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Mycoplasma mastitis transmission and treatment

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بسم الله الرحمن الرحيم

﴿ اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ *
خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ * اقْرَأْ
وَرَبُّكَ الْأَكْرَمُ * الَّذِي عَلَّمَ بِالْقَلَمِ
*عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ ﴾
(العلق: 1 – 5)

صدق الله العظيم

Dedication

I dedicate my graduation to the one who taught me that love has no life and that giving has no limits to my dear mother, and to the candle that burned to illuminate my life path, my dear father.

At the beginning of my saying, thanks be to Gad, give my graduation and the fruit of my labor. Whoever smiles in my face is always my dear mother. I ask God to protect it for me from all evil and to my father, may God have mercy on him, for he has been a support and aid to me throughout his life.

I thank my mother for standing by my side throughout my life and staying with me throughout all stages of my academic life and her concern and her efforts for my sake. I thank my beloved sister, and I thank all my friends and my companions in the past years. I have graduated with my diploma and a lot of people whom I love with all my heart and appreciate their efforts.

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Introduction

The first reported case of mycoplasma mastitis was that of Hale and coworkers [1]. This Connecticut research group described the difficulties in isolating the pathogen that infected approximately 30% of a dairy herd. They had success when they allowed incubation of milk cultures to proceed for 5 days under 10% CO_2. They named the isolated organism *Mycoplasma agalactiae* var. *bovis*, currently known as *M. bovis*. This first described outbreak was remarkable in that it affected a large proportion of the herd, spread to multiple quarters of the same cow, and the agent was difficult to culture. Shortly after this report Carmichael and coworkers of NY [2] as reported by Jasper[3] and Stuart and coworkers of Britain [4] reported mycoplasma mastitis cases. One can imagine that following the report by Hale and coworkers [1], researchers [1,4] and others applied the culture techniques described and were able to isolate *Mycoplasma* sp. from cases of mastitis s that might have previously been considered idiopathic. Thus 50 years ago it was apparent that mycoplasma mastitis was a problem, perhaps an emerging problem.

Today it is recognized that mycoplasma mastitis affects cattle around the world [5,6]. *Mycoplasma* sp. are categorized as contagious mastitis pathogens [7] and it appears that mycoplasma mastitis is a growing problem in the USA [3,8,9,10]. Moreover, given the difficulty in culturing the pathogen that was first noted 50 years ago, there is reason to suspect that cases of mycoplasma mastitis are under-reported [11]. In this review the epidemiology of mycoplasma mastitis will be discussed, followed by a discussion of the host- pathogen interaction, and elements associated with control of the disease. A focus of the paper will be the presentation of recent findings that would explain why mycoplasma may be an emerging mastitis pathogen. Mastitis is a well-recognized and costly disease of dairy cattle. Most farmers are well acquainted with traditional causes of mastitis such as

Staphylococcus aureus **and** ***Streptococcus agalactia*****. The widespread adoption of standard mastitis control practices such as teat dipping, dry cow therapy, appropriate treatment, judicious culling and good milking preparation has allowed many dairy farmers to control contagious forms of mastitis. In a recent study,** ***Staph aureus*** **and** ***Strep ag*** **accounted for only 8% of clinical mastitis cases in Ontario dairy herds.4 While these traditional forms of mastitis are now controllable, mastitis continues to require management attention .**

Mycoplasmas **are the smallest and simplest self-replicating bacteria. The mycoplasma cell contains the minimum set of organelles essential for growth and replication: a plasma membrane, ribosomes, and a genome consisting of a double-stranded circular DNA molecule . Unlike all other prokaryotes, the mycoplasmas have no cell walls, and they are consequently placed in a separate class** ***Mollicutes*****(*****mollis*****, soft; *****cutis*****, skin). The trivial term mollicutes is frequently used as a general term to describe any member of the class, replacing in this respect the older term mycoplasmas.**

Mycoplasmas have been nicknamed the "crabgrass" of cell cultures because their infections are persistent, frequently difficult to detect and diagnose, and difficult to cure. Contamination of cell cultures by mycoplasmas presents serious problems in research laboratories and in biotechnological industries using cell cultures. The origin of contaminating mycoplasmas is in components of the culture medium, particularly serum, or in the flora of the technician's mouth, spread by droplet infection. [24]

2. literature review

2.1- Mycoplasma :

Mycoplasmas are a group of very small organisms that can be cultured from multiple body sites of both sick and healthy cattle. Some common species of mycoplasma include *M. bovis* (most commonly cultured from the udder), *M. alkalescens* (commonly cultured from the respiratory tract, *M. bovigenitalium* (commonly cultured from reproductive tract) and *M. canadense* (commonly cultured from joints). While many of these organisms have been isolated from bovine mastitis, *M. bovis* is the most common mycoplasma species isolated from milk samples in Wisconsin. *Mycoplasma* species are very small bacteria and are unique because they lack a bacterial cell wall. This means they are neither Gram-negative nor Gram positive. Several *Mycoplasma* species cause mastitis, including *M. bovis* (most common mastitis isolate), *M. alkalescens*, *M. bovigenitalium*, and *M. canadense*. These organisms may be isolated from both sick and healthy animals, from the respiratory and reproductive tracts and other sites. *Mycoplasma* is a tiny bacterium that can cause mastitis, metritis, pneumonia, drooped ears, and lameness in dairy cattle. While this bacterium has existed for more than 100 years, the current disease was first recognized in the 1960s and 1970s, and has only recently become a problem in Virginia. There has been a steady rise in the frequency and severity of disease associated with *Mycoplasma* in the last ten years. *Mycoplasma* is a highly contagious disease that can have devastating economic effects on a dairy farm due to decreased milk production, additional veterinary costs, culling of cows, calf loss, and treatment cost. All dairy animals can be infected, including calves, heifers, dry cows and lactating cows. [15,12].

2.2- EPIDEMIOLOGY :

Mycoplasma **species are pathogens associated with several cattle diseases, primarily otitis media, inflammation of the urogenital tract, arthritis, pneumonia and mastitis. The most prevalent species causing these diseases is *M. bovis* . With respect to mycoplasma mastitis, *M. bovis* is the predominant causative agent and *M. californicum* and *M. bovigenitalium* appear to the next most common**

Jasper summarized the agents associated with cases of clinical mycoplasma mastitis during a 14 year period and found that *M. bovis* and *californicum* were the most common. The third most common was *M. alkalescens* which comprised approximately 12% of intramammary infections followed by *M. bovigenitalium* at 5% . Kirk and coworkers surveyed bulk tank milk from a cooperative of 267 dairies in CA monthly for 6 years. The annual prevalence of tanks with *Mycoplasma* sp. knwn to be mastitis agents ranged from 1.2%-3.1% of tank samples. They reported that *Mycoplasmas bovis, californicum,* and *bovigenitalium* were most consistently the mycoplasma mastitis agents isolated. Boonyayatra et al. examined milk samples from 248 cases of clinical mastitis from a variety of sources over several years and reported 85% were *M. bovis*, 5% were *M. californicum* and only 1% were *M. bovigenitalium*. In the surveys reported in Table 1 it is clear that *M. bovis* and *M. californicum* appear to be the two most prevalent mycoplasma mastitis pathogens. Other species that have been noted as causes of mycoplasma mastitis include: *M. arginini, bovirhinis, canadense*, dispar, bovine group 7, and F-38 [18].

2.3- Prevalence :

Prevalence of contagious mastitis pathogens estimates have been made through culture and analysis of bulk tank milk samples. The major contagious mastitis pathogens identified this way in the USA are: *Staphylococcus aureus, Streptococcus agalactiae* and *Mycoplasma* sp.; with herd level prevalence of 43.0%, 2.6%, and 3.2% . In this survey , the herd size affected the prevalence of only

mycoplasma mastitis; with the prevalence of other contagious mastitis pathogens unaltered by the number of cows per herd. In large herds (>500 cows) the prevalence of mycoplasma mastitis was 14.4%. Results from a previous study were similar as it was reported that the percentages of mycoplasma positive bulk tanks from herds with less than 100, 100-499, and >500 cows was 2.1, 3.9, and 21.7 . In the later survey regional differences were noted with 9.4% of the operations in the West having one positive mycoplasma bulk tank culture, with operations in the Northeast and Midwest with less than 3% and the Southeast at 6.6%. Presumably the regional differences are a function of herd size as herds are largest in the West and smallest in the Northeast and Midwest .

Based on bulk tank surveys the prevalence of mycoplasma mastitis varies across the globe. In the European Union countries of Belgium, France, and Greece the range in prevalence was <1% to 5.4% of herds . Yet surveys done in Mexico [24], Iran . and Australia indicate prevalence estimates as high as 55% to 100% of herds. In New Zealand, McDonald and coworkers surveyed 244 herds and could not detect *Mycoplasma* sp. in any bulk tank samples suggesting a very low prevalence. The wide variation in global prevalence may be a function of exposure to these agents. Importation and mixing of cattle has been reported to lead to outbreaks of mycoplasma diseases. For example, the first reported case of mycoplasma cattle disease in Ireland occurred in 1993 and was attributed to the relaxation of import controls within the European Union . Exposure of naïve cattle to this agent led to the appearance and then a significant increase in bovine mycoplasma diseases . [16].

Herd replacement cattle exposed to cattle outside the herd, either imported or reared off-site, increased with increasing herd size, a biosecurity risk factor. It was found that herd size and culling were risk factors for increased herd prevalence of mycoplasma mastitis. Presumably this is a result of herd expansion, the entrance of new cattle with symptomatic or asymptomatic carriage of new strains of *Mycoplasma* sp. into the herd. Thus the elevated prevalence of

mycoplasma mastitis in herds, and herds of some countries, where cattle movement into and out-of a herd is common, could explain the increased prevalence of this disease.

Cow level prevalence is more difficult to estimate. It has been reported that in Britain less than 1% of cows are affected by mycoplasma mastitis . Mycoplasma mastitis has most often been reported as a clinical disease. A survey of clinical mastitis in NY indicates that *Mycoplasma* sp. are the cause of 1.5% of cases . [13].

2.4- Transmission :

***Mycoplasma* sp. that have been associated with mastitis have been considered contagious in nature, transmitted mostly at milking time from a reservoir, the infected udder; via fomites, hands of a milker, milking unit liners, or udder wash cloths; to an uninfected cow . Strict milking time hygiene practices of: disinfectant of udders before milking using single service towels; use of rubber type gloves by milkers; post-milking unit disinfection; and disinfection of teats post-milking; were very effective in controlling the traditional contagious mastitis pathogens of *S. aureus* and *S. agalactiae* . It has been assumed, but not tested, that such practices would be effective in the control of mycoplasma mastitis. [14].**

Mycoplasma species can spread from one bovine body site to another presumably via lymph or peripheral blood systems. *Mycoplasma* sp. associated with mastitis have been isolated from the blood of cattle . In outbreaks with mycoplasma mastitis it is not unusual to find cases of mycoplasma arthritis . Similarly a field outbreak of mycoplasma associated bovine respiratory disease was associated with outbreaks of arthritis . The link between arthritic mycoplasma disease events and mastitis or pneumonia is indicative that internal somatic spread of this agent is not uncommon. Often multiple organ sites of cattle can be colonized and it is clear that the strain causing the disease is most often the same strain that is widely disseminated throughout the body . This is also been shown by Jain et al.1969 who experimentally induced intramammary infections with *Mycoplasma* sp. in lactating

cows and found that the apparent strain inoculated was shed at the mucosal surfaces of the eyes, nose, vagina and rectum, within hours to days after inoculation. With this experiment they also demonstrated vertical transfer of the agent as a calf, born during the trial from one experimentally infected cow, became colonized by the by the agent . Moreover in an outbreak of mycoplasma mastitis, the agent was found colonizing the nares of cattle, both cows and/or calves . The strain causing mastitis was found from nasal swab samples collected from cows and calves . Thus transmission of *Mycoplasma* sp. associated with bovine mastitis may occur within the cow internally, from one infected organ site to the udder or reverse; and between cows from indirect udder to udder contact at milking time; or perhaps by shedding of the pathogen through external mucosal surfaces of an infected or colonized animal to a naïve animal. [15].

Transmission of *Mycoplasma* sp. from environmental sources to the udder has been discussed . In this review the authors report on two studies, one in Italy and one in Germany, where it was found that *M. bovis* survived in and on multiple surfaces at various temperatures for up to 8 months. Materials studied were those that could be typically found on dairies including sponges, stainless steel, wood, rubber, glass, and water. Justice-Allen and coworkers in Utah discovered that mycoplasma could live for up to 8 months in a sand pile. The sand originated from a herd with an outbreak of mycoplasma mastitis. Mycoplasma was also isolated in sand from two other dairies. The authors suggested that sand could be a reservoir for mycoplasma mastitis. However in a separate investigation where there appeared to be a link between sand bedding and a clinical mastitis outbreak it was found that the strains of *Mycoplasma* sp. in the bedding had a completely different fingerprint than those causing mastitis. Utah researchers investigated the possible transmission of *M. bovis* from sand to naïve dairy calves during a 105 d trial. Although calves housed on sand bedding with *M. bovis* carried this agent for periods of time during the trial, there was no evidence of carriage beyond transient colonization and no specific antibody titers formed against the agent. The authors

concluded that there was no evidence that the contaminated bedding would serve as a source of *M. bovis* disease transmission to naïve dairy calves. Thus although it is clear that environmental sources could serve as a reservoir for mycoplasma mastitis, there is no evidence to support that *M. bovis* transmission from the environment to a cow is a likely mechanism involved in mycoplasma mastitis. [18].

2.5- Clinical Signs :

Mycoplasma causes a clinical contagious mastitis that usually appears as a swollen quarter that is sensitive to the touch and has decreased milk production. This is followed by abnormal milk 1 to 3 days later. Often, the milk initially has visible particles that progress to pus, eventually becoming watery with fine particles that form sediment. Affected cows generally do not appear sick and maintain good appetites. Frequently, more than one quarter is affected. *Mycoplasma* often will invade quarters that are already infected with other organisms. The incidence of *Mycoplasma* mastitis also appears to be greater in the winter (Gonzalez *et al*., 1992). Somatic cell counts (SCC) are often elevated in cows infected with *Mycoplasma*, but normal SCC are possible. This characteristic means that it is possible for cattle that are not showing clinical signs and have normal SCC to be a source of infection for other cows. Cows nearly always have subclinical infections with *Mycoplasma* after recovering from clinical cases. Cows that have never been noted to have clinical cases may also have subclinical *Mycoplasma* infections.

2.6- Diseases caused by Mycoplasma.

M. bovis lives naturally in the respiratory tract of cattle throughout the world.3 Most respiratory tract colonizations of Mycoplasma do not produce symptoms of disease but M. bovis is an important cause of respiratory disease in calves and feedlot cattle. Mycoplasma has also been implicated in joint infections, occasional abortions and ear infections in calves. [22].

2.7- Characteristic of Mycoplasma Mastitis .

The classic symptoms of mycoplasma mastitis have been described :

- **Multiple quarters involved**
- **Dramatically decreased milk production**
- **Cows appear otherwise healthy but have severe mastitis**
- **Milk has sandy or flaky sediments in watery or serous fluid**

However, cows can develop subclinical infections with mycoplasma and have normal appearing milk.1 These subclinically infected cows may have intermittent periods of abnormal milk or their milk may continually appear normal. Somatic cell counts of subclinically infected cows will be increased. Cows that have had mycoplasma cultured from their milk should be considered to be permanently infected regardless of the visual appearance of their milk. [21].

2.8- Mycoplasma Mastitis Diagnosis .

Bacteriologic culture of milk is required for the diagnosis of mycoplasma mastitis. Milk samples from infected quarters, composite milk samples from infected cows or bulk tank samples can be submitted for culturing. Not every mastitis laboratory performs cultures for mycoplasma because special techniques must be used to grow this organism. The Wisconsin Animal Health Laboratory is one Wisconsin laboratory that performs mycoplasma cultures. Even at laboratories that offer mycoplasma culture, the culture is not performed unless it is specifically requested. To detect mycoplasma, milk is plated on different media and incubated for 7 days in a special incubator. In milk samples obtained from individual cows, a negative mycoplasma culture usually means that the organism is not present. However, intermittent shedding of the organism has been reported, so false negative cultures may rarely occur.3 Bulk tank culturing is a good way to monitor a herd for the introduction of mycoplasma mastitis. Detection of as few as one infected cow in bulk tank milk from a 1000 cow dairy has been reported.1 Like cultures of individual cow milk samples, periodi shedding patterns may lead to an occasional false negative bulk tank sample in a herd with infected cattle. [20].

2.9- Mycoplasma Mastitis Spread .

Mycoplasma mastitis is classified as a contagious mastitis pathogen because the reservoir for the infection is other infected cattle, including calves. In contrast to other forms of contagious mastitis, mycoplasma infection can spread from the respiratory system to the udder. The spread can occur due to transmission through the air or through the blood stream. A history of respiratory disease or ear infections in calves occasionally precedes outbreaks of mycoplasma mastitis. A common source of infection is the purchase of cows subclinically infected with mycoplasma mastitis. Non-lactating animals are also at risk as they can be subclinically infected prior to freshening. After calving, these animals may never develop clinical mastitis but may shed high levels of mycoplasma organisms in their milk.1 Transmission between cows can occur during the milking process or through contamination of cow contact areas in the environment. [23].

2.10- Mycoplasma be Controlled .

The first step in controlling mycoplasma mastitis is recognizing that the disease is present in Wisconsin dairy herds. A strong association between the introduction of new cattle and outbreaks of mycoplasma mastitis has been reported.1 Mastitis biosecurity programs can be used to decrease the risk of purchasing infected cattle. Bulk tank cultures from the herd of origin should be requested for non-lactating purchased cows and somatic cell counts and composite milk samples from individual cows should be reviewed prior to purchasing lactating cows. Cows that calve after purchase should be isolated until a negative composite milk sample is obtained. Herds that are routinely purchasing cattle should submit bulk tank milk for mycoplasma twice monthly. The management of sick and fresh cows also contribute to the spread of this organism. Fresh cows should not be housed in the same pens or milked with the same equipment as sick cows. The feeding of waste milk to calves is another source of transmission of this disease throughout the herd. Calves fed infected milk may develop pneumonia,

joint infections and head tilts related to ear infections. When mycoplasma is found in a bulk tank or individual cow culture, the number of infected cows must be determined. Depending upon herd size, there are several strategies that can be considered. If resources allow or the herd is small, composite samples from all cows should be submitted for culture. In larger herds, group milk samples can be submitted by sequentially culturing the bulk tank during milking. Individual milk samples can be obtained from cows only in the infected groups. [24].

There is no treatment for cows that develop mycoplasma mastitis. Antibiotics are totally ineffective for this organism. Cows that are infected with mycoplasma should always be considered as infectious, regardless of their production level, appearance of their milk or subsequent negative milk culture. In most cases, infected cows should be promptly culled. The only exception to this rule is when a culling is financially unacceptable because a large proportion of a herd is infected. In this case a herd specific strict segregation plan should be developed.

2.11- Treatment :

Most experts agree that there is no effective treatment for *Mycoplasma* mastitis. If other organisms are present, they should be cultured and treated. If the immune status of the animal is good, cows may eventually eliminate the infection, but the time period for this to occur is extremely variable. Additionally, infected cows may develop normal milk but still be subclinically infected (and therefore able to shed the bacteria) well into the next lactation (Byrne *et al*., 2005). [19].

2.12- Prevention :

An important aspect of prevention is the thorough screening of animals before they enter the herd. Requesting cultures for *Mycoplasma* on the individual cow or from bulk milk tank samples before purchasing new cows is highly recommended. Three to five samples should culture negative for *Mycoplasma* over the 1 to 2 months prior to adding the new animal to your herd. Although this seems like a lot of testing, the consequences of introducing *Mycoplasma* into a dairy herd can be disastrous. The source of the cow introductions should be considered in

determining the number of cultures performed. Pregnant heifers can carry *Mycoplasma* in their udders

and become clinically infected after they calve. Consequently, heifers should also be cultured. At freshening, the measures taken to prevent other contagious causes of mastitis (such as *Staph aureus* and *Strep agalactiae*) will also help in the prevention of *Mycoplasma* infections. [16].

Recommended preventative measures include :

- **Automatic milking detachers**
- **Sand bedding**
- **Dip cups instead of spraying**
- **Pre- and postdipping**
- **Good overall sanitation during udder preparation and milking**

Damaged teat ends make it easier for organisms to invade the udder. If there are a large number of damaged teat ends (approximately 20 percent to 25 percent), or a sudden increase in the number of damaged teat ends, the milking equipment vacuum should be checked. It is also important to use commercially prepared, single-use mastitis preparations and only place the very tip of the applicator into the teat. Again, good hygiene during the treatment procedure is critical. Bulk-tank cultures can be performed on a routine basis (ex. every month) to ensure *Mycoplasma* is not likely present on the farm. [17].

Summary :

Mycoplasma **is a costly and difficult problem to deal with once it has entered the herd. It causes a contagious mastitis in the lactating dairy herd as well as a severe otitis, pneumonia, and arthritis in calves and heifers. New arrivals, heifers, and lactating cows with mastitis should all be screened for *Mycoplasma*. Monthly bulk-tank samples can also be screened for *Mycoplasma.* Routine screening is particularly important for open herds. *Mycoplasma* should be suspected when there is a large number of mastitis cases that are unresponsive to treatment or if unresponsive pneumonia, otitis, and arthritis occurs in the calves. Working closely with a veterinarian to set up a monitoring and/or treatment program specifically for the herd is recommended for all modern dairies.**

Over the past several years, sophisticated molecular-based techniques such as PCR, along with older technology such as serology, and culture, augmented by knowledge obtained from the complete genome sequence, have been applied in epidemiologic investigations, animal models of disease, evaluation of diagnostic tests, and clinical trials of antimicrobial agents. As a result, our understanding of *M. pneumoniae*'s cell biology, mechanisms of cytadherence, disease production, evasion of host defenses, disease transmission, contribution to chronic lung diseases, emergence of antimicrobial resistance, and efficacy of new antimicrobial treatments have improved. Despite these many advances, much is still unknown about this microorganism, which is among the smallest of all bacteria. Most *Mycoplasma* infections never have a microbiological diagnosis because rapid, sensitive, specific, and reasonably priced methods for its direct detection are not readily available in physician offices or hospital laboratories. A reliable and user-friendly nucleic acid amplification method for *Mycoplasma* detection in clinical specimens adapted for performance in clinical diagnostic laboratories would be of immense importance both for patient diagnosis and management and for epidemiological research. Development of a safe vaccine that offers protective immunity might also go a long way towards reducing the extent of *M. pneumoniae* infections, particularly in high-risk populations such as the military, schools, hospitals, and other institutions where large numbers of people dwell in close proximity, but this seems unlikely to be developed in the foreseeable future. [5,6].

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