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Use of Bulk Tank and Milk Filter Cultures in Screening for *Streptococcus agalactiae* and Coagulase-positive Staphylococci

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ABSTRACT

The use of a single bulk tank milk culture and a single milk filter culture was studied for their suitability as screening tests for coagulase-positive staphylococci and *Streptococcus agalactiae*. Bulk tank and bulk tank milk filter cultures were compared to quarter milk cultures taken from individual cows at 49 Ohio dairy herds selected from all Ohio dairy herds by a stratified random sampling scheme. Individual cow quarter milk samples were collected from a sample of all milking cows using a sampling scheme designed to detect an organism present in 2% of quarters, with 95% confidence intervals between 1 and 3%. Seventeen (35%) herds had one or more cows positive for *S. agalactiae* and 34 (69%) had one or more cows positive for coagulase-positive staphylococci. Using the results of individual cow sampling as the standard, the sensitivity for *S. agalactiae* was estimated as 23.5% for a single milk filter sample and 35.3% for a single bulk tank milk sample. The sensitivity for coagulase-positive staphylococci was estimated as 52.9% for a single milk filter culture and 41.2% for a single bulk tank milk culture. Based on these results and those of others, it appears that a single bulk tank or milk filter sample has a relatively low sensitivity for both coagulase-positive staphylococci and *S. agalactiae*, making these poor screening tests for the presence of these pathogens within a dairy herd.

Sampling milk from the bulk tank has been investigated as a means of detecting specific pathogens present in the milking herd (*2-4,6,7,11-13,15*). Bacteria present in bulk tank milk come primarily from the cows' udders but also possibly from teat skin or milking equipment. For an obligate intramammary pathogen like *Streptococcus agalactiae*, the bovine udder is recognized as the only reasonable source (*4*), and isolates in the bulk tank can usually be assumed to have originated in the bovine udder. Other mastitis agents such as coliforms and environmental streptococci are common environmental flora. Their isolation from the bulk tank does not necessarily mean they came from the bovine udder. Coagulase-positive staphylococci (CPS) isolated from the bulk tank are usually considered evidence of bovine intramammary infection; however, it is possible that this organism could also have originated from the environment or teat infections outside the mammary gland (*4,14*). Robertson et al. (*14*) reported that CPS could be isolated from numerous environmental sites and nonmammary body sites on dairy farms. However, these may not be the same strains of CPS which commonly infect the mammary gland (*14,17*).

There is a need for an inexpensive screening test for *S. agalactiae* and CPS within dairy herds. Bulk tank milk samples are routinely collected by milk marketing organizations. Many such organizations have begun to provide microbiologic tests as a service to the dairy producer. Routine screening for specific pathogens, such as CPS and *S. agalactiae*, are being made available in much the same way that tests are currently performed for bacterial count and somatic cell count. Also, practicing veterinarians or other dairy consultants may be interested in identifying or ruling out these pathogens as possible causes of udder health problems occurring in the herd. Culture of the disposable filter (located in the milk line immediately before entry into the bulk tank) has also been attempted for isolation of specific pathogens (*9*).

The purpose of this study was to evaluate the suitability of a single bulk tank milk culture and a single bulk tank filter culture as screening tests for identification of *S. agalactiae* and CPS within a herd of dairy cattle.

METHODS

A computerized list of all Ohio dairy herds licensed to sell milk was used by Ohio Agricultural Statistics to select a stratified random sample of herds for study (*1*). The sampling scheme was designed to sample an approximately equal number of herds from each of three herd-size strata and approximately the same proportion of herds from each of 13 geographical regions.

The project was performed in conjunction with the 1988-1989 Ohio National Animal Health and Monitoring System survey conducted as a cooperative effort by the U.S. Department of Agriculture, the Ohio Department of Agriculture, and The Ohio State University. The methodology for this project is described in more detail elsewhere (*1*).

Each participating dairy producer was visited at milking time between October 26, 1988 and March 28, 1989, with the exception of one herd which was visited in early May of 1989. At each herd visit, individual-quarter milk samples (IQMS) were obtained from lactating cows based on a sampling scheme designed to detect an organism present in 2% of the quarter samples, with 95% confidence limits of between 1 and 3% (*5*). The sampling scheme was employed so that the ability to detect organisms

would be approximately equal for herds of all sizes. For example, our sampling scheme prescribed that we sample 79% of the cows from herds with 50 lactating cows, 65% from herds with 100 lactating cows, and only 48% from herds with 200 lactating cows. In stanchion barns, cows to be sampled were selected by a systematic sampling scheme. For example, to obtain a 66% sample, every third cow was skipped. In milking parlors, similar systematic sampling schemes were used to balance right side versus left side, early milking versus late milking, and front of parlor versus the rear of the parlor. All IQMS were collected following milking and the use of a germicidal teat dip (*10*). Teats were dried with individual paper towels and swabbed with cotton soaked in 70% alcohol before a milk sample was obtained in a separate tube for each quarter.

Immediately after obtaining the IQMS, a bulk tank milk sample was collected. Also, the used milk filter was placed in a sterile jar with about 100 cc of bulk tank milk. The jar was immediately shaken for about 5 s to dislodge bacteria from the filter.

All IQMS, bulk tank milk samples, and bulk tank filter samples were immediately cooled, transported to the mastitis laboratory, and plated within 24 h after collection. Samples were shaken and .01 ml streaked on one quadrant each of sheep blood esculin in agar, thallium, crystal violet, toxin medium (TKT), and mannitol salt medium. Agar plates were incubated at 37°C and evaluated at 1 and 2 d. Colony morphology, hemolysis, catalase reaction, and Gram stain were used for the first level of identification, according to NMC recommendations (*10*). Coagulase-positive staphylococci were identified as being gram-positive cocci, catalase positive, and coagulase positive. *S. agalactiae* was identified as gram-positive cocci, catalase negative, esculin negative, and CAMP-positive (Christe, Atkins, Munch-Peterson Test). All IQMS which grew three or more different organisms were judged to have been contaminated at collection.

Each herd was classified as positive or negative for *S. agalactiae* and CPS by the IQMS, the bulk tank milk sample, and the milk filter sample. For both *S. agalactiae* and CPS, a Fisher's exact test was used to compare the results of the IQMS to those from culture of the bulk tank milk and the milk filter (*5*). Using the results of the IQMS survey as the standard, the sensitivity and specificity of the bulk tank milk and filter cultures were estimated for each of the two organisms. Because an IQMS was not obtained from every quarter contributing to the bulk tank, an exact calculation of these statistics could not be calculated.

RESULTS

Seventeen herds (34.7%) were positive for *S. agalactiae* on the IQMS survey, 5 were positive on the milk filter survey, and 7 were positive on the bulk tank milk survey (Table 1). Using the IQMS survey results as the standard, the sensitivity of the milk filter culture was 23.5% (4/17) and the specificity was 96.9% (31/32). Positive predictive value (PPV) was 80% (4/5) and negative predictive value (NPV) was 70.4 (31/44). The Fisher's exact was p=.0432. The sensitivity of the bulk tank milk sample for *S. agalactiae* was 35.3% (6/17) and the specificity was 96.9% (31/32). PPV was 85.7% (6/7) and NPV was 73.8% (31/42). The Fisher's exact was p=.0048.

Thirty-four herds (69.4%) were positive for CPS on the IQMS survey, 19 were positive on the milk filter survey, and 15 were positive on the bulk tank milk survey (Table 2). Using the IQMS survey results as the standard, the sensitivity of the milk filter culture was 52.9% (18/34) and

TABLE 1. *Comparison of individual quarter milk sample cultures to milk filter cultures for* S. agalactiae.

Milk filter culture

		+	-	Total		
	+	4	13	17	Sensitivity	= 23.5%
IQMS[1]	-	1	31	32	Specificity	= 96.9%
Total		5	44	49	PPV[2]	= 80.0%
					NPV[3]	= 70.4%
					Prevalence[4]	= 34.7%

Bulk tank culture

		+	-	Total		
IQMS	+	6	11	17	Sensitivity	= 35.3%
	-	1	31	32	Specificity	= 96.9%
Total		7	42	49	PPV	= 85.7%
					NPV	= 73.8%
					Prevalence	= 34.7%

[1]Individual quarter milk samples.
[2]Positive predictive value.
[3]Negative predictive value.
[4]Prevalence as determined by IQMS.

TABLE 2. *Comparison of individual quarter milk sample cultures to milk fillter cultures for Coagulase-positive* Staphylococcus.

Milk filter culture

		+	-	Total		
	+	18	16	34	Sensitivity	= 52.9%
IQMS[1]	-	1	14	15	Specificity	= 93.3%
Total		19	30	49	PPV[2]	= 94.7%
					NPV[3]	= 46.7%
					Prevalence[4]	= 69.4%

Bulk tank culture

		+	-	Total		
IQMS	+	14	20	34	Sensitivity	= 41.2%
	-	1	14	15	Specificity	= 93.3%
Total		15	34	49	PPV	= 93.3%
					NPV	= 41.2%
					Prevalence	= 69.4%

[1]Individual quarter milk samples.
[2]Positive predictive value.
[3]Negative predictive value.
[4]Prevalence as determined by IQMS.

the specificity was 93.3% (14/15). PPV was 95% (18/19) and NPV was 46.7% (14/30). The Fisher's exact was p=.0432. The sensitivity for CPS of the bulk tank milk sample was 41.2% (14/34) and the specificity was 93.3% (14/15). PPV was 93.3% (14/15) and NPV was 41.2% (14/34). The Fisher's exact was p=.0048.

DISCUSSION

The percentage of herds having at least one cow infected with *S. agalactiae*, as determined from the IQMS survey, was 34.7%. This is slightly lower than the 55% prevalence reported by Godkin using composite cow cultures, the 42.4% prevalence reported by Godkin using four consecutive bulk tank cultures, and the 39.1% prevalence reported by Sears who used composite individual cow cultures (*3,15*). Bulk tank surveys have reported prevalence of herds infected with *S. agalactiae* as 47%, 47%, and 44% (*4,6,15*). Older studies summarized by Postle reported prevalence rates for *S. agalactiae* of 89% from Wisconsin, 76% from New York, and 84% from Connecticut (*13*). Some survey reports show the results analyzed as individual cow prevalence rather than as the percentage of farms with at least one infected animal (*8,16*).

Every quarter of every cow was not sampled, thus it is possible that some herds with a very low prevalence of *S. agalactiae* may have been incorrectly classified as having no quarters infected with this organism. As such, this might provide some explanation for the lower herd prevalence of *S. agalactiae* found in our survey when compared with the above reported results of other investigations. However, our sample size was designed to detect an organism present in 2% of quarters with 95% confidence limits of 1-3%. As such, only herds with a very low prevalence of *S. agalactiae* infected cows would be expected to escape detection. Therefore, the magnitude of this potential misclassification bias should be small. It appears more probable that the observed differences in the prevalence of infected herds cited above are largely real and are due to numerous regional and environmental differences.

Fewer surveys for CPS have been reported in the literature. In Ontario, Brooks et al. reported a herd prevalence of 83.3% of 74 herds infected with *Staphylococcus aureus* on the basis of whole herd culture (*2*).

For screening tests, high sensitivity is a more important consideration than is specificity since the objective is to identify high-risk herds for more definitive (high specificity) testing. However, it must be considered that the one false-negative herd (positive on bulk tank or filter culture but negative on the IQMS herd) shown in Tables 1 and 2 may, in fact, be a truly infected herd mistakenly classified as negative. This could have occurred because the IQMS survey sampling scheme sampled only a portion of the cows in most herds. If this one herd were reclassified, the sensitivity of the milk filter culture for *S. agalactiae* would increase slightly from 23.5% (4/17) to 27.8% (5/18). This small change would be inconsequential for most decision-making users of these diagnostic tests. The sensitivity for CPS would change by even less were the one false negative to be considered a true positive.

Except for the preceding consideration, calculations of sensitivity in the current study could be considered as maximum values because the IQMS survey sampled only a portion of the cows contributing to the tank. For example, 17 herds showed one or more quarters positive for *S. agalactiae*. If all quarters from all cows were sampled, more herds may have been found to have positive cows. This increase in the number of herds positive on IQMS would tend to decrease the calculated value for sensitivity.

Godkin and Leslie (*3*) and Sears et al. (*15*) have shown that sensitivity can be improved by taking multiple bulk tank samples (*3,15*). For *S. agalactiae*, Godkin and Leslie studied 250 herds with multiple bulk tank cultures, of which 106 were eventually found to be positive on one or more samples. Only 12 herds were positive on the first bulk tank culture. Second, third, and fourth samples yielded an additional 30, 39, and 25 positive herds, respectively (*3*). Sears et al. (*15*) compared single samples for *S. agalactiae* with duplicate and triplicate samples. Only 71% of positive herds surveyed were found positive on a single sample, and 95% were found positive on duplicate samples (*15*). These results indicate that multiple sampling schemes might hold promise for improving the sensitivity of bulk tank culturing.

Specificity is a less important consideration when evaluating a screening test. Because of our sampling scheme, it was possible to obtain a positive bulk tank or milk filter sample from a farm which did not yield a positive isolate from the individual cow cultures. As such, specificity in this study was found to be less than 100%. If all quarters from all cows had been cultured instead of only a sample, the specificity should theoretically be 100%, i.e., false positives should not exist. This assumption of 100% specificity is reasonably valid for *S. agalactiae* where the bovine udder is thought to be the only source of the organism. However, for CPS, other sources such as wound or teat infections are possible, although probably not common (*4*).

The PPV and NPV are perhaps the most important consideration for the dairy producer, veterinarian, or consultant who is performing a diagnostic test. The predictive value of a positive test is the probability that the positive test result is a true positive. The predictive value of a negative test is the probability that a negative test result is a true negative. One important consideration in the use of PPV and NPV is that they are very dependent on the prevalence of disease. This means that values of PPV or NPV taken from the literature would not apply to a particular herd if the expected prevalence of disease in that population differed substantially. Sensitivity and specificity depend primarily on the diagnostic test itself and can thus be applied to different situations with varying disease prevalence. If the test user knows the test sensitivity and specificity and can approximate the disease prevalence for his locality, he or she can then calculate values of PPV and NPV.

When interpreting the results of bulk tank or milk filter cultures, the test user will usually want to assume a PPV of 100%. That is, if the bulk tank or filter culture is positive, it will be assumed that the herd definitely has at least one infected cow. Interpretation of a negative test result is more

difficult, and the NPV is helpful in this consideration. A NPV of 47% means that there is only a 47% chance that the herd with a negative test result is truly negative. As mentioned above, this poor NPV can possibly be improved by taking repeated samples from the same herd on consecutive days (*10,15*).

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