Bovine Mycoplasmosis

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Significance

Mycoplasmal diseases remain among the most intractable infectious diseases of cattle. Difficulties in diagnosis, poor response to treatment, few preventive measures and increased incidence have converted these diseases into a new frontier for the bovine practitioner. On this frontier, unknown factors and scarce resources create conditions of great danger, and perhaps also opportunity. A regional context is in order. In Asia, Africa, the Middle East, and parts of southwestern Europe contagious bovine pleuropneumonia, caused by Mycoplasma mycoides subsp. mycoides continues to be a problem. Rapidly spreading into new regions, or to regions previously cleared of this disease, the disease represents a continuing world-wide threat. Introductions of this endemic disease into North America via infected bovidae have been prevented by prolonged quarantine observations.

In the United States several diseases of bovines that are caused by mycoplasmas can be recognized. Feedlot pneumonia, arthritis, and mycoplasmal mastitis are diseases that can cause severe losses at this time. Pneumonia, arthritis and otitis of young dairy calves can also be significant. Some mycoplasmas can play a significant role in enzootic pneumonia of dairy or beef calves. Finally, some diseases of minor importance have been recognized, such as ulcerative vulvovaginitis, ureaplasmal infertility and bovine mycoplasmal conjunctivitis. This paper will only provide an overview of the major bovine mycoplasmal diseases of North America.

Clinical Overviews

Feedlot pneumonia and arthritis is a disease produced by Mycoplasma bovis. It is characterized by subacute pneumonia that may be accompanied by lameness with multiple swollen limb joints. It presents at three to six weeks after shipment to feedlots or backgrounding operations. Moderate febrile responses are noted, and response to most antibiotics is poor. Affected animals lose condition rapidly, with some appearing cachectic. Coughing is seen sporadically. Lung lesions include marked septal edema and may also include multiple small coagulative necrosis (abscessing) lesions centered on bronchioles. Joints have clear and abundant fluid and thickened capsules, tenosynovitis, and bursitis is prominent. Cattle from all regions of the United States have presented with this condition, and all ages and sizes can be affected. Morbidity can vary from 10 to over 80%, and overall mortality (including culling) has reached 10%.

Mycoplasmal mastitis is generally produced by M. bovis infection, although occasional infections with Mycoplasma californicum can be clinically indistinguishable. Acute presentations involving several cows are seen when mycoplasmal mastitis first affects a herd. Affected cows are febrile but alert, multiple quarters are swollen, warm, and milk production is dramatically decreased, with only serous, or serous with flakes secretion produced. Cows may also present with pneumonia and lameness. Up to 10% of the cows in a herd may die or need to be culled in such an outbreak. More commonly, the infection is endemic in the dairy, with multiple chronic shedders that maintain acceptable to high production of milk. With poor milking hygiene and stress, flare-ups are seen involving a few cows that have usually only one quarter affected. Mycoplasmal mastitis in a dairy is usually associated with problems in calves, such as pneumonia and arthritis, particularly if discard milk is used to feed them.

In young dairy calves, disease caused by mycoplasmal mastitis agents (M. bovis, M. californicum) presents at three to four weeks of age. Calves will have febrile...
reactions and pneumonia, and may be lame with markedly swollen limb joints. Otitis media (droopy ears and tilted head) may be seen occasionally, or may be the primary sign affecting a large number of calves. Subcutaneous infection at decubital sore sites results in palpable crepitant edematous swellings that can abscess. Affected calves may recover, but will evolve into "poor doers" or cripples. More importantly, all will remain nasal shedders of mycoplasma for many months, and will be a source of infection for replacement heifers.

Enzootic pneumonia can be caused by *Mycoplasma dispar* or *M. bovis*. Infection with either of these mycoplasmas may be clinically indistinguishable, or concurrent. Since only *M. bovis* gives systemic infections, lameness, subcutaneous swellings or otitis media concurrent with chronic pneumonia would suggest the presence of *M. bovis*.Calves affected are from two to six months of age. Mortality can be very variable, but may reach high levels if stress and overcrowding are persistent problems.

**Etiological Agents and Pathogenesis**

Mycoplasmas are very small wall-less bacteria. They are somewhat sensitive to drying and osmotic shock, yet their small size allows them to be more resistant to environmental insults than mammalian cells. Most mycoplasmas are resistant to penicillins and beta-lactam antimicrobials. Resistance to other antimicrobials varies from strain to strain for any species of mycoplasma. Mycoplasmas also can rapidly acquire resistance to antimicrobials due to their rapid rate of mutation and genetic rearrangement.

Mycoplasmas such as *M. bovis, M. dispar* and *M. californicum* are strictly extracellular yet require a significant concentration of cholesterol to grow. This forces them to live attached to epithelial cells (*M. dispar*), unless they can translocate across mucosal surfaces and access blood (*M. bovis, M. californicum*), or damage endothelium and cause edematous lesions so they can access cholesterol bound to plasma albumin (*M. bovis*).

Virulence factors of mycoplasmas are poorly described to date. Attachment of *M. dispar* is affected by a capsular polysaccharide and surface proteins. Noncapsulated mycoplasmas, such as *M. bovis* and *M. californicum*, use multiple surface proteins to attach, and some of these can be rapidly changed or suppressed from the mycoplasmal surface by the agile genetic mechanisms of these mycoplasmas. Because of this, vaccines stimulating immune responses to mycoplasmal surface proteins cannot fully prevent colonization. The capsule of *M. dispar* has been shown to inhibit alveolar macrophage functions, so this mycoplasma can act as a primary agent and reduce respiratory tract defenses against bacteria of low pathogenicity, such as *Pasteurella multocida*. An immunosuppressive effect of *M. bovis* is currently being studied. In addition, *M. bovis* has been shown to induce tumor necrosis-alpha production by alveolar macrophages. This can lead to endothelial damage, edema, and if systemic, to caquexia.

Young calves are protected from tracheal infection by *M. dispar* by maternal antibody for a few weeks. After that, the mycoplasma rapidly colonizes the trachea and bronchii and significantly reduces ciliary escalator activity. This is a very frequent scenario in beef and dairy calves. The organism cannot translocate, so the infection is self-limiting but persistent. A lymphoid activation results, with prominent cuffing around bronchioles. With the bronchial lumen obstructed, lung atelectasis occurs, typically in the caudal aspects of cranial lung lobes.

Colostrum provides very little protection against *M. bovis*. Nasal colonization with this mycoplasma, although persistent, may not progress downward. With virulent strains, and in the presence of a debilitated respiratory tree, trachea and bronchii are colonized. This rapidly leads to mycoplasmemia which may be transient or persistent, and is always accompanied by a mild febrile response. Persistence in blood for several days is needed to produce lesions in joints, synovial sheaths of tendons, and bursae. The interaction of *M. bovis* with endothelial linings is still poorly described, but must play crucial roles in establishing mammary infections, middle ear infections, or subcutaneous edema lesions. Independent of systemic effects, persistent bronchiolar colonization with *M. bovis* leads to the attraction of large numbers of neutrophils to these sites. Some strains of *M. bovis* produce multiple small (5 mm in dia.) coagulative necrosis lesions centered around bronchioles, and bronchioles are filled with purulent material that can be expressed out of them on cut sections of lung. All strains of *M. bovis* produce septal edema, visceral pleuritis and interstitial pneumonitis. It is thought this is in response to the strong tumor necrosis-alpha production elicited by the infection. Affected lung tissue can contain massive amounts of mycoplasmas up to the time of death of the animal. Mastitis caused by *M. bovis* is most often caused by ascending teat infection under conditions of poor milking hygiene. Fresh heifers are at greatest risk of infection, with upper respiratory fomites as a source, or more infrequently they can localize a mycoplasmemia (requires several days of febrile mycoplasmemia). An affected quarter will release massive amounts of mycoplasma in the first days of the acute response. Thereafter, milk production returns to normal in 10 to 50% of animals. These "normal" quarters maintain foci of *M. bovis* colonization for months and presumably after drying. The mammary gland is very susceptible to mycoplasmal infection, and species (this presumably also applies to strains) that can colonize the
Case 1 – Enzootic Pneumonia

A beef herd of 250 purebred Hereford cows (with 30% first-calf heifers) calved on open pasture during a stormy spring. Severe flooding and calf scours forced the producer to bring calves into his barn for treatment. Many calves subsequently developed pneumonia. Mannheimia haemolytica and M. bovis were cultured from submitted lungs. Antibiotic treatment resulted in control of the losses associated with M. haemolytica pneumonia. Enzootic pneumonia cases continued with staggered deaths that resulted in a composite mortality of 60% of the calf crop over six months of time. There were no reports of lameness, and no presence of coagulative necrosis lesions recorded in lungs. These calves uniformly yielded M. bovis from lungs. Examination of the M. bovis isolates recovered over time revealed that a single genotype was involved in this outbreak.5 Antimicrobial sensitivity tests done on the isolates did not identify any useful drug from among those approved at the time. Enzootic pneumonia caused by M. bovis in beef calves of one to seven months of age is not commonly reported, and this case presents exceptional environmental and management conditions. More common are presentations involving M. dispar, where coughing and febrile responses without anorexia are noted. These cases have low mortality, and mortality is commonly caused by associated viral or bacterial pathogens. Response to treatment can be seen with antibiotic therapy directed against the bacterial pathogen. In these cases the involvement of M. dispar was suspected after necropsy results.

Case 2 – Feedlot Pneumonia and Arthritis

A midwestern feedlot received 200 light-weight beef calves (ave. 450 lb) during early fall from a western ranch. After processing with inactivated vaccines, cattle were treated uneventfully for respiratory disease during the first 10 days. Shortly after, some calves became lame, and by 3 weeks, 25% of them were lame, febrile, coughed, and had therapy-resistant pneumonia. Lame calves had swollen hocks and knees. Several animals had subcutaneous swellings on their backs that were edematous on palpation, and some ulcerated and drained serous exudate. Several of the affected calves lost condition rapidly, and 15 of them died. On necropsy, severe bronchopneumonia was seen, with numerous small coagulative necrotic lesions disseminated throughout the lungs. M. bovis was recovered from noses of affected and healthy calves, and from lung lesions. Immunohistochemistry proved that the small necrotic lesions were associated with M. bovis antigen accumulations. All deaths occurred during weeks three through six after arrival. At about seven weeks, a group of home-raised calves were placed in the feedlot with nose-to-nose contact with the affected group. No disease was noted in this group of calves, and nasal swab samples were obtained from them at 12 weeks. Isolates of M. bovis obtained from the lungs and noses of affected calves were of a single genotype, demonstrating that a single introduction of infection was made. Healthy home-raised calves yielded 2 different genotypes of M. bovis from their noses, the one associated with the outbreak as well as a different one.

Diagnosis and Management - Pneumonia

Since mycoplasmas can colonize noses of cattle without causing disease, it is important to determine if there is lung colonization. It should also be noted that mycoplasmal pneumonia will take much longer to establish than bacterial pneumonia, so cattle affected from the third week and later, are more likely to be suspect. Presentations involving M. bovis are currently suspected in all antibiotic-resistant, late pneumonia diseases. The best etiological diagnosis is secured when lung tissue is submitted fresh and refrigerated for immunohistochemistry. Formalin-fixed tissue may lose antigenic signal due to excessive formalin exposure. Since most diagnostic labs do not offer immuno-histochemistry testing for M. bovis, other methods are used. Mycoplasma can be cultured from lung homogenates or bronchial swab samples, but should be speciated since other non-pathogenic mycoplasmas may also be cultured from these samples. Speciation is accomplished by indirect immunofluorescence or immunoblotting using specific antisera. PCR can help determine whether M. bovis is present or not, and this gives satisfactory results in older calves and feedlot samples, where M. bovis is currently the primary pathogen. In young dairy calves with pneumonia, other mycoplasmal pathogens may be involved or may co-infect with M. bovis. Culture and identification of pathogenic species is necessary in these cases. Some labs use PCR complemented with restriction fragment analysis to speciate mycoplasmas obtained from cases. The procedure is time-consuming since each suspected species has to be grown in purity prior to PCR. Antimicrobial susceptibility is performed by broth microdilution,12 and in the case of M. bovis requires the use of redox indicators to detect growth. Antimicrobial susceptibility testing in feedlot outbreaks is of minor value, since isolates of M. bovis can be expected to be highly resistant, and the information is of little retrospective value. Susceptibility testing in enzootic pneumonia cases, particularly dairy calves, may be of value. Isolates of M. bovis from dairy herds are susceptible to more antimicrobials, and can come from the same source over time.
All evidence indicates that stress is a necessary component to allow *M. bovis* to colonize the lower respiratory tract. Minimizing stress can be a significant management strategy. Treatment of calves affected with enzootic pneumonia may be successful in reducing mortality if started early (shortly after grouping hutch-raised calves) and with an appropriate antibiotic.

It may be more beneficial to identify the source of the infection. This could be a particular dairy supplying calves to the calf raising farm, or the use of unpasteurized, or poorly pasteurized discard milk. Pooling of colostrum may also increase risk of transmission to calves.

Autogenous bacterins have been shown to provide immunity if multiple doses of vaccine are used. It is imperative to continue evaluating deaths to assure that the mix of pathogens does not change significantly over time.

Early treatment with antibiotics that control *M. bovis* may be important in feedlots. Autogenous bacterins cannot be successfully used in feedlots. Commercial bacterins have been recently used, and two doses given prior to shipping may be needed to obtain protection.

**Case 3 - Acute Mastitis**

A midwestern dairy expanded by purchasing 130 cows which were added to the home group of 100. All cows were milked in a new parlor. Within one month, 33 cows presented with acute mastitis involving multiple quarters, with severe agalactia and/or altered secretion (completely serous to small white grains in milk). Several cows had pneumonia and some became lame and had swollen joints. During the next month 29 cows died or were culled (did not return to production or were pneumonic or lame), including 9 that were culled because *M. bovis* was cultured from composite 4 quarter milk samples obtained from them. A full herd sampling done at four months revealed that cows were still shedding low levels of *M. bovis* in milk. The owner opted not to cull these, and bulk tank samples became negative after six months from expansion. Of note in this case is that almost equal numbers of home cows were strongly seropositive for *M. bovis*, as incoming cows were affected. Also, serum samples obtained two months after commingling indicated that all cows were strongly seropositive for *M. bovis* by ELISA. This provides evidence that both herds were exposed to *M. bovis*, most likely through nasal colonization. Stress of commingling and adaptation to the new parlor probably increased nasal shedding as well as reduced udder defenses.

**Diagnosis and Management - Mastitis**

Bulk tank samples should be routinely cultured for mycoplasmas in large herds or during expansions. Milk samples can be frozen or shipped refrigerated for arrival within one day. Although only 2% of dairies have mycoplasmal mastitis, very few are seronegative due to nasal colonization or antigenic cross-reactivity between mycoplasma species. Culture from milk and identification of species by indirect fluorescent antibody or immunoblotting is the accepted diagnostic standard. PCR or PCR complemented with restriction analysis is also used. In all cases, diagnosis requires species identification since non-pathogenic mycoplasmas (Acholeplasmas) can be recovered from normal milk. When a dairy yields positive bulk tank milk samples, further culture tests can help identify shedders for culling. To reduce costs, samples from milking strings can be pooled for testing, and only those pools that are positive require individual cow testing. A nested PCR utilizing a procedure for extraction of nucleic acid from milk has enabled detection of 1 *M. bovis/ml* milk, even after the milk is treated with preservatives. Thus, DHIA milk samples can also be used to screen for *M. bovis* and aid in a culling program. Serology has been used to confirm acutely affected herds. ELISA serology is still questioned due to the antigenic cross-reactivity among certain species of mycoplasmas of cattle. In areas of low mycoplasma exposure, a seronegative status can be trusted in selecting well isolated replacement dairy heifers. Treatment of cows has not been successful, and vaccination has not led to protection.

**Conclusions**

Mycoplasmal diseases, particularly those due to *M. bovis*, are increasingly recognized as significant problems. Evolving understanding of the transmission patterns as well as the pathogenesis of disease can be combined with new diagnostic approaches to provide options for managing these diseases.

**References**