

# REALISTIC MILK CULTURE PROGRAMS FOR HERD EXPANSION

Rubén N. González and David J. Wilson  
Cornell University  
Ithaca, New York

## Introduction

Dairy farms in the United States of America as well in other milk producing countries in the world have been following a rapid trend toward larger and fewer operations. New free stall barns or dry lots, and new or expanded milking parlors have been a common feature of this expansion in the U.S. Our state of New York has not been an exception to that tendency of having a greater number of cows per farm with fewer producers. While in 1970 there were nearly 20,000 dairy farms, currently there are around 8,000 dairy farms. However, cattle numbers in those 30 years have remained almost unchanged.

Biosecurity measures should be adopted to prevent a plethora of diseases, mainly infectious diseases, from being introduced and having a negative economic impact in dairy herds during the expansion process. As the public has recently become aware through the threat of foot and mouth disease, infectious diseases can enter a herd because of purchased additions, other animal species, humans, and fomites. Among the diseases to consider that can be introduced into a herd during the expansion process, contagious mastitis because of *Streptococcus agalactiae*, *Staphylococcus aureus* and *Mycoplasma* spp. are commonly found on U.S. dairy farms. However, there are two other mastitis pathogens that can enter a herd with new additions and can cause usually minor economic problems (*Arcanobacterium pyogenes*) or serious management and economic problems (*Prototheca* spp.).

In our daily work, we are frequently asked by those seeking to purchase cattle several questions related to the subject of this paper such as: 1) Can cows be selected only by using recent somatic cell count (SCC) evaluations provided by the Dairy Herd Improvement Association (DHIA) or similar organizations? 2) Before purchase, can prospective additions be submitted to the California Mastitis Test (CMT) and culture only those cows that scored 1 to 3 in the CMT test? 3) Is it reliable to perform only one bulk tank milk (BTM) analysis instead of several analyses over a pre-determined period for contagious mastitis agents? 4) If a whole herd is going to be purchased should quarter or composite milk samples from cows be taken for culture? 5) Is it necessary to routinely culture for *Mycoplasma*? 6) Once new additions are on the premises, which is the best way to monitor them? We sense when we are asked those questions that from the perspective of dairy producers, veterinary practitioners, extension personnel, and others it implies that they want to know how major problems can be prevented while doing the least amount of sampling and culture testing. Frequently, ambitious growth plans do not include a plan for testing prospective additions (4,12,18,19) or how to properly manage those additions when they are on the premises. We have documented elsewhere (18,19) producers suffering economic losses as a result of failure to properly address those subjects. Some time honored recommendations still apply (18,19).

Admittedly from the focused point of view of mastitis workers, while acknowledging the complexity of problems faced by dairy producers during expansion, this paper will provide a concise analysis of the aforementioned frequently asked questions.

### Selecting Cows By Using a Recent SCC Evaluation

The use of SCC is an effective method to indirectly identify cows with an intramammary infection (IMI). It measures the degree of inflammation in the mammary gland but does not identify the etiologic agent that causes that inflammation. It does not also indicate if a cow with  $LS > 4.0$  currently has an infection or the infection has already been cured and the high SCC is a sequela of that infection. A single high SCC test does not necessarily indicate that subclinical mastitis in a cow is caused by any of the contagious agents of the disease. As reported earlier (18,19), sensitivity of SCC  $LS > 4.5$  for detection of contagious mastitis agents was 72% for *Strep. agalactiae*, 62% for *Staph. aureus*, and 63% for *Mycoplasma* while the predictive value of a positive test was 11% for *Strep. agalactiae*, 15% for *Staph. aureus*, and 0.002% for *Mycoplasma*. Therefore, most of the cows with  $LS > 4.5$  that were analyzed did not have major contagious pathogens. Furthermore, when undertaking diagnostic and research work at the Quality Milk Promotion Services (QMPS), we detected three herds with subclinical infections because of *M. bovis* where cows consistently shed the organism in milk for several months with  $LS$  ranging between 2.4 and 3.2 (8). This feature is more frequently seen in herds that have cows with *Staph. aureus* IMI. It has been suggested that biological and therapeutic pressure in a specific herd could select strains inducing a low immune response (17). We speculate that there are strains of *Mycoplasma* that do not trigger the classical immune response also, including the migration of huge numbers of blood leukocytes toward the site of inflammation. However, it is important to remember that when these low SCC cows are introduced into a different herd, the spread of contagious mastitis to the resident herd may cause major clinical mastitis and/or high SCC problems.

Cows with *Prototheca zopfii* IMI were followed up in several New York dairies for up to four lactation periods. Almost all those cows consistently had  $SCC\ LS < 4$  after recovering from the clinical episode of the infection (R.N. González, G.J. Bennett, D.J. Wilson, unpublished observations). *Prototheca* spp. caused chronic IMI in cows for which there is not treatment available and spontaneous recovery has not been reported.

The conclusion is that SCC is not an acceptable method for screening potential herd additions for contagious mastitis.

### The California Mastitis Test and Culture of Cows With Scores 1 to 3

Since it was first described in 1957 (16), the CMT has been frequently overvalued as a procedure for detecting cows with IMI. However, its authors clearly stated that the CMT was only an indicator of inflammation in the udder, that it could not distinguish among causes of inflammation and that it was not a substitute for bacteriological culture. Moreover, it was reported that milk samples that scored negative (N) or trace (T) had SCC ranges of 0-200,000 cells/ml and 150,000-500,000 cells/ml. They compared CMT reactions and bacterial flora of

mammary glands and determined that the sensitivity of the test for *Staph. aureus* and *Strep. agalactiae* was 66% and 72%, respectively. Therefore, the CMT did not detect approximately one-third of the infected cows with those contagious mastitis agents. No improvement has been reported for detection of contagious mastitis agents since then. Furthermore, cows with current CMT scores of N and T can actually have mastitis (LS between 3.6 and 5.3) and may be infected with any contagious mastitis agent, or *A. pyogenes* (R.N. González, D.J. Wilson, G.J. Bennett, unpublished observations) or *Prototheca spp* (7).

### Reliability of Single and Consecutive Bulk Tank Milk Cultures

Testing BTM has been an accepted practice for many years, particularly with respect to the detection of contagious mastitis agents in a herd. We have frequently discouraged potential dairy cattle buyers from using a single culture of BTM to screen for contagious mastitis agents. Although a positive culture result indicates the presence of infected quarters in the herd (9) with *Strep. agalactiae*, *Staph. aureus*, *Mycoplasma*, *A. pyogenes* and possibly *Prototheca*, a negative result does not necessarily indicate that the herd is negative to infections caused by those pathogens.

From March 1992 to November 2001, staff of the QMPS Central laboratory visited a total of 4237 dairy herds located in New York and Northern Pennsylvania. Milk samples from all lactating cows were aseptically collected and together with a BTM sample submitted for bacteriological culture. Herd prevalence (percentage of herds visited with at least one cow found positive) was 17.8% for *Strep. agalactiae* and 86.2% for *Staph aureus*. Across all herd sizes, 2251 herds visits had at least one cow positive for *Strep. agalactiae*. Of those herd visits, 1590 BTM also yield a positive culture for the organism. Therefore, sensitivity of a single BTM culture was 70.6%. For *Staph. aureus*, 3642 herds had at least one cow positive while 2154 herds had at the same time a positive BTM culture for this bacterium (sensitivity = 59.1%).

In the case of *Mycoplasma spp.*, 26 of the 2409 herds visited between March 1992 and December 1998 had a BTM sample positive for the organism (prevalence = 1.1%). Sensitivity of one bulk tank culture was 33%.

The aforementioned information clearly shows that the use of a single BTM sample would not detect the presence of *Strep. agalactiae* in an infected herd 30% of the time. The possibility for *Staph. aureus* to remain undetected in an infected herd when a single BTM sample is used is even higher: 41%.

We recommend serial cultures to detect the presence of contagious mastitis pathogens in BTM. However, if at least three BTM samples are collected 3-4 days apart, are cultured for contagious mastitis organisms, and all are negative, the probability that cows contributing milk to the tank are negative is 97.3% for *Strep. agalactiae*, 93.1% for *Staph. aureus* and 70.0% for *Mycoplasma*. Seven consecutive daily cultures are even better to increase the predictive value of negative cultures for *Staph. aureus* and *Mycoplasma spp.*

*Prototheca* has been found to sometimes dramatically increase BTM standard plate count (9). Although the organism seems to be ubiquitous in some dairy environments and may have access to BTM because of manure contamination of teats, isolation from BTM usually means that there are cows with *Prototheca* intramammary infections in the herd (9).

The presence of *A. pyogenes* in BTM may be due to cows with clinical and subclinical infections or due to blind quarters milked by mistake by untrained milkers. The development of IMI caused by this organism is still not well understood. Occasionally, subclinical or clinical IMI because of *A. pyogenes* may be the cause of elevated bacteria counts in BTM (R.N. González, D.J. Wilson, unpublished observations).

### Culture of Quarter or Composite Milk Samples From Individual Cows

An IMI occurs when a microorganism invades the mammary gland and reaches the tissue suitable for its establishment and multiplication. Therefore, bacteriological culture of milk is still the accepted "gold standard" for the detection of the causative agent of that IMI. However, because of several factors already discussed elsewhere (2,8,9,16,13), the procedure may fail to isolate the pathogen. The accuracy of a test is defined as its ability to measure what it is supposed to measure. In bovine mastitis, the accuracy culturing quarter or composite milk for diagnosis of IMI has been estimated by comparing single milk culture results with "true" intramammary infection status based on multiple culture results (18). Four measures of diagnostic test accuracy are sensitivity (Se), specificity (Sp), predictive value positive (PV+) and predictive value negative (PV-). In our case, Se is the probability that a culture result will be positive when the cow tested actually has an IMI by a determined organism. Specificity is the probability that a culture result will be negative when the cow tested does not have an IMI by a determined organism. The PV+ of a culture test is the probability that a cow that cultures an organism will actually have an IMI with that organism. The PV- is the probability that a cow that shows a negative culture for an organism will not have an IMI.

For the isolation of *Strep. agalactiae*, the culture of quarter milk samples (0.01 ml inoculum) collected before and after milking yielded the following results, respectively (3): 98.8% and 97.7% Se, 100% and 98.8% Sp, 100% and 98.8% PV+, and 98.8% and 97.4% PV-. For composite milk samples (0.01 ml inoculum), results were as follows: 96.5% and 97.7% Se, 100% and 98.85% Sp, 100% and 98.8% PV+, and 96.4% PV- for both before and after milking. The accuracy of cultures using a larger inoculum (0.5 ml) was not significantly improved.

In another experiment, researchers cultured quarter milk samples (0.01ml inoculum) to determine the Se and Sp of bacteriological culture to isolate *Staph. aureus* from infected glands. Using a positive result on either pre-milking or post-milking samples as the definitive diagnosis for *Staph. aureus* (14), it was determined that pre-milking Se was 91% and post-milking Se was 81%. Following the same procedure, Sp were 92% for pre-milking and 96% for post-milking sampling, respectively. A more recent study (2), which included a variety of samples and inoculum sizes from several studies, showed that Se of bacteriological culture for *Staph. aureus* ranged from 54% to 92% while Sp ranged from 86% to 99.8%. Both Se and Sp were higher when a 0.1 ml milk inoculum was plated. For quarter milk samples that were collected for 6

consecutive days and 0.1 ml of milk was plated, Se and Sp were 90.9% and 99.8%, respectively (1). For single composite milk samples and a milk inoculum of 0.1 ml, Se was 92% and Sp 86% (11).

To our knowledge, there have not been studies related to Se and Sp for culture of *Mycoplasma*. Variable duration of clinical signs and shedder status has contributed to the difficulty of establishing those parameters in naturally infected cows. However, for group of cows with IMI because of *M. bovis* that were followed up for several months by culturing 0.1 ml of quarter milk samples, the Se of a single milk sample was 24% (8). *Mycoplasma bovis* was shed intermittently and periods without shedding the organism ranged between 2 and 20 days (8).

Cows infected with *Prototheca* may also become intermittent shedders (7) and there are not figures available for Se and Sp of bacteriological culture. However, the ecology of this organism presents a serious threat for a dairy operation. It has been reported that *Prototheca* can cause IMI, and also can be isolated from fecal samples from cows with or without a *Prototheca* IMI (7). This suggests that the organism can be brought into a dairy herd through the purchase of animals with infected mammary glands or as a resident of the digestive tract of animals of different ages, including calves (7).

There are some technical limitations for bacteriological culture to provide the most accurate information of the health status of a cow's mammary gland. However, the culture of individual cow milk samples (including heifers not yet milking) is by far the safest way that a dairy producer has to avoid purchasing contagious mastitis (18), principally, and *Prototheca* mastitis.

#### Routine Culture for *Mycoplasma*

Investigations undertaken at QMPS have shown that in almost all the herds that faced a *Mycoplasma* mastitis problem, the organisms were introduced when replacements (virgin heifers, pregnant heifers, cows or even calves) were purchased and commingled with the existing herd without quarantine and bacteriological testing (5,8). It has been frequently seen that purchased heifers were the origin of severe a *Mycoplasma* mastitis outbreak in previously *Mycoplasma*-free herds, the heifers showing clinical mastitis immediately after calving (5,8). Therefore, always test replacements for *Mycoplasma* mastitis, but keep in mind that a single milk culture has sensitivity of only 24%, probably due to intermittent shedding of the organism in milk.

#### New Additions on the Premises

If animals are not tested before or at the time of purchase, as in the case of dry cows, heifers and calves, they should be tested as they calve to safeguard against mixing cows with contagious mastitis (5,8,18,19). The same recommendation applies for *Prototheca* and *A. pyogenes*, because infection with these two organisms may arise during the dry period (6,10).

## Sample Collection

It is strongly recommended that the buyer or his representative obtain his/her own samples from animals or BTM following the procedures for collection and handling of milk samples published by the National Mastitis Council (9).

## Diagnostic Laboratory

It is important that prospective buyers evaluate the diagnostic capabilities of the laboratories that they may use to test samples. They need to be sure that the laboratory has sound standard operating procedures to produce consistent and reliable results (within the limitations of bacteriological diagnosis).

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