Effect of leaving milk trucks empty and idle for 6 h between raw milk loads

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ABSTRACT

The US Pasteurized Milk Ordinance (PMO) allows milk tanker trucks to be used repeatedly for 24 h before mandatory clean-in-place cleaning, but no specifications are given 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 between loads, especially in warm weather. Initial studies were conducted using 5-gallon milk cans (pilot-scale) as a proof-of-concept and to demonstrate that extended idle time intervals could contribute to compromised raw milk quality. Based on pilot-scale results, a commercial hauling study was conducted through partnership with a Pacific Northwest dairy co-op to verify that extended idle times of 6 h between loads have minimal influence on the 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 commercially pasteurized whole milk (load 2) to measure cross-contamination. For the commercial-scale study, a single tanker was filled with milk from a farm known to have poorer quality milk (farm A, load 1), emptied, and refilled immediately (0 h) or after a delay (6 h) with milk from a farm known to have superior quality milk (farm B, load 2). In both experiments, milk samples were obtained from each farm's bulk tank and from the milk can or tanker before unloading. Each sample was microbiologically assessed for standard plate count (SPC), lactic acid bacteria (LAB), and coliform counts. Selected isolates were assessed for lipolytic and proteolytic activity using spirit blue agar and skim milk agar, respectively. The pilot-scale experiment effectively demonstrated that extended periods of idle (>3 h) of soiled hauling vessels can significantly affect the microbiological quality of raw milk in subsequent loads; however, extended idle times of 6 h or less would not measurably compromise milk quality in subsequent loads in commercial tankers.

Current tanker sanitation practices appear to be sufficient for maintaining raw milk SPC, LAB, and coliform levels, which are important measures of milk quality.

Key words: 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 use this automated method for pipelines and tanks. By the early 1960s, hauling transitioned from milk cans to bulk milk tanker trucks (Erba et al., 1995). 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 h before a mandatory clean-in-place cleaning (FDA, 2015). Repeated tanker usage maximizes milk collection efficiency and reduces water, chemical, and energy usage. Tanker usage frequency varies depending on size and location of the processing facility but can be typically categorized as (1) high-frequency hauling, where several loads will be collected within 24 h from producers within a short distance from the processing facility; and (2) low-frequency hauling, in which a tanker may remain empty and dirty (parked/idle or en route) for an extended period between loads (Darchuk et al., 2015a,b; Kuhn et al., 2018). Members of the dairy industry are concerned that extended periods between loads at elevated temperatures could promote microbiological growth in the tanker that could affect subsequent loads of milk.

The PMO hauling regulations have been demonstrated to be sufficient when standard practices are followed.

Received June 22, 2017.

Accepted October 18, 2017.

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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 and did not identify a measurable effect in subsequent loads for SPC, preliminary incubation count, or thermophilic spore count. Times between loads varied and were dependent on route management of participating industry members. Bulk farm and tanker milk samples were collected and tested in an attempt to determine potential impact; however, quantifying the negative effects of hauling on the commercial scale is challenging because of the typically low microbial load of raw milk coupled with the dilution factor (surface area-to-volume ratio) when the tank is refilled. Milk cans provide a pilot-scale simulation of a tanker due to their similar geometry and material surface (stainless steel). Milk cans lack insulation and have an increased surface area-to-volume ratio relative to a commercial tanker, which creates a situation where microbiological growth in residual milk will be maximized and could result in a measurable negative effect on quality of the subsequent load. We hypothesized that the negative effect of extended idle time between loads could be demonstrated in milk cans. These pilot-scale studies provided evidence to support a commercial-scale verification study to test the hypothesis that extended idle times of <6 h would not compromise raw milk quality.

MATERIALS AND METHODS

Experimental Design

Cross-Contamination in Milk Cans—Pilot Scale. Five-gallon (19 L) stainless steel milk cans (Hamby Dairy Supply, Maysville, MO) were used as hauling vessels to demonstrate the effect of the worst-case scenario on milk quality. Raw milk was collected from the Oregon State University (OSU) dairy farm (Corvallis). Before 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 min before unloading at OSU's Arbuthnot Creamery (Corvallis). The raw milk was held in the milk cans at ambient temperature (~27°C) for 1.5 h 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 (Liquid 90, Ecolab, St. Paul, MN) and sanitized (Mikroklene, Ecolab) or were left uncleaned. The empty milk cans (cleaned/sanitized and uncleaned) were then held at 30°C for up to 20 h. At predetermined time points (3, 6, 10, and

20 h), milk cans ($n = 3$ /time point per replicate) were filled with 19 L 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 before 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 h).

Milk cans were numbered and randomized for each sample day. Each time point was completed in triplicate, with 3 cans per replicate, totaling 9 cans per time point. For each day, 2 of the 8 cans served as a control. Microbiological analyses for this portion of the study were conducted in the Food Safety Systems laboratory at OSU.

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 Pacific Northwest dairy processing company. The truck would then either (1) immediately (within 30 min) collect milk from a second farm (farm B), or (2) stand idle and uncleaned for 6 h before collecting milk from the second farm (farm B). This experiment was repeated over the course of 6 consecutive days with the 2 scenarios (1 and 2) being randomized across the 6 d. Farms were selected based on previous trends in the microbiological quality of their raw milk (Table 1) with farm A being a historically high-count farm and farm B being a historically low-count farm (E. Kuhn, L. Meunier-Goddik, and J. G. Waite-Cusic, unpublished data).

Milk samples were collected using 90-mL sterile snap vials from the farm bulk tanks and tankers. All samples were stored at <4.4°C for up to 24 h before conducting microbiological testing. Microbiological testing took place in the Pacific Northwest dairy processing facility's quality laboratory.

Microbiological Testing. Milk samples were removed from 4°C storage and shaken thoroughly before 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, MN), tryptic soy agar (Acumedia), de Man, Rogosa, and Sharpe agar (Difco, Sparks, MD), spirit blue agar (SBA; HiMedia, Mumbai, India) with lipase reagent (Difco), and skim milk agar (SMA; HiMedia). Media were incubated and enumerated as shown in Table 2.

Colonies representing the morphological and enzymatic variability of microbial populations from SBA (n

Table 1. Historical microbiological data (24 mo; n = 106 farms) used to select route for industry hauling experiment

Farm	Daily milk production (kg)	N	Microbial test ¹	Mean ²	SD	Minimum	Maximum
A	7,700	729	IBC	31.7	47.8	10	1,101
(high-count producer)			PIC	66.5	432.3	8	10,676
B	9,000	982	IBC	8.8	9.0	2	209
(low-count producer)			PIC	13.3	72.5	2	2,081

¹Counts enumerated by FOSS BactoScan (Foss, Hillerød, Denmark) and reported as individual bacteria count (IBC/ μ L) and preliminary incubation count (PIC/ μ L).

²Farm A microbial counts (IBC and PIC) were significantly greater than farm B microbial counts ($P < 0.001$).

= 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 nonproteolytic (**P**−) based on SMA growth and classified based on a gradation of lipolytic activity on SBA from nonlipolytic (**L**−) to highly lipolytic (**L**+++). Following characterization, isolates were transferred to tryptic soy broth (Acumedia) and incubated at 32°C for 24 h. Broth cultures were mixed with an equal volume of 30% (vol/vol) glycerol in cryogenic tubes and stored at −80°C.

Data Analysis

Microbiological counts were calculated as colony-forming units per milliliter and log-transformed before statistical analyses. The significance of treatments was determined by comparing their microbiological counts using one-way ANOVA with post-hoc (Tukey) tests as appropriate. Statistical tests were performed using JMP Pro 12 (SAS Institute Inc., 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 pilot-scale (milk can) study had baseline bacterial counts of 3.9 ± 0.2 log cfu/mL (SPC), 2.9 ± 0.3 log cfu/mL lactic acid bacteria (**LAB**), and 2.4 ± 0.2 log cfu/mL coliforms (Figure 1). At the time of unloading following the emulated hauling scenario, 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, which 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-pasteurized whole milk was selected for load 2 because its reduced background microflora would increase the likelihood of detecting cross-contamination from load 1. The 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 (FDA, 2015). The SPC of the HTST milk used in this study was 1.5 ± 0.1 log cfu/mL, and

Table 2. Media types used to enumerate and characterize milk samples

Microbiological test	Medium	Incubation conditions	Growth interpretation
Standard plate count	Tryptic soy agar	32°C, 48 h	Enumerated all growth for SPC
Lactic acid bacteria	de Man, Rogosa, and Sharpe agar	32°C, 72 h	Enumeration of lactic acid bacteria
Coliforms	Coliform Petrifilm ¹	32°C, 24 h	Enumerated red colonies with gas bubbles as coliforms
Lipolytic bacteria	Spirit blue agar	21°C, 72–96 h	Nonlipolytic 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 halos deep royal blue color
Proteolytic bacteria	Skim milk agar	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

¹3M Petrifilm (St. Paul, MN).

Figure 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 dairy farm bulk tank; load 2 was commercially pasteurized (HTST) whole milk. Milk cans were incubated at 30°C for 3, 6, 10, or 20 h before the addition of pasteurized milk (load 2). Time points were randomly assigned and each sample time was completed in triplicate on 3 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 microbiological test; shared letters are not significantly different ($P > 0.05$).

LAB and coliform counts were negligible (0.1 and 0.0 log cfu/mL, respectively). As anticipated, emulated hauling had no effect 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 effect on the microbiological contents of the HTST milk (load 2) when milk cans were not cleaned and sanitized between loads (Figure 1). Extension of the incubation times of dirty milk cans before filling with load 2 led to significant increases in the bacterial counts (SPC, LAB, and coliforms; Figure 1). The SPC of load 2 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 occurred from load to

load. The SPC of load 2 following 3- and 6-h incubations did not differ significantly from one another ($P > 0.05$), implying that residual milk microflora had not yet reached the exponential growth phase. Load 2 SPC for 6, 10, and 20 h significantly increased with each incubation time point. The LAB counts followed a similar trend. Interestingly, coliform counts were undetectable (<1 cfu/mL) for HTST milk after a 3-h incubation of empty fouled cans, but increased steadily between 6 h (1.5 ± 0.3 log cfu/mL) and 20 h (5.1 ± 0.4 log cfu/mL). Measurable contamination between loads was demonstrated. These data demonstrate that increasing the time between milk collections can lead to microbial growth in the hauling vessel and could have a negative effect on subsequent loads.

Figures 2 and 3 display relative percentages of proteolytic and lipolytic activities, respectively, for isolates from each milk can sampling point ($n = 1,152$ total

Figure 2. Characterization of milk can microflora proteolytic enzyme activity ($n = 1,152$ total isolates), expressed as the relative total percentage for each milk sample category.

isolates). Isolate data are not shown for HTST whole milk (load 2) samples because counts on SMA and SBA were lower than the limit of detection (<1 cfu/mL). The majority of isolates at all time points were nonproteolytic and nonlipolytic to weakly lipolytic.

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 4). As expected, microbial counts of milk from farm A were similar before (farm A bulk tank) and after hauling (farm A unloading; load 1). The counts of raw milk from farm B for SPC, LAB, and coliforms 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 1), microbial counts from farm A's bulk tank raw milk were significantly higher than those from farm B's bulk tank raw milk throughout this study (P -values of 0.0003, 0.03, and <0.0001 , respectively, for SPC, LAB, and coliform counts). 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 log cfu/mL, 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 (combined 0-h and 6-h load 2 unloading) compared with bulk tank samples (farm B; 0.8 to $1.4 = 0.7$ -log increase; $P = 0.03$).

Proteolytic and lipolytic enzyme activities of isolates from farm A and farm B bulk tank and tanker unloading milk are displayed in Figures 5 and 6, respectively. Interestingly, microbial populations in raw milk from farm A and farm B exhibited distinctly different enzyme activity profiles (Fisher's Exact test 2-sided P -value <0.001). Farm A bulk tank isolates were predominantly nonproteolytic (70.6%) and nonlipolytic to weakly lipolytic (nonlipolytic = 27.8%, weakly lipolytic = 43.8%). The relative distribution of enzyme activity of isolates from farm A at bulk tank and unloading were nearly identical, as expected. The majority of farm B

Figure 3. Characterization of milk can microflora lipolytic enzyme activity ($n = 1,152$ total isolates), expressed as the relative total percentage for each milk sample category.

isolates were proteolytic (56.3%) and highly lipolytic (68.8%). This distribution was not significantly different at unloading (load 2), regardless of time between loads, indicating that load 1 did not significantly affect the distribution of microbial populations with these phenotypes. Given the dilution factor and the surface area-to-volume ratio, these data confirm that a 6-h idle time for tankers will not have a measurable effect on the microbial quality of raw milk of subsequent loads.

DISCUSSION

Our results from the pilot-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 se-

verity of contamination, except for SPC between 3 and 6 h. The dilution effect creates a challenge for hauling research because tankers are reported to retain ~0.02% residual milk volume (~8 L) after unloading, which is diluted with subsequent milk loads (Darchuk et al., 2015a,b). We overcame this challenge in our experiment because milk cans have a surface area (SA):volume (V) ratio 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) because the cans were constructed of a single layer of stainless steel without insulation. In contrast, commercial tankers are designed to insulate and maintain milk temperature, and are constructed of an internal stainless-steel tank layered with thick polystyrene and shelled with stainless steel. However, the internal temperature of a tanker can rapidly increase upon unloading and idle time,

especially because tankers require airflow to prevent implosion during unloading. Although tanker design is adequate for maintaining milk temperature when the tanker is full, Teh et al. (2011, 2012, 2013) reported that internal surfaces of empty milk tankers reached 20°C in New Zealand during summer months and demonstrated that tanker contaminants are capable of producing thermostable lipolytic enzymes at such temperatures. Our collaborating industry partner allows tankers to stand idle (empty and dirty) for a maximum of 6 h or they must be cleaned and sanitized before next use. The data from our pilot-scale and commercial-scale experiments demonstrate that this practice is 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 affect 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 depen-

dent 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 2 farms. Interestingly, farm A had significantly higher microbiological counts, but farm B's microbial population had a relatively higher proportion of isolates that demonstrated proteolytic and (or) lipolytic activity. Several psychrotrophic genera produce proteolytic and lipolytic enzymes, which can remain active after pasteurization. Both SMA and SBA were incubated at 21°C to isolate and enumerate organisms likely capable of producing these enzymes at cold temperatures (~7°C; Frank and Yousef, 2004). Although 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* spp. or *Acinetobacter* spp. and those that produce both lipolytic and proteolytic enzymes are typically *Microbacterium* spp. Isolates with proteolytic

Figure 4. 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; $n = 6$ sample days. Letters (a, b) indicate statistical differences between samples, within a microbiological test; shared letters are not significantly different ($P > 0.05$).

Figure 5. Commercial tanker study proteolytic enzyme activity. Proteolytic activity of isolates from farm A and farm B were significantly different (Fisher's Exact test; 2-sided P -value <0.0001). Bulk tank indicates samples were collected from the on-farm raw milk bulk tank (i.e., before hauling). Unloading indicates samples were collected from the tanker at the time of receiving at the commercial processing facility (i.e., after hauling). Load 1 indicates the first load of raw milk (from farm A) in the tanker. Load 2 indicates the second load of raw milk (from farm B) in the tanker. Time references indicate whether the tanker was refilled with load 2 after delivery of load 1. Empty/dirty indicates that the tanker was empty and dirty (no clean-in-place treatment) between loads 1 and 2.

activity alone are less common. Lactic acid bacteria (*Lactococcus* spp. and *Leuconostoc* spp.) do not typically produce significant proteolytic or lipolytic enzyme activity (Hantsis-Zacharov and Halpern, 2007).

Using microbiological media that demonstrate enzyme activity may be an important and meaningful tool for the dairy industry to characterize raw milk quality. Interpretation of proteolytic activity on SMA was straightforward. Freshly prepared SMA is opaque milky white in appearance. Nonproteolytic colonies do not change the appearance of the medium, whereas proteolytic colonies create transparent halo clearings under and around each colony. Interpretation of lipolytic activity on SBA is more subjective, and morphological interpretations vary in the literature. Prepared SBA is slightly transparent and light blue, and has a

sheen due to the presence of tributyrin (milk glyceride). The manufacturer of SBA (HiMedia) describes spirit 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-Zacharov and Halpern (2007) described a royal blue zone around colonies as being characteristic of lipolytic activity, whereas Frank and Yousef (2004) described clear zones with or without a deep blue color around or under each colony as indicating lipolytic activity. Trmčić et al. (2015) used a 3-point measurement scale: – for no visible zone of cleaning, + for <2 mm of clearing, and ++ for ≥ 2 mm of clearing; however, the color change of agar was not mentioned. In our preliminary work, we found that colonies produced 4 types of reactions on SBA that we described herein. We speculate that L+

likely represents weakly lipolytic or a false indicator of lipolysis because the medium becomes transparent around colonies but with no defined halo or clearing. In contrast, colonies that did not change the appearance of the medium were designated L⁻. Colonies were classified as L⁺⁺ if well-defined halos were apparent around each colony with a matte appearance in contrast to the sheen of the media's original appearance; L⁺⁺⁺ indicated a royal blue reaction, as described in the literature, although 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 accurate interpretation.

CONCLUSIONS

Raw milk was traditionally transported in 38-L milk cans and we demonstrated that the lack of insulation and increased surface area of milk cans resulted in measurable contamination with extended idle time between uses. Tankers have revolutionized dairy processing capabilities and are an essential component in the modern dairy industry. The PMO does not specify a maximum time that tankers can be empty and dirty between loads, just that they must be cleaned every 24 h. In our commercial study, we were able to demonstrate that a 6-h idle time between loads is unlikely to have measurable effect on subsequent load quality; however,

Figure 6. Commercial tanker study lipolytic enzyme activity. Lipolytic activity of isolates from farm A and farm B were significantly different (Fisher's Exact test; 2-sided P -value <0.0001). Bulk tank indicates samples were collected from the on-farm raw milk bulk tank (i.e., before hauling). Unloading indicates samples were collected from the tanker at the time of receiving at the commercial processing facility (i.e., after hauling). Load 1 indicates the first load of raw milk (from farm A) in the tanker. Load 2 indicates the second load of raw milk (from farm B) in the tanker. Time references indicate whether the tanker was refilled with load 2 after delivery of load 1. Empty/dirty indicates that the tanker was empty and dirty (no clean-in-place treatment) between loads 1 and 2.

as observed in the milk cans, the severity of the negative impact increases with time. This is the first study to demonstrate a measurable negative effect on raw milk microbiological quality (SPC, LAB, and coliforms) due to hauling. Although this effect was measured in the pilot-scale experiment, this proof-of-concept study allowed us to estimate the potential negative impact in commercial tankers under worst-case conditions. Additionally, it is necessary to explore better options for rapidly assessing raw milk quality; specifically, a means for rapid proteolytic and lipolytic enzyme analysis could be a beneficial approach for the dairy industry.

ACKNOWLEDGMENTS

The authors thank Dairy Research Institute (Rosemont, IL) for funding this project. We also thank our industry partners for participating in our research, and the OSU Arbuthnot Dairy Center for use of their facility. Thank you to Javier Jaspar-Hernandez and Julia Jones (Department of Food Science and Technology, Oregon State University, Corvallis) for assisting with microbiological testing.

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