Analysis of Bulk Tank Milk to Determine the Bacterial Flora of the Mammary Gland of Lactating Cows in a Dairy Herd

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Introduction

To properly evaluate a herd mastitis problem, it is necessary to determine the type and extent of bacterial infections. Bacteriological examination of quarter milk samples from all cows in the herd will provide the most accurate evaluation. However, in large herds this is a time consuming and somewhat costly procedure. Various attempts have been made to reduce the number of cultures required for evaluation. The use of composite samples, screening tests to select cows with high somatic cell counts, and the culturing of bulk tank milk have all been used (1,4). The National Mastitis Council, in its tape and slide set, "Procedures for Handling the Mastitis Problem Herd" (3), recommends the use of bulk tank samples for this purpose. However, California workers in 1971(2) indicated an extremely poor correlation between the bulk tank counts of *Streptococcus agalactia* and *Staphylococcus aureus* and the percentage of infected cows in the herd. In our attempt to utilize counting procedures, we found it difficult to identify organisms when pour plate techniques were used and difficult to count the colonies because of overgrowth when milk was spread on the surface of agar plates. In an attempt to overcome some of these difficulties, the following technique using differential media was developed.

Materials and Methods

Milk samples were collected from the bulk tank daily for five consecutive days. The samples were collected by aspirating 5 to 10 ml into a sterile syringe after the tank agitator had been running for 10 to 15 minutes. All samples were frozen until the time of processing. At the time of examination the samples were thawed and each sample was mixed with a vortex mixer for one minute. Two ml were removed from each sample and used to make a composite sample. The composite sample was again mixed for 30 seconds on the vortex mixer. Dilutions of 1:10 and 1:100 were made. Each dilution was again mixed with a vortex mixer for 30 seconds. One tenth ml of undiluted milk and of each dilution was then placed on an agar plate, resulting in final dilutions of 1:10, 1:100 and 1:1000. A sterile glass spreader rod was used to spread the materials smoothly over the agar surface. The following media were used: blood agar, TKT-ferric citrate, tellurite glycine, and MacConkey. The plates were then incubated at 37°C for 18 to 24 hours. Counts were taken from plates with between 30 and 300 colonies present. This technique allows the determination of the total bacteria count and the number of certain specific organisms: *Streptococcus agalactia*, streptococcus other than *Strep. agalactia*, pathogenic staphylococci, non-pathogenic staphylococci and coliforms.

Results

It was determined in earlier trials that this technique resulted in greater reliability than the use of single samples on blood agar plates. The correlation between the numbers of organisms and percentage of infected quarters has not been determined on a large number of herds. But, in general, correlation exists which is helpful in herd analysis work. The following examples indicate the usefulness of this technique in evaluating problems and arriving at a specific bacteriologic diagnosis in mastitis problem herds without the cumbersome and expensive procedure of individual quarter sampling.

Herd Number 1

This was a 30-cow herd with a long history of chronic mastitis. Somatic cell counts of 1.5 million cells/ml had been reached several times. Previous bacteriologic sampling in the herd indicated a staphylococcus problem. Treatment based on sensitivities had been conducted but the results were poor.

The milking equipment was considered to be under capacity and not functioning optimally. The vacuum pump had a capacity of 15 cfm (New Zealand) and

three bucket units were being used on a ¾ inch line. The vacuum controller was also sticking.

Results of the California Mastitis Test (CMT) taken by the dairy plant fieldman indicated 50% of the quarters had scores 2 and 3 (scale negative, trace, one, two and three).

The results of our bulk tank culture method are listed in Table 1. These results are suggestive of a *Staphylococcus aureus* infection since it was the only organism isolated in significant numbers. This diagnosis is further supported by the previous cultures, high CMT scores and the clinical mastitis observed in the herd. The problem was considered to be related to the inadequate milking equipment and the retention of chronically infected cows in the herd with little attempt to control cow to cow spread.

Herd Number 2

This herd had a consistently high somatic cell count; occasionally, during the previous year, it exceeded 1.5 million cells/ml. Sanitation in this herd appeared to be excellent, however, the management practices were average. One or two chronically infected cows were being maintained in the herd and most of the time there were 2 to 5 cows in the herd with mild clinical mastitis. A severe leak in the vacuum system at the attachment of the pulsators to the stall cocks due to excessive wear, which was causing fluctuation and inadequate vacuum at the teat end, was found and repaired. Bulk tank samples and individual cow samples were taken for cultures (Table 1). Because the bulk tank and individual samples indicted primarily a *Streptococcus agalactia* problem with a secondary staphylococcus problem, it was recommended that the streptococcus-infected cows be treated. The staphylococcus cows were also treated by the owner (against our advice). Bulk tank and quarter samples were taken three weeks after the treatment period (Table 1). The *Streptococcus agalactia* count was reduced markedly with only a slight reduction in staphylococcus coagulase positive population. At this time the bacterial count from the Dairy Quality Control Laboratory was 3,000 and the Wisconsin mastitis test was 12. In both samples the bulk tank test results correlated with the results of the individual quarter samples.

Herd Number 3

This was an experiment station herd which had an occasional case of clinical mastitis and a relatively low infection rate. Sanitation in this herd was excellent. Results of concurrent bulk tank and quarter samples are presented in Table 1. The bulk tank results appear to correspond with the low quarter infection rate and the excellent level of sanitation.

Herd Number 4

This 70-cow herd had a previous *Streptococcus agalactia* problem which had been almost completely resolved. Recently, most clinical mastitis has been caused by coliforms based on cultures and clinical signs. The level of sanitation in this herd was poor. All cows were washed with a cloth towel which was returned to a pail of water with inadequate detergent and disinfectant and the udder and teats were not dried. The lots were often poorly scraped.

The milking equipment showed adequate vacuum pump capacity and proper pulsater function and was considered adequate.

The results of the bulk tank sample are presented in Table 1. On the basis of the bulk tank sample, we concluded that this herd had a mixed problem. *Streptococcus agalactia* counts were considered elevated and indicate there was *Streptococcus agalactia* infection and in addition there were environmental organisms (non ag strep) and coliform bacteria present in quite high numbers. The influence of the poor sanitation on the bulk tank counts is quite evident.

Herd Number 5

This was a 200-cow herd, in a total confinement environmental barn. A moderate level of clinical mastitis was present and some cases appeared

Table 1. Comparison of Numbers of Bacteria in Colony Forming Units Obtained from Bulk Tank Samples and Individual Quarter Samples.

Herd No.	1	2	2	3	4	5
		Before Rx	After Rx			
Approximate herd size	25	60	60	60	70	200
Sanitation	Good	Excellent	Excellent	Excellent	Poor	Poor
Bulk Tank Results						
Total count*	3,700	5,300	1,500	540	31,000	48,000
Strep. ag.	50	1,300	50	0	200	1,700
Non-ag. Strep.	0	950	310	60	21,000	9,400
Coagulase Pos. Staph.	540	340	220	0	60	380
Coagulase Neg. Staph.	0	220	120	0	40	—
Coliforms	12	10	0	0	12,000	990
Quarter Sample Results						
Uninfected	—	153 (74%)	197 (93%)	- - - - - - - - -		
Strep. ag.	—	42 (20%)	3 (1%)	- - - - -		approx. 20%**
Non-ag. Strep.	—	0	0	- - - -		
Coagulase Pos. Staph.	—	13 (6%)	12 (6%)	- - - -		
Coagulase Neg. Staph.	—	0	0	- - -		

*All numerical values are colony forming units per ml.
**Based on 25 cow subsample of the herd.
-Not done.

clinically to be coliform mastitis. Previous subsamples of the herd had indicated approximately a 20% level of *Streptococcus agalactia* infection.

General sanitation of this barn was poor. Carpeting which could not be properly cleaned was used in the free stalls. Milking sanitation was considered to be average. The milking equipment was adequate in capacity and design but the owner had been having some problems with maintenance of the electronic parts of the system. The cows were washed with cold water and drying of the teats after washing was not adequate or consistent.

The bulk tank results from this herd are listed in Table 1. We interpret these results as indicating a mixed infection with the major problem being *Streptococcus agalactia*. The high total bacterial count and the elevated numbers of non-agalactia streptococcus and coliforms are indicative of a poor level of sanitation in this herd.

Discussion

The technique described in this report using multiple samples and differential media appears to increase the reliability of bacteriologic analysis of milk from bulk storage tanks and allows for the determination and a rough quantitation of the major pathogens as well as giving an indiciation of the level of sanitation in the herd.

Limited comparisons of various techniques were made in the process of developing this test and several factors appear to be critical:

1. Sample handling is very important. Some samples kept at room temperature for as little as one hour showed a great increase in bacterial numbers. Freezing appears to be the most practical method of assuring sample quality. The freezing, in addition to its beneficial effect of stabilizing bacterial numbers, appeared to result in a lysis of phagocytic cells and a release of bacteria so they would replicate when the sample was incubated.
2. Mixing of the sample is also important. Use of the vortex mixer as described increased repeatability of results.
3. The use of differential media was found to be necessary and the only way to differentiate the bacteria present. Colonies are difficult to identify because of overgrowth when only blood agar is used. Use of differential media results in some disparity of counts due to the inhibitory effects of the media but in general appears to give much more satisfactory and reproducible results.
4. Use of five consecutive daily samples also appears to be important. Considerable daily variation occurs in a herd but the use of five samples appears to give a reasonably accurate picture. The use of a composite of five daily samples was compared to the mean and geometric mean of individual samples and tends to be between the two values, with a tendency to be closer to the geometric mean.

The exact correlation between bulk tank counts and actual infection rates may show some variation due to the difference in rates of shedding of bacteria and other variable factors in counting techniques and growth characteristics of organisms in differential media. But the use of this technique appears to give results with enough reliability to allow some conclusions to be made in problem herds and allows general evaluation of the herd. The technique can be employed with a minimum of time and expense and can serve as an indication as to what further tests and evaluation should be made.

References

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