Evaluation of Bulk-Tank Culture for the Identification of Major Contagious Mastitis Pathogens on Dairy Farms

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Introduction

From May 1997 to December 1998, 40 Ontario dairy veterinarians and 60 dairy producers participated in the Sentinel Herd project. Composite milk samples from all lactating cows were collected and cultured every 4 months. Bulk-tank milk samples were collected from each farm on the day of the fourth herd culture. The objective of this analysis was to describe the prevalence of bacterial pathogens in the bulk-tank samples, the variability of culture results among the repeated samples, and the association of the bulk-tank culture results with the results of the corresponding cow-milk cultures.

Materials and Methods

Three bulk-milk samples were obtained from the same bulk tank and frozen at minus 4° F (-20° C) for one to two months. Samples were thawed for one hour at room temperature and then vortexed for 15 seconds immediately prior to further processing. Each bulk sample was then split in triplicate, which resulted in a set of 9 culture samples for each bulk tank. For each culture the following four media were inoculated using a sterile cotton swab soaked in milk: 5% sheep blood in Columbia Agar Base (Becton Dickinson), MacConkey Agar (Becton Dickinson, Cockeysville, Maryland), Baird-Parker Agar (Oxoid, Ottawa, Ontario) and Edwards Medium (Oxoid) with added staphylococcal hemolysin. The milk inoculum (approximately 0.1 ml) was spread on the agar plates using an Iso-plater (Fischer Scientific). All media were incubated in humidified room air at 95° F (35° C) for 48 hours.

In addition, milk samples were also incubated at 95° F (35° C) for 18 hours after the initial plating. Re-incubated milk samples again were inoculated onto Baird-Parker Agar and Edwards Medium in the manner described above using an inoculum of 0.001 ml. These are referred to as replate cultures, while the initial plating of un-incubated milk samples are referred to as primary cultures. After 48 hours' incubation, bacterial species isolated on the blood and MacConkey agars were quantified and also identified based on previously published criteria. Growth on Baird-Parker Agar and Edwards Medium were evaluated for the presence of *Staphylococcus aureus* and *Streptococcus agalactiae,* respectively, on both the primary and replate cultures.

Results

With regard to the major contagious pathogens, direct replicate plating of approximately 0.1 ml. of bulk-tank milk led to the detection of one or more colonies of *S. aureus* in 32 of 56 uncontaminated sets of milk samples (contaminated samples were received from 3 of the farms). The application of the re-incubation and replating procedure lead to the identification of *S. aureus* in one or more samples from 23 of the 24 farms negative on primary plating, plus all 3 of the farms with originally contaminated samples. Based on the results from both the direct plating and the enrichment plating methods, 58 of 59 farms (98%) were *S. aureus* positive. Only 1 of 59 herds (2%) was positive for *S. agalatiae*.

Three milk samples were taken from each bulk tank and plated in triplicate. Therefore, 9 sets of cultures were done from each herd. The variation in the *S. aureus* results was negligible among the samples or among the replicate cultures from the same samples. Hence, repeatability of the enrichment method for detecting *S. aureus* was high. Only 3 of 58 positive herds were negative on any of the 9 sets of enriched sub-samples. In one of these 3 herds, 1 of 9 sub-samples was positive for *S. aureus,* while in the other 2 positive herds, 7 of 9 sub-samples were positive. The repeatability of the bulk-tank culture process for identifying *S. agalactiae* could not be fully assessed, as only 1 herd had any of the bacteria isolated. In this sense, among negative herds the repeatability was high.

Results of bulk-tank culture were then compared to the individual cow culture data from the samples collected on the same day as the bulk-tank samples. For presumed *S. aureus* isolations, there were a significant number ($p<0.01$) of discordant pairs of results: (in 16 of 59 farms there was disagreement between conclusions drawn from the cow cultures versus the conclusions drawn from the bulk-tank culture. In 15 of 16 cases, the

bulk-tank culture suggested the organism was present, but the individual cow cultures from the herd were all negative. Interestingly, in the 1 negative bulk-tank herd, 1 cow out of 83 sampled was indeed positive for *S. aureus*. In 5 other instances where there was disagreement, 2 or more subsamples of the replicated bulk-tank cultures were negative, yet *S. aureus* was isolated from several cows in the herd.

Conclusions

The bulk-tank cultures using enrichment methods with Baird Parker medium appear to hold promise as a screening test in assessing udder health and possibly milk quality issues on the dairy farm. Further study of this data is ongoing to determine why some herds were positive for contagious bacteria at the bulk-tank level, yet culture-negative at the cow level. Re-sampling of cows and consideration of the herd history and previous herd cultures will likely be important additional evidence to help interpret bulk-tank cultures for contagious bacteria. Further study of the types and combinations of bacteria, and the determination of realistic cut points may be needed before the utility of bulk-tank cultures for assessment of environmental bacteria is known.