ON-FARM CULTURE AND TREATMENT DECISIONS

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Introduction

In spite of considerable improvements in milk quality, mastitis continues to be the most frequent and costly disease of dairy cows, but unless the cow is acutely ill, few veterinarians are directly involved in administration of mastitis treatments. Mild and moderate cases account for about 85% of all mastitis cases (Oliveira et a., 2014) and these cases are usually detected and treated by farm personnel without direct veterinary supervision. In the US, several studies have indicated that many dairy veterinarians are only marginally involved in mastitis control (Richert et al., 2013). Only 24% of dairy farmers (n = 180) enrolled in a milk quality program in Wisconsin indicated that they used their herd veterinarian to plan milk quality programs (Rodrigues et al., 2005). In a companion survey, most dairy veterinarians (n = 42) interested in participating in a mastitis control program indicated that they spent <10% of their professional time actively working to improve milk quality (Rodrigues and Ruegg. 2004). There are many economic and societal reasons for veterinarians to increase their involvement in mastitis control. Mastitis is the most common reason that antimicrobials are used on dairy farms (Pol and Ruegg, 2007, Saini et al., 2011) and much of the antimicrobial usage cannot be justified as many clinical cases are culture negative (Oliveira et al., 2013). The occurrence of mastitis has profound economic impacts through reduced milk production, increased milk discard, premature culling and reduced reproductive efficiency (Fetrow, 2000, Barker et al., 1998, Schrick, 2001, Fuenzalida et al., 2015). Involvement of veterinarians in development and implementation of a milk quality herd plan can result in increased demand for veterinary services and improved economic performance for the dairy farm. The purpose of this paper is to provide an introduction of how veterinarians can use milk culture data to improve mastitis control programs.

Use of Milk Culturing on Dairy Farms

Mastitis is caused by bacterial infection of the mammary gland and appropriate treatment and control programs are based on understanding the etiology of the infection. Historically, mastitis was primarily caused by *Staphylococcus aureus* and *Streptococcus agalactiae*, but in many regions, emphasis on improved milk quality has resulted in effective control of these pathogens. However, as dairy farms have increased in size and adopted intensive management strategies, an increasingly diverse group of pathogens have been associated with the occurrence of mastitis. Of 793 cases of clinical mastitis that were cultured from 50 Wisconsin dairy herds, the most common bacteriological diagnoses were no bacterial growth (25% of cases) and *E coli* (21% of cases); a further 11% of cases were caused by a variety of opportunistic organisms for which no approved antimicrobials can be expected to be effective (Oliveira et al., 2013). Regardless of etiology, almost all of the cases were treated symptomatically for 4-5 days using intramammary products (Oliveira and Ruegg, 2014). Many of the treatments were not appropriate but without diagnosis of etiology it is difficult to know when antimicrobial therapy is indicated. While clinical signs may be suggestive of some pathogens, detection of mastitis is based on observation of non-specific signs of inflammation and it is impossible to diagnose the etiology based on observation of the milk, gland or animal. Thus, almost all mastitis experts recommend the use of milk culturing to direct mastitis control programs. In spite of this advice, relatively few farmers use a culturing program to make treatment decisions (Hoe and Ruegg, 2005; Table 1).

Table 1. Frequency of submitting samples for culturing by Wisconsin Dairy Farmers

	Percent of responders				
Question	<50 Cows	51-100 Cows	101-200 Cows	>200 Cows	Overall
Number of Herds	279	202	42	37	
Frequency of submitting milk samples for culture (n = 547)					
All or some clinical cases	28%	33%	50%	70%	35%
Some or most cows with high SCC	28%	24%	23%	30%	27%
Some or all fresh cows	16%	13%	12%	25%	15%
Rarely submit milk cultures	56%	49%	45%	28%	51%

The use of diagnostic tests (such as milk cultures) is cost effective only when the results are closely linked to management decisions and the value of culturing milk from cows with clinical mastitis has been clearly identified by owners of larger herds. Of 325 large Wisconsin dairy herds recently surveyed, use of culture of milk from most or all clinical cases was 20%, 22%, 52% and 75% of herds containing 200-499, 500-999, 1000-2000 and >2000 cows,

respectively (Rowbotham and Ruegg, 2015 unpublished). Traditionally, microbiological examination of milk has been a technical task performed by trained microbiologists at a remote diagnostic laboratory. While accurate, submission of milk samples to remote laboratories results in a delay in receiving the results, reducing the ability to make real-time decisions about treatment. Anecdotal data suggests that farmers don't use of milk culturing because they don't know how to use the results or recognize the economic value of the decisions that are made as a result of the test. The challenge for veterinarians is to supervise or provide diagnostic services in a manner that can be easily used for on-farm decision making. If the etiology is determined rapidly, that knowledge can be used to direct mastitis therapy and result in economic benefits such as reduced antimicrobial usage and reduced milk withholding periods (Lago et al., 2011a,b).

Practical Aspects of Collecting and Using Milk Culture Data

Milk samples may be individually collected from affected quarters (quarter milk samples) or combined from all four glands into a single vial (composite milk samples). Quarter milk samples are more sensitive in detection of bacteria from subclinical infections as compared to composite samples, although the sensitivity of composite samples increases with the number of infected glands per cow. Mastitis occurs when teats are exposed to pathogenic bacteria that are able to overcome teat end defenses and stimulate a detectable immune response. Mastitis is therefore almost always caused by a single type of bacteria and laboratory protocols indicate that the recovery of >2 types of bacteria from a single milk sample is defined as contamination during collection (NMC, 1999). The use of composite milk samples, almost always results in a greater proportion of isolation of CNS and more contaminated samples and this method of sample collection should be reserved for screening for subclinical infections caused by organisms such as *Streptococcus agalactiae* and *Mycoplasma bovis*. When the goal of culturing is to identify cows with subclinical infections caused by contagious pathogens, pooling of 5-10 aseptically collected milk samples is another strategy that is used in some herds to further reduce sampling costs. When either of these strategies is used it is important to recognize the potential for false negative outcomes is increased. In all instances, sensitivities increase as the number of infected mammary gland quarters increase. When using pooled or composite samples, veterinarians should routinely review cow histories to identify cows with increased SCC that are typical of cows with subclinical infections.

Cows are generally more cooperative before milking and more likely to stand still to allow collection of a clean sample. To maximize the possibility of recovering bacteria from the sample, milk should be collected after the teats have been prepared for milking (application of pre-milking disinfectant and drying) but before the units are attached. Unless each teat is vigorously scrubbed with alcohol before sampling, it is likely that the milk sample will be contaminated with teat skin commensals (such as CNS or *C. bovis*). After collection, milk samples need to be cooled immediately and should be cultured on farm as soon as possible or submitted to the laboratory within 24 hours of collection. If samples cannot be processed within 24 hours, they should be frozen until transported to the lab. Freezing for periods of <2 weeks has minimal effects on recovery of most mastitis causing bacteria but can reduce recovery of *Mycoplasma spp*.

When specific bacterial diagnoses are needed, samples should not be processed in an on-farm laboratory and it is important that the milk samples are submitted to a laboratory that has experience working with mastitis bacteria. Standardized laboratory methods for identification of mastitis pathogens have been well defined (National Mastitis Council, 1999) and should be the basis for most identifications. While the methods used in most mastitis laboratories are simple, evaluation of results is improved by technicians who are experienced in interpretation of the nuances of working with bovine milk samples. In most laboratories the objective is to rapidly identify likely mastitis pathogens and complex methods are not generally needed. In general, technicians inoculate media that contains nutrients required for bacterial growth and the inoculated media is placed in an incubator that contains the appropriate atmosphere and temperature to encourage growth of the target organisms. In most mastitis laboratories, 10 to 100 μL of each milk sample are inoculated onto a portion of a blood agar plate. The inoculum volume determines the lower limit of detection. For example, one colony observed on a plate inoculated with 0.01-mL (10-μL) is equivalent to approximately 100 CFU/mL of milk while one colony observed using a 0.1-mL (100-μL) inoculum is equivalent to approximately 10 CFU/mL. The use of larger inoculum volumes increases sensitivity but also increases the possibility of contamination.

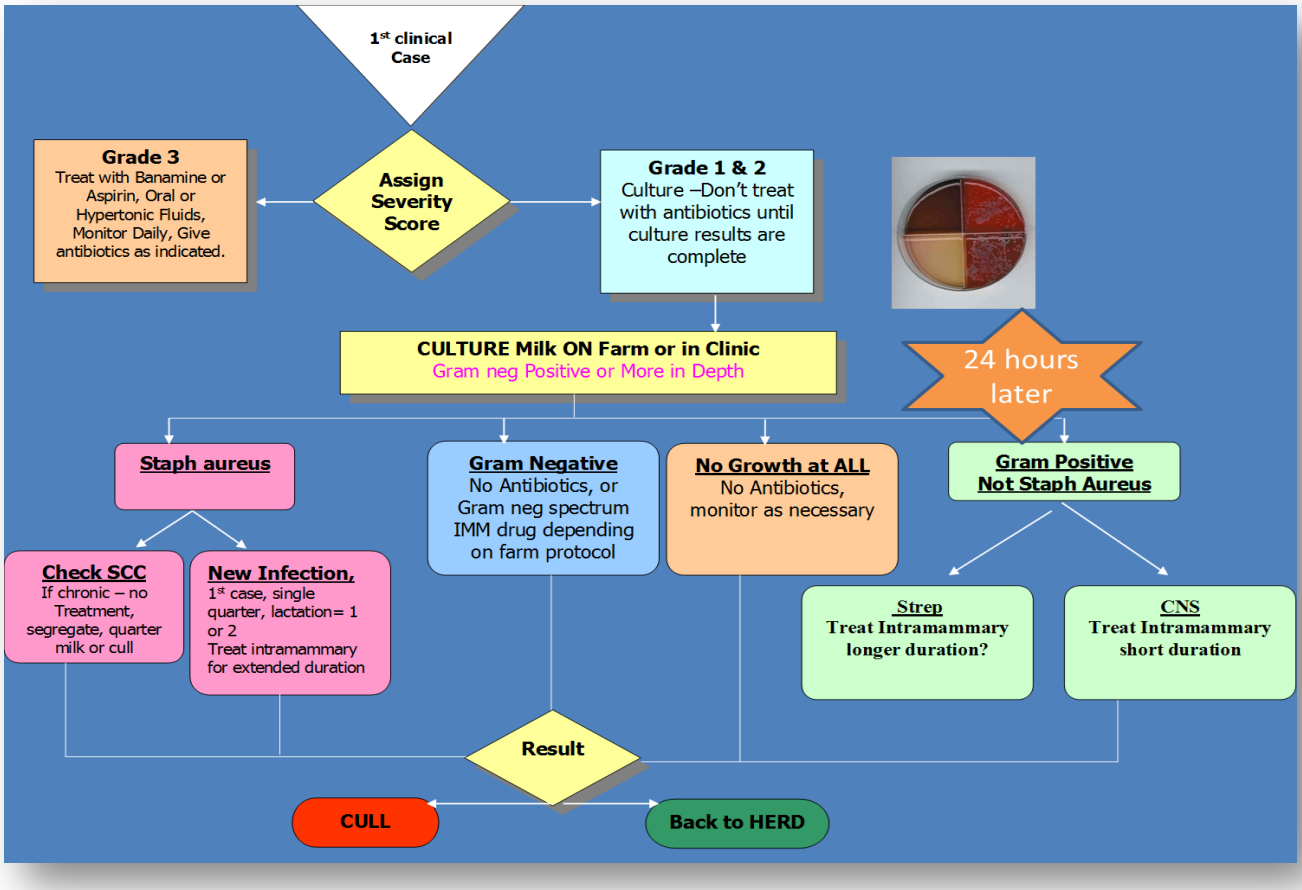
After inoculation, agar plates are incubated at 37°C and observed for growth at 24 and 48 hours. While there is no absolute definition of IMI, the presence of at least 100-300 cfu/mL is usually required to define an infection. Identification of bacteria is made based on phenotypic characteristics of the colonies and the result of additional

laboratory tests. *Staphylococcus aureus* is usually differentiated from other staphylococci based on a positive coagulase reaction and other typical phenotypic characteristics, such as hemolysis. *Streptococci* are usually identified using the Christie-Atkins-Munch-Petersen (CAMP) test and esculin reactions. When milk samples originate from cases of clinical mastitis, MacConkey agar is usually also inoculated to facilitate the rapid identification of Gram-negative, lactose-fermenting organisms (coliforms). Additional biochemical tests are required for final identification at the species level. Identification of *Mycoplasma* spp. requires the use of media containing specific nutrients not found in general medias and incubation in a CO_2 enhanced environment.

Use of on Farm Culturing to Make Mastitis Treatment Decision.

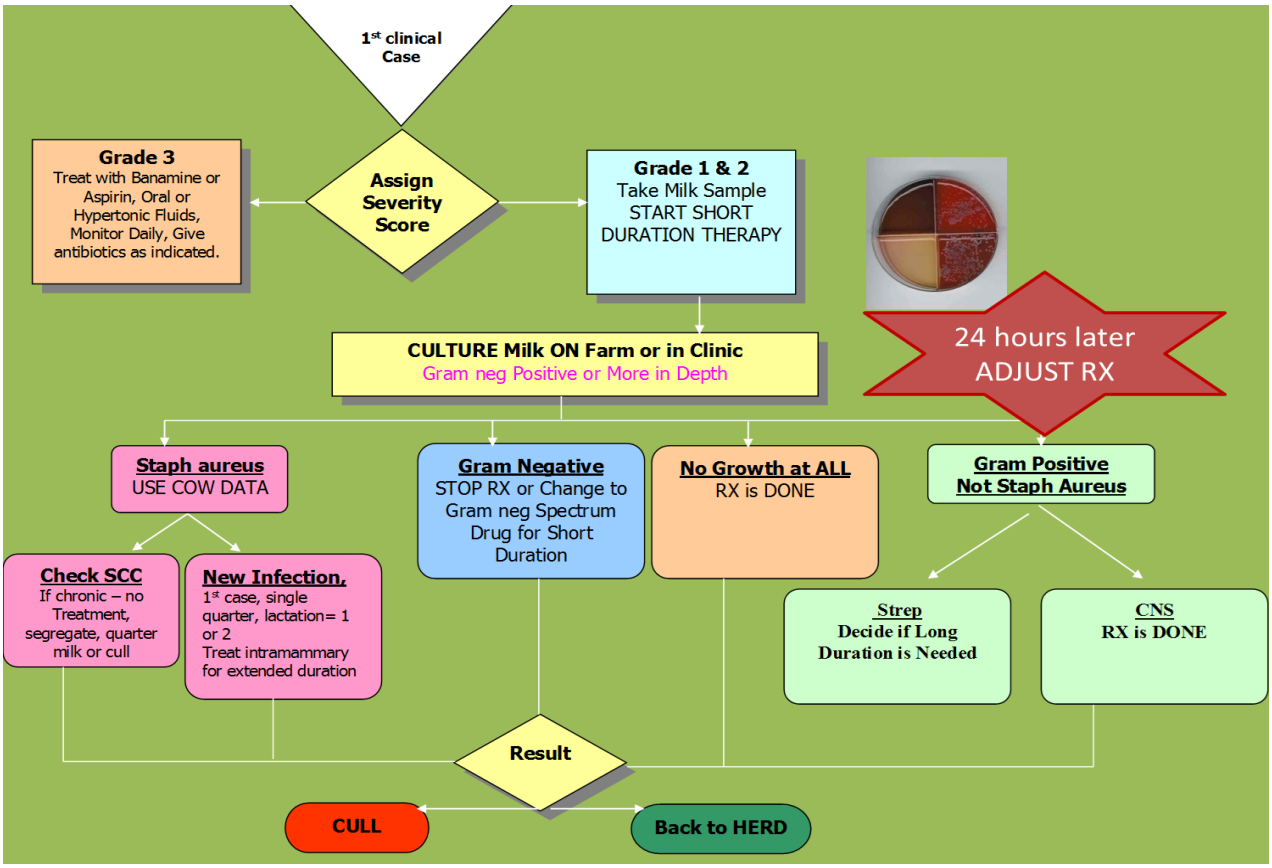
As dairy herds have increased in size and developed specialized labor forces, mastitis treatment plans that include the use of on-farm culturing have been developed (Hess, et al., 2003). In general, upon diagnosis of a clinical case of mastitis, the cow is examined and assigned a severity score and a milk sample is obtained. Recording of standardized severity scores can help veterinarians better define the pattern of clinical mastitis on individual farms. One severity scoring system uses a 3-point scale that combines the appearance of milk with the progression to additional clinical signs (1 (mild mastitis) = abnormal milk only; 2 (moderate mastitis) = abnormal milk & abnormal udder; 3(severe) = systemic symptoms) (Pinzon-Sanchez et al., 2011). This system is practical, simply recorded and can be an important way to assess detection intensity. In most herds, the distribution of clinical mastitis by severity is approximately 40-50% mild cases, 40-50% moderate cases and 5-15% of the cases scored as severe (Oliveira et al, 2013). If the proportion of severe cases is excessive it is a signal that detection intensity and case definition should be investigated. If the case is scored as a grade 1 or 2 (mild to moderate mastitis), then the use of OFC program to direct treatment may be considered. In some selective treatment programs, no antibiotic treatment is given until results of the OFC are known (generally24 hours) (Figure 1)

Figure 1: Selective therapy based on use of on-farm culture and 24 hour delay before therapy.



Alternatively, a farmer may begin treatment (after collection of the milk sample) but plan to readjust (change the duration or drug or end therapy) therapy 24 hours later, after the initial culture results are known (Figure 2).

Figure 2: Selective treatment based on immediate therapy and revision after diagnosis is known



Laboratory methods used for OFC are not usually the same as those that are used in professional diagnostic laboratories and are often performed by farm personnel who may have some technical expertise but lack formal training in microbiological methods. On most farms, OFC methods are based on the use of laboratory shortcuts and have a goal of rapidly reaching a presumed diagnosis to guide treatment. The most basic method of OFC is the use of biplates (usually containing a media selective for Gram-positive and Gram-negative bacteria), triplates (often includes a media selective for *Staph aureus*) or quadplates (often includes a media selective for *Streptococci* spp.). Growth on a selective media is used to differentiate cases as caused by Gram-positive or Gram-negative bacteria, culture-negative cases or in some instances specific pathogens. After 24 hours of incubation, culture plates are observed and the treatment protocol is specified based on the culture outcome. Typical agars that are used include: MacConkey agar (selective for growth of Gram-negative bacteria); TKT agar (selective for growth of Streptococci) and Factor, Baird-Parker or KLMB medias (selective for growth and differentiation of some Staphylococci spp.). Other medias may include chromogenic media that are identify *Staph aureus* or Klebsiella spp.. Studies have indicated that 24 hour interpretation of selective medias used in OFC systems is about 80% accurate in differentiating Gram-positive and Gram-negative pathogens as compared to diagnostic laboratories (Lago et al., 2011a). The use of OFC to make more specific pathogen diagnoses is not as accurate and requires additional training of personnel.

Most smaller herds (< 200 cows) do not have sufficient cases of mastitis to develop the expertise needed for OFC and one alternative is to offer rapid culturing using selective media within the local veterinary clinic. In these instances, farmers usually collect a milk sample and immediately initiate treatment. They will bring the milk sample to the local veterinary clinic and stop or modify treatment duration or change the drug after receiving a

preliminary microbiological diagnosis from their veterinarian in about 24 hours. Development and oversight of a selective treatment program guided by rapid culture results is an ideal way for veterinarians to increase involvement in mastitis decision making. Some veterinary practices provide increase their involvement by offering complete technical support for OFC systems. The use of veterinary technicians to supervise these programs may also increase veterinary involvement and oversight of mastitis treatments. When OFC is used, veterinary technicians can visit farms to restock supplies, train farm personnel and provide oversight and quality control.

Evaluating and Using Results.

The interpretation of results of milk cultures is dependent on the organism, sampling method and laboratory procedures. The techniques used in OFC programs are simple but it is quite possible for mistakes to occur and veterinarians should be involved in reviewing plates and records of microbiological results. The most common mistake is related to failure to aseptically collect the milk sample. Detection of contamination is more difficult using selective media as growth of some bacteria is suppressed, thus a simple rule is that growth on both the Gram-positive and Gram-negative media is a good indication that sample technique needs to be reviewed. Another common problem is the over-diagnosis of infection. Most OFC programs use a swab for inoculation with an estimated volume of about 0.1mL. The occurrence of 1 or 2 colonies of growth on such a plate is likely due to contamination and in many OFC programs, one requirement for a diagnosis of infection is growth of at least 3-5 colonies. One way veterinarians can supervise results of OFC is to review the proportion of culture-negative samples. On many farms, at least 25-30% of samples obtained from clinical cases will be culture negative. If all samples are microbiologically positive, that is a good sign that oversight of sampling and interpretation of the results is needed.

Increasing the use of diagnostic methodologies is an excellent way for veterinary practitioners to improve the efficiency and efficacy of mastitis treatments. The use of OFC to direct treatment of clinical mastitis gives farmers the opportunity to make better treatment decisions and reduce costs associated with milk discard and treatment of microbiologically negative cases (Lago et al., 2011a,b). A positively controlled clinical trial evaluating OFC used in a selective treatment program demonstrated that there were no significant differences in either long-term or short-term outcomes for cases of mastitis that received treatment based on results of OFC as compared to cases treated immediately without regard to diagnosis. (Lago et al., 2011a,b). In this study, antimicrobials were not administered to cases that were culture negative or Gram negative thus the use of intramammary antimicrobials was reduced by approximately 50% as compared to cases which were treated without prior diagnosis. Even more dramatic results were obtained in a study conducted on a large Michigan dairy. In this study, OFC was used to direct treatment of cows with mild to moderate cases of clinical mastitis. Cows that were infected with Gram-positive organisms received antimicrobial treatment while cows with no organism isolated or Gram-negative organisms were excluded from therapy. This reduced the number of treated cows by 80% 152 (Hess et al, 2003). It is apparent that selective treatment of mild clinical mastitis based on OFC or IVCC does not affect long-term outcomes, but does decrease antibiotic use. If farms do not routinely culture milk from cows with clinical mastitis, it is still important to determine the most frequent clinical mastitis pathogens in the herd to make appropriate treatment and prevention recommendations. It is particularly important to determine if *Mycoplasma* species or opportunistic pathogens that are nonresponsive to antibiotic therapy are contributing to clinical mastitis.

Conclusion

Development of an appropriate mastitis control strategy and selection of antibiotics for treatment of mastitis must be based on a presumptive diagnosis of the type of pathogen that is most likely responsible for the infection. While some farms are using on-farm culture systems to help define treatments, other farms may benefit if veterinarians provide similar services in a limited in-veterinary clinic laboratory. Even when on farm culture or veterinary clinic based rapid culturing cannot be provided, it is still extremely important to have at least some culture data routinely collected so that treatment protocols can be appropriately defined. When contagious mastitis is part of the problem, milk samples from fresh cows should be cultured to identify cows that are candidates for post-calving treatments, segregation or other interventions. The most cost-effective treatment protocols are those that are targeted for specific pathogens and provision of these services is an excellent way to increase veterinary involvement in mastitis control on dairy farms.

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