Mycoplasma Mastitis in Dairy Cattle

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Introduction

Mastitis is defined as inflammation of the mammary gland, usually due to microbial infection. Many organisms have been known to cause mastitis including bacteria, fungi, and yeast. Mastitis is the most economically important disease of the dairy industry, the condition has been estimated to cause as much as two billion dollars in lost income for United States dairy producers at a cost of $181 per cow per year.[1] The biggest losses are due to lowered production, but discarded milk, drugs, veterinary costs, and premature culling also contribute to the losses.

More than 130 different microorganisms have been isolated from the mammary gland of the bovine with the majority of infections due to staphylococci, streptococci, and coliforms. However, mycoplasmas have begun to cause significant problems in some dairies. The first reported cases of mycoplasma mastitis were in Europe in 1960. Since that time it has been found all around the world, including the United States. Traditionally, California was most affected, but the disease has now become a problem across the entire country.[2]

The Organism

Mycoplasma are the smallest prokaryotic cells that can self-replicate. Their genomes are simple and one-fifth the size of the average bacteria. These organisms do not have the genetic capability to produce a cell wall, and are enclosed in a plasma membrane. Mycoplasmas can take many individual forms, including cocci, spirals, filaments, and rings, but they all grow in a characteristic "fried egg" microcolony. These bacteria are considered Gram negative but stain better with Giemsa rather than Gram stain.[3]

Over 100 Mycoplasma species have been identified to date, with more yet to be named.[2] Of the Mycoplasma species discovered, eight have been isolated from the bovine udder. By far the most frequent isolate recovered is *M. bovis,* but others such as M *canadense, M. bovigentalium,* and *M. californicum* have also been cultured .[4]

Mycoplasma require special medium on which to grow. Beef infusion is the basic medium with 20% serum, yeast extract, DNA, and other growth factors added. Along with the special medium, penicillin and other antibiotics are added to aid in selection of Mycoplasma versus other bacteria.[3] Because these organisms lack a cell wall, beta-lactam antibiotics can be added to the culture media and the growth of mycoplasma is not affected.

In vitro, mycoplasma species are susceptible to antibiotics which act in other ways besides inhibiting cell wall synthesis. However, the organism in vivo must function as a parasite in close contact with host cells. Because of this close contact, these microorganisms can obtain precursor molecules from the host and thus be fairly resistant to the antibiotics that act by inhibiting bacterial protein synthesis.[5]

Clinical Signs

As stated, the species most often implicated in mastitis is *Mycoplasma bovis.* This microorganism is a common inhabitant of the bovine upper respiratory tract, and is often involved in Bovine Respiratory Disease Complex and Enzootic Pneumonia of calves.[6]

Some other less common diseases which have been linked to *Mycoplasma bovis* are arthritis/synovitis, genital tract infections, abortion, and otitis media.[7] However, this discussion will be limited to

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mastitis.

Cows should be suspected of having a *Mycoplasma* species infection if they have severe purulent mastitis and no other systemic signs. Cows typically have mastitis in more than one quarter, with a sharp drop in milk production, sometimes approaching agalactia. Milk appearance can be tannish to brown with flaky sediment.[6] However, milk appearance is not a sensitive indicator for early infection and researchers have discovered that 10^6 to 10^8 colony forming units of *Mycoplasma bovis* per milliliter of milk can be shed for two to three days preceding any changes in milk appearance. The California Mastitis Test will be negative the first two to three days of infection but from three to seven days post infection both CMT and milk appearance will indicate mastitis. At some point during the initial week of infection the cow's milk production will drop dramatically and not rebound for months.[2] Despite the involvement of more than one quarter and severe inflammation the infected cows rarely go off feed or elicit a fever.[8]

Mycoplasma bovis can occasionly infect quarters where other pathogens already occur, allowing for variation in clinical signs depending on the other bacteria involved. Also on occasion cows can shed this organism and never show clinical signs, complicating control and outcome of an outbreak. It should be noted that because of a cow's ability to shed *Mycoplasma bovis* for variable amounts of time, she should be considered positive for life.[9]

Pathogenesis

Along with the common mastitis bacteria *Staphylococcus aureus* and *Streptococcus agalactiae, Mycoplasma bovis* is considered a contagious pathogen, thus infection is spread during the milking procedure. Because as few as 70 colony forming units per milliliter can cause infection, *M. bovis* can be spread easily from cow to cow with poor milking hygiene, contaminated equipment, and via milkers' hands.[10]

Although the majority of tissue damage is due to the cow's own immune response, mycoplasma themselves can produce direct damage. Metabolic end products and toxins can damage host cell membranes, and cause tissue damage.[5] The host's own immune response causes tissue destruction by a large inflammatory reaction of neutrophils, macrophages, plasma cells, eosinophils, and fibroblasts. Initially the alveoli and milk ducts fill with neutrophils and the epithelium of the ducts begin to divide, eventually filling the duct. Meanwhile, alveolar epithelium undergoes the opposite reaction: cells involute, milk production drops, and the cistern fills with exudate. Where the reaction is severe, the alveoli and ducts are replaced by fibrous scar tissue and are permanently lost; the areas less severely affected may begin producing milk again but at a lower level. Cows typically have numerous abscess in their mammory glands, ranging in size from microscopic to ten centimeters in diameter.[5] Generally, the host's immune response to *Mycoplasma bovis* is considered reactive versus protective. The reactive response leads to necrosis of mammary tissue and established foci of infection, causing reduced milk secretion. In addition, the abscesses are known to harbor viable *Mycoplasma bovis,* which may be responsible for long-term intermittent shedding by infected cows.[5,11]

Diagnosis

If cows are showing the clinical signs discussed earlier, quarter and composite milk samples from cows suspected of infection should be submitted for culture. Enriched media is used to isolate this organism, and a special request is often required by the veterinarian in order for the laboratory to conduct the culture for *Mycoplasma sp.* Samples should be kept cold or may be frozen for up to two weeks before arrival at the laboratory. The milk is then plated directly on to the mycoplasma media and incubated at 37°C in a five to ten percent CO^2 atmosphere. Usually growth can be seen in two to three days, but some mycoplasma require up to six or seven days of incubation before growth is seen. Another helpful diagnostic step is routine culturing of the bulk tank; this can help moni-

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tor a herd for early infection. If culture is positive, individual cows can be cultured in order to identify individuals with mastitis.

Culturing is the test of choice for confirming a suspected case. However, in order to identify the species of mycoplasma, different tests are required. Imunofluorescence and immunoblotting on colonies transferred to a nitrocellulose membrane are the common methods used to differentiate species. Because *Mycoplasma bovis* is considered the most pathogenic mycoplasma that causes mastitis, it is important to identify isolates down to the species in order to take the appropriate control measures and reduce spread to naïve cows.[2]

Control

Because no effective treatment exists, the most important aspects of a mycoplasma mastitis outbreak have to do with controlling its spread. Fomites such as the milking unit, milkers' hands, and wash clothes are capable of transmitting the organism from cow to cow, so milking hygiene is an important factor in reducing its spread. Cleanliness techniques such as single use towels, pre- and post-milk teat dipping, wearing of rubber gloves, and disinfection of the unit between cows have reduced the spread within a herd.[5]

In addition to the sanitary practices during milking, other steps may be necessary to reduce transmission between infected cows and clean cows. Culturing milk from all mastitis cases and any fresh cows for *Mycoplasma sp.* and then separating and milking the positive cows last will help control spread. Also, using single use mastitis treatment tubes for clinical cases of mastitis is beneficial.[5]

Antibiotic treatment at dry-off for the other common mastitis pathogens is a rewarding and encouraged practice. However, this same procedure can be a source of spread of *Mycoplasma bovis* and careful measures should be followed not to use contaminated intramammary mastitis tubes. Because mycoplasma are resistant in vivo to most commonly used drugs, antibiotic treatment on clinical cases is often futile and not recommended.[12]

Experimental vaccines for prevention of mycoplasma arthritis have given researchers hope in prevention of mastitis through vaccination. The few cows that are able to clear the infection on their own are less inclined to become reinfected. However, attempts at protection through parenteral and intramammary immunization have been disappointing. Both modified live and killed *Mycoplasma bovis* vaccines have been used and neither was proven to be effective. These experimental trials have elicited a humoral response, but this has not been proven to be protective against mastitis.[13]

Many herd outbreaks of mycoplasma mastitis can be linked to the arrival of new animals, putting expansion herds at a greater risk. Culturing milk from newly arrived animals will help to identify carriers and assist farmers in making decisions before the infection is widespread. Because one animal can shed large amounts of the microorganism, screening of all new animals is important. Once an animal has cultured positive, she should be segregated and milked last or culled immediately. In addition, some researchers believe respiratory shedding of *Mycoplsma bovis* may infect the mammary gland of naïve animals, so some experts recommend culturing nasal secretions from any new animals.[2]

Because mycoplasma organisms are shed in very high numbers from infected animals, bulk tank cultures can be a very sensitive test of farm infection. A single cow shedding the pathogen can be detected from the bulk tank culture on a farm of 400 head.[14] This sensitivity gives veterinarians and farmers an early warning of a possible outbreak. If the bulk tank is positive, further culturing of individual cows is necessary and the control measures discussed earlier need to be implemented to prevent widespread infection on that farm. Also, it should be noted that a cow that cultures positive is considered positive for life.[8]

Milk from cows infected with *Mycoplasma bovis* should be considered a possible source of infection to calves consuming the milk. *Mycoplasma bovis* was

recently isolated from a group of Holstein calves in Michigan diagnosed with otitis media. Investigators believe the calves consumed milk from infected cows on this known positive herd and this led to colonization of the nasopharynx with extension into the auditory tubes and tympanic bullae. The same group of calves also had an increased incidence of respiratory infections due to *Mycoplasma bovis*.[15] The common practice of feeding calves discarded milk from cows that have been treated for mastitis should be discouraged on farms known to be infected with this pathogen.

Conclusion

Mycoplasma mastitis is an emerging disease in the dairy industry. Once thought to be primarily confined to California's dairies, it is being diagnosed with increasing frequency across the country. Existing herds buying outside replacements and expanding herds are at increased risk of introducing the problem. Although the traditional mastitis bacteria are still the most economically important problems to the dairy industry, mycoplasma mastitis has devastated some farms undergoing expansion. Cows with multiple quarter mastitis and no systemic signs of illness should be considered possible cases and milk culture should be used to confirm the diagnosis. Once a herd is diagnosed as *Mycoplasma bovis* positive, steps should be taken to control its spread. Because the organism is shed in such high numbers in the milk, separating and milking the positives last along with strict milking time hygiene are beneficial in controlling spread within a herd. Culturing any new additions to a herd before they are mixed with the original animals will be of great benefit in preventing introduction to a naïve herd. Finally, due to its ability to resist antibiotics in vivo and its high degree of infectivity, culling of any cows testing positive for mycoplasma should be considered.◆

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