REVIEW ARTICLE

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Culture of bulk tank milk as a mastitis screening test: A brief review

M. Ann Godkin, Ken E. Leslie

Abstract

The culture of a sample of bulk tank milk may be a useful technique by which to screen herds for major mastitis pathogens. *Staphylococcus aureus* and *Streptococcus agalactiae*, if identified on a culture of a sample of bulk milk, reliably indicate infection of the udder. Environmental bacteria, such as the other streptococci and coliforms, are unlikely to be indicative of the proportion of cows infected with these organisms.

Samples of bulk milk are readily obtainable and can be rapidly and inexpensively cultured to screen large numbers of herds for mastitis-causing bacteria, yet the performance of the test has only recently been formally assessed for its ability to correctly classify herds according to infection status.

A single culture of bulk tank milk has been found to be a test with low sensitivity and high specificity for determining the presence of *S. agalactiae* or *S. aureus* in the herd. This means that many infected herds will be called negative, but few uninfected herds will be classified as positive.

The literature assessing the performance of bulk tank milk culture in comparison with other mastitis screening tests, the use of bulk milk culture for prevalence surveys, and factors affecting these results is discussed.

Résumé

Culture d'échantillons de lait prélevés dans le réservoir comme épreuve de dépistage pour la mammite

La culture d'un échantillon de lait prélevé à même le réservoir peut être une aide technique valable pour dépister la présence des principaux agents pathogènes responsables de la mammite dans le troupeau. L'identification sur culture des bactéries *Straphylococcus aureus* et *Streptococcus agalactiae* indique de façon fiable la présence d'infection de la glande mammaire. Les bactéries provenant de l'environnement telles les coliformes et autres *streptococcus* ne sont pas représentatives de la proportion des vaches infectées par ces organismes. Les échantillons de lait provenant du réservoir sont facilement disponibles et peuvent être cultivés rapidement et à faible coût pour dépister la présence de bactéries responsables de la mammite dans un grand nombre de troupeaux. La validité de l'épreuve n'a été formellement évaluée que tout récemment pour déterminer son habileté à classer correctement les troupeaux selon le statut de l'infection. Une culture unique d'un échantillon de lait provenant du réservoir est un test hautement spécifique, mais peu sensible pour déceler la présence de *S. agalactiae* ou de *S. aureus* dans le troupeau. Ceci signifie que plusieurs troupeaux infectés seront de faux négatifs, mais que peu de troupeaux non infectés seront de faux positifs. Les auteurs présentent une revue de littérature comparant cette épreuve aux autres méthodes de dépistage de la mammite et discutent de l'utilisation de ce test dans les études de prévalence ainsi que des facteurs influençant les résultats.

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Persistently elevated, herd somatic cell counts (SCC) indicate a high prevalence of subclinical infection within the herd (1), and suggest a failure to adopt practises recognized as effective in the control of contagious mastitis (2). Somatic cell counts, widely utilized as screening tests for mastitis at the individual cow and herd levels (3), provide no information about the identity of specific bacterial pathogens. The lack of a rapid, accurate, regular monitoring system for the types of mastitis-causing pathogens active in a herd may be a limiting factor in motivating the implementation of specific control efforts.

Milk samples from bulk tanks are collected routinely for milk quality evaluation in most jurisdictions. The Standard Plate Count, a nondifferential culture of a standard volume of bulk tank milk, has been routinely used in official testing programs to assess the overall level of bacterial contamination of milk (4).

Culture, with concurrent identification of bacterial species, has been used as a qualitative test in research and voluntary monitoring programs for the presence of mastitis-causing pathogens (5–9). Quantitative bulk tank milk results have been applied to predict the preva-

Animal Industry Branch, Ontario Ministry of Agriculture and Food, Wellington Place, R.R. 1, Fergus, Ontario N1M 3S1 (Godkin); Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1 (Leslie).

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lence of cows infected with specific pathogens within the herd (5,6,9).

The first purpose of this article is to review the scientific literature concerning the performance of bulk milk culture as a test for the classification of herds according to the types and prevalence of mastitis pathogens in comparison to a variety of other mastitis screening tests. Studies were gathered by computer-aided search of the Agricola database from 1970 to December 1989, the Current Contents database from January 1990 to March 1993 using Procite, from personal reference files, and from the reference lists of assembled papers.

Second, this article highlights the limited amount of information collected using standard epidemiological methods for assessing test performance, in spite of the use of this test in herd surveys and udder health monitoring programs. We also make suggestions of techniques to improve the objective evaluation of the culture of bulk tank milk as a screening test for mastitis.

Properties of screening tests

To evaluate the use of bulk tank milk culture as a test for mastitis-causing pathogens, the general characteristics of the test must be determined. The sensitivity, defined as the ability of the test to correctly identify infected herds, and specificity, defined as the ability of the test to correctly identify negative herds, are two measurements of test function which can be used to assess test performance (10). The calculation of sensitivity and specificity depends on the comparison of culture results to a validated test or "gold standard" for mastitis (10). The lack of a generally accepted gold standard for mastitis has resulted in the assessment of the agreement between the culture of bulk tank milk and a number of other screening tests. Assessment of agreement allows only the estimation of relative sensitivities and specificities (11).

The predictive value of a positive or negative test may be used to enumerate the reliability of test results (10). The predictive value of a positive test is the percentage of positive test results which are truly positive. Conversely, the predictive value of a negative test is the percentage of negative test results which are truly negative (10). The predictive values are influenced by the sensitivity and specificity of the test and the prevalence of infection in the population being tested (11). Culture of the bulk tank is usually used in surveys of populations for which the prevalence of infection is unknown (5,6,12–15). Occasionally, populations of herds with a known prevalence of specific pathogens, or with higher prevalence of clinical and subclinical mastitis infections, have been surveyed (6,9,16,17). However, the deficiency of information concerning prevalence of pathogens within test populations has prevented the calculation of these test parameters, and in turn, contributed to the lack of objective assessment of the validity of bulk tank culture as a test for mastitis (18,19).

Mastitis pathogens

Bacteria present in milk samples from the bulk tank may originate from infected udders, from teat and udder surfaces, or from a variety of other environmental sources (16,20,21). *Staphylococcus aureus* or *Streptococcus agalactiae*, major causes of contagious mastitis in dairy herds, reside primarily in the udder of infected cows (18). When found on a culture of bulk tank milk, these mastitis-causing pathogens are strong indicators of the presence of intramammary infections in the herd (5). The number of these organisms found on culture is determined by the number of bacteria shed, the number of infected cows, the milk production from infected cows and herd mates, and the severity of infection (18).

Environmental bacteria, such as *Streptococcus uberis*, *Streptococcus dysgalactiae*, and coliforms, may enter milk from intramammary infections, but also from nonspecific contamination (4,20,22). The presence of these organisms on culture of milk from the bulk tank may relate to the general level of environmental and milking hygiene in the herd (20); however, their presence has been shown to be independent of the prevalence of mastitis (5). Udder infections with these environmental pathogens are predominantly of short duration and characterized by clinical disease which makes their inadvertent introduction to the bulk tank less likely (23).

Microbiological techniques

Numerous bacteriological techniques have been used to isolate and identify organisms in milk from bulk tanks. Although standard methods exist for the culture of milk from individual cows and quarters (24), none have been established for the culture of milk from bulk tanks (8). A variety of ancillary tests (25), or the presumptive identity from conventional blood esculin agar (8,26), have been used to identify microorganisms isolated from bulk milk.

Growth, identification, and classification of specific pathogens have been found to be enhanced by the use of selective media. Thallium sulfate toxin (TKT) medium has been used to improve the recovery and facilitate the identification of streptococci from samples of bulk tank milk (6,9). In a study comparing the use of the selective TKT medium to a nonselective medium for the identification of herds positive for *S. agalactiae*, it was found that twice as many herds were declared positive using the selective technique (6). This observation illustrates that comparisons between studies using different bacteriological media may not be valid. Bacterial growth may be affected by these differences in media, hence the identification, enumeration, and classification of pathogens may not be similar between studies. Appropriate evaluation of the media used to selectively promote the growth of important pathogens, and thereby increase the uniformity of the test, is needed.

The differences in classification protocols for positive and negative culture outcomes across studies has inhibited the assessment of the reliability and predictive ability of this test. The minimum colony numbers required for a positive result are rarely defined (5,17,25,27,28) and may vary widely among diagnostic laboratories.

The likelihood of a misclassification of culture results is influenced by the colony numbers used to classify the cultures as positive or negative. For example, false negative results (a herd with infected cows but classified as negative on culture of bulk milk) are more likely to occur if the herd must have five or more colonies present to be classified as positive, than if one or more colonies is the criterion used. Fewer positive herds will be identified but, also, fewer herds will wrongly be identified as positive.

Similarly, false positive results are more likely (a herd with uninfected cows but whose culture of bulk milk is classified as positive) if the classification rests on the presence of only a single colony. Some negative herds will be called positive, but fewer positive herds will be wrongly called negative and missed.

Mistakes in colony identification are more likely to occur on bulk tank cultures than on individual cow cultures as a greater variety and larger number of organisms are often present in the former (25).

The appropriate number of colonies used to classify the outcome of a culture of bulk milk will depend on the objectives of the culturing program. The application of the results of this test will determine whether it is more important for the test to correctly identify positive or negative herds. For example, the impact of missing a herd infected with contagious pathogens (a false negative result), when regional eradication is the goal, will be greater than wrongly identifying uninfected herds (a false positive result).

Some researchers (9), have speculated that culturing larger volumes of bulk milk would facilitate the recovery of organisms and increase the sensitivity of the test. Techniques like this, which could increase the test sensitivity without concurrently increasing the false positive rate, require further exploration.

Sampling strategies

Various numbers of samples and sampling intervals have been employed to reduce the coefficient of variation of culture of the bulk tank as a test for the presence of specific pathogens in the herd. The culture of multiple samples from the same bulk tank during a specified time period has been examined (9,7,14,18,25). In a recent Ontario survey, four consecutive weekly samplings identified 11%, 39%, 76%, and 100%, respectively, of herds considered positive for *S. agalactiae* (9). Sears *et al* (14) cultured three samples from each of 998 herds to estimate the prevalence of *S. agalactiae* in Mississippi. One, two, or three samplings identified 71%, 94%, and 100%, respectively, of herds ultimately classified as positive. Monthly culturing of bulk tank samples obtained from 379 Irish herds found 38% positive for *S. agalactiae* at least once during the year (18). Of these positive herds, 34% were positive once, 16% twice, and 8% positive three times. In a repeated study, of a population of 526 smaller herds determined to have a similar prevalence of *S. agalactiae*, these researchers found 44% of the positive herds were positive once, 12% were positive twice, and 7% positive three times (13).

Parallel interpretation of repeated cultures, the classification of a herd as positive if a pathogen is isolated on any one of a number of samples, may be a technique which can be used to reduce the false negative rate (10,29). Appropriate sampling intervals, relative to herd size and management, disease characteristics, economics, and practicality must be considered in future research proposals.

Comparisons with other indices of milk quality

Culture of bulk tank milk has been compared to bulk tank milk SCC (16,18,26,30–32), herd summaries of SCC of individual cows (33), and herd summaries of cultures of individual cows (5,6,9,34). The underlying variability in the ability of these other tests to determine the incidence and prevalence of mastitis within the study populations has further hindered the objective assessment of the usefulness of bulk milk culture.

The frequency of positive bulk tank milk cultures has been compared to the herd average bulk tank SCC. Herds with a higher mean bulk tank SCC have been found to have *S. agalactiae* isolated more frequently on monthly samples (18). A highly significant correlation of 0.75 was found. This high correlation suggests that herds with a low and high prevalence of *S. agalactiae* infected cows were identified by culture of bulk milk. However, in a follow-up study, in which tank samples of 51 herds were cultured monthly for one year, a lower correlation of 0.53 was found between the frequency of herd isolation and the percent of quarters infected with *S. agalactiae* (25). In the latter study, the authors suggested that some positive herds, particularly those with a low prevalence of infection, would not be detected even with the increased sensitivity resulting from repeated sampling. Previous research, conducted in an experimental herd of 40 cows, has suggested that more than 5% of milking quarters must be shedding *S. agalactiae* for the organism to be isolated on 80% of repeated cultures of bulk tank milk (6).

Correlations between the numbers of colonies of specific pathogens in bulk tank samples and the bulk tank SCC have been determined (26,31). For *S. agalactiae*, the correlations between colony numbers and SCC in two large surveys of randomly selected herds were 0.51 (31) and 0.37, respectively (26). Samples with higher SCC were more likely to have greater numbers of *S. agalactiae* cultured. For *S. aureus*, lower correlation coefficients of 0.36 (31) and 0.37 (26) were reported.

These generally low correlations between herd SCC and specific pathogen colony numbers may occur because herd SCC can be influenced by the presence of cows infected with pathogens not belonging to the group of bacteria under investigation, or because herd SCC is affected by herd milk yield (35), or the numbers of bacteria shed may vary periodically (36).

The presence of ubiquitous organisms in cultures of bulk tank milk, such as "other streptococci" (the nonagalactiae species), coliforms, or staphylococcus species, has not been found to be significantly correlated to the bulk tank SCC (16,26,31).

The lower correlations associated with the attempted culture of *S. aureus*, in comparison to those achieved for *S. agalactiae*, may be attributed to the higher rate of false negative cultures that have been shown to occur (36). Cows and herds infected with *S. aureus* may have negative culture results because fewer organisms are shed (29), bacteria are present in the milk cyclically (36), L-form bacteria not recovered by conventional laboratory techniques may be produced (37), or intracellular organisms may not be recovered (36).

Summaries of SCC for individual cows within herds were compared to culture of bulk tank milk samples for *S. agalactiae* and *S. aureus* (33). The presence of either of these two pathogens was assessed for relationship to the rolling herd high SCC prevalence (the 12-month average of the percentage of cows with milk SCC in excess of 283,000 cells/mL). High SCC prevalence was significantly greater when either pathogen was

present, although only 14.6% of the variation in the outcome was explained.

Culture of the bulk tank has been compared to summaries of cultures of individual cows to determine the test's ability to predict the prevalence of various pathogens within herds (5,6,25). The frequency of positive monthly bulk milk cultures for *S. agalactiae* was found to have a correlation of 0.53 with the prevalence of infected quarters within herds, and 0.48 with the prevalence of infected cows (25). When colony numbers of major pathogens found on a single culture of the bulk tank were compared to the herd prevalence of infected cows, correlations of 0.68, 0.44, 0.62, 0.28, 0.22, and 0.25 were reported for *S. agalactiae*, *S. aureus*, *Mycoplasma* spp., other streptococci, coagulase-negative staphylococci, and coliforms, respectively (5).

In two studies, the parameters of culture of a single bulk milk sample as a test for determining the presence of major mastitis pathogens, in comparison to classification by individual cow culture results, have been calculated (9,34). In the first study, the sensitivities and specificities found were 20.5% and 94.0% for *S. agalactiae*, 9.2% and 100.0% for *S. aureus*, 44.8% and 100.0% for other streptococci, and 36.5% and 80.0% for coliforms (9). Similarly, in the second study, sensitivities and specificities determined for *S. agalactiae* and *S. aureus* were 35.3% and 96.9% and 42.2% and 93.3%, respectively (34).

These two studies illustrate that a single culture of bulk tank milk should be considered a test of low sensitivity and high specificity for the major contagious pathogens *S. agalactiae* and *S. aureus*. The finding of *S. agalactiae* in a culture of bulk tank milk reliably identified a herd as containing infected cows but failed to identify 79.5% and 64.7% of infected herds. For *S. aureus*, the test failed to identify 90.8% and 58% of herds as containing infected cows. Few false positive results, herds positive on bulk tank culture but not identified as having positive cows, were reported.

Relationships with clinical mastitis

Culture of bulk tank milk has been examined for use as predictor of the rate of clinical mastitis (15,22). One report indicated that culture of the bulk tank was of little use in predicting the type of incidence of clinical mastitis (15). The purposive selection of herds with superior management may have influenced these results. In another study, the bacteriology of the bulk tank was monitored on a weekly basis for one year in 10 herds (22). These herds were selected for the production of superior quality milk, as evidenced by their low SCC and bacterial counts. Statistically significant correlations were found between the streptococcal count in the bulk tank and the rate of streptococcal clinical mastitis (0.35), and between the gram-negative bacterial count of the bulk tank and the incidence of clinical coliform mastitis (0.46). However, only a small amount of the observed variation in the rates of clinical mastitis, as indicated by the low coefficients of determination of 0.12 and 0.21 for streptococci and coliforms, respectively, was explained by the bulk tank cultures (22).

For clinical episodes of mastitis, the incidence rate, rather than the prevalence, has previously been shown to more accurately reflect the dynamics of environmental pathogens (38). Thus, the relationship between the occurrence of clinical mastitis and bulk tank bacteriology requires further clarification.

Prevalence surveys by bulk milk culture

Although the performance of the culture of bulk tank milk as a test for mastitis has not been fully examined, the ease and rapidity of examining large numbers of readily available milk samples has made this technique attractive for the survey of populations of herds for the prevalence of major mastitis-causing pathogens. Studies estimating the prevalence of the endemic contagious pathogens *S. agalactiae* and *S. aureus* and of the epizootic pathogens such as *Nocardia* spp. and *Mycoplasma* spp., have been conducted.

In the United States, the prevalence of herds infected with *S. agalactiae*, as determined by bulk tank culture, has been reported to be 44% of 998 herds in Mississippi submitting three samples on a single occasion in 1982 (14), 4% of 4189 herds in South Dakota sampled repeatedly over two years commencing in 1970 (13), 47% from a single sampling of 2931 herds in Vermont in 1986 (26), 89% in 490 herds sampled weekly for several months in southern Wisconsin (6), and 28% in a survey of 353 herds in California collected biweekly for a nondefined period of time (31). The prevalence of *S. agulactiae* in a 1973 survey of 379 large Irish herds sampled monthly for one year has been reported as 38.5% (18), whereas a second survey of 526 smaller herds in 1976 found 37.6% positive (7). In Canada, a survey of 250 dairy herds in Ontario, sampled weekly for four weeks, found that 106 (42.4%) herds were positive for *S. agalactiae* (9). Although a range in infection rates is evident, to date there is little evidence from these reports of cultures of bulk tank milk that extensive progress is being made towards improvement of udder health through the elimination of the relatively manageable pathogen, *S. agalactiae*.

Surveys to determine the prevalence of *S. aureus* found 76%, 33%, and 50% of herds from Ontario, Vermont, and California, respectively, to be positive (9,26,31).

The culture of bulk tank milk has been used to estimate and monitor the change in prevalence, over time, of mastitis pathogens whose prevalence in a population is believed to be increasing. In California, Vermont, and New York (5,39–41) culture of bulk tank milk samples has been used to periodically determine the prevalence of herds infected with *Mycoplasma* spp. In Canada, during a recent epizootic of *Nocardia* spp. mastitis, culture of bulk tank milk samples was used to monitor the prevalence and incidence of herds containing cows infected with *Nocardia* (42,43), and to select case and control herds for research into practices predisposing to infection (44).

In summary, it is clear that infection with mastitis-causing bacteria remains an important problem for the dairy industry. Efficient techniques which will identify the presence and extent of infection at the herd level will aid in the implementation of control programs targeted towards specific pathogen groups (41). Producers can be motivated to improve udder health through the use of regular programs which monitor and report herd infection rates.

A single culture of a sample of bulk tank milk has been found to have a high specificity but a low sensitivity as a test for the contagious mastitis pathogens. Many infected herds will not be detected but few herds will erroneously be called infected. The lack of a uniformly applied, well-documented sampling and test methodology, as well as a lack of consistency in the methods of evaluting the test (including the gold standard chosen for comparison), has hampered the implementation of culture of bulk tank milk as a routine screening procedure for mastitis.

Future research should be aimed at standardizing a protocol for the culture of bulk tank milk to provide a screening test with known performance parameters. Acceptable limits of sample age, handling, and quality, as determined for samples used for regulatory testing, should be defined. The frequency of sample collection needed to maximize the sensitivity of the test for pathogens such as *S. agalactiae* and *S. aureus* needs to be determined. Finally, epidemiological methods of test evaluation such as calculation of the sensitivity, specificity, and positive and negative predictive values, should be used to standardize interpretation of test results.

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