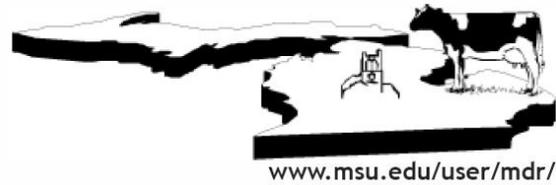


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Detecting *Mycoplasma* Mastitis

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Introduction

Mycoplasmas are normal inhabitants of the upper respiratory airways, urinary and genital tracts, and the digestive system; however, they can become infectious and cause high impact diseases in dairy herds including mastitis and metritis. In dairy cattle, *Mycoplasma bovis* is the most commonly isolated mycoplasma species (spp.). This species is very invasive and capable of colonizing different organs including the ear canal, the joints and the udder. Other species such as *Mycoplasma californicum* and *bovigenitalium* are also common pathogens in dairy cattle. *Mycoplasma* spp. cause disease in cattle of all ages; in calves for example, they cause pneumonia, arthritis, and otitis. In dairy heifers and cows, they cause clinical and subclinical mastitis, metritis, and pneumonia.

Signs of *Mycoplasma* Mastitis

Mycoplasmas are considered contagious mastitis pathogens since they are spread from infected to uninfected cows by milkers' hands and milking machines. *Mycoplasma* mastitis herd outbreaks usually are characterized by an increase in the number of severe clinical mastitis cases that are unresponsive to treatment. Cows with mycoplasma mastitis usually have more than one quarter infected, reducing considerably their milk production and elevating their somatic cell counts (SCC). Abnormal udder secretions are common and may vary from watery milk with a few clots to a thick colostrum-like material.

Table 1: Several key procedures at the farm, during sample transportation, and at the diagnostic laboratory before agar inoculation will assure the highest achievable sensitivity and specificity of mycoplasma detection techniques.

At the farm	Sample Storage and Transportation
Train dairy personnel in correct milk sampling procedures	If sample transit time is: <ul style="list-style-type: none"> • Less than 72 hr refrigerate at 36-42 °F • More than 72 hr freeze at -68 °F
Target high shedder groups such as fresh cows and clinical mastitis cases	If samples will be frozen use cryoprotectants <ul style="list-style-type: none"> • dimethyl sulfoxide (DMSO) 10% volume to volume • Glycerol 10-30% volume to volume

Cows chronically-infected with *Mycoplasma bovis* may show a tannish-colored secretion with sandy or flaky sediments that resembles cooked cereal in a whey-like fluid. *Mycoplasma mastitis* usually becomes chronic because these bacteria are resistant to the majority of antibiotics currently available in food animals. The reason is that mycoplasmas lack a cell wall and therefore are resistant to antimicrobials that target this structure such as penicillin.

In the case of subclinical mastitis, cases are characterized by progressive hardening and reduction in the size of the affected quarter. In some cases, multiple abscesses within the affected quarter may be found and this characteristic may cause intermittent shedding of mycoplasmas in milk from the affected quarter. In herds with high SCC, mycoplasmas should be considered as one of the possible causes of subclinical mastitis.

Mycoplasma Mastitis Herd Outbreaks

During a mycoplasma mastitis outbreak, priority should be given to identifying infected cows for segregation and/or culling as soon as possible. This is because antibiotic treatment does not suffice. Furthermore, mycoplasma is highly contagious and rapid identification will reduce the spread of the infection among the lactating herd. However, mycoplasma identification is difficult because these organisms are sensitive to drying, pH changes, and extended refrigeration or freezing periods. The other problem is that infected cows have intermittent bacterial shedding that further complicates detection. Different procedures can be implemented at the farm and when submitting the milk samples to improve the diagnosis of mycoplasma (Table 1 above).

Sample Collection and Storage

Mycoplasma screening should start with cows presenting clinical mastitis and cows within the first week of lactation. Both groups are more likely to be shedding mycoplasma organisms, as compared with mid- and late-lactation cows.

In the case of an open herd, milk from new animals should always be tested for mycoplasma growth before commingling with the herd. Routine bulk tank milk sampling should usually precede individual sampling. Depending on the size of the lactating herd and *Mycoplasma*

prevalence in the dairy operation, these samples should be repeated at least once a month for small herds, and weekly, for large herds.

Aseptic milk sample collection, usually following udder preparation and sanitizing the teat end with alcohol pads, is important to minimize contamination with bacteria from the environment. Good milk sampling procedures alone will increase mycoplasma recovery; this is because environmental bacteria growth will acidify milk and impair mycoplasma growth.

The amount of time between sample collection and culturing should be kept to a minimum. Since immediate culturing is far from practical in commercial herds, it is very likely that samples will require some type of storage. If the time period from sample collection until laboratory processing is less than 72 hr, milk samples should be refrigerated at 36-41 °F.

On the other hand, if the storage time is longer than 72 hr, samples should be frozen at -68 °F. In this case, addition of a cryoprotectant, either glycerol 10 to 30% volume to volume, or dimethyl sulfoxide (DMSO) 10% volume will improve mycoplasma recovery.

Use of the Non-culture Based Mycoplasma Tests

Different PCR mycoplasma detection tests are available to producers through DHI and animal health diagnostic laboratories. Currently, the use of these tests may be limited because they are targeted to identify *Mycoplasma bovis* only. Because of their sensitivity, cost, and speed of results, PCR testing is an attractive method for detection of this *Mycoplasma* spp. in milk samples from bulk tanks.

In the case of mycoplasma mastitis outbreaks, PCR testing could be used for screening milk samples from cow groups, thus reducing processing time. Positive PCR results should be interpreted carefully, and ideally confirmed by culture, because of the possibility of false positives.

During a mycoplasma mastitis outbreak, identification of mycoplasma positive cows should be accompanied by a complete critical analysis of milking routines, lactating herd teat condition, milking equipment performance, maternity pen management, protocols for new cow and heifer management, etc. *Mycoplasma* is prevalent in many Michigan dairy herds, therefore good preventive procedures are essential to minimize the risk of mycoplasma mastitis outbreaks.