Field Validation of a Milk Line Sampling Device for Monitoring Milk Quality and Udder Health

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ABSTRACT

The objective of this study was to investigate the ability of a milk line sampling device to obtain a representative sample by comparing SCC and bacterial culture results between milk line and bulk tank samples for milk harvested from the same group of cows at the same milking. A total of 42 paired milk line and bulk tank samples were collected at separate milking events from 21 different herds. Concordance correlation coefficients showed a high level of agreement between the two sample types, with values ranging between 0.74 and 0.99 for all parameters and bacterial species measured. ANOVA showed that SCC and bacterial culture results for Streptococcus agalactiae, Staphylococcus aureus, Streptococcus non-agalactiae, Coliforms, and coagulase-negative staphylococci were neither numerically or statistically different between milk line and bulk tank samples. KAPPA analysis showed that overall agreement beyond chance between milk line and bulk tank samples in determining whether a herd was positive or negative for either Strep. agalactiae or Staph. aureus were 100 and 75%, respectively.

While further research is needed to fully assess the utility of this tool for the purpose of bacterial culture, the results of this study suggest that the strategy of milk line sampling is a very promising monitoring tool. This sampling strategy should provide producers with inexpensive and timely information that will help to improve programs for monitoring milk quality and udder health in commercial dairy herds.

(Key words: milk line sampling, somatic cell count, bacterial culture, mastitis)

INTRODUCTION

Dairy producers, veterinarians, milk quality specialists, and milk processors have a wide range of monitoring tools and sampling strategies available for the purpose of monitoring udder health and milk quality in dairy herds. The use and interpretation of individual cow or bulk tank SCC data have been well described and widely adopted by the dairy industry, and therefore, will not be reviewed in this paper (Dohoo and Meek, 1982; Leslie et al., 1983; Hueston and Heider, 1984; Reneau, 1986; Fetrow et al., 1987; Fetrow et al., 1988; Azzam et al., 1989; Radostits et al., 1994; Kelton and Godkin, 2000). Another monitoring tool, bacterial culture, has traditionally been performed using either quarter samples, cow-composite samples, or bulk tank samples. The use and interpretation of quarter or cow-composite samples for bacterial culture have also been well described (Radostits et al., 1996; Kelton and Godkin, 2000), and will not be reviewed here.

Bulk tank culture results have been criticized as having a relatively low sensitivity for detecting contagious mastitis pathogens, such as Streptococcus agalactiae or Staphylococcus aureus, within the herd (Bartlett et al., 1991; Godkin and Leslie, 1993). It has been suggested that this low sensitivity may be improved by performing repeated cultures over time, by plating larger volumes for culture, or by using enhanced culture methods (Farnsworth, 1992; Godkin and Leslie, 1993; Kelton and Godkin, 2000). Despite the limitation of low test sensitivity, bulk tank cultures have a very high test specificity for contagious mastitis pathogens. As such, they are generally acknowledged to be a useful screening tool because a positive culture for either Strep. agalactiae, Staph. aureus, or Mycoplasma bovis is a reliable indicator of IMI (Gonzalez et al., 1986; Pankey et al., 1987; Bartlett et al., 1991; Godkin and Leslie, 1993; Farnsworth, 1993). As for predicting the number of infected quarters or cows, while one study demonstrated a moderate-to-high correlation ($r^2 = 0.71$) between the bacterial counts (cfu/ml), and the percentage of infected cows shedding Strep. agalactiae (Gonzalez et al., 1986), bacterial counts, interpreted by themselves, are generally not highly predictive of the prevalence of infected quarters or cows within the herd, particularly for Staph. aureus and M. bovis (Gonzalez et al., 1986; Godkin and Leslie, 1993).
Bulk tank cultures also have strengths and weaknesses as a monitor for environmental mastitis. The primary reservoir for environmental pathogens is not the infected mammary quarter, but rather the soil, bedding, and manure from the cow’s environment which contaminates the teat skin before milking (Smith et al., 1987). Therefore, while the environmental bacteria counts from bulk tank cultures are acknowledged to come, in part, from IMI (Smith et al., 1985; Godkin and Leslie, 1993), the correlation between bulk tank environmental bacteria counts and the prevalence of environmental IMI has generally been reported to be poor or zero (Gonzalez et al., 1986; Hogan et al., 1988; Godkin and Leslie, 1993). More useful, perhaps, is that bulk tank environmental bacteria counts have been correlated with factors related to the general level of environmental hygiene (e.g., bedding, cow cleanliness, and milking system cleaning and sanitation) and the adequacy of udder preparation procedures, i.e., cleanliness of teat skin at time of attachment of the milking unit (Cullen, 1966; Eberhart et al., 1979; Galton et al., 1982; Gonzalez et al., 1986; Panki et al., 1987; Hogan et al., 1988). In particular, Galton et al. (1982) demonstrated that premilking udder preparation is a very important factor in determining the level of contamination of milk with environmental bacteria. The milking machine is a great washing machine to remove bacteria from the teat skin. Thus, environmental bacterial counts from bulk tank cultures may be most useful to producers as a tool to monitor environmental hygiene and to monitor whether there is adequate cleaning and drying of teat ends before attaching the milking unit (Pankey et al., 1987; Farnsworth, 1992).

Sampling strategies for monitoring SCC or bacterial culture data have traditionally been limited to either individual cow samples (e.g., SCC from samples collected by DHIA field staff on test day, or bacterial culture results collected aseptically at the quarter or cow-level) or bulk tank samples (e.g., SCC routinely tested and reported by milk processors after each pick-up or bacterial cultures). Both these sampling strategies come with their own set of advantages and disadvantages.

Advantages for measuring individual cow SCC results from samples submitted through DHIA testing include the ability to monitor individual cow performance, to calculate and monitor group or herd average performance, and to monitor variation among individuals or groups of cows over time. A major disadvantage of this sampling strategy, however, is the relatively infrequent testing schedule (usually once/month or less frequently), in which case producers may be unable to quickly detect and respond to changes in animal or group performance. However, more frequent individual cow testing through DHIA to provide more timely information is probably too labor-intensive and costly to warrant routine adoption. Similarly, while individual quarter or composite-cow sampling for bacterial culture is useful both to describe the etiology of clinical mastitis in the herd and to identify cows infected with contagious mastitis pathogens for the purpose of specific cow-level interventions (e.g., treatment, segregation, or culling), this process can become labor-intensive, time-consuming, and costly if performed routinely as part of a surveillance system for the entire milking herd (Kelton and Godkin, 2000).

Similarly, the strategy of sampling bulk tanks for either SCC determination or bacterial culture also carries its own set of advantages and disadvantages. Because bulk tank analysis of SCC is provided with every shipment of milk, producers receive frequent, timely, and inexpensive information about the average herd performance. However, because the analysis is of a single pooled sample, producers are unable to accurately measure the average performance of any one group of cows and are unable to measure variation among different groups. Also, while the presence of a positive culture for contagious mastitis pathogens such as Strep. agalactiae, Staph. aureus, or M. bovis may indicate that at least one cow in the herd is infected and shedding at high enough levels to be detected, the bacteria count, interpreted by itself, usually does not give producers any estimate for the prevalence of infected cows or quarters in the herd, nor does it help to narrow the search for infected cows without performing individual cow cultures on the entire milking herd.

More recently, some larger herds have begun to adopt a new sampling technique, the milk line sampling of individual groups of cows. This sampling device is typically placed in the milk line past the receiver and past the plate cooler, if one is present in the system. Milk line sampling may be particularly attractive to larger herds because it allows producers to monitor the performance of several different groups of cows within the herd. And, because relatively few samples are submitted at any one milking, the overall program cost is relatively inexpensive. As such, producers can opt for more frequent sampling schedules. This should improve the effectiveness of the monitoring program by allowing the producer to more quickly detect changes in any one group’s performance.

Milk line sampling may have tremendous potential to improve our monitoring programs by compensating for some of the disadvantages previously described for sampling at either the individual-cow level or the bulk tank level. However, little independent research has been performed to ensure that the sampling systems currently available can collect a sample that is truly
representative of all the cows in a particular group of interest. If the milk line sampler were functioning properly and collecting a consistently metered sample, one would expect that the SCC and bacterial culture results from the milk line sample should be nearly identical to the milk entering the bulk tank from that same group of cows. The objective of this study was to determine whether a milk line sampling device could obtain a representative sample by comparing SCC and bacteriological culture results between milk line and bulk tank samples, for milk harvested from the same group of cows at the same milking.

MATERIALS AND METHODS

Herd Enrollment and Sampling Period

Twenty-one dairy herds in Minnesota and western Wisconsin were selected to participate in the study based on their willingness to participate, and to achieve a broad range of SCC levels and mastitis pathogen profiles. In particular, special effort was made to include a spectrum of herds both infected with, and free of, the contagious mastitis pathogens *Strep. agalactiae* and *Staph. aureus*. Sample collection occurred between February and August 2001. Repeated sampling occurred at separate milkings in some herds.

Sample Collection and Analysis

Sampling occurred during the milking immediately following a previous milk pick-up (i.e., sampling started with an empty bulk tank). Before beginning milking, the QMI Safe Septum Sani-Elbow (Quality Management, Inc., Oakdale, MN) was placed in the milk line past the receiver jar and past the plate cooler, if one was present in the system. The sampler was positioned such that the sampling port was on the bottom or side of the line, where possible. A 16-gauge, 1.5-inch needle was placed through the sampler diaphragm with the bevel of the needle toward the flow of milk in the line. If the port could only be positioned on the top of the line, a 16-gauge, 3-inch needle was used to reach across the elbow and allow the bevel opening to be on the bottom of the line, in order to get a sufficient flow rate. A sterile fluid administration set was then attached to the needle to collect milk, by gravity flow, into a sterile collection container (sterilized bag or 1-gallon bottle). The flow regulator on the fluid line was used to establish a consistent flow rate throughout the sample collection process. Flow regulation was handled differently depending on whether the flow in the line was intermittent or continuous: If milk flow was continuous, a steady drip or stream was collected. If milk flow was intermittent, flow regulation was set so that approximately 10 to 15 ml was collected each time the milk pump operated. A total sample volume of 200 ml or greater was collected, at an even sampling rate, over the course of the entire milking. Samples were kept cool while being collected, by placing the collection container in an insulated cooler surrounded by ice or ice packs. Upon completion of the milking process, the collection container was mixed thoroughly and a subsample was collected from the collection container into a sterile 30-ml vial. A bulk tank sample was also collected into a sterile 30-ml vial.

Upon completion of collection, the paired samples were refrigerated and then transported directly to the Udder Health Laboratory at the University of Minnesota. Here, one subsample from both the line and bulk tank samples was submitted directly to the DQCI Laboratory (Dairy Quality Control Institute, Mounds View, MN) for routine analysis of SCC with a Bentley somatic cell count instrument based on flow cytometry. The second subsample for each sample type was used for bacteriological culture for *Strep. agalactiae*, *Staph. aureus*, *Strep. non-agalactiae*, coliforms, and coagulase-negative staphylococci.

Microbiological Analysis

Each subsample of milk was kept cold during all analysis procedures. The sample was mixed thoroughly, and a 1:10 dilution was made in sterile brain/heart infusion broth. Two hundred microliters of undiluted milk was placed on each of the following media: factor (selects for gram-positive bacteria and identifies *Staph. aureus* readily), MTTK (selects for streptococci only and identifies *Strep. agalactiae*), and MacConkey agar plates. The 200 μl was spread evenly over the surface to the petri plate with an L-shaped daily rod. Next, 200 μl of the 1:10 dilution was placed on a second set of plates and spread evenly over the entire surface of the respective plate. Both sets of plates were incubated at 37°C. If the bacteria could not be accurately counted using the above dilutions, an additional dilution was performed.

After 24 h of incubation, the plates were removed from the incubator and allowed to stand for 30 min at room temperature to allow the esculin reaction to occur in the TKT plates. Each species of bacteria was counted on all inoculated plates and recorded. The colonies on each plate were counted again and recorded after an additional 24 h of incubation (48 h).

The counts were averaged to obtain an organism count per milliliter for each bacteria species. The identity of any streptococcal organism that was in question was identified using biochemical reactions or group specific antisera. Staphylococci were identified using coagulase reaction, biochemical tests, or latex agglutination.
test specific for *Staph. aureus*. Gram-negative bacteria were identified using biochemical reactions.

**Statistical Analysis**

Descriptive statistics were derived for both milk line and bulk tank samples, describing the mean, variation, and range for SCC, LS, and both the cfu/ml and Log₂ (cfu/ml) for *Strep. agalactiae*, *Staph. aureus*, *Strep. non-agalactiae*, coliforms, and coagulase-negative staphylococci. Whereas a log-base 10 transformation is commonly used for transforming nonnormally distributed data, the authors used a log-base 2 transformation for both SCC and bacteria counts, as people working with udder health data are more familiar with this calculation (e.g., is used for determining LS).

A concordance correlation coefficient was calculated to measure agreement between the two sample types for each parameter or pathogen, and a scatter plot was created for LS and Log₂ (cfu/ml) for each bacterial species, plotting milk line against bulk tank sample results. Next, ANOVA (Proc Mixed in SAS, Version 8.1, 2001) was used to determine, for each parameter or pathogen of interest (outcome variable), whether the measure differed depending on the sample type (explanatory variable). In addition to the main effect of sample type (milk line vs. bulk tank sample), each model was also controlled for random herd effects, given that repeated sampling had occurred in some of the study herds. Statistical significance for all comparisons was set at $P < 0.05$.

Finally, a $2 \times 2$ KAPPA value was calculated to measure overall agreement beyond chance, between milk line and bulk tank samples, in determining whether a herd was positive or negative for either *Strep. agalactiae* or *Staph. aureus*.

**RESULTS**

A total of 42 paired samples were collected from 21 herds, with a mean of two separate sampling events occurring for each herd (median = 1, SD = 2.7, range = 1 to 12). The final number of paired samples collected in this study should have enabled us to detect the following minimum differences between bulk tank and line sample results, should a difference have truly existed:
- LS: difference of >0.50
- Log₂ (*Staph. aureus*, *Strep. non-ag.* or coagulase-negative staphylococci): difference of >1.0
- Log₂ (*Strep. agalactiae* or Coliforms): difference of >2.0

Descriptive statistics for both milk line and bulk tank samples, describing SCC, LS, and both cfu/ml and Log₂ (cfu/ml) for each bacterial species are presented in Table 1. Ten of the 21 study herds and 18 of the 42 samples collected were positive for *Strep. agalactiae*, based on bulk tank culture. Sixteen of the 21 study herds and 31 of the 42 samples collected were positive for *Staph. aureus*, based on bulk tank culture. Concordance correlation coefficients comparing bulk tank versus line samples showed a high level of agreement between the two sample types, with values ranging between 0.74 and 0.99 for all parameters and bacterial species measured (Table 1). ANOVA, while controlling for repeated measures at the herd level, indicated no difference between bulk tank and line sample results for either SCC, LS, or any bacterial species studied ($P > 0.05$; Table 1). Note that the ANOVA results reported in Table 1 considered analysis of all 42 paired samples for all outcome parameters of interest. ANOVA was also performed separately for only those samples that were positive for either *Strep. agalactiae* ($n = 18$ paired samples) or *Staph. aureus* ($n = 31$ paired samples) based on bulk tank culture. The results of the latter analysis showed no association between sample type and either Log₂(*Strep. agalactiae*) (estimate = −0.083, SD = 0.38, $P = 0.83$) or Log₂ (*Staph. aureus*; estimate = 0.011, SD = 0.35, $P = 0.98$). Concordance correlation values between the two sample types for only those samples that were positive for either *Strep. agalactiae* or *Staph. aureus* were 0.97 and 0.89, respectively. The $2 \times 2$ KAPPA values showed that overall agreement beyond chance between milk line and bulk tank samples in determining whether a herd was positive or negative for either *Strep. agalactiae* or *Staph. aureus* were 100 and 75%, respectively.

Graphs plotting bulk tank versus line sample results for LS and for Log₂ (cfu/ml) of *Strep. agalactiae*, *Staph. aureus*, *Strep. non-agalactiae*, Coliforms, and coagulase-negative staphylococci are presented in Figures 1 through 6, respectively.

**DISCUSSION**

SCC and bacterial culture results from milk line and bulk tank samples were compared both from a scientific perspective to describe numerical and statistical differences, as well as from the perspective of a producer interpreting and making management decisions with either raw or categorized data. High concordance correlation coefficients indicated that agreement between the two sample types ranged between good and excellent for the various mastitis pathogens of interest. Similarly, when reviewing either the simple or the adjusted (estimated) difference of means (estimates from ANOVA), only a very small, statistically nonsignificant ($P > 0.05$), difference was seen to exist between milk line and bulk tank sample results for any of the parameters examined (Table 1). The $2 \times 2$ KAPPA values showed
Table 1. Comparison of SCC, LS and bacterial culture results between milk line and bulk tank milk samples.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample Type</th>
<th>Mean (± SD) (range)</th>
<th>Concordance correlation coefficient</th>
<th>ANOVA Results est. difference (± SD) (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bulk tank</td>
<td>Milk line</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCC (× 1000 cells/ml)</td>
<td>289 (175)</td>
<td>286 (162)</td>
<td>0.92</td>
<td>-3.05 (16.82) (0.86)</td>
</tr>
<tr>
<td></td>
<td>(2-794)</td>
<td>(81-753)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS</td>
<td>4.22 (1.10)</td>
<td>4.28 (0.85)</td>
<td>0.79</td>
<td>0.066 (0.15) (0.95)</td>
</tr>
<tr>
<td></td>
<td>(0.6-99)</td>
<td>(2.7-5.91)</td>
<td></td>
<td></td>
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<tr>
<td>Raw bacteria counts (cfu/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Strep. agalactiae</em></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>2347 (7746)</td>
<td>2559 (9020)</td>
<td>0.88</td>
<td>156.67 (888) (0.96)</td>
</tr>
<tr>
<td></td>
<td>(0-84,000)</td>
<td>(0-48,000)</td>
<td></td>
<td></td>
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<tr>
<td><em>Staph. aureus</em></td>
<td>104 (252)</td>
<td>73 (162)</td>
<td>0.89</td>
<td>-31.81 (28.62) (0.27)</td>
</tr>
<tr>
<td></td>
<td>(0-1350)</td>
<td>(0-850)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Strep. non-agalactiae</em></td>
<td>1362 (1681)</td>
<td>1432 (2084)</td>
<td>0.87</td>
<td>46.53 (238.0) (0.85)</td>
</tr>
<tr>
<td></td>
<td>(103-6600)</td>
<td>(90-11,600)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coliforms</td>
<td>3576 (13,211)</td>
<td>2350 (7031)</td>
<td>0.74</td>
<td>-1295.52 (1620.78) (0.43)</td>
</tr>
<tr>
<td></td>
<td>(0-64,000)</td>
<td>(0-89,200)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>160 (239)</td>
<td>144 (175)</td>
<td>0.92</td>
<td>-16.56 (15.74) (0.29)</td>
</tr>
<tr>
<td></td>
<td>(38-1600)</td>
<td>(15-1200)</td>
<td></td>
<td></td>
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<tr>
<td>Log₂ (bacteria counts (cfu/ml))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Strep. agalactiae</em></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>2.34 (3.60)</td>
<td>2.36 (3.57)</td>
<td>0.99</td>
<td>-0.009 (0.17) (0.96)</td>
</tr>
<tr>
<td></td>
<td>(0-11.41)</td>
<td>(0-11.90)</td>
<td></td>
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</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>1.43 (1.87)</td>
<td>1.29 (1.69)</td>
<td>0.93</td>
<td>-0.12 (0.27) (0.65)</td>
</tr>
<tr>
<td></td>
<td>(0-6.75)</td>
<td>(0-6.09)</td>
<td></td>
<td></td>
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<tr>
<td><em>Strep. non-agalactiae</em></td>
<td>5.91 (1.55)</td>
<td>5.85 (1.66)</td>
<td>0.94</td>
<td>-0.09 (0.16) (0.57)</td>
</tr>
<tr>
<td></td>
<td>(3.04-9.04)</td>
<td>(2.85-9.86)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coliforms</td>
<td>2.24 (3.32)</td>
<td>3.61 (3.15)</td>
<td>0.84</td>
<td>0.29 (0.39) (0.47)</td>
</tr>
<tr>
<td></td>
<td>(0-12.32)</td>
<td>(0-11.61)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>3.16 (1.06)</td>
<td>3.11 (0.99)</td>
<td>0.77</td>
<td>-0.043 (0.16) (0.79)</td>
</tr>
<tr>
<td></td>
<td>(1.60-6.70)</td>
<td>(0.26-6.58)</td>
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</table>

that good-to-excellent agreement existed between milk line and bulk tank samples in determining whether a herd was positive or negative for either *Strep. agalactiae* (KAPPA = 100%) or *Staph. aureus* (KAPPA = 75%). These test characteristics indicate that milk line samples should produce very similar conclusions regarding the group status for contagious mastitis pathogens. Sampling strategies for monitoring either SCC or bacterial culture data, as part of herd programs to monitor udder health and milk quality, have traditionally

Figure 1. Bulk tank versus milk line sample results for linear score.

Figure 2. Bulk tank versus milk line sample culture results for *Streptococcus agalactiae*.
been limited to either individual cow samples (e.g., SCC from samples collected by DHIA field staff on test day, or bacterial culture results collected aseptically at the quarter or cow-level) or bulk tank samples (e.g., SCC routinely tested and reported by milk processors after each pickup or bacterial cultures). The advantages and disadvantages of these sampling strategies were discussed in the introduction. The milk line sampling strategy addresses many of these aforementioned limitations, and therefore, has the potential to be a useful additional surveillance tool to improve udder health and milk quality monitoring programs in a variety of ways. First, it should allow producers to conveniently monitor the performance of several different groups of cows within the herd, something that cannot be achieved from a pooled bulk tank sample. And, because relatively few samples are submitted at any one milking, a routine testing program is relatively inexpensive. As such, producers can opt for more frequent sampling schedules. More frequent monitoring should result in an increased sensitivity, or ability to detect a recent change in, any one group’s performance, whether that be evidence that a recently implemented strategy has been effective in solving a problem, or detecting a breakdown in the control strategy (Kelton and Godkin, 2000).

A second potential benefit of milk line sampling may be an increased overall test sensitivity for the detection of contagious mastitis pathogens, such as Strep. agalactiae, Staph. aureus, or M. bovis, given that there will
be less of a dilution effect in a milk line sample (i.e., sample only one group of cows) as compared to the significantly larger dilution effect produced in a bulk tank sample (i.e., sample the entire milking herd). This hypothesis requires further investigation.

A third particularly useful aspect of milk line sampling may be its use to narrow the search for cows that are infected with contagious mastitis pathogens to their group of origin. Historically, if a producer identified the presence of a contagious mastitis pathogen in the herd through a positive bulk tank sample, finding those individual infected cows for a specific management intervention (i.e., treatment, segregation, or culling) would require a whole herd culture of all individual cows in the milking herd, an expensive, labor-intensive, and time-consuming process. If the culture of milk line samples from individual groups could be used to narrow the search to one or two positive groups, this would have the potential to save the producer considerable time, labor, and expense.

A fourth potentially very useful aspect of milk line sampling may be to use counts or concentrations of environmental bacteria, such as coliforms or Strep. non-agalactiae, as a tool to monitor changes in environmental hygiene and milking management. One would expect that the same relationships would hold true for milk line samples as exist between bulk tank culture results and both environmental hygiene and milking procedures (reviewed in introduction). More specifically, if environmental hygiene (e.g., bedding maintenance, environmental cleanliness, and adequacy of system cleaning) is held relatively constant for at least a short period of time, then comparing environmental bacteria counts from milk line samples among individual milkings (e.g., a.m. vs. mid-day vs. p.m.) may be a particularly useful tool to monitor adequacy of udder preparation procedures both between different milking shifts and within the same milking shift over time.

A potential limitation of milk line sampling that was not investigated in this study because the samples collected represented an entire milking is the possible limited opportunity for the carryover of milk in the line from a previous group to comingle with milk from the current group of cows being sampled, potentially leading to inaccurate results. It is likely that a relatively small volume of carryover milk, once diluted with the relatively large volume collected from the current group, is unlikely to greatly influence either SCC results or the presence or level of counts of environmental pathogens such as Strep. non-agalactiae or coliform species. However, the issue of carryover milk could be very important when considering its potential impact on the accuracy of milk line culture results (i.e., presence or absence) for the contagious mastitis pathogens, Strep. agalactiae, Staph. aureus, and M. bovis. If a group sample is culture positive for a contagious mastitis pathogen, it may be difficult to differentiate whether those bacteria came from the current group, from carryover milk from the previous group, or both. If false positives do occur due to contamination of the current sample with positive milk from the previous group, the specificity of using line sampling as a test to detect these mastitis-contagious mastitis pathogens in a given group would be reduced. One potential method of minimizing or preventing such carryover from occurring might be to allow the milk line to be flushed with milk from the current group for a few minutes, before opening the flow regulator on the fluid line to start collecting a new sample from the current group. However, while this technique might be successful in improving the specificity of the test (i.e., avoid false positives), it could result in a reduction in sensitivity (i.e., produce false negatives). For example, if the first few cows milked in the current group, and whose milk was flushed through without being sampled, happened to be the only cows in the current group sampled that were infected with one of the contagious mastitis pathogens of interest, then the group would incorrectly be identified as negative. The impact of carryover milk between successive groups, and the accuracy and subsequent interpretation of milk culture results from milk line samples, should be addressed, if possible, in future studies.

CONCLUSIONS

The results of this field study demonstrate that milk samples collected from the QMI Sani-Elbow line sampling device, properly installed and functioning, provide SCC and bacterial culture results that agree well with bulk tank sample results for milk harvested from the same group of cows during the same milking. These results should not be extrapolated when considering the function of other available line sampling devices. The potential for carryover milk from a previous group to impact the accuracy of culture results in a successive group remains to be investigated, particularly with reference to its impact on test sensitivity and specificity for the detection of contagious mastitis pathogens. However, the results of this study suggest that the strategy of milk line sampling is a very promising sampling strategy. It should assist in providing producers with inexpensive and timely information that will improve programs for monitoring milk quality and udder health in commercial dairy herds.

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