Short communication: Microbial quality of raw milk following commercial long-distance hauling

Emily M. Darchuk, Lisbeth Meunier-Goddik, and Joy Waite-Cusic¹

Department of Food Science and Technology, Oregon State University, Corvallis 97331

ABSTRACT

Hauling is a critical part of the commercial milk supply chain, yet very few studies have aimed to understand its effect on raw milk quality. This study focused on the effect of extended-duration tanker use during hauling on raw milk quality at a commercial facility. Standard tanker use [cleaned-in-place (CIP) once per 24 h] served as a control and an incremental between-load water rinse with sanitizer treatment (RS) was evaluated to mitigate any effect from extended duration hauling. During this study, 1 commercial truck with 2 trailers was monitored for 10 d. The truck collected milk at a large dairy farm, transported the milk to a manufacturing facility, and then returned to the same farm for a second load. Each round-trip journey took between 10 and 12 h, allowing for 2 loads per 24-h use period. Following the second delivery, the truck was cleaned by CIP treatment starting a new treatment day. Producer samples were collected from the raw milk bulk tank on the farm before loading milk into the tanker. The same milk was sampled directly out of the tanker truck before unloading at the manufacturer. Effect on individual bacteria count, thermophilic spore count, and preliminary incubation count was quantified through common industry tests. Surface sponge swabs were also used to monitor tanker sanitation and the efficacy of cleaning treatments. Results did not identify a negative effect on raw milk quality due to extended duration hauling. Whereas the addition of RS did not provide any measurable quality benefits for the microbial milk quality, swab results demonstrated that the RS treatment was able to reduce surface bacteria in the tanker, although not to the same level as the full CIP treatment. Based on this study, current CIP practices for long distance milk hauling appear to be effective in mitigating any measurable effect on raw milk quality.

Key words: hauling, milk tanker, bacteria, cleaning

Short Communication

Within the United States, all Grade A dairy products are regulated by the Pasteurized Milk Ordinance (PMO). As stated in the PMO, milk tanker trucks can be used repeatedly for a full 24 h between clean-in-place (CIP) treatments (Food and Drug Administration, 2013). Although individual truck utilization varies, routes can involve extended-duration hauls where each load of milk is hauled for long durations and only a few loads can occur per each 24-h period. Extended-duration hauls may also include situations where a truck remains soiled and empty for extended periods between loads. Residual milk remaining in the truck may lead to microbial growth, as well as the formation of biofilms that could negatively affect the microbiological quality of subsequent load quality (Teh et al., 2014), making extended-duration hauling an industry practice that poses potential risk to raw milk quality.

The objective of this study was to understand the effect of extended-duration hauling practices on raw milk microbiological quality within an industrial setting. The study was outlined to measure (1) the effect of hauling loads of milk for long distances during standard intervals between CIP treatments and (2) the effect of an incremental between-load water rinse with sanitizer treatment to remove milk residue.

This study was performed within the standard operations of a commercial dairy manufacturing plant. Samples were analyzed using microbiological methods commonly used by dairy manufacturers to ensure that the study was representative of industry practices. The study was conducted during summer to ensure maximum heat exposure. Average temperature was 23°C with a highest day temperature of 36°C and a lowest night temperature of 12°C.

Milk was hauled within 1 double trailer tanker truck with a flexible transfer hose to connect the 2 compartments. Each milk trailer consists of 2 cylinders fabricated from stainless steel metal sheets welded around a 38.1-mm polystyrene core, which acts both as support and as an insulator between the internal and external diameter of the tank. The trailers are not refrigerated, but instead rely on insulation alone to preserve milk

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¹Corresponding author: Joy.waite-cusic@oregonstate.edu

Table 1. Outline of milk and swab sample location, collection frequency, and analysis method

Sample location	Frequency	Analysis ¹
Producer milk	Duplicate samples were collected from every bulk tank that was loaded into tanker (n = 40)	IBC, TSC, PI
Tanker milk	Duplicate samples were collected from the front and back trailer of every tanker load that was delivered to plant (n = 80)	IBC, TSC, PI
Tanker surface swab	Swabs were taken from the front and back trailer ceiling ² following every load delivered to the plant. When a cleaning treatment occurred a second set of swabs were taken before a truck continued on route (n = 74).	APC
Rinse water	Samples were taken throughout the study from the clean-in-place tank or outlet pipe (n = 6)	APC

¹IBC = individual bacteria count (Bactoscan FC, Foss, Hillerød, Denmark); TSC = thermophilic spore count; PI = preliminary incubation count; TEMPO (bioMérieux, Marcy l'Etoile, France); APC = aerobic plate count (Petrifilm, 3M, St. Paul, MN).

²Location of swab sample rotated with every load and before and after cleaning treatment.

temperature. These trailers were transported by a truck that carried the transfer pump and hose, which loaded the milk from the farm bulk tank into the trailer compartment. Prior to the study, all equipment had passed regulatory inspection.

To reduce variability in producer milk quality, one route was repeated for the duration of this study. This route consisted of milk from 1 farm, which was collected twice daily. Prior to the first load, the truck underwent a CIP treatment at the manufacturing plant. Following CIP, the tanker would travel to the farm, which was located approximately 5 h away. All milk was loaded from a single bulk tank, filling both trailer compartments of the truck. Once loaded, the truck would return to deliver the milk to the same manufacturing plant. Following delivery of the first load, the truck would either return to the same farm without any cleaning treatment [standard use (SU)] or a water rinse followed by a sanitizing spray (RS) would occur before the second farm pick up. Milk picked up in the second load was from the same herd, but from a subsequent milking. Regardless of treatment, following the delivery of the second load the truck would undergo a CIP treatment and a new treatment day would begin. All cleaning treatments, including CIP, were conducted in the receiving bay of the plant immediately after unloading milk and before continuing on to the next load.

This study investigated the addition of a between-load water rinse (RS), which was incremental to the standard operating procedure of a 24-h CIP (SU). The truck underwent each treatment for multiple days, creating 7 replicated days for the RS treatment followed by 3 replicated days of the SU treatment over the 10-d study.

Incremental treatments were partial stages of the full CIP cycle that used existing chemicals, receiving bays, and equipment. The RS treatment consisted of 2 to 3 min of ambient water rinse followed by a sanitizing spray containing a blend of peroxyacetic acid and hydrogen peroxide (Oxonia Active, Ecolab US, St. Paul,

MN). Water samples were taken from the CIP system throughout the study to ensure no contamination of the tanker occurred due to the RS treatment.

Samples were collected daily (Table 1) at both the farm and plant. Milk was sampled from the raw milk bulk tank on the dairy farm and the same milk was sampled directly out of the tanker truck before unloading at the manufacturer. Prior to the study, training of both the receivers and haulers was conducted to ensure that sampling and cleaning procedures were consistent throughout the study. Samples were shipped by courier to the corporate laboratory once every 24 h and were tested upon arrival. All samples were kept below 7°C during storage and transport and were tested within 36 h of sampling.

Haulers followed PMO regulations when collecting producer samples from the farm bulk tank (Food and Drug Administration, 2013). Receivers took tanker samples using a sanitized stainless steel dipper from the top hatch of the front and back tanker trailer. A different dipper was used for each compartment of the truck to avoid cross contamination.

Sponge-stick swabs moistened with Lethen broth (3M US, St. Paul, MN) were used after unloading milk to measure residual bacteria left on the internal surface of tank. For every load, a 900-cm² area (30 × 30 cm) was swabbed per the manufacturer's instructions. Following CIP or RS treatment, a second swab was taken to measure the efficacy of the clean. Receivers were trained to rotate the area of the ceiling swabbed with each incoming load and before and after cleaning treatments.

Milk samples were evaluated using the same microbiological techniques as was described in detail within Darchuk et al. (2015). Briefly, all milk samples were analyzed for individual bacteria count (IBC), thermophilic spore count (TSC), and preliminary incubation (PI) most probable number. Individual bacteria counts of all milk samples were conducted using a Bactoscan FC (Foss, Hillerød, Denmark). Thermophilic spores

were quantified using the method described by Wehr and Frank (2004). Preliminary incubation was conducted by adding a diluted sample to a TEMPO Total Viable Count vial (bioMérieux; Marcy l'Etoile, France). The vials were incubated at $13 \pm 1^\circ\text{C}$ for 18 h followed by $32 \pm 1^\circ\text{C}$ for 48 h and enumerated using a TEMPO reader following the manufacturer's instructions.

Both the rinse water and sponge swabs were evaluated for aerobic plate count (**APC**) using Petrifilm (3M US) incubated at $32 \pm 1^\circ\text{C}$ for 48 h. Petrifilms were enumerated using an automated counter (3M Petrifilm reader).

The effect on milk quality due to extended-duration hauling was defined as the difference in the microbiological count between the producer sample (collected from the farm bulk tank) and the tanker milk sample (collected from the tanker following transport). Differences in the effect of hauling on raw milk quality between load 1 and 2 within a treatment (RS or SU) were then investigated and were considered significant at $P < 0.05$. Likewise, differences in the effect on raw milk quality due to the cleaning treatment (RS) compared with SU were considered significant at $P < 0.05$. All samples below the limit of detection for PI ($<1,000$ cfu/mL) and TSC (<10 cfu/mL) were scored as 500 and 5 cfu/mL, respectively.

Statistical analysis on the data was conducted using R software (R Development Core Team, 2013). The effect on tanker milk quality due to extended tanker use was determined through a Welch's *t*-test comparing the difference between the tanker and producer milk of the first and second load of milk for both the SU and RS treatment. The effect of cleaning treatment on tanker milk quality was determined through a Welch's *t*-test comparing the difference between the tanker and producer milk between treatments. Prior to conducting statistical analysis, results for duplicate samples from both front and back trailers were averaged so 1 tanker output value could be compared with the single producer input value for every load.

Producer milk quality during this study was good, with over 95 and 68% of the loads testing below 10,000 cfu/mL in IBC and PI, respectively (Figure 1). Statistical analysis found no significant differences in milk quality between pre- (producer samples) and posthauling (tanker samples) with *P*-values for differences in milk quality being $P = 0.228$ for IBC, $P = 0.7071$ for TSP, and $P = 0.7923$ for PI counts (Figure 2). Also, no significant difference in effect to milk quality was observed between load 1 and 2 in either the SU or RS treatments, demonstrating no difference between the pre- and posthauling milk quality over the course of a 24-h period.

Following CIP treatment, the average surface bacteria count of a clean truck before starting a route was $1.23 \log \text{cfu}/900 \text{cm}^2$. A tanker, after delivering 1 load of milk, had an average surface bacteria count of $4.00 \log \text{cfu}/900 \text{cm}^2$. The use of RS reduced the surface bacteria count to $2.68 \log \text{cfu}/900 \text{cm}^2$ (Figure 3), which was significantly different to the clean CIP surface ($P = 0.003$) and to the soiled surface ($P = 0.001$).

The difference in microbiological counts between the producer and tanker samples did not increase significantly between loads, suggesting that the extended use of a tanker truck has no effect on raw milk quality as measured by common industry test methods. No significant increase in microbiological counts over time was observed for the standard-use variable (CIP once every 24 h); therefore, the addition of an RS treatment provided no measurable benefit to raw milk quality. As the data provided no convincing evidence that hauling has a significant effect on milk quality in any of the microbiological parameters measured (IBC, TSC, PI), current industry practices based on PMO regulations appear to be effective (Food and Drug Administration, 2013).

A similar outcome was found when investigating frequent-use hauling, during which tanker trucks were used for the same period (up to 24 h between CIP), but each load of milk had a short duration within a tanker and more loads were hauled per day (Darchuk et al., 2015). It is reasonable to find similar results between frequent-use and extended-duration studies, as the lack of measureable effect from hauling is largely due to the low levels of residual milk remaining in the tank after pumping and the limited sensitivity of test methods used within industry. This suggests that, regardless of

Figure 1. Producer milk quality categorized as percentage of producer milk samples within individual bacterial count (IBC) and preliminary incubation (PI) most probable number quality categories ($n = 20$).

Figure 2. Effect of cleaning treatments on tanker microbiological growth. Raw milk individual bacterial count (IBC; a), preliminary incubation (PI) most probable number (MPN; b), and thermophilic spore count (TSC; c) results displayed as the average difference between the tanker and corresponding producer milk across treatments and each load within the 24-h cycle. Rinse and sanitize treatment was replicated 7 times and the standard use treatment was replicated 3 times. Both treatments consisted of 2 loads per 24-h use period with all milk sourced from the same farm.

the type of hauling, 24-h continual use of tanker trucks can occur without negatively affecting raw milk quality based on the metrics we investigated.

Within our study, swab data showed that RS treatments could reduce residual surface bacteria counts, but it was not as effective as a full CIP treatment. Although the RS treatment was able to reduce surface bacteria, there is not enough evidence to suggest that its use would provide a quality benefit to industry that would be worth the resource usage required to implement it after every load. This observation is based on hauling conditions where raw milk quality in subsequent loads is relatively similar. It is possible that an RS treatment could be beneficial in the scenario where the preceding load was of inferior quality. Thus, RS could be a useful tool when hauling deviates from general operating conditions. It is important to note that all chemicals should be used according to manufacturers' specifications.

Our study focused on milk used for powder production; TSC is of great concern in powder production because of the possibility for growth within processing systems. In future studies, it would be of interest to investigate microbiological quality of milk destined for other uses, including fluid milk. Quality of fluid milk would be greatly affected by other microbial organisms, such as psychrotrophs, which were indirectly estimated through the PI count.

Although biofilm formation within a tanker is a concern, the risk of its development within a truck is less than other areas of a dairy plant. Biofilm formation within a tanker is expected to be less likely due to the cold temperature, low shear, and smooth surface area that the raw milk is exposed to within a stainless steel

Figure 3. Before and after average surface bacteria count from the rinse and sanitize cleaning treatment. Data reported is the average aerobic plate count (APC) of a soiled tanker before ($n = 14$) and after ($n = 14$) a water rinse and sanitizer treatment as measured by sponge swab sampling. The dotted line represents the average starting APC count of a clean tanker post-clean-in-place (CIP) treatment ($n = 20$).

tanker truck (Marchand et al., 2012). Also, it is important to note that internal tanker temperature likely increased significantly during the second return to the dairy farm while driving empty and soiled. The risk of biofilm development is also managed through regular CIP treatments, but overall tanker sanitation is only as good as the efficacy of any given facility's regular practices. A previous study investigating plant-to-plant differences in tanker sanitation (Darchuk et al., 2015) found efficacy differences in CIP and incremental cleaning treatments between facilities. As biofilm-forming bacteria isolated from tanker trucks have been found to have negative effects on raw milk quality (Teh et al., 2014), variability in truck sanitation could lead to sporadic downstream milk quality issues that would be difficult for a company to trace.

Within our study, extended use of tanker trucks (24-h CIP) did not appear to have a negative effect on raw milk quality. The results of this study, along with a previous study investigating high-frequency hauling, suggest that regardless of how a tanker is used, 24 h of continuous use can occur without a negative effect on milk quality. Based on the results of our study, there is not sufficient evidence to suggest the addition of a between-load RS treatment could provide any operational benefit in terms of improved quality. We suggest that industry instead focus on effective CIP treatments at all manufacturing facilities.

As this study was an initial investigation, its goal was to provide a preliminary look into the effect of extended-duration hauling. Continued research into the effect of commercial hauling practices is recommended, as further investigation utilizing more sensitive test methods may find relevant effects that we were unable to detect, such as counts of psychrotrophs and lipolytic

bacteria, final product shelf-life (Teh et al., 2014), as well as flavor analysis of the milk. Based on the limited scope of our study and the variability in plant-to-plant operations, this sole study should not be used as a justification to reduce cleaning treatments at any individual facility.

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