, as observed in the milk cans, the severity of negative impact increases with time. This is the first study to demonstrate a measurable negative impact on raw milk microbiological quality due to hauling, and while on a small-scale, this proof- of-concept allows us to estimate the potential negative impact that could occur in commercial tankers under worst-case conditions. Additionally, there is necessity for exploring better options for rapidly assessing raw milk quality; exploration of rapid proteolytic and lipolytic analysis could be beneficial to industry.

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Chapter 5. Conclusions

The transition from 10-gallon milk cans to tankers was a cornerstone in dairy processing; the ability to readily transport mass amounts of raw milk to processing facilities allowed from increased production sizes. Our overarching hypothesis was that with adequate sanitation and operation practices, milk hauling was unlikely to compromise raw milk quality, therefore preserving the on-farm quality. This is important because theoretically raw milk may be up to 7 days old before processing; 72 h on-farm bulk tank + 24h tanker + 72 h storage silo. As we observed, elevated initial counts due to weaknesses in sanitation can increase the likelihood of psychrotrophic bacteria; producers of thermo-stable proteolytic and lipolytic enzymes. Seven days at a high concentration could be detrimental to dairy product quality.

Baseline raw milk microbiological quality is established at the farm level; it is imperative that counts are as low as possible to mitigate elevated counts through the dairy processing continuum. In our historic data study, we found that producer microbiological counts were the dominating factor on raw milk quality, and that negative impact due to hauling is sporadic and occurs infrequently. In cases where a tanker load is made up of more than one producer, the impact of a high count producer is likely diluted by the other producers on the load - making the impact unmeasurable. The co-op in our case study demonstrates an exceptional monitoring program of on-farm bulk tank and tanker unloading milk quality. Emphasis on daily routine milk quality allows processors to quickly capture elevated counts and take corrective action, which ultimately prevents issues from persisting for long periods of time. Daily monitoring requires rapid enumeration technologies such as flow cytometry since traditional culture-based enumeration is not efficient due increased labor, supplies, incubation time and space. It is challenging to pinpoint sources of contamination in milk hauling practices because there are numerous potential culprits. However, daily monitoring notifies processors and producers when a problem arises and allows corrective action to be taken.

While impact from hauling is unmeasurable using standard industry microbiological testing methods, this does not necessary mean that negative impact is not happening. The work produced by Teh et al. (2011,2012,2013) where several genera of microflora obtained from commercial tankers produced thermo-stable enzymes, inspired us to explore novel culture-based techniques to consider proteolytic and lipolytic activity as a potential measurement of raw milk quality. As demonstrated in literature, raw milk microbiological counts do not directly correlate with shelf-life (Martin et al., 2011), but since many co-ops implement premium incentive programs there is need for a rapid method for determining raw milk quality that better correlates with product quality. It would be interesting to have a technology similar to flow cytometry that could measure microbial enzyme activity in raw milk.

In our survey study, we were able to capture a snapshot of the variability in hauling sanitation and operating practices that occurs in the Northwest dairy industry. Ultimately the actions of handling, transporting, and receiving milk are fundamentally the same; however, there are many practices that vary among facilities but fit within PMO regulations. This can be attributed to different styles and materials of equipment, size and layout of milk reception at a given facility, and differences in training amongst personnel.

Milk hauling is an essential function in the dairy processing continuum. Repeated usage of tankers between cleans promotes efficiency; more milk can be collected per day, while reducing resources such as chemicals, water, energy. Modern day milk hauling practices appear to be adequate, as long as sanitation and operational practices are implemented. Many of the recommendations in this thesis are simple to implement, but require persistence to be effective. Processors and producers need to understand the importance of their role in milk quality. Best practices at every step of the process yield excellent quality dairy products.

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Figures

Figure 2.1. Box-and-whisker plot of individual producer on-farm bulk tank and tanker unloading IBC and PI-IBC. Outliers are omitted from this figure and will be discussed in subsequent figures.

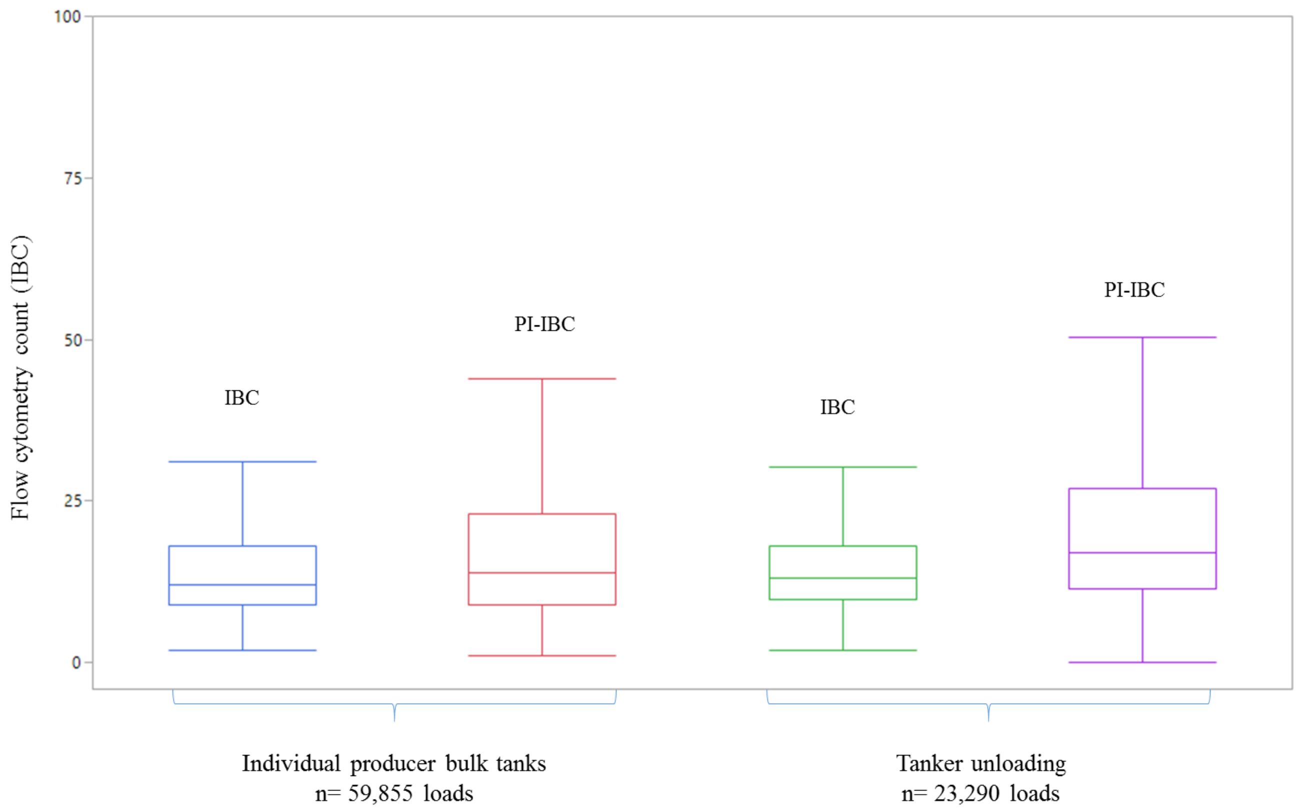


Figure 2.2. Distribution of historic producer PI-IBC data. Producers ordered ascending from lowest to highest mean PI-IBC (overall mean 36.3 PI-IBC). All outliers (counts > than box plot's 3rd quartile x 1.5) are removed for clarity of figure and are discussed in subsequent figures. Thirteen of 106 producers are significantly different (one-way ANOVA); *ten producers with significantly greater counts (H, M, O, L, D, C, B, A, G, N) and **three producers (I,E,F) with significantly lower counts.

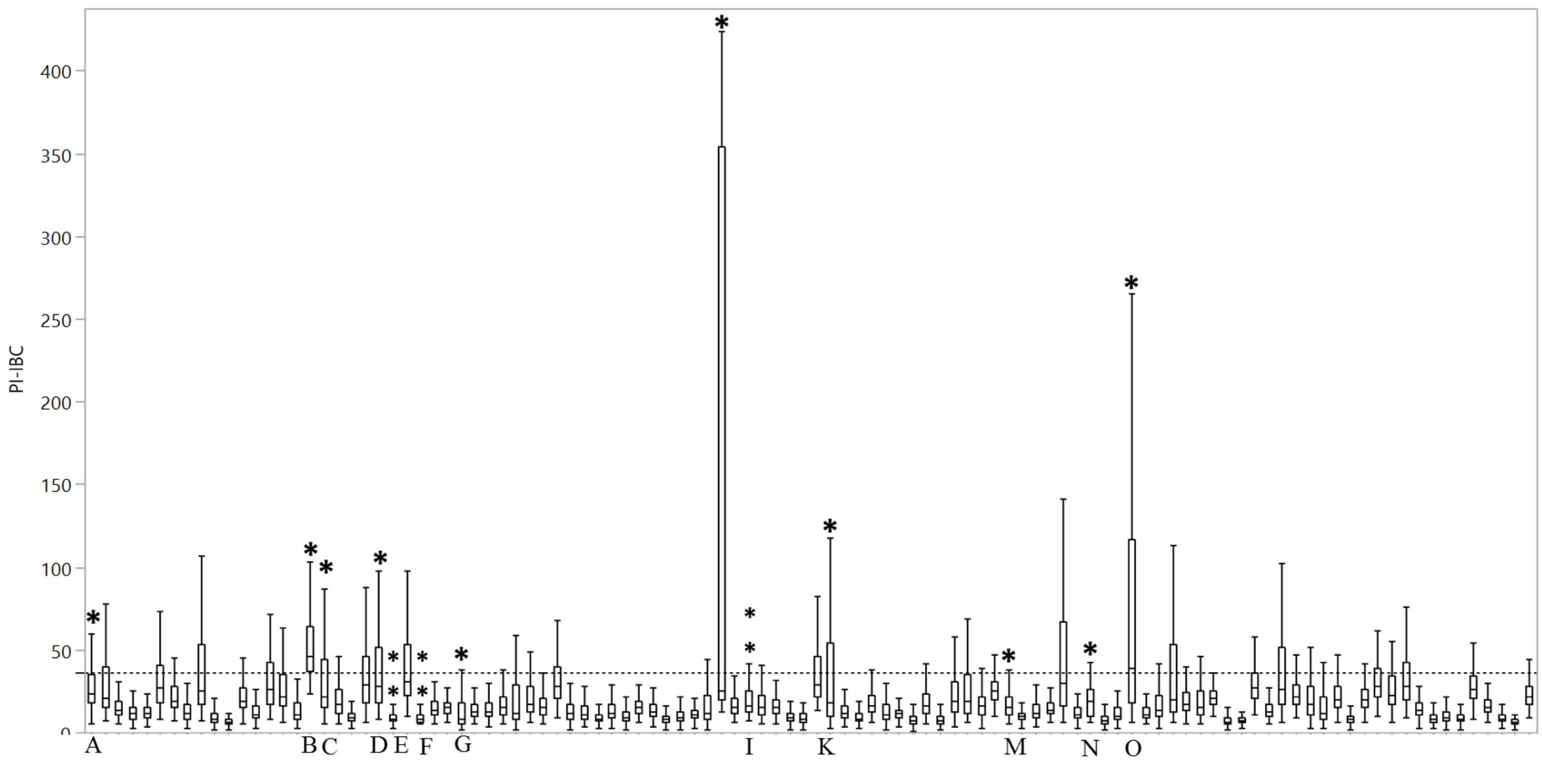


Figure 2.3. Individual producer PI-IBC outliers (>305; top 1%) for 2014-2015 were compared by one-way ANOVA,* indicates significant difference in number of outliers by producer compared to total number of outliers. Six producers with the most instances of outliers (C,D,H,K,O,A) has historically higher counts, as indicated in figure 2. Two producer account for 2.2% of the outliers.

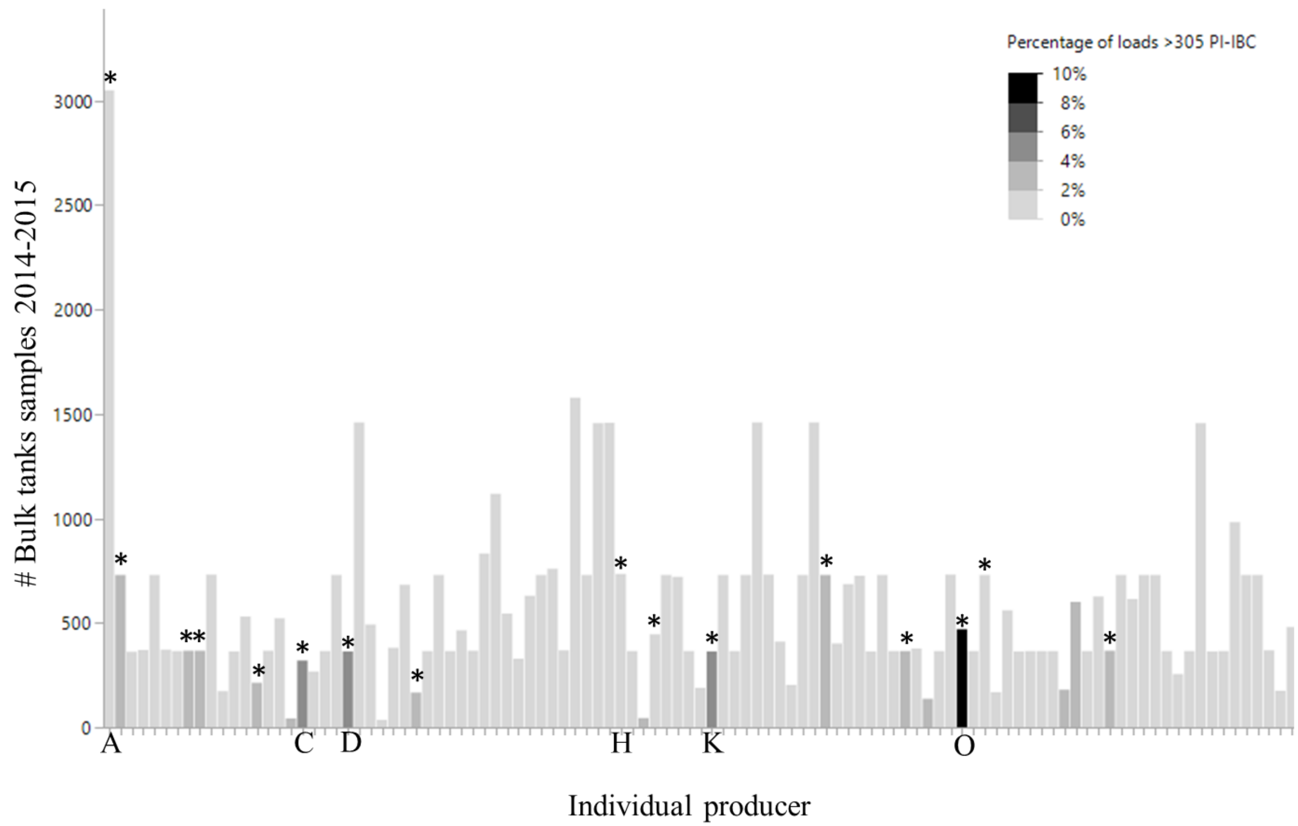


Figure 2.4. Snapshot of a small producer with historically good milk quality (J; n = 446 bulk tank loads, mean IBC: 14.9 ± 0.3 and PI-IBC 54 ± 9.8). In total this producer had 15 loads in the top 1%, and the majority (13/15) occurred in October 2015. Concurrently there was a spike in mean PI-IBC (492.0 ± 114.5), and 41.9% of loads for the month were classified as outliers for the month.

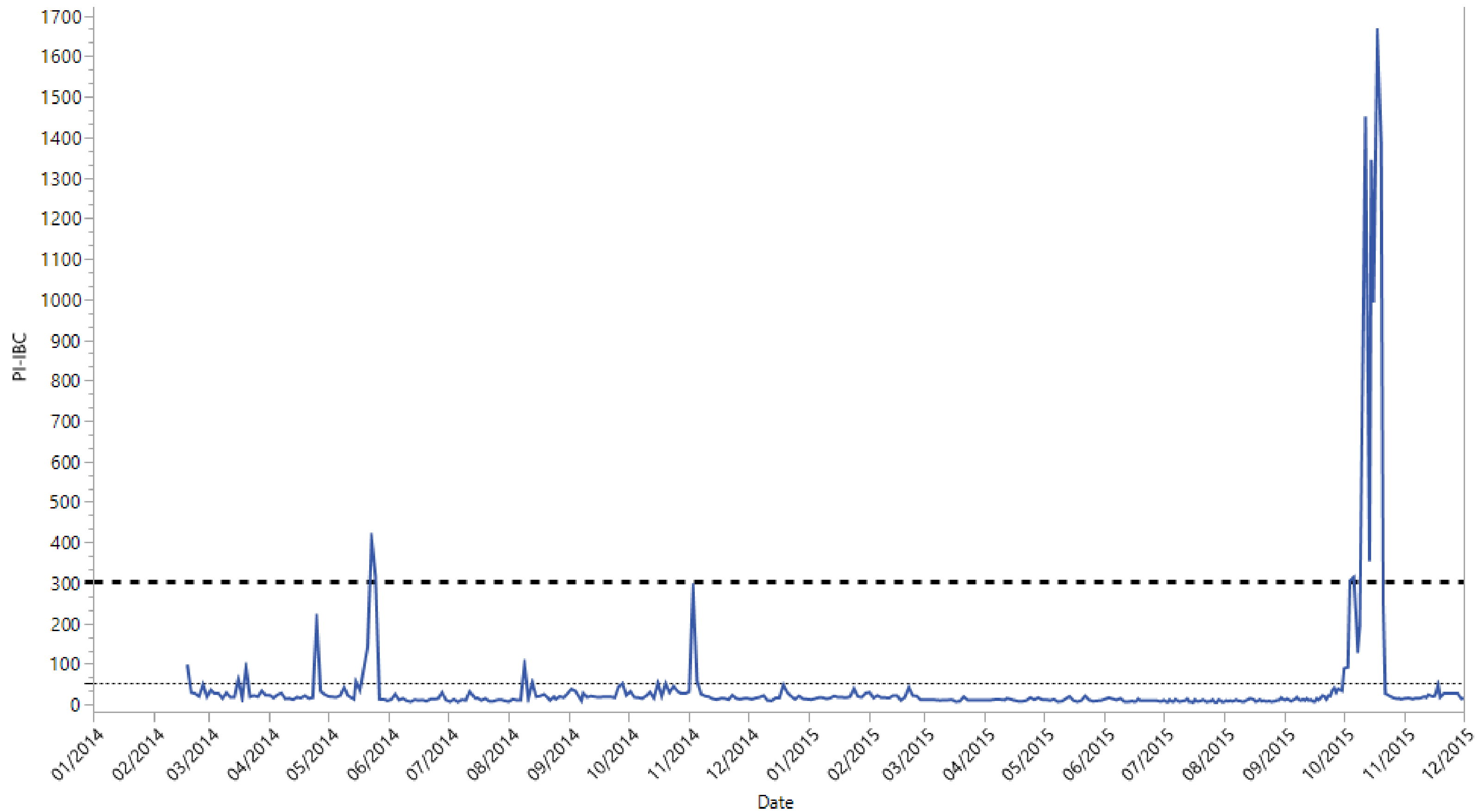


Figure 2.5. Snapshot of a small producer (O; n=470 bulk tank loads) has frequent elevated PI-IBC (mean 200 ± 30.6), and 13.2% (62/470) of bulk tank samples were in the top 1 %. Outliers occurred in 15 of 24 months, and accounted for approximately 10% of outliers for the entire co-op.

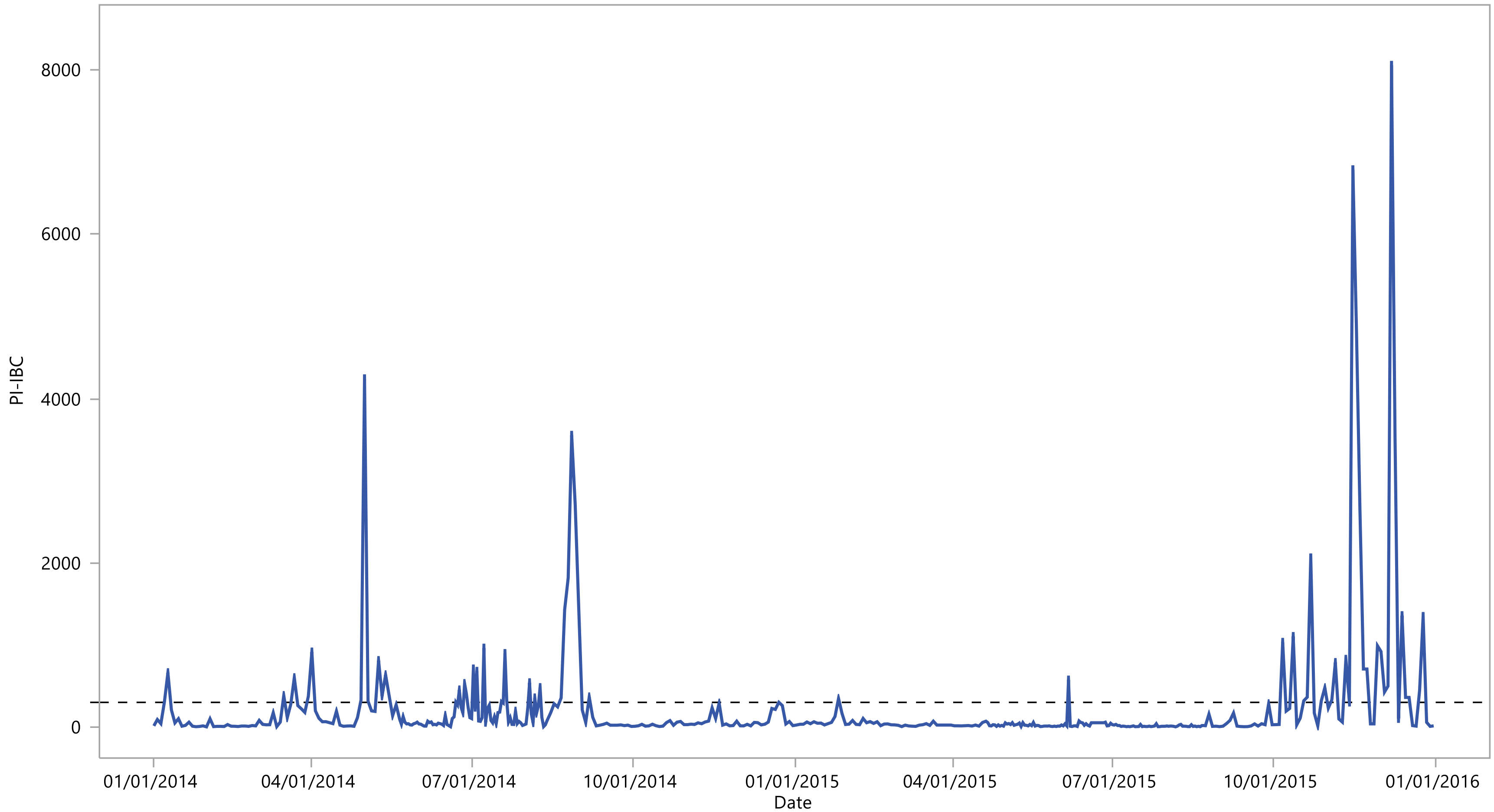


Figure 2.6a. Historical trends of the largest producer (A) in the co-op (n=3049 bulk tank loads). Mean PI-IBC milk quality 66.5±7.8 (indicated by dashed line). July 2015 and November 2015 were the worst months for this producer (498.6 ± 34.1 and 374.3 ± 57.2, respectively); mean PI-IBC exceeded the top 1% outlier cutoff (> 305) for both months.

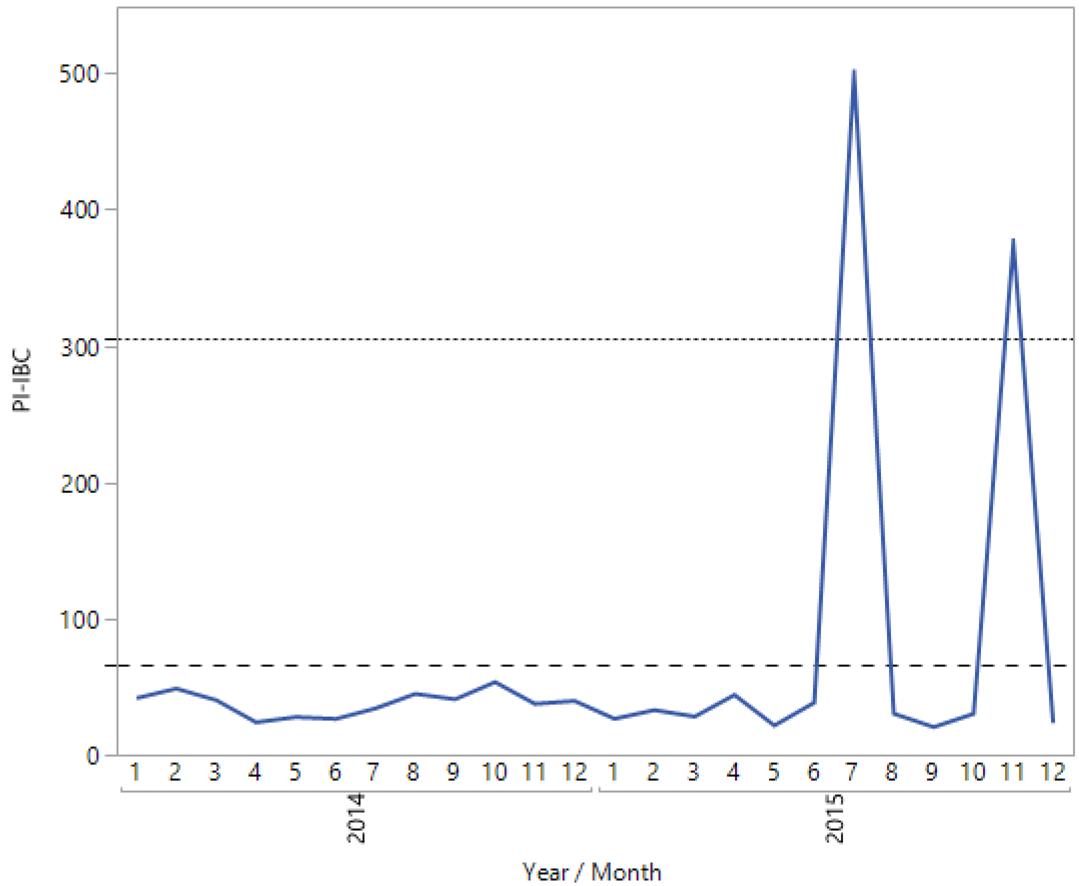


Figure 2.6b. Investigation of November 2015 elevated PI-IBC for producer A. Mean PI-IBC for the month exceeded the top 1% PI-IBC cutoff. Counts began to creep up early in the month, then spiked and returned to "normal" (e.g. below producer overall mean PI-IBC; 66.4 ± 7.8).

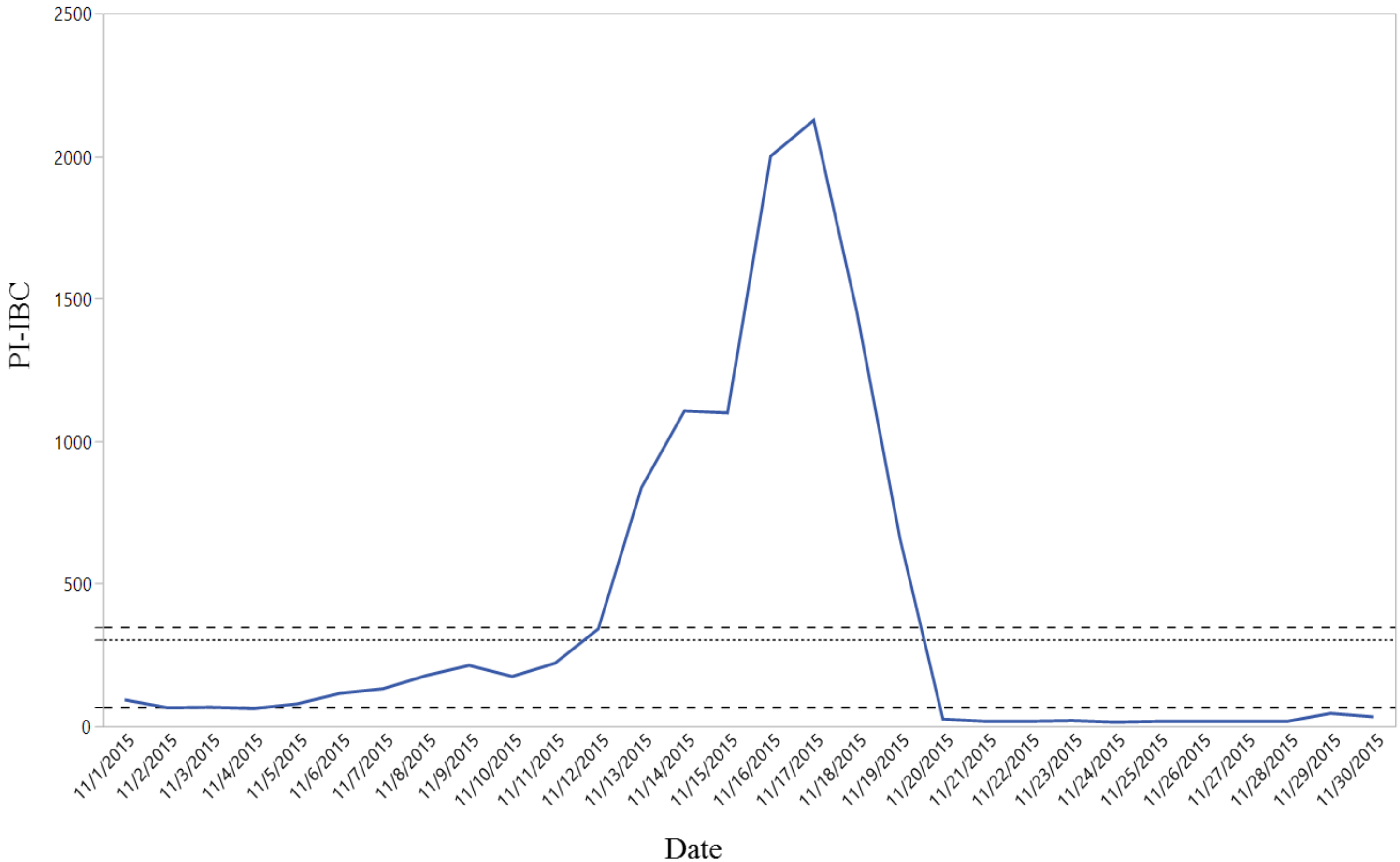


Figure 2.7. Top 1% of individual producer and tanker unloading PI-IBC. Data sorted by number of bulk tanks per tanker load to see when dilution by number of bulk tanks overcame one high sample per load P-values are for matched pairs t-test by number of bulk tanks. P-value represents 2-sided non-equivalence (P > |t|).

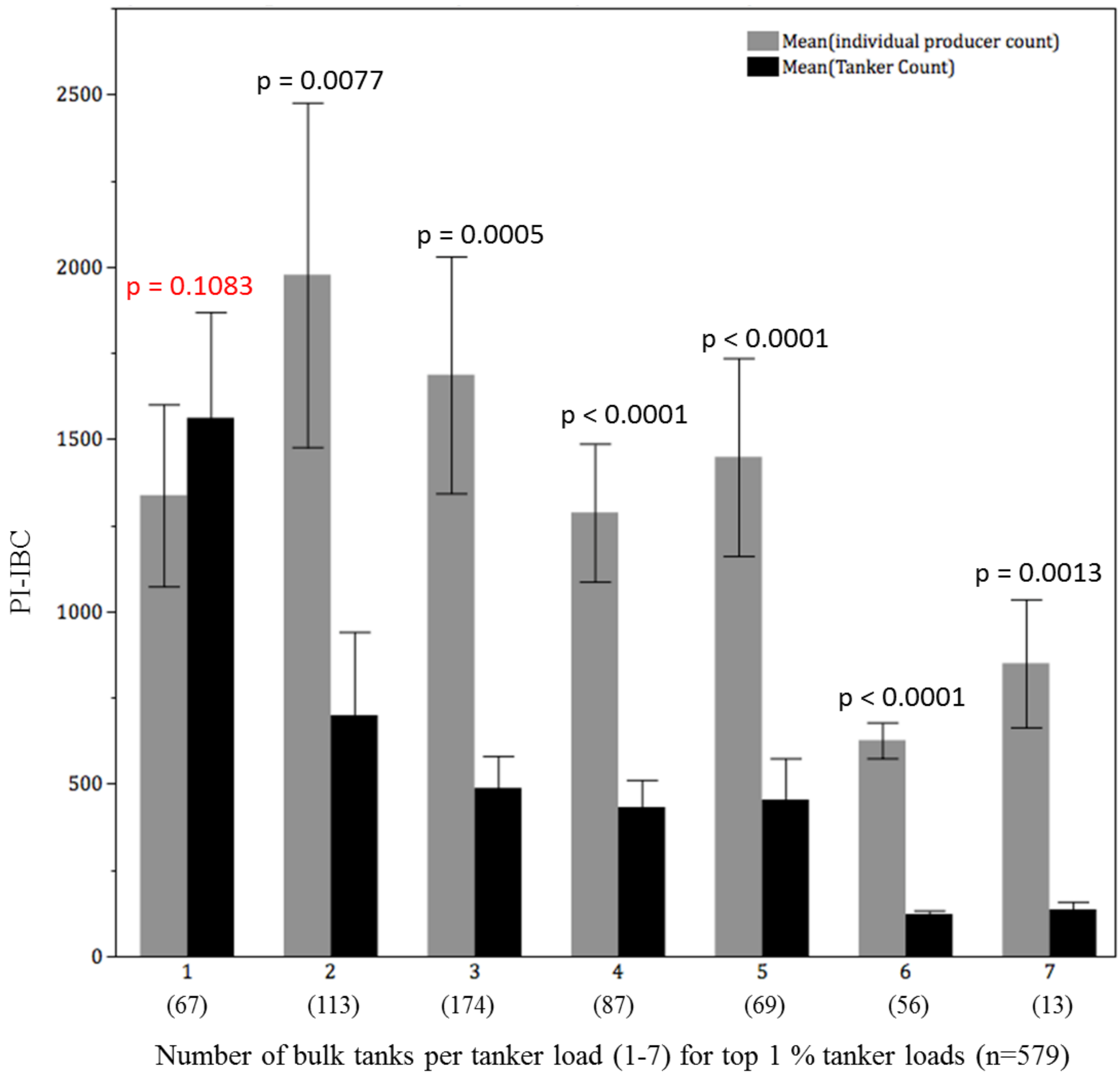


Figure 2.8. Snapshot of negative impact due to hauling during January 13-15 2014; producer on-farm bulk tank PI-IBC was low and tanker unloading counts increased in subsequent loads (manifest 172736 through 172738).

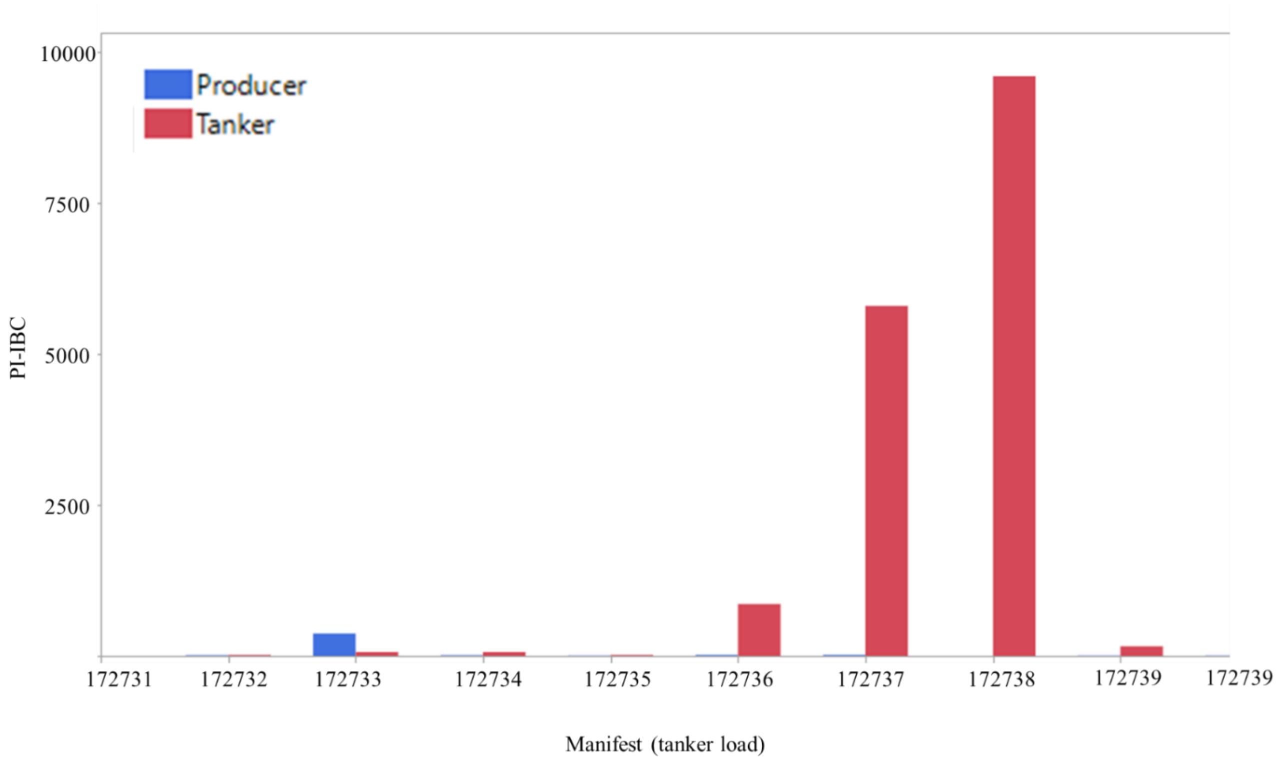
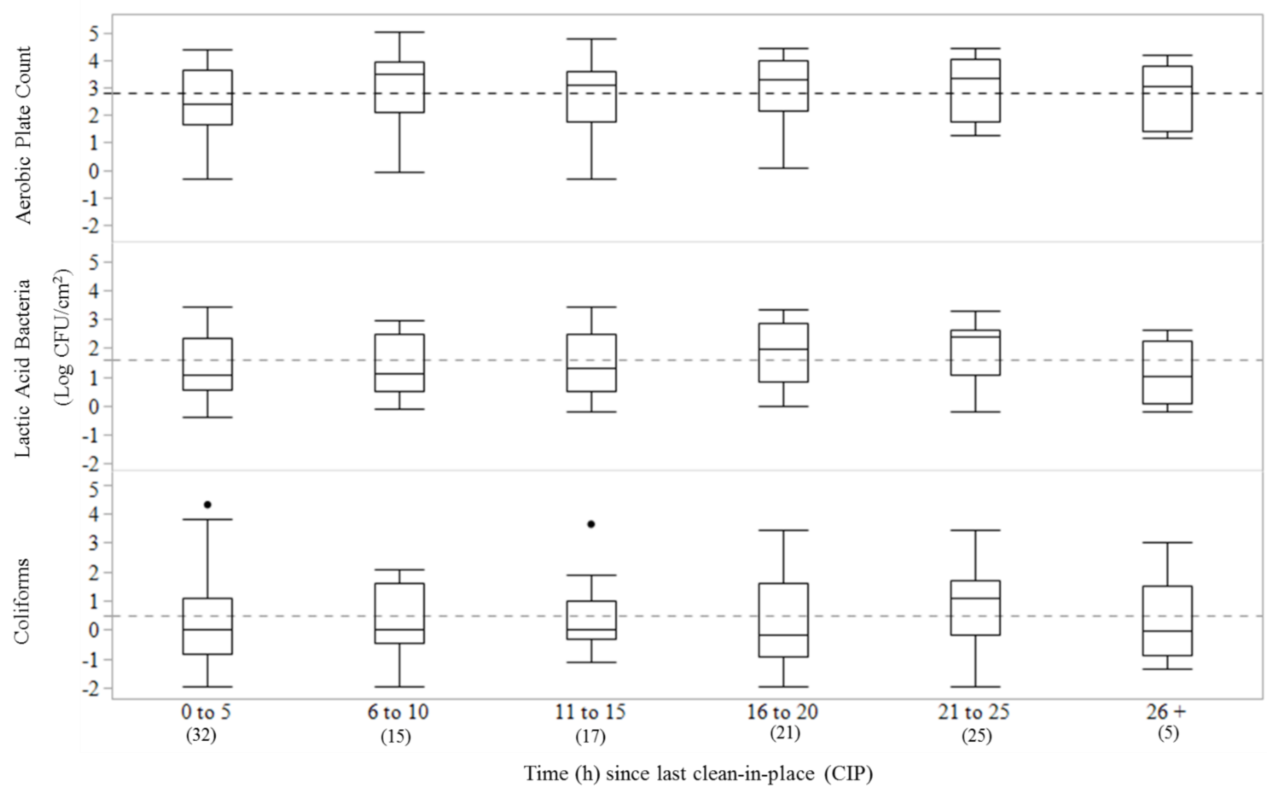


Figure 3.1. Box-and-whisker plot of facility B microbial concentrations (log cfu/100 cm2) of aerobic plate count, lactic acid bacteria, and coliforms on the internal surfaces of tanker farm hoses as a function of time (h) since last clean-in-place of tanker (n = 115 hoses). Sample size distribution is listed below each hourly interval. Dashed line for each microbial group indicates mean log cfu/100 cm^2. Limit of detection 1 cfu/100 cm^2.



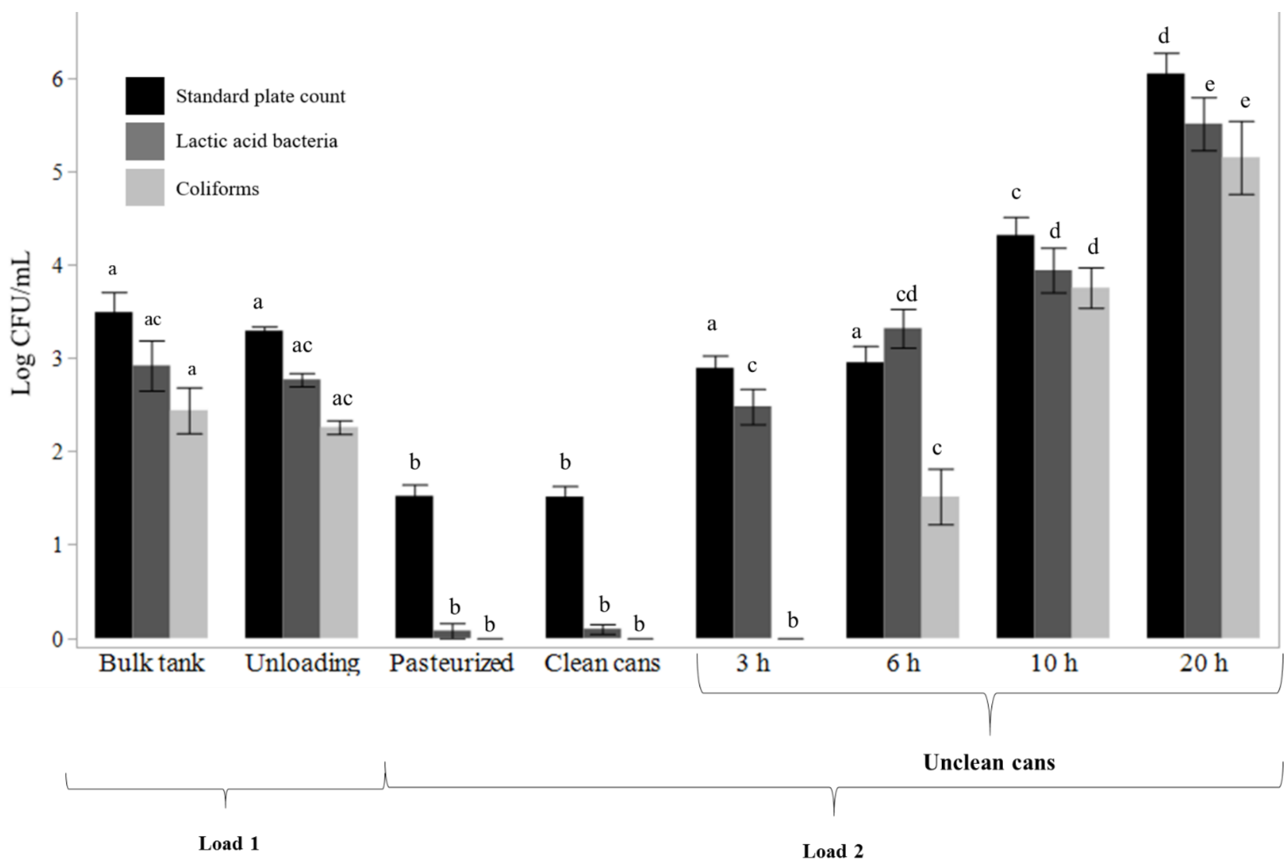


Figure 4.1. Influence of the residual milk from the initial load and idle time on microbial contamination of the subsequent milk load. Limit of detection > 1 CFU/mL. Load 1 was raw milk collected from a local dair farm bulk tank. Load 2 commercially pasteurized (HTST) whole milk. Milk cans were incubated at 30°C fo 3, 6, 10, or 20 h prior to the addition of pasteurized milk (load 2). Time points were randomly assigned ar each sample time was completed in triplicate on three different days (n = 9 cans). Two control cans were used every sample day (n = 12 cans). Error bars represent ± 1 SE. Statistical lettering grouped by microbia type; shared letters are not significantly different.

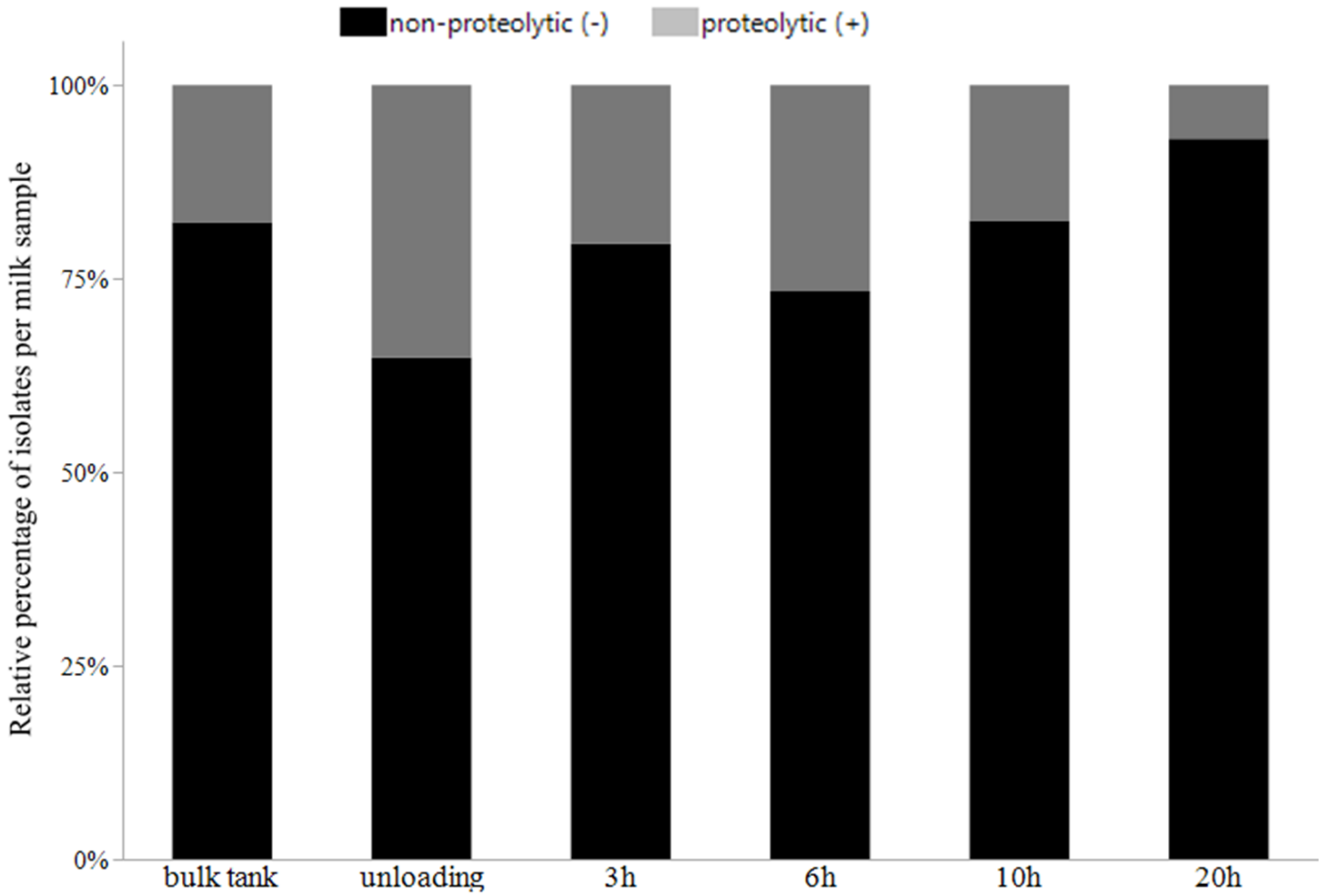


Figure 4.2a and b. Characterization of milk can microflora enzyme activity (n=1152 total isolates) as the relative total percentage for each milk sample category. Total number of isolates per sample: bulk tank (96), unloading (591), 3h (39), 6h (210), 10h (144), and 20h (72).

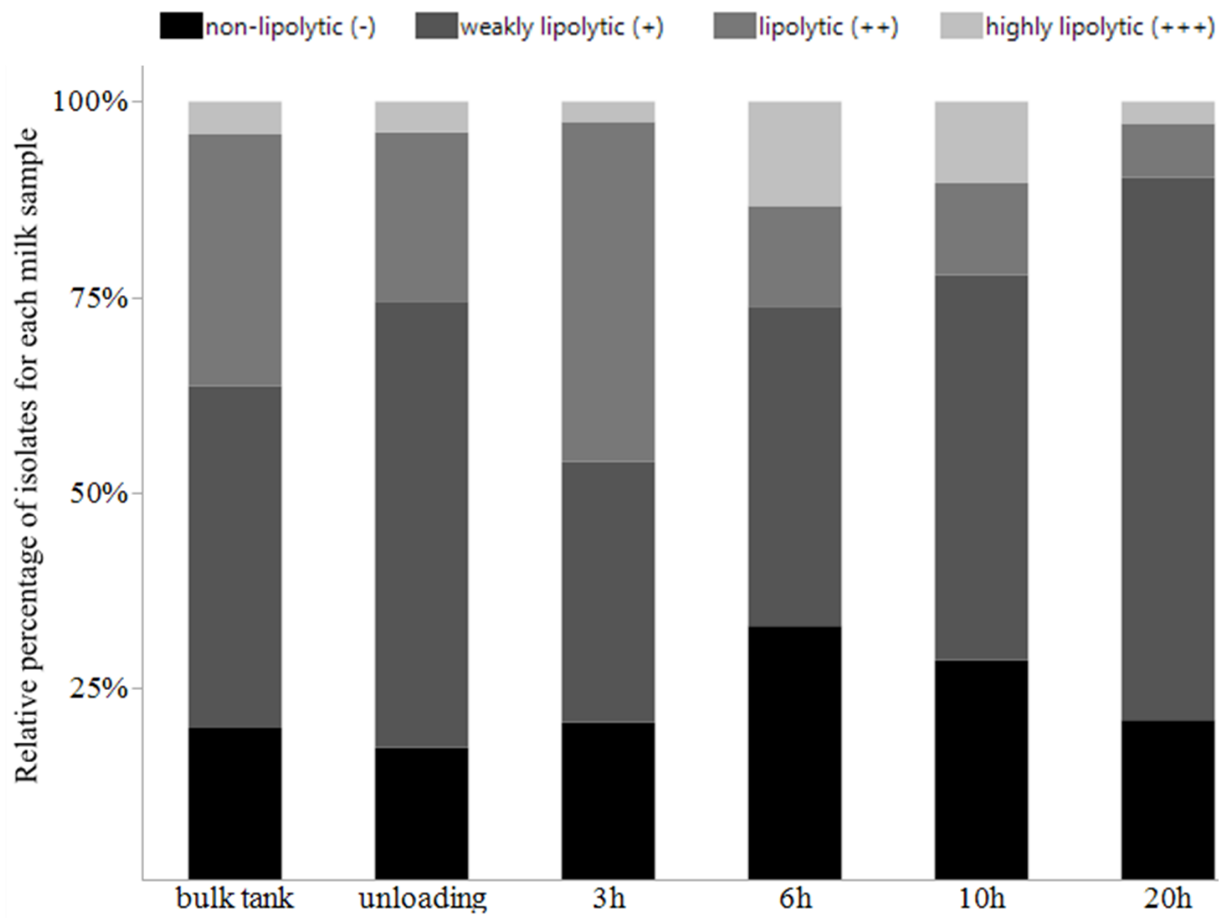


Figure 4.2b Characterization of milk can microflora enzyme activity (n=1152 total isolates) as the relative total percentage for each milk sample category. Total number of isolates per sample: bulk tank (96), unloading (591), 3h (39), 6h (210), 10h (144), and 20h (72).

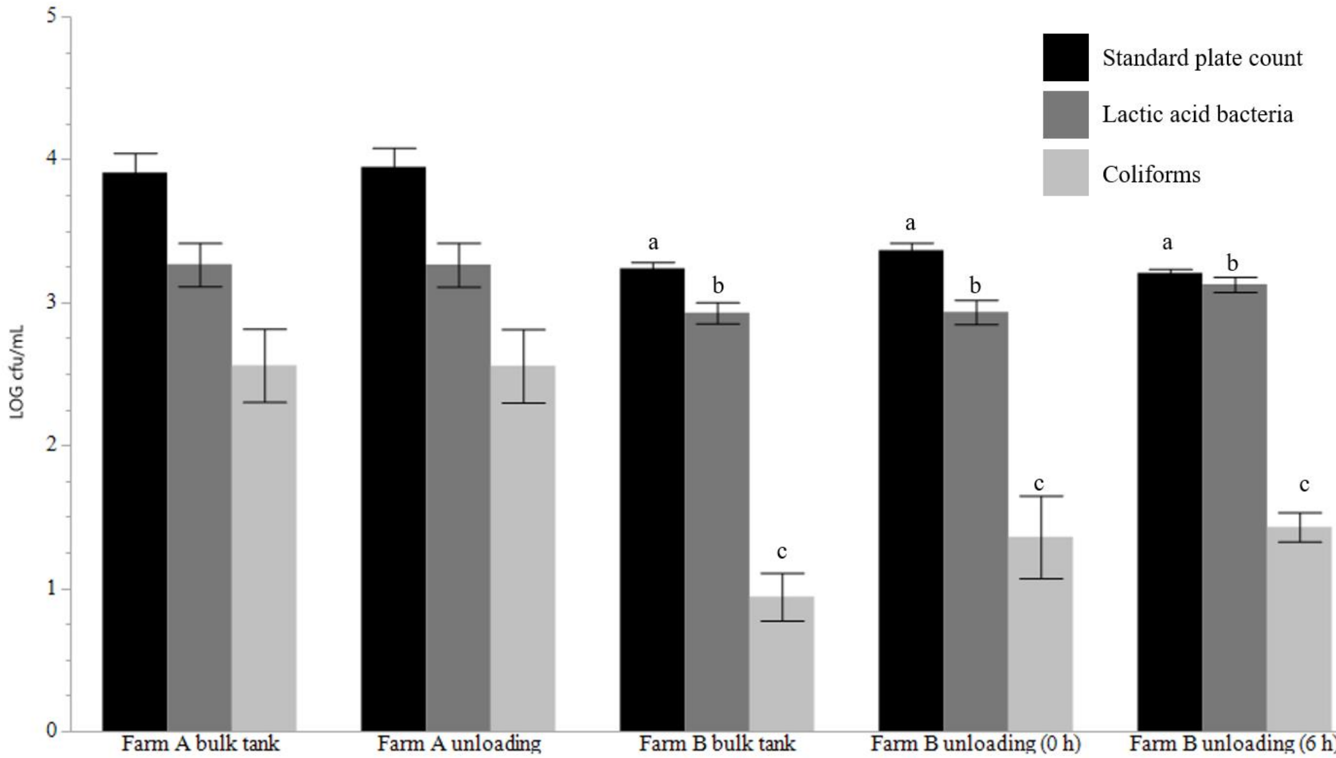


Figure 4.3. Commercial tanker standard microbiological counts for Farm A and Farm B bulk tank and tanker unloading milk samples. Limit of detection >1 CFU/mL. Error bars represent ± 1 SE.

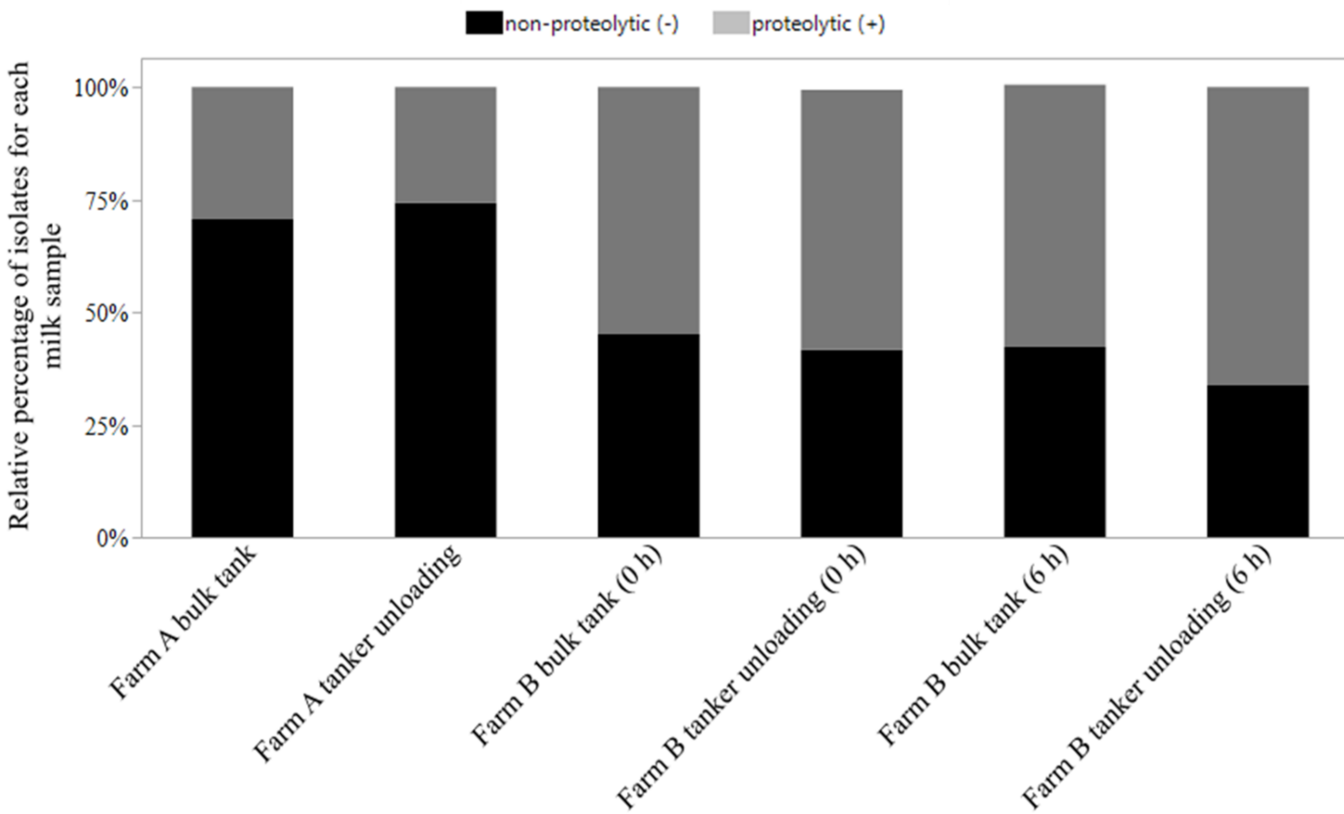


Figure 4.4 a and b. Commercial tanker study isolate enzyme activity (n=144 total isolates per milk sample; 24 isolates per day). Farm A and B proteolytic activity is significantly different (Fisher's Exact test; 2-sided p-value < 0.0001).

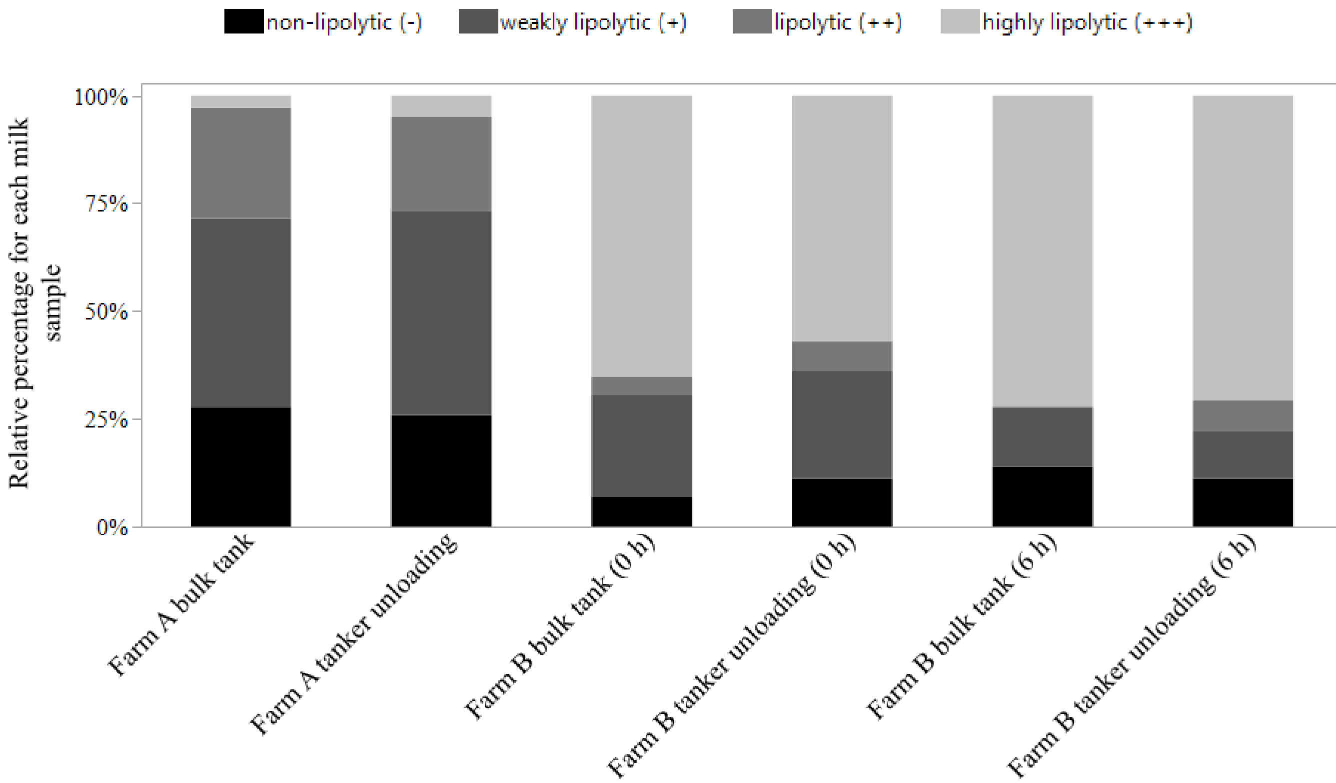


Figure 4b. Commercial tanker study lipolytic enzyme activity (n=144 total isolates per milk sample; 24 isolates per day). Farm A and B lipolytic activity is significantly different (Fisher's Exact test; 2-sided p-value < 0.0001).

Tables

Table 2.1. Individual bacteria count (IBC) and preliminary incubation count (PIC) top 1% (outliers) of historic counts of individual producer, producer load average of a given load, and tanker unloading.

Sample	**Top 1%**	
	IBC	PI-IBC
Individual producer	69	305
Producer load average	73	288.1
Tanker	65.9	405

Table 3.1. Milk hauling and receiving practices used by commercial dairy facilities in the northwest region of the United States.

Survey Topic	Number of facilities (n = 11) 78% response rate
Overview of Milk Receiving Operation	
Receiving bay layout	
Full enclosure	5 (45%)
Partial enclosure- overhead cover	6 (55%)
Number of receiving bays	
1	2 (18%)
2	7 (64%)
3	2 (18%)
Number of receiving hoses	
1	2 (18%)
2	5 (45%)
3	1 (9%)
4	2 (18%)
5	1 (9%)
Receiving hose storage	
Stored on ground	0 (0%)
Stored hanging	11 (100%)
Receiving hose style	
Clamp + cap + gasket	4 (37%)
Threaded cap	5 (45%)
Other	2 (18%)
Daily tanker receipt	
0-5	2 (18%)
6-10	5 (45%)
11-30	4 (37%)
Milk receiving sample collection	
Aseptic	1 (9%)
Dipper	10 (91%)
Frequency of replacing cleaning equipment such as buckets and brushes	
Routine; 2-4 weeks	1 (9%)
As needed	10 (91%)
Frequency of replacing gaskets, farm hoses, and other parts susceptible to deterioration	
Routine; 3-6 months	2 (18%)
As needed	9 (82%)
Frequency of small part cleaning/sanitizing	
Hourly/between tankers	2 (18%)

Daily only	2 (18%)
At least once a day; as needed	7 (64%)
Cleaning and Sanitation Practices	
Tanker CIP schedule	
After each load	4 (37%)
Once per 24 hours	7 (63%)
Routine Maintenance	
Validate CIP system[a]	
Daily	1 (9%)
Monthly	1 (9%)
Quarterly	3 (27%)
Annually	6 (55%)

[a] Validate CIP system was the terminology used in the survey tool. Responses are presented as reflected on the survey; however, it is should be noted that the definition of "validate" is often misunderstood by personnel; therefore, this should be considered in the interpretation of answers to this survey question.

Table 3.2. Microbiological load of receiving bay hoses at two commercial dairy facilities during normal operation.

Facility	Dates of Collection	Ambient Temperature	Number of Samples	Aerobic Plate Count	Lactic Acid Bacteria	Coliform Count
				(log cfu/100 cm^2)		
Facility A	September 2016	24℃	9[a]	3.7 ± 0.1[b]	ND[c]	1.4 ± 0.2
Facility B	March 2017	22℃	56[d]	2.1 ± 0.2	0.8 ± 0.1	0.6±0.1

[a]Receiving bay hoses (n = 3) at Facility A were sampled after daily standard use (~24 hours) over three consecutive days.
[b]Mean ± standard error.
[c]Not determined. Samples collected from receiving bay hoses at Facility A were not analyzed for lactic acid bacteria.
[d]Receiving bay hoses (n = 4) at Facility B were sampled hourly over an eight-hour period for two consecutive days. No significant difference in counts over time.

Table 4.2. Media types used to enumerate and characterize milk samples.

Purpose	Media	Incubation conditions	Growth interpretation
Standard Plate Count (SPC)	Tryptic Soy Agar (TSA)	32°C, 48 h	Enumerated all growth for SPC
Lactic Acid Bacteria (LAB)	MRS Agar	32°C, 72 h	Enumeration of lactic acid bacteria
Coliforms	Coliform Petrifilm	32°C, 24 h	Enumerated all growth as coliforms
Lipolytic bacteria	Spirit Blue Agar (SBA)	21°C, 72-96 h	*Non-lipolytic reaction* **L-** no reaction; agar remains pale blue, no halo clearings or deep royal blue colonies *Lipolytic reactions* **L+** agar transitions from pale blue to colorless **L++** matte halo clearing around colony, agar transitions from pale blue to colorless **L+++** colonies and/or halos deep royal blue color
Proteolytic bacteria	Skim Milk Agar (SMA)	21°C, 72-96 h	**P-** no clearing around colony; agar remains opaque white **P+** clearing around colony; agar transitions from opaque white to colorless

Table 4.3. Historical microbiological data (24 months) used to select route for industry hauling experiment.

Farm	Daily Milk Production (kg)	N	Microbial Test[a]	*Mean	St. Dev	Min	Max
A Historically Low Count Producer	7,700	729	IBC	31.7	47.8	10	1101
			PIC	66.5	432.3	8	10676
B Historically High Count Producer	9,000	982	IBC	8.8	9.0	2	209
			PIC	13.3	72.5	2	2081

[a]Counts enumerated by FOSS BactoScan and reported as individual bacteria count (IBC) and preliminary incubation count (PIC).

*Farm A microbial counts are significantly greater than Farm B's count (P value <0.001).

Appendices

Appendix 1. Milk Hauling Survey Questionnaire

OSU is conducting research on milk hauling and receiving practices and investigating ways to improve current practices to help contribute to better milk quality. Please answer the following questions to the best of your abilities to us help gain insight into current milk receiving practices. We greatly appreciate your participation in our research. We plan to use this research to communicate best practices to the industry. Your responses will be kept anonymous and please let us know if you have any questions or concerns.

1) Describe the general layout of the receiving bay. (enclosure type, number of receiving stations)
2) Does your facility clean and sanitize tanker trucks after receiving each milk load or once every 24 hours as the PMO allows?
3) On average, how many trucks come through receiving on a daily basis?
4) How many receiving hoses are there, and how are they stored when not in use? (Include description of receiving hose cap. (Clamp + cap + gasket or threaded cap, etc.))
5) How frequently are small parts, such as gaskets, O-rings, pipe/valve/hose covers cleaned and sanitized throughout the day?
6) Sample collection method during receiving (choose one)

   In-line   aseptic   dipper
7) What type of cleaning and sanitizing agents are used to clean small parts, and how frequently is the wash water and sanitizer changed out throughout the day?
8) How frequently are wash brushes and buckers replaced?
9) How frequently is the CIP system validated?
10) How frequently are gaskets, receiving hoses, and any other parts that are prone to deterioration replaced?
11) Have there been any instances of rejecting tankers at receiving, other than testing positive for antibiotic residues, out of range temperature, improper paperwork, or negative organoleptic attributes.
12) Please provide any additional information in regards to milk hauling and receiving practices that could be beneficial to this research. This includes areas of concern or in need of improvement, recommendations for best practices, etc.

Appendix 2. Milk can experimental design schematic

Samples	D1	D2	D3	D4	D5	D6	Total
Load 1 bulk tank	1	1	1	1	1	1	6
Load 1 receiving (raw)	8	8	8	8	8	8	48
Load 2 receiving (fouled cans, pasteurized)	1	1	1	1	1	1	6
3h	0	3	0	3	0	3	9
6h	0	0	3	0	3	3	9
10h	0	0	3	3	3	0	9
20h	6	3	0	0	0	0	9
Load 2 receiving (control, cleaned + sanitized)	2	2	2	2	2	2	12

Experiment repeated for 6 days to randomize time points. Each sample day a bulk tank milk sample was obtained to determine baseline microbial counts. Eight milk 19-liter milk cans were used for hauling. All 8 milk cans were sampled prior to unloading to capture variation between cans. Each time point for fouled cans was done in triplicate for each sample day. Every sample day contained two control cans, which were cleaned and sanitized after load 1 receiving.

Appendix 3. Research presentations and posters

Oral presentations

Kuhn. E, Meunier-Goddik, L., and Waite-Cusic, J. 2017. OSU Milk Hauling Research Update. Oregon Dairy Industries Conference (Salem, OR).

Kuhn. E, Meunier-Goddik, L., and Waite-Cusic, J. 2016. Worst Case Milk Hauling Scenarios. BUILD Conference (Logan, UT).

Kuhn. E, Meunier-Goddik, L., and Waite-Cusic, J. 2016 Milk Hauling Research Update. Oregon Dairy Industries Conference (Salem, OR).

Kuhn. E, Meunier-Goddik, L., and Waite-Cusic, J. 2015. Investigation into the Impact of Industry Hauling Practices on Overall Milk Quality. BUILD Conference (Twin Falls, ID).

Poster presentations

Emulating worst-case milk hauling practices on a small-scale to demonstrate negative impact on raw milk quality

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Oregon State University College of Agricultural Sciences

INTRODUCTION

Negative impact from hauling is challenging to capture

- The Pasteurized Milk Ordinance (PMO) mandates milk hauling practices, including allowance of repeated tanker usage for 24 h between mandatory clean-in-place (CIP).
- Industry says that negative impact from hauling occurs sporadically, but at what frequency?
- Standard industry hauling practices are unlikely to impact raw milk microbiological quality (Darchuk et al., 2015). However, impact is difficult to measure due to scale and dilution factor.
- **Worst-case hauling conditions** = extended idle time (empty + dirty) between loads in hot weather conditions.

OBJECTIVE

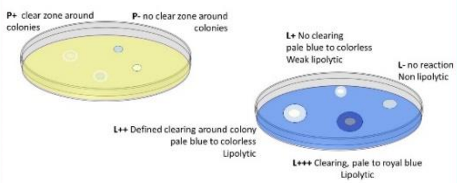
Demonstrate measurable negative impact on milk microbiological quality on small-scale under worst-case condition. If successful, this proof-of-concept will be used for commercial-scale hauling experiment.

MATERIALS AND METHODS

Industry standard microbiological testing

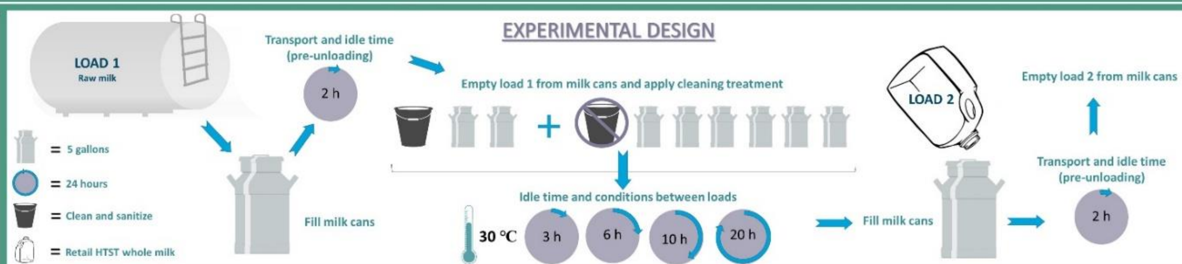
Samples were enumerated for standard plate count, lactic acid bacteria, and coliforms.

Characterize enzymatic activity of isolates



Skim milk agar and spirit blue agar were used to characterize proteolytic (P-/+) and lipolytic (L-/+/++/+++) activity of isolates from each sample location.

EXPERIMENTAL DESIGN



RESULTS AND DISCUSSION

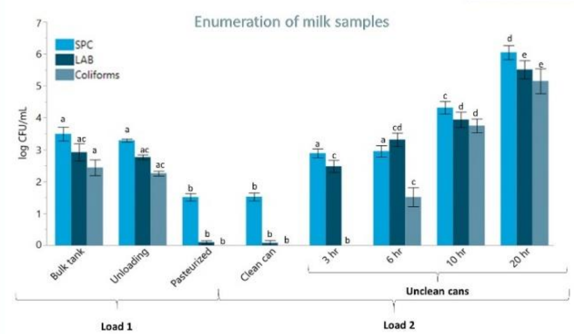


Figure 1. Comparison of mean microbiological counts for load 1 and load 2. **SPC** indicates standard plate count, **LAB** indicates lactic acid bacteria. Limit of detection > 1 CFU/mL. Error bars represent ± 1 SE. Load 1 microbiological counts did not increase during hauling. Load 2 was measurably contaminated by load 1 unclean cans, and severity of impact increased with time. Load 2 hauled in clean milk cans was not measurably influenced. Statistical lettering is grouped by microbial type.

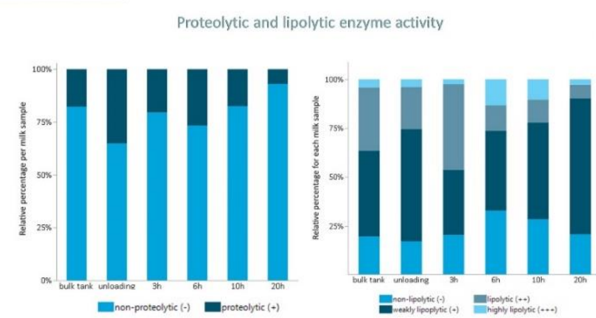


Figure 2. Enzyme activity of isolates on skim milk agar (proteolytic) and spirit blue agar (lipolytic). Load 2 milk samples obtained from retail container and from clean cans did not have any isolate growth. Appears that as time increased, a single colony morphology dominated the load microflora. The majority of isolates for our study are non proteolytic and non to weakly lipolytic; however, this is contingent on the milk source microbiota.

ACKNOWLEDGMENTS

This project was funded by Dairy Research Institute. Thank you to our industry partners for their insight, support, and collaboration. An additional thank you to Javier Gaspar-Hernandez for assisting with the microbiological testing.

REFERENCES

- Darchuk, E., Waite-Cusic, J., Meunier-Goodik, L. 2015. Effect of commercial hauling practices and tanker cleaning treatments on raw milk microbiological quality. J. Dairy Sci. 98:7384-7393.
- Kuhn, E., Meunier-Goddik, L., and Waite-Cusic, J. 2017. Impact of Milk Hauling Practices on Microbiological Quality. ADSA 2017. Pittsburgh, PA.

CONCLUSION

Worst-case hauling practices on a small-scale yields measurable contamination on subsequent milk loads. This proof-of-concept demonstrates the potential negative impact that commercial milk hauling could have on raw milk microbiological quality. Our findings paves the way to scale-up to a commercial tanker study (Kuhn et al., 2017)

2017 IFT Conference (Las Vegas, NV)

ADSA Annual Meeting, Pittsburgh, PA 2017

#70406

Impact of Milk Hauling Practices on Microbiological Quality

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ABSTRACT

The Pasteurized Milk Ordinance (PMO) allows for milk tanker trucks to be used repeatedly for 24 hours before mandatory clean-in-place (CIP) cleaning. There are no specifications for length of time a tanker can be empty between loads. We partnered with a Pacific Northwest dairy company to investigate if extended idle time between loads influences microbiological populations in subsequent loads of milk. This processor does not allow tanker trucks to sit idle between loads for more than 6 hours. Two farms were selected to participate in the study based historical microbiological data, quantified using Foss Bactoscan and reported as individual bacteria count (IBC) and preliminary incubation count (PIC). Historically, farm A had substantially poorer milk quality than Farm B. The study occurred over six consecutive days; for three days farm B milk was collected immediately after unloading Farm A, and the other three days farm B milk was collected 6 hours after unloading. Samples were obtained from each farm bulk tank and from the tanker prior to unloading. Each sample was microbiologically assessed for standard plate count (SPC), lactic acid bacteria (LAB), and coliforms. Colony isolates were assessed for lipolytic and proteolytic activity using spirit blue agar (SBA) and skim milk agar (SMA), respectively. We have demonstrated that the processor's current hauling parameters are adequate; there was not a significant difference in microbiological counts and enzyme activity in farm B's tanker sample when comparing 0 and 6 hours between hauling.

INTRODUCTION

Tanker use frequency greatly varies

The Pasteurized Milk Ordinance (**PMO**) does not specify a limit in either the number of farms nor number of loads for tanker use per 24 hours. Number of loads hauled per day and farms per load greatly varies. Depending on the route, daily tanker use ranges from single-use, high frequency short distance hauling to low frequency long distance hauling.

Industry has concerns about the potential negative contribution of low frequency hauling when the tanker may remain idle and "dirty" for extended periods of time. Tankers are well insulated when full of cold milk; however, during unloading the tanker must have air pulled through it to prevent implosion, this introduces outside ambient air that increases the inside temperature of the tanker.

Residual milk in the tanker harbors natural microflora that could rapidly multiply during this idle period and negatively influenced the microbial quality of subsequent loads of milk.

OBJECTIVE

Investigate if extended idle time between loads influences microbiological populations in subsequent loads of milk.

ACKNOWLEDGMENTS

The authors would like to thank our industry partners for their collaboration. This project was funded by Dairy Research Institute.

EXPERIMENTAL DESIGN

Creating a route using producer historical quality data

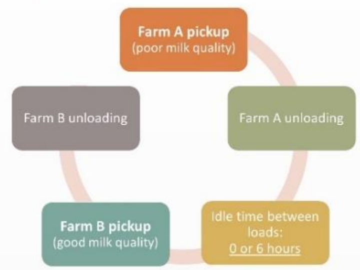


Table 1. Producers selected for route based on historical data analysis. A producer with historically high counts was selected for farm A to increase the likelihood of negatively impacting farm B milk quality.

Farm	Historic milk quality	n bulk tank samples (2 years)	Mean IBC*	Mean PI-IBC*
A	Poor	729	47.8	432.3
B	Good	982	8.8	13.2

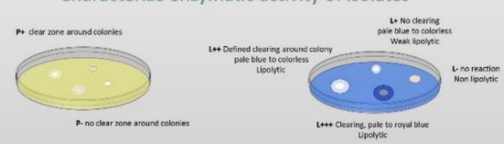
*Farm A Mean individual bacteria count (IBC) and preliminary incubation count (PI-IBC) is significantly greater than Farm B counts.

MATERIALS AND METHODS

Industry standard microbiological testing

Samples were enumerated for standard plate count (SPC), lactic acid bacteria count (LAB), and coliform count.

Characterize enzymatic activity of isolates



Skim milk agar (proteolytic) and **spirit blue agar** (lipolytic) were used to characterize proteolytic (P-/+) and lipolytic (L-/+/++/+++) activity of isolates from each sample location.

RESULTS AND DISCUSSION

Enumeration of milk samples

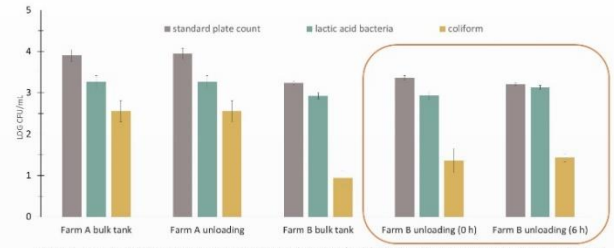


Figure 1. Farm A and Farm B mean microbial counts (LOG CFU/mL) for bulk tank and tanker unloading samples (n=6 per sample point). Farm B milk quality was not measurably impacted by 6 hours idle time. There is no significant difference in Farm B milk quality when compared to immediate (0 h) pickup. Error bars represent ± 1 SE.

Proteolytic and lipolytic enzyme activity

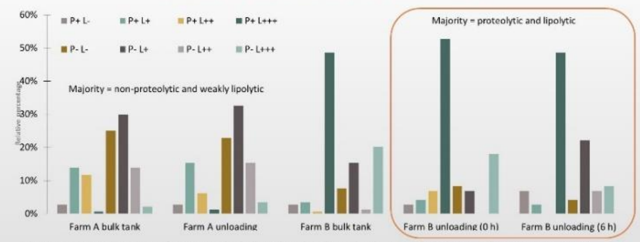


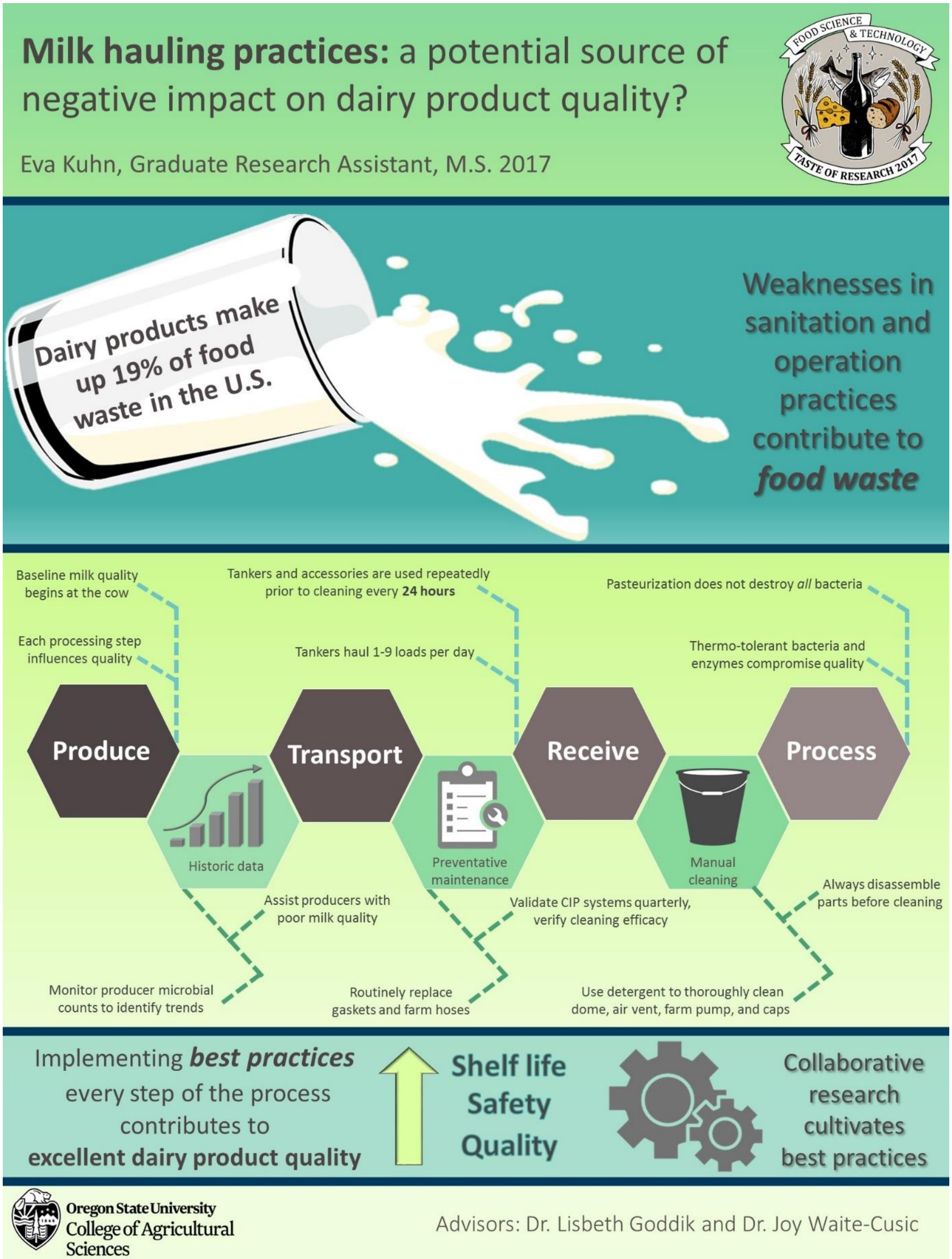
Figure 2. Enzymatic activity of Farm A and B bulk tank and tanker unloading sample isolates. Isolates are categorized based on both proteolytic and lipolytic activity and expressed as relative percent composition and count for a given sample set (n=144). Idle time (when tanker is empty and dirty) between loads did not impact Farm B milk quality.

Farm A milk quality was significantly poorer than Farm B's; however, Farm B's microbial population contained a larger portion of proteolytic and lipolytic bacteria. While the majority of bacteria are destroyed in pasteurization, their enzymes may be thermo-resistant and could potentially compromise shelf life and sensory qualities.

CONCLUSION

We have demonstrated that 6 hours between loads does not negatively impact subsequent loads of milk and that the processor's current hauling parameters are adequate to reduce this potential impact. Additionally, enzyme activity should be considered as another layer to determining raw milk quality.

2017 ADSA Conference (Pittsburgh, PA)



Milk hauling practices: a potential source of negative impact on dairy product quality?
Eva Kuhn, Graduate Research Assistant, M.S. 2017
FOOD SCIENCE & TECHNOLOGY
TASTE OF RESEARCH 2017
Dairy products make up 19% of food waste in the U.S.
Weaknesses in sanitation and operation practices contribute to food waste
Baseline milk quality begins at the cow
Each processing step influences quality
Tankers and accessories are used repeatedly prior to cleaning every 24 hours
Tankers haul 1-9 loads per day
Pasteurization does not destroy all bacteria
Thermo-tolerant bacteria and enzymes compromise quality
Produce
Transport
Receive
Process
Historic data
Preventative maintenance
Manual cleaning
Assist producers with poor milk quality
Monitor producer microbial counts to identify trends
Validate CIP systems quarterly, verify cleaning efficacy
Routinely replace gaskets and farm hoses
Always disassemble parts before cleaning
Use detergent to thoroughly clean dome, air vent, farm pump, and caps
Implementing best practices every step of the process contributes to excellent dairy product quality
Shelf life
Safety
Quality
Collaborative research cultivates best practices
Oregon State University
College of Agricultural Sciences
Advisors: Dr. Lisbeth Goddik and Dr. Joy Waite-Cusic

Hauling and Receiving Practices at Dairy Processing Facilities

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ABSTRACT

Milk hauling is an overlooked portion of the dairy industry and its impact on raw milk quality is not well characterized. Practices are mandated by the Pasteurized Milk Ordinance; however, gaps exist in the specifics of practices that could impact downstream milk and milk product quality. Description and classification of hauling and receiving practices and their relative impact could allow for the prioritization of improved practices that could improve quality throughout the dairy industry. The objective of this study was to identify current practices that could negatively impact the microbiological quality of raw milk during hauling and receiving. This objective was approached from two angles:1) an industry survey was conducted to characterize milk hauling and receiving practices, and 2) a database that represented two years of differences in IBC and PI (PIC) counts between receivers and producers (n =23,285 tanker loads) was analyzed to identify and quantify hauling situations that have a negative impact on milk quality. Dairy processing facilities (n = 14) were asked to participate in the survey, of which 10 responded (78% response rate). The majority of facilities utilized repeated tanker use per 24 hours; however, facilities that only receive a few tankers a day washed after each load. Frequency of CIP system validation greatly varied among facilities. This suggests that facilities that do not frequently validate could potentially have underlying CIP issues that could have a negative impact on tanker cleaning efficacy. For the database analysis, negative impact was defined as the top 2.5% of instances (n=583) where the tanker and producer load average difference was ≥7.67 IBC/mL and ≥61.5 PIC/mL. Negative impact was more pronounced in PI counts. There was not an identifiable trend in seasonality. The analysis demonstrated that in instances of negative impact, the load typically included milk from a producer with historically high counts. This study suggests that CIP validation frequency and route management may need increased attention to minimize the impact hauling and receiving practices have on raw and downstream product quality.

SCOPING THE PROBLEM

Milk quality begins at the farm and subsequent practices cannot improve milk quality; only implementing adequate sanitary handling and operating practices can mitigate negative impact. Negative impact related to hauling and receiving practices may be sporadic and difficult to pinpoint. Gaps exist in the Pasteurized Milk Ordnance (PMO), especially for practices pertaining to connection points between transportation. Better characterizing these practices can aid in contributing to better milk quality. Route management is another potential action that could mitigate negative hauling impact by reducing repeated tanker use between cleanings when these farms are included in routes.

OBJECTIVES

The objectives of this research were to:
1) Characterize industry hauling and receiving practices.
2) Identify and quantify negative impacts related to hauling from standard industry microbiological tests.

ACKNOWLEDGEMENTS

We would like to acknowledge DRI for funding this research. We also would like to thank our industry partners for their participation. We look forward to continuing to work with industry to contribute to a better understanding of milk hauling and receiving practices and their impacts on downstream milk quality.

2016 ADSA Conference (Salt Lake City, UT)

ASAS-ADSA-CSAS-WSASAS 2016 JAM
JULY 19 - 23, 2016 • SALT LAKE CITY, UTAH

Hauling and Receiving Practices at Dairy Processing Facilities

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OSU Oregon State UNIVERSITY

OBJECTIVE 1: METHODOLOGY AND FINDINGS

Industry Hauling and Receiving Survey

14 dairy processing facilities were either surveyed during onsite visit or by responding to a written questionnaire. Questions were based on type and frequencies of sanitation and operating procedures related to milk hauling and receiving practices.

Figure 2. Highlights of industry responses to milk hauling and receiving practices survey

"As needed". Processors reported that cleaning and replacement of parts such as gaskets, clamps, outlet valves, hose, and cleaning and sanitizing agents for manual cleaning addressed as needed. As needed is stated in the PMO but is subjective and needs to be better defined.

Cleaning frequency. Majority of facilities utilize repeated taker use per 24 hours, strengthens necessity to develop better knowledge on impact of repeated tanker use , which could be used to improve tanker related operational and sanitation practices.

Milk sampling technique. Stainless steel dippers are more commonly used for collecting tanker milk samples at receiving. Aseptic and inline samplers are alternative methods however; they are expensive and can skew microbial counts if the sample port is contaminated.

CIP system validations. Processors reported CIP validation frequencies ranging from daily to annually, indicating the commonly incorrect interchange between validation and verification activities. This highlights the importance of educating processors on using the correct terminology to improve communication between regulatory agencies.

OBJECTIVE 2: METHODOLOGY AND FINDINGS

Tanker and Producer Microbial Count Analysis

Analyzed two year dataset (n=23,285 tanker loads) obtained from an industry facility by comparing differences in IBC and PI counts for producers and corresponding tanker loads and characterized outliers (top 2.5%), defined by greatest difference in elevated tanker counts (tanker > producer average), indicating an increased microbial count during hauling.

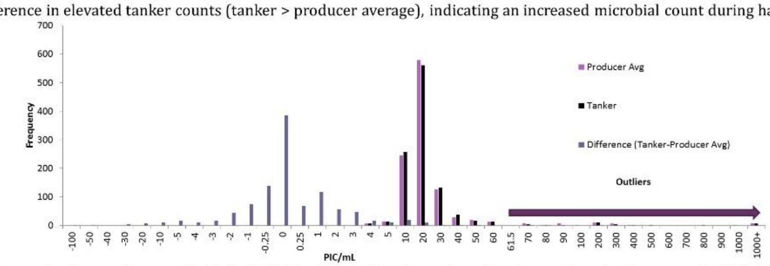

Figure 1. Example month (July 2015) of distribution of preliminary incubation counts (PIC/mL)

Producer average indicates average PIC/mL for producer samples for a given tanker load.

Table 1. Distribution of outliers for PIC 2014-2015 differences (tanker-producer average).

Seasonality did not have an impact on outlier distribution

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2014	6.92%	5.54%	0.79%	0.90%	5.74%	0.10%	0.76%	1.87%	0.63%	0.31%	0.33%	0.52%
2015	0.91%	0.43%	0.49%	0.31%	0.49%	0.00%	0.73%	0.19%	3.50%	12.96%	13.36%	5.02%

July 2015: **8/1089** tanker loads were outliers (>61.5 PIC/mL)

Historically high count producers make up **50%** of impacted tanker loads (range=1696-9508 PIC/mL).

Milk pickup frequency did not influence the likelihood of being a historically high count producer.

RECOMMENDATIONS

It is recommended that producers and processors should emphasize on continuous improvements to promote best practices in areas such as preventative maintenance, CIP validations, and consistency in standard operating procedures.

PLANNED FUTURE WORK

Investigate worst case scenario hauling situations and their impacts on downstream quality by measuring thermo-resistant enzyme activity of tanker microflora.