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## **Mycoplasma: What we know about mastitis and systemic disease**

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Several mycoplasma species can cause diseases in dairy cows. Mastitis, pneumonia and arthritis agents include *Mycoplasma bovis* and *Mycoplasma californicum*. Several species may cause sporadic mastitis, and ureaplasmas can cause vulvovaginitis and infertility. The most frequently reported mycoplasma problem in cows is disease caused by *M. bovis*. Many of the mycoplasma species, pathogens and non-pathogens, may also be recovered in samplings from cows affected by other diseases or stressing factors. It is therefore useful to recognize well characterized clinical syndromes that are associated with the important mycoplasmal diseases of dairy cows.

*Acute mycoplasmal mastitis, pneumonia, and polyarthritis-* A Western Iowa dairy herd of 130 cows was comingled with 180 purchased cows. Within a week, a few cows were reported with single non-secreting quarters. Rapid progression to full agalactia followed, and affected cows were febrile. By 15 days, there were 12 cows with severe mastitis. This represented cows from the home herd as well as the purchased herd, early and late lactating. Cows responded to antibiotic treatment with slight recovery of secretion, then relapsed with severe mastitis, and most of them also presented polyarthritis involving knees, hocks and shoulder joints, as well as signs of pneumonia. Culture from affected quarters yielded *M. bovis*, and segregated milking of all abnormal milk cows was instituted. At one month of comingling there were 20 cows dead or salvaged, and bulk tanks from "clean strings" tested negative. At 3 months 25% of affected herd cows were culture-positive on composite samples. At 3.5 months, tank samples from the healthy herd gave positive cultures and it was decided to culture all cows. The culture-positives (5/200) were culled. Overall losses (all causes) by 4 months were 110/310 cows. Tank samples taken at 5, 6, and 7 months after comingle were negative. Serology done on all cows at 6 months after comingle revealed that only 70% of cows were seropositive.

*Chronic mycoplasmal mastitis-* Herds yielding bulk tank samples that are persistently or intermittently positive for mycoplasmas have been described. Generally, this outcome follows an acute mycoplasmal outbreak. In a study following a herd up to a year after an outbreak, the percentage of mycoplasma shedder cows in the herd closely followed that of cows with non-secreting quarters. A third of cows with non-secreting quarters were found to be shedders[1]. It should be noted that chronic shedders were shown to produce as much milk as healthy cows. In another herd followed over 8 years, 25 bouts of mycoplasmal infection were detected during the period. These were initiated by infections in freshened cows in over a third of the bouts. Significant increase in environmental mastitis cases could be correlated with these mycoplasmal mastitis bouts [2].

*Disease in dairy calves-* Dairy calves can be affected by mycoplasmal diseases of several different types. In a study of calves raised by 18 herds of 25 to 180 cows, pneumonia at 4 to 5 weeks of age was significantly associated with seroconversion to *Mycoplasma dispar* and recovery of this organism from transtracheal wash samples [3]. Mortality in these calves was

low, at 2% of calves. Typically, these calves present with persistent cough of one month or more, are febrile, but continue eating. *M. dispar* infection is prevalent, with nearly 100% of calves becoming exposed during the first 6 months of life [4]. In contrast, in large California dairy calf ranches, pneumonia is seen at 3 to 4 weeks of age in association with recoveries of *M. bovis* from lungs. Here mortality can rise to 7% of calves, and can be refractory to antibiotic treatment. Calves raised on crude discard milk from dairies affected by mycoplasmal mastitis can present with pneumonia and polyarthritis evident from 4 weeks of age onwards. Most commonly this involves *M. bovis*, although severe presentations with *M. californicum* have been described [5]. While mortality is variable, and generally under 5%, morbidity may be over 80%. Operations raising large numbers of dairy calves in barns often describe epizootic presentations of mycoplasmal otitis media in calves of 3 to 5 weeks of age [6]. These calves initially present conjunctivitis, later droopy ears and head tilt. Pneumonia and polyarthritis can also be seen in advanced cases. As with all other mycoplasmal diseases, the calves eat well and have low grade fever. Finally, calves with decubital subcutaneous abscesses have been described in calf ranches feeding discard milk. These calves became affected at 3 weeks of age, and later also developed polyarthritis and pneumonia [7].

*Transmission and epidemiology*- Nose to nose contact appears to be a major route of transmission. In one study with *M. bovis*, this transmission was noted within 5 days, and involved 100% of contact calves by 10 days. Transmission by milking environment may be much faster. Experimental infection of udders with mycoplasmas results in shedding quarters within one day [8]. Confirming this, clinical signs appear in cows within days of introduction of the infection into herds [9]. The infectious dose for nasal transmission has not been precisely determined, but is presumed to be similar to the udder quarter infectious dose of 100 CFU [10]. Nasal secretions and milk can contain high concentrations of mycoplasmas, often  $10^6$  times the infectious dose. This high concentration during acute infection stages assures efficient transmission, as well as seeding of the environment. Environmental contamination with mycoplasmas is a concern. Significant persistence of mycoplasmal inocula on dry surfaces has been reported, in particular for *M. bovis* [11]. The importance of this persistence in establishing new outbreaks in cattle has not been determined.

*Pathogenesis*- As studies on pathogenesis of *M. bovis* infections have advanced, it has become clear that this mycoplasma is very different in attributes from other mucosal-associated mycoplasmas (such as *Mycoplasma hyopneumoniae* of swine). Attachment of *M. bovis* to mucosal surfaces appears to be mediated by phase-variant surface proteins and multiple other surface proteins. With a phase-variant protein in ON phase (expressed), the mycoplasma attaches to ciliated epithelial cells [12]. After rapid switch to OFF, the mycoplasma can invade epithelium and access the bloodstream. A number of other host, and agent phenomena are also presumed to be involved in this epithelial translocation, and these remain to be studied. Arthritis and heifer mastitis are understood to follow seeding of these tissues from blood, although detection of *M. bovis* in blood is difficult in field cases.

We have shown that about 50% of *M. bovis* strains induce abscess formation. These 3-6 mm abscesses are found disseminated in lung tissue, in synovial membrane walls, and in middle ears. Unlike other bacterial abscesses, these structures are not heavily walled off and a prominent mononuclear reaction is found in the periphery. Lung abscesses are formed by progression from

occluded bronchi, and in these bronchi, there is minimal damage to respiratory epithelial cells. We have shown that an epithelial cytotoxic factor was produced by strains of *M. bovis* that do not produce abscesses, while abscessing strains do not produce this, providing a lab marker to differentiate strains of divergent pathogenicity. For yet unknown reasons, *M. bovis* uses its phase variant surface proteins to interact with phagocytic cells. The proteins are in ON phase in neutrophil-rich abscess areas, and in OFF phase in mononuclear cell-rich areas. In addition, *M. bovis* will switch to OFF phase when found in blood after circulating antibody responses are produced by the host. These observations fit with the notion that the mycoplasma uses phase variation of surface proteins to avoid antibody-complement-mediated lysis, and to avoid phagocytosis by macrophages.

Cachexy sequelae are described for infections with *M. bovis*. Calves that do not respond to repeated therapy rapidly lose body condition, even though they continue eating. The observation that *M. bovis* induces strong production of tumor necrosis factor alpha (TNF- $\alpha$ ) when cultured with lung macrophages is tied in to this [13]. We have observed prolonged lung colonization, of more than  $10^8$  organisms/gram lung tissue for more than 30 days, and this would presumably lead to high and persistent levels of circulating TNF- $\alpha$ , a hallmark of cachexia. The absence of cell wall in *M. bovis* explains the lack of anorexia in these chronic cases, since there are no polymers to induce febrile responses.

An important histologic lesion in affected lungs is septal thickening with edema and lymphatic and vascular thrombosis. All strains of *M. bovis* apparently produce these lesions. However, the vasculitis appears limited to lung (and joint) lesion areas, and is not associated with systemic vascular alterations. The significance of this localized vasculitis has not yet been determined, aside from providing a valuable diagnostic sign.

A historical observation has been that *M. bovis* is immunosuppressive to bovine lymphocytes in vitro [14]. We have revisited this and observed that *M. bovis* kills all classes of lymphocytes in vitro, and may produce local to systemic and non-specific immunosuppression [15]. The phenomenon may also be caused by at least two activities, a lymphotoxic property and an immunosuppressive one. The implications for immune responses to unrelated antigens during *M. bovis* infections is currently under study.

Overall, the pathogenesis of *M. bovis* infections can be subdivided into those aspects related to surface phase variation (attachment, invasion, evasion from host defenses), and those that are associated to toxic activities (abscess formation, vasculitis, immunosuppression, and indirectly, cachexia). These pathogenic aspects of *M. bovis* are important determinants that set the stage for unresponsiveness to therapy, as well as the technical difficulties encountered in the development of vaccines.

**Diagnosis-** Since both acute or chronic mycoplasmal mastitis can cause serious economic loss, detection of udder-pathogenic mycoplasmas is of concern. Several PCR approaches have been reported for the detection of *M. bovis* in milk samples, while comparable tests for other pathogenic species are lacking at this time. Because of this, culture of milk samples is required, even though *M. bovis* is the most prevalent pathogenic mycoplasma. Routine periodic testing of bulk tanks should be considered for large herds, or herds undergoing expansion. In addition,

individual cows presenting mastitis that do not yield bacterial cultures, or are unresponsive to antibiotic treatment, should be tested also. Samples for mycoplasma culture can be stored frozen, or shipped refrigerated if testing can be done within two days. These samples should be handled by laboratories that can provide this specialized service, and can determine that the species of mycoplasma recovered is pathogenic. After a herd is declared positive, additional testing may be needed to segregate an “infected sub-herd” that will be milked last. This is done by pooling composite milk samples from strings of 10 or more cows. Tank samples containing milk from even a few acute shedder cows mixed with several hundred healthy cows will test positive.

In calf operations, presentations with significant mortality refractory to treatment should be subjected to etiological diagnosis. Swollen joints can be tapped to provide samples that often yield mycoplasmas in pure culture. These samples, as well as lung fragments can be sent refrigerated for culture or detection. Again, *M. bovis* is the most frequent pathogen of significance, and specific PCR detection of this species can provide economical diagnosis. Nasal swabs or tracheal wash samples are less valuable, since pathogenic mycoplasmas may be found in the upper respiratory tract without playing a role in disease. Serological diagnosis has been used for *M. bovis* on a herd basis. The ELISA test can present some problems with false positives, and is not usable with calves that have been vaccinated. The problem of how to screen incoming replacement heifers is still unsolved, at least by individual animal screening.

*Prevention and Control*- Closed dairy herds that have not had clinical forms of mycoplasma mastitis require programs to prevent entry of infection. These herds need to have full control of their replacement heifer program, and should test all semen batches for mycoplasmas. In infected or at-risk herds decisions need to be made to either cull all positive cows, or minimize spread and allow the infection to gradually subside. Aggressive initial culling of all clinical and test-positive cows can eliminate the acute shedders, thus reducing the herd and environmental burden of infection. Early and continuing segregation of an “infected subherd” can result in more rapid attainment of negative bulk tank samples for the dairy. Since chronic cows can shed mycoplasmas intermittently in milk, a positive status should be considered irreversible for that cow’s lactation. A proportion of positive cows will become shedders again in the following lactation. In one study following an acute mastitis episode, cows in second lactation were at much higher risk of having mycoplasma mastitis than those in other lactation cycles [1]. Thus, testing of freshening cows no earlier than the third day of lactation, followed by segregation if needed, can reduce flare-ups after an acute infection. Colostrum samples, even in infected herds, tend to be negative by culture. Immunity to reinfection is specific for the species of mycoplasma involved, and is short-lived. Vaccination of dry cows has not been shown to prevent infections, although it may not have negative effects.

Calves are infected with mycoplasmas by contact with the dam at birth, or ingestion of contaminated discard milk. In calf operations that have a single source of infection, it may be useful to complete an etiological diagnosis as well as an antimicrobial profile for the pathogenic mycoplasma involved. After assuring that all discard milk used as feed is pasteurized, calves can be treated with appropriate antibiotics by injectable and/or oral route to provide medication coverage for 10 days. All calves at risk should be treated to reduce mycoplasma burden on the premises. In calf operations that have multiple sources of infection (calf ranches), it has been found useful to apply multistrain autogenous bacterins in three to four weekly doses starting soon

after birth. For complete suppression of pneumonia, calves will need to be treated as indicated above as supplement to the vaccination program. Heifer calves can benefit from vaccination with commercial bacterins several weeks prior to shipping to dairies, and revaccination during dry periods is a recommended follow-up.

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