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Mycoplasma bovis Infections in Cattle

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Mycoplasma bovis is a pathogen causing respiratory disease, otitis media, arthritis, mastitis, and a variety of other diseases in cattle worldwide. It is increasingly recognized by the veterinary and livestock communities as having an important impact on the health, welfare, and productivity of dairy and beef cattle. *M. bovis* diseases can be difficult to diagnose and control because of inconsistent disease expression and response to treatments and vaccines, and large gaps in our understanding of the epidemiology and pathophysiology of these diseases. There are limited data on which to base evidence-based decisions for treatment and control, and the literature contains differing clinical biases and opinions. This document is intended for veterinarians dealing with cattle and is focused on the cattle production systems of North America. The goal of the consensus statement panel was to encourage an evidence-based approach to *M. bovis* problems. The scientific literature was critically reviewed, including peer-reviewed journal articles and reviews obtained by database searches using the terms "*Mycoplasma bovis*" or "mycoplasma + cattle." Where other data were lacking, conference proceedings were reviewed as a source of expert opinion.

Key words: Arthritis; Mastitis; Otitis media; Pneumonia.

How Important Is *Mycoplasma bovis* as a Bovine Pathogen?

The ability of *M. bovis* to cause mastitis,[1] respiratory disease,[2] and arthritis[3] is demonstrated in experimental infection studies, although variation in disease severity is common. In natural infections, *M. bovis* can be isolated in pure culture from the mammary gland of cows with mastitis[4] and from the joints, tendon sheaths, or periarticular tissues of cattle with arthritis, tenosynovitis, or chronic pneumonia and polyarthritis syndrome (CPPS).[3,5–8] *M. bovis* is the predominant pathogen isolated from the middle ear of calves with otitis media.[9,10] However, the role of *M. bovis* in the multifactorial bovine respiratory disease (BRD) complex is not as easily defined. At the group level, seroconversion to *M. bovis* is associated with increased risk of being treated for BRD.[11] *M. bovis* is often isolated from the lungs of cattle with pneumonia,[5,12] and identified within lesions using immunohistochemistry (IHC).[8] However, *M. bovis* can also be isolated from the lungs of some cattle without clinical disease or lesions, and so variable disease expression appears to be a key feature of both natural and experimental infections. *M. bovis* is often present in the upper respiratory tract (URT) of cattle without clinical disease. Therefore, although *M. bovis* alone can cause natural and experimentally induced clinical disease, the presence of *M. bovis* does not always result in disease, and clinical disease does not appear necessary for the maintenance and dissemination of *M. bovis* in the cattle population.

Abbreviations:

BAL	bronchoalveolar lavage
BRD	bovine respiratory disease
CPPS	chronic pneumonia and polyarthritis syndrome
IHC	immunohistochemistry
IKC	infectious keratoconjunctivitis
MIC	minimum inhibitory concentration
PCR	polymerase chain reaction
URT	upper respiratory tract

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What Is the Prevalence/Incidence of M. bovis *Infection and Disease?*

In a 2002 survey of 871 dairies in the United States, 6.8% were *M. bovis* positive on a bulk tank milk culture.[13] In other studies, mycoplasma was identified in bulk tank milk samples in 7–20% of dairies sampled.[14,15] Because mycoplasmas are shed intermittently and mastitic milk is withheld from the bulk tank, these values likely underestimate true prevalence. Individual cow prevalence and the incidence of clinical mycoplasma mastitis vary widely between herds and studies.[4,14,16,17] For dairy calves, there are limited data on the prevalence of *M. bovis*. In a study published over 30 years ago, the nasal prevalence of *M. bovis* in Californian dairy calves up to 8 months of age was 34% in herds with *M. bovis*-associated disease and 6% in nondiseased herds.[18] More recent longitudinal studies indicate that almost all calves in diseased herds become infected with *M. bovis*.[19]

In beef cattle, the prevalence of *M. bovis* in nonstressed calves is generally low (0–7%) in lungs,[12] nasal swabs,[20] or by serology.[21] Conversely, prevalence is often high even in the absence of clinical disease for comingled calves, transported calves, or calves at a feedlot.[22] Based on necropsy studies, it appears that *M. bovis* can contribute substantially to morbidity and mortality in feedlot cattle.[8,23,24] In 1 recent study, 28% of cattle necropsied in 3 Canadian feedlots had CPPS, a disease attributed largely to *M. bovis*.[24]

What Are the Economic and Other Consequences of M. bovis *Infections?*

Although *M. bovis* is an important contributor to mastitis and BRD, both major cattle diseases, there are few available estimates of the costs of *M. bovis* infection. One report estimated costs to the US beef industry of US$32 million per year as a result of loss of weight gain and carcass value, and US$108 million per year to the US dairy industry as a result of *M. bovis* mastitis.[25] However, given the limitations of prevalence data, these numbers should be interpreted with caution.

Costs of mycoplasma disease include reduced production, drugs and labor for treatment, death and culling losses, implementation of diagnostic and control measures, and a portion of the cost of non-pathogen-specific preventive measures. Because *M. bovis*-associated disease tends to be chronic, costs per case are typically high relative to other pathogens.[16,23] In addition to economic costs, there are important animal welfare consequences of *M. bovis* infections, given that the associated disease is often chronic and poorly responsive to treatment.

What Do We Know about the Epidemiology of *M. bovis* Infections?

Colonization, Persistence, and Shedding

M. bovis is well adapted to colonization of mucosal surfaces, where it can persist without causing clinical disease. The URT mucosa is the primary site of *M. bovis* colonization in cattle after URT exposure.[2] After intramammary exposure the mammary gland appears to be the major site of colonization.[1] Regardless of the route of exposure, *M. bovis* can be isolated from multiple body sites during early infection, particularly the URT, mammary gland, conjunctiva and urogenital tract,[17,26] and bacteremia during *M. bovis* infection has been documented.[1,2,27] The URT mucosa and the mammary gland appear to be the most important sites of persistence and shedding of *M. bovis*.[1,17] Although many cattle shed *M. bovis* for a few months or less,[17,18] some cattle can shed *M. bovis* intermittently for many months or years.[17,18,28] The factors responsible for intermittent shedding have not been determined. Cattle with clinical disease usually excrete especially large numbers of *M. bovis*.[18] Stressful events such as transportation, comingling, entry into a feedlot, and cold stress are associated with increased rates of nasal shedding of *M. bovis*.[22,29] Chronic asymptomatic infection with intermittent shedding of *M. bovis* appear critical to the epidemiology of infection, especially the maintenance of *M. bovis* within a herd and exposure of naive populations.

How is M. bovis *Transmitted and What Is Known about Risk Factors for Infection and Disease?*

The introduction of asymptomatically infected animals is thought to be the primary means by which *M. bovis*-free herds become infected.[4] Transmission is delayed until, and if, shedding occurs; this delay can make it difficult to identify the source of infection and mycoplasma disease outbreaks occur in seemingly closed herds.[30]

Once present in a herd, *M. bovis* can be readily transmitted from infected to uninfected cattle. In dairy cattle, *M. bovis* has traditionally been regarded as a contagious mastitis pathogen, with udder-to-udder spread being the major means of transmission.[4,31,32] Whether URT transmission with internal dissemination to the mammary gland is important in the epidemiology of *M. bovis* mastitis has not been determined, but *M. bovis* can be isolated from nasal secretions of cows with mastitis.[17] For young calves, ingestion of infected milk is an important means of *M. bovis* transmission. Calves fed infected milk have much higher rates of nasal colonization than those fed uninfected milk,[18] and feeding of contaminated milk or nursing of cows with *M. bovis* mastitis has been associated with disease in calves.[7,18] However, other means of transmission must also be important, as the disease can occur in calves that are fed milk replacer or pasteurized milk. Once established in a multiage facility, *M. bovis* is very difficult to eradicate, suggesting ongoing transmission from older to incoming calves; calves could also become infected from adults in the calving area. Congenital *M. bovis* infections appear to occur infrequently.[26]

Transmission of *M. bovis* in respiratory secretions is considered important in the epidemiology of infection, although there is little experimental data to support this contention. *M. bovis* might be transmitted in respiratory secretions via aerosols, nose-to-nose contact, or indi-

rectly via feed, water, housing, or other fomites. The importance of aerosols in calf-to-calf transmission of *M. bovis* is unknown, but *M. bovis* has been isolated from air in barns containing diseased calves,[32] and calves can be experimentally infected by inhalation of *M. bovis*.[2] It therefore seems prudent to assume that infection can occur via this route. Fomite-mediated transmission of *M. bovis* in respiratory secretions is likely given that fomites can be important in the transmission of mycoplasma mastitis.[4,31,32] Mycoplasmas are susceptible to desiccation and sunlight, but *M. bovis* can survive for long periods in protected environments with greatest survival in cool, humid conditions.[33] *M. bovis* has been shown to persist for months in recycled sand bedding,[34] and has been found in cooling ponds and dirt lots on dairies.[35] Further studies are needed to determine the role of environmental reservoirs in *M. bovis* epidemiology.

What Is the Role of Coinfection with Other Pathogens?

In some studies,[36] but not others,[23] an association between bovine viral diarrhea virus infection and *M. bovis* has been observed. Bacterial coinfections in *M. bovis*-associated pneumonia[8,23,37] and otitis media[9] are extremely common; for example, *M. bovis* was isolated from the lungs of 82% of feedlot calves with fibrinosuppurative pneumonia from which *Mannheimia haemolytica* was isolated.[8] In other surveys of feedlot pneumonia *M. bovis* was commonly identified in combination with *Histophilus somni*,[23] and coinfection with *Pasteurella multocida* is common in younger calves.[37]

What Microbial Characteristics Are Important in *M. bovis* Pathogenesis?

M. bovis has characteristics that enable it to colonize and persist on mucosal surfaces, to invade tissues, and to persist at sites of disease despite an aggressive immune response. Molecules involved in adherence, antigenic variation, invasion, immunomodulation, biofilm formation, and production of toxic metabolites are likely to be important in pathogenesis, but exactly how *M. bovis* interacts with the host is poorly understood.

Mycoplasmas lack a cell wall, and exposed membrane proteins form the primary interface with the host. These membrane proteins facilitate adherence to mucosal surfaces, although *M. bovis* adhesions are not yet well characterized. *M. bovis* has a large family of immunodominant variable surface lipoproteins (Vsps), which undergo high frequency phase and size variation in vitro and in vivo,[38–40] and exhibit extensive strain variation in their coding sequences.[38] Particular Vsp variants can be selected by exposure to antibodies.[41] These characteristics impart a vast capacity for antigenic variation in *M. bovis* populations that likely contributes to immune evasion and persistence and provides a challenge for vaccine development.

M. bovis has several other properties that enhance pathogenesis. After adherence, many mycoplasmas, including *M. bovis*,[42] generate products such as phospholipases, hydrogen peroxide, and superoxide radicals which damage host cells. *M. bovis* can also form biofilms in vitro that impart increased resistance to desiccation and heat stress.[43]

What Role Do Immune Responses Play in the Progression of *M. bovis* Infections?

Passive Immunity

There is a strong association between failure of transfer of passive immunity and increased risk and severity of BRD in calves. However, the role of maternal immunity in protection against *M. bovis*-associated disease is unknown. In 1 study, there was no association between postcolostral serum antibody titers against *M. bovis* and pneumonia in 325 dairy calves.[44]

Host Immune Responses

Innate immune responses are critical in the early phase of mycoplasma infections. Alveolar macrophages in particular are important in the early clearance of mycoplasmas from the lung. However, inappropriate activation of alveolar macrophages by mycoplasmas can promote an excessive inflammatory response. Detrimental inflammatory responses in *M. bovis* infections have been partly attributed to excessive TNF-α production by alveolar macrophages.[45] Activation of macrophages results in the recruitment of neutrophils to sites of inflammation, and neutrophils are a prominent cell type in the lungs, middle ear, and joints of *M. bovis* infected calves.[8,9] Excessive neutrophil recruitment with the subsequent release of large amounts of inflammatory mediators can occur, and the extent of neutrophil recruitment is directly correlated with the severity of mycoplasma disease. Although bovine neutrophils are able to kill opsonized *M. bovis*, unopsonized *M. bovis* can adhere to neutrophils and inhibit respiratory burst activity.[46]

Despite a substantial body of work examining adaptive immune responses to mycoplasma infections, the optimal responses for protection and the types of responses contributing to disease remain poorly defined. Adaptive responses that are in place at the time of exposure can help control new infections. For example, prior *M. bovis* mastitis seems to protect cows from developing the severe mastitis that is typically observed on primary infection; most reinfections result in subclinical or mild disease.[47] However, adaptive responses are often ineffective at eliminating established mycoplasma infections, and ongoing, ineffective responses result in chronic inflammation. Exactly how mycoplasmas manage to avoid clearance by the host is not well understood. However, mycoplasmas can induce a broad range of immunomodulatory events that might induce ineffective immune responses, and variation of surface antigens could help mycoplasmas to avoid clearance mediated by adaptive responses.

Experimental respiratory infection of calves with *M. bovis* usually elicits a strong humoral response characterized by high levels of serum IgG_1 and very little IgG_2,[48] and local mucosal IgG and IgA responses.[49] Similarly, *M. bovis* inoculation of the mammary gland results in serum IgG and local mucosal IgG and IgA

responses.[47,50] Humoral responses of naturally infected cattle are more variable.[51] Together with innate responses, humoral immune responses appear to be important in protection from *M. bovis*. Systemic antibody is particularly important in preventing disseminated infections, and serum IgG *M. bovis* titers are correlated with protection from arthritis.[2] On mucosal surfaces, however, local antibody is likely to be more important. For example, anti-*M. bovis* antibody concentrations in milk, but not in serum, are correlated with resistance to reinfection in cows following *M. bovis* mastitis.[47] IgG concentrations in bronchoalveolar lavage (BAL) fluid have been correlated with resistance to *M. bovis*-associated respiratory disease.[49]

It is widely accepted that mycoplasma respiratory infections have substantial immunopathological components, characterized by large accumulations of lymphocytes in affected tissues, the production of proinflammatory cytokines, and lung inflammation. Mycoplasmas, including *M. bovis*, can also modulate some inflammatory responses.[47] However, little is known about the cytokine environment in the lungs of calves with *M. bovis* infections. In 1 study,[48] peripheral blood mononuclear cells from *M. bovis*-infected calves secreted IFN-γ and IL4 in response to *M. bovis* antigen, and there was a strong systemic IgG_1 response with little IgG_2 produced. These findings indicate that *M. bovis* induces a mixed Th1-Th2 cytokine response, although the lack of IgG_2 production was more consistent with a Th2-biased response.

What Clinical Signs Are Associated with Mycoplasma Infections?

Mastitis

The herd presentation of mycoplasma mastitis varies from endemic subclinical disease to severe clinical mastitis outbreaks.[31] Many infections are subclinical, and a subset of subclinically infected cows do not have a marked increase in somatic cell count or reduced milk production. Cows of any age or stage of lactation are affected, including prepubertal heifers[27] and dry cows.[52] When the disease is clinical, signs are nonspecific; classically more than one quarter is affected, there is a drastic decrease in milk production and signs of systemic illness are relatively mild. The mammary gland might be swollen but is not usually painful; secretions vary from mildly abnormal to gritty or purulent, and are sometimes brownish in color.[31] A history of mastitis that is resistant to treatment with antimicrobials is common, and clinical disease can persist for several weeks. Return to production is possible but slow.[31] Arthritis, synovitis, joint effusion or combinations, or respiratory disease in mastitic or nonmastitic cows can accompany *M. bovis* mastitis.[26,30,31]

Pneumonia

M. bovis-associated pneumonia occurs in any age cattle, including dairy and beef calves, beef cattle after arrival at a feedlot, and adults. Clinical signs are nonspecific and include fever, tachypnea, dyspnea, and decreased appetite, with or without nasal discharge and coughing.[26,37,53] Poor weight gain is observed in chronically affected animals.[37] Mycoplasma pneumonia can be accompanied by cases of otitis media, arthritis, or both, in the same animal or in other animals in the herd. CPPS, where animals develop polyarthritis in association with chronic pneumonia, occurs in beef cattle several weeks after feedlot entry.

Otitis Media

M. bovis-associated otitis media occurs in dairy or beef calves as enzootic disease or as outbreaks, and also occurs sporadically in feedlot cattle. In early or mild cases calves remain alert with a good appetite, but as disease progress they become febrile and anorectic. Clinical signs are because of ear pain and cranial nerve VII deficits, especially ear droop and ptosis.[9,10] Ear pain is evidenced by head shaking and scratching or rubbing ears. Epiphora and exposure keratitis can develop secondary to eyelid paresis. Clinical signs can be unilateral or bilateral, and purulent aural discharge can be present if the tympanic membrane has ruptured. Concurrent cases of pneumonia, arthritis, or both are common. Otitis interna and vestibulocochlear nerve deficits can occur as sequelae; head tilt is the most common clinical sign, and severely affected animals can exhibit nystagmus, circling, falling, or drifting toward the side of the lesion and vestibular ataxia.[9,10] In advanced otitis media-interna, meningitis can develop.[9,10] Spontaneous regurgitation, loss of pharyngeal tone, and dysphagia have also been reported, indicative of glossopharyngeal nerve dysfunction with or without vagal nerve dysfunction.[54]

Arthritis, Synovitis, and Periarticular Infections

Cattle of any age can be affected by *M. bovis* arthritis. Cases tend to be sporadic and are often concurrent with cases of pneumonia or mastitis, although outbreaks of *M. bovis* arthritis as the predominant clinical manifestation have been reported in calves[6,7] and dairy cows.[30] CPPS is described in feedlot cattle.[8] Clinical signs are typical of septic arthritis, including acute nonweight bearing lameness with joint swelling, pain, and heat on palpation. The animal might be febrile and anorectic. Involvement of tendon sheaths and periarticular soft tissues is common.[5,30] Large rotator joints (hip, stifle, hock, shoulder, elbow, and carpal) are commonly affected, although other joints such as the fetlock or even the atlantooccipital joint can be involved. Poor response to treatment is a common feature.[5,30]

Other Diseases

Keratoconjunctivitis. *M. bovis* can be isolated from the conjunctiva of healthy and diseased cattle.[27,55] Its involvement in infectious keratoconjunctivitis (IKC) is seldom reported, and it is mainly considered a predisposing or coinfecting agent. However, it was the only pathogen isolated in 1 outbreak of IKC in calves, where IKC was followed by cases of pneumonia and arthritis.[55]

Meningitis. Meningitis can occur as a complication of mycoplasma otitis media-interna. *M. bovis* has also been isolated from the cerebral ventricles of young calves with clinical signs of meningitis in conjunction with severe arthritis, suggesting disseminated septic disease.[6]

Decubital Abscesses. In 1 report, 50 calves developed *M. bovis*-infected decubital abscesses over the brisket and joints; some calves had concurrent *M. bovis*-associated pneumonia.[56]

Cardiac Disease. *M. bovis* was identified concurrently with *H. somni* in the hearts of 4 of 92 feeder calves dying from myocarditis.[57] In another report, a heifer with clinical signs of cardiac insufficiency was found to have mural and valvular endocarditis with *M. bovis* isolated from the chronic active fibrinopurulent endocarditis.[a]

Genital Disorders. In isolated and predominantly experimental cases, *M. bovis* has been associated with genital infections and abortion in cows[26] and seminal vesiculitis in bulls.[58] However, there is little evidence to support an important role for *M. bovis* in naturally occurring bovine reproductive disease.

How Are *M. bovis* Infections Diagnosed?

Rapid and accurate diagnosis of *M. bovis* infections is compromised by the low sensitivity and, in some cases, specificity of the available tests, and subclinical infections and intermittent shedding complicate diagnosis.

Detection of Antibodies against M. bovis

M. bovis-specific serum antibodies can be detected by indirect ELISA,[59] usually by 6–10 days after experimental infection. However, in natural infections, individual animal titers are poorly correlated with infection or disease; not all diseased animals develop high titers, titers can remain increased for months,[21] and maternal antibody results in high titers in calves.[51] On a group level, however, seroconversion or high titers are predictive of active *M. bovis* infection.[11] Serology is therefore best applied in surveillance or as part of a biosecurity program.[21,60] Antibody titers in milk have been used to identify *M. bovis*-infected mammary glands.[61]

Detection of M. bovis *in Clinical Material*

Mycoplasma culture requires complex media, specialized equipment, and technical skill. Growth is often apparent by 48 hours, but 7–10 days incubation is recommended before samples are called negative. The sensitivity of culture for the detection of *M. bovis* in clinical material is quite low. Intermittent and low-level shedding, uneven distribution of *M. bovis* throughout diseased tissue, suboptimal sample handling or culture conditions, and the presence of mycoplasma inhibitors in samples likely contribute to low sensitivity. Sensitivity of milk culture for diagnosis of mycoplasma intramammary infection has been reported as approximately 50% for bulk tank samples and $<30\%$ in individual cows without clinical mastitis,[28,31,62] although it is higher in cows with clinical mastitis. The sensitivity of *M. bovis* culture for other clinical material has not been reported. Sensitivity can be enhanced by repeated sampling, optimal sample handling, and the use of various laboratory techniques.[28,63] Mycoplasmas isolated in culture should be speciated by antibody-based tests (immunofluorescence or immunoperoxidase tests) or, preferably, polymerase chain reaction (PCR).

M. bovis can be detected directly in clinical specimens by PCR.[64,65] PCR can be especially useful for stored samples; PCR had a similar sensitivity to culture for detection of *M. bovis* in fresh milk but was much more sensitive than culture in milk frozen for 2 years.[66] Real-time PCR systems with high sensitivity and specificity have been described for the detection of *M. bovis* in clinical samples.[67,68] Other techniques, including denaturing gradient gel electrophoresis PCR and melting-curve analysis of PCR products, appear promising for the simultaneous detection and differentiation of multiple mycoplasma species.[68,69] A monoclonal antibody-based sandwich ELISA (sELISA) kit for the detection of *M. bovis* in clinical material is available in Europe;[b] sensitivity and assay time are better than conventional culture when samples are preincubated in broth.[70] *M. bovis* can be detected in situ by IHC on formalin fixed, paraffin-embedded tissues.[8] An indirect fluorescent antibody test for detection of *M. bovis* in fresh, frozen lung tissue has also been described.[71]

For the diagnosis of *M. bovis* pneumonia in the live animal, transtracheal wash or BAL is preferable to URT samples,[72] although isolation of *M. bovis* is not well correlated with respiratory disease in the individual animal. Aspirates of affected joints or tendon sheaths can be submitted for *M. bovis* detection. In live calves with otitis media, the sensitivity or specificity of URT *M. bovis* culture has not been reported, and samples are not typically collected from the middle ear of live calves. Imaging (radiography, computed tomography) has been used as an aid in the diagnosis of otitis media/interna in calves.[10,54]

How Should Samples Be Collected and Handled?

Optimal sample handling is vital to ensure mycoplasma survival. Because mycoplasmas are cell-surface associated, it is important to swab vigorously when sampling. Wooden-shaft cotton swabs should be avoided as they can inhibit mycoplasma growth. Swabs should be placed immediately into aerobic bacterial (Ames without charcoal, Stuart's, or Eaton's) or mycoplasma transport media. Tissue samples should be formalin fixed for histopathology and IHC or placed in plastic bags on ice for culture. When tissue cannot be processed rapidly after necropsy, postmortem BAL samples or swabs of lesions might be preferable; mycoplasmas remain viable in BAL fluid for a few days at 4°C, whereas isolation from lung tissue decreases markedly over a few hours because of the release of mycoplasma inhibitors from disrupted tissue.[72] Samples should be refrigerated, or frozen if time to processing will exceed 2 days. Significant reductions in mycoplasma recovery rates occur with increased time to processing, regardless of whether samples are refrigerated or frozen, and best recovery rates are achieved

when samples are processed fresh within a few hours of collection.[73]

What Are the Typical Necropsy Findings in M. bovis*-Associated Disease?*

With the exception of mastitis, *M. bovis*-associated disease is best diagnosed by necropsy; a definitive diagnosis is based on demonstration of *M. bovis* in affected tissues by IHC or by culture, PCR, or sELISA. Although some *M. bovis* lesions are characteristic, many are grossly indistinguishable from other pathogens. Additionally, *M. bovis* pneumonia can resemble contagious bovine pleuropneumonia, a foreign animal disease. Therefore, tissues should be submitted to a diagnostic laboratory for verification of field necropsy findings.

Pneumonia. The presence of *M. bovis* in pneumonic lungs must be interpreted together with histopathology and other findings, given that *M. bovis* can be isolated from lungs of cattle without lesions. Macroscopically, affected lung often contains multiple necrotic foci filled with dry yellow to white caseous material.[53] These raised nodular lesions can be a few millimeters to several centimeters in diameter. Interlobular septae can contain linear necrotic lesions. Extensive fibrosis is common, and necrotic sequestra can be present. Acute fibrinous to chronic fibrosing pleuritis occurs in some cases. Histologically, naturally occurring *M. bovis* pneumonia is characterized as subacute to chronic bronchopneumonia that can be suppurative and is usually necrotizing.[8,53] IHC staining reveals large amounts of *M. bovis* antigen, especially at the periphery of lesions.[8] Mixed infections often complicate the characterization of lesions, and IHC can be useful in determining *M. bovis* involvement in these cases.

Other Infections. A diagnosis of mycoplasma mastitis is usually made clinically rather than at necropsy, but lesions are characterized as mild to severe fibrinosuppurative to caseonecrotic mastitis.[1] In mycoplasma otitis media, the affected tympanic bullae contain suppurative to caseous exudate and have often undergone extensive osteolysis.[9,54] During field necropsy the ventral aspect of the bulla can be opened and swabbed or aspirated for culture. Joints with *M. bovis* arthritis contain nonodorous fibrinous to caseous exudate accompanied by fibrosis.[5,8] Periarticular involvement is common and can involve tendons, synovial sheaths, muscle, and connective tissue.[5,8] Affected periarticular tissues contain foci of caseous necrosis, linear necrotic lesions, and extensive fibrosis. IHC reveals *M. bovis* antigen at the edges of necrotic lesions and within exudates.[5,8]

What Treatment Is Appropriate for *M. bovis*-Associated Disease?

Should Mycoplasma Mastitis be Treated?

As early as the 1970s researchers reported that mycoplasma mastitis responded poorly to intramammary or systemic antimicrobial treatment, and this remains the case today. Treatment of cows with mycoplasma mastitis is not recommended. Cows that spontaneously resolve clinical mastitis or become culture negative often remain intermittent, subclinical shedders, and should be regarded as permanently infected.

What Do We Know about Antimicrobial Resistance of M. bovis*?*

Because mycoplasmas lack a cell wall, the β-lactam antimicrobials are not effective against these pathogens. Similarly, mycoplasmas do not synthesize folic acid and are therefore intrinsically resistant to sulfonamides. Mycoplasmas as a class are generally susceptible to drugs that interfere with protein (tetracyclines, macrolides, linosamides, and florfenicol) or DNA (fluoroquinolones) synthesis. However, *M. bovis* is resistant to erythromycin.[74,75]

Is Antimicrobial Susceptibility Testing Useful to Guide Treatment of* M. bovis *Infections? Antimicrobial susceptibility testing of large *M. bovis* populations can be useful to make generalizations about resistance. However, the value of antimicrobial susceptibility testing in making evidence-based herd-, or individual-level treatment decisions for *M. bovis*-associated disease has not been determined. Susceptibility testing for mycoplasmas in animals is not currently standardized and should be interpreted with caution.

Which Isolates Should Be Collected for Antimicrobial Susceptibility Testing? Isolates obtained from the site of infection from representative early, untreated cases should be used. Samples collected at necropsy are ideal. If live cattle with respiratory disease are sampled, BAL samples should be used; antimicrobial susceptibility data of paired *M. bovis* isolates obtained from nasal swabs and BALs were found to differ considerably.[72]

What Methods Are Appropriate for Antimicrobial Susceptibility Testing of* M. bovis*? Microbroth dilution, agar dilution, and the *E*-test[c] can be used to determine minimum inhibitory concentrations (MICs) for *M. bovis*. There are currently no MIC testing control standards for veterinary mycoplasmas, although the Clinical Laboratory Standards Institute is in the process of developing these. Breakpoints have not yet been determined and so MIC results cannot be defined as susceptible, intermediate, or resistant.

What Data Are There on the Antimicrobial Susceptibility of* M. bovis *Isolates? Selected data on the antimicrobial susceptibility profiles of *M. bovis* isolates are presented in Table 1; most isolates originated from the respiratory tract of diseased cattle.[74–77] The MICs of tilmicosin and spectinomycin tend to have a bimodal distribution, and many isolates have high MICs for tetracyclines, findings that are suggestive of acquired resistance. Resistance to the fluoroquinolones and florfenicol appears uncommon, although enrofloxacin resistance has been identified in a subpopulation of Israeli *M. bovis* isolates.[76] MICs of tulathromycin have been reported for 63 European *M. bovis* isolates with the MIC_{50} being 4 μg/mL, MIC_{90} > 64 μg/mL, and MIC range 0.125 to > 64 μg/mL.[78] However, tulathromycin was efficacious in the treatment of calves infected with a strain of *M. bovis* that had an MIC of >64 μL/mL, so the clinical relevance of tulathromycin MIC values is unknown.[79]

Table 1. Selected minimum inhibitory concentration (MIC) values for *Mycoplasma bovis*.[a]

	Rosenbusch[b]	Gerchman[c]	Ayling[d]	Francoz[e]
Enrofloxacin				
MIC_{50}	0.25	0.16	—	0.19
MIC_{90}	0.5	0.63	—	0.25
MIC range	0.03–4	0.08–2.5	—	0.047–0.5
Florfenicol				
MIC_{50}	1	—	4	—
MIC_{90}	4	—	16	—
MIC range	0.06–8	—	1–64	—
Oxytetracycline/tetracycline[f]				
MIC_{50}	2	4	32	4
MIC_{90}	16	8	64	8
MIC range	0.125 to > 32	0.5–16	1–128	0.094 to > 256
Spectinomycin				
MIC_{50}	2	2	4	2
MIC_{90}	4	> 1,024	> 128	> 1,021
MIC range	1 to > 16	0.5 to > 1,024	1 to > 128	0.38 to > 1,021
Tilmicosin				
MIC_{50}	64	128	> 128	—
MIC_{90}	> 128	> 128	> 128	—
MIC range	0.5 to > 128	0.5 to > 128	4 to > 128	—

[a]All values are reported as μg/mL.
[b]Rosenbusch et al.[75] 223 US isolates, microbroth dilution method.
[c]Gerchman et al.[76] 17 Israeli isolates, microbroth dilution method except for spectinomycin, where the *E*-test was used.
[d]Ayling et al.[77] 62 UK isolates, microbroth dilution method.
[e]Francoz et al.[74] 55 Canadian isolates, *E*-test.
[f]Data from the Francoz study are for tetracycline, other data are for oxytetracycline.

What Other Information Can Be Used in Selecting Treatment for Cattle with M. bovis*-Associated Disease?*

There is little information on how pharmacokinetic and pharmacodynamic data, where available, should be applied in the treatment of *M. bovis* infections.

Two antimicrobials are currently approved in the United States for treatment of BRD associated with *M. bovis*; these are tulathromycin[d] and florfenicol.[e] Another macrolide, gamithromycin,[f] is approved for treatment of *M. bovis*-associated BRD in Canada. Oxytetracycline, tilmicosin, and tylosin have a theoretical basis for efficacy against *M. bovis* and are approved in the United States for treatment of BRD. Spectinomycin is no longer available for treatment of BRD in the United States. Enrofloxacin is only approved for treatment of BRD associated with *M. haemolytica*, *P. multocida*, and *H. somni*, and extralabel use is prohibited in the United States. However, in countries where fluoroquinolones and spectinomycin do carry appropriate labels, these drugs could be considered for treatment of *M. bovis* infections.

Some controlled trials have evaluated the efficacy of antimicrobials for the treatment of experimentally induced *M. bovis*-associated disease. In an industry-sponsored study, calves that developed respiratory disease after experimental *M. bovis* infection were treated with tulathromycin.[79] Treated calves had lower temperatures, lower rate of removal from the trial for welfare reasons, and lower lung lesion scores than control calves. In another study, tilmicosin given at the onset of clinical disease was associated with reduced numbers of *M. bovis* in the lungs of calves experimentally infected with *M. haemolytica* plus *M. bovis*.[80] Calves treated for 10 days with oral valnemulin or oral enrofloxacin beginning 10 days after experimental infection with *M. bovis* had improved clinical scores and fewer *M. bovis* recovered from their lungs compared with untreated calves.[81]

There is little information on the treatment of naturally occurring *M. bovis*-associated disease in cattle, despite a huge volume of literature on the treatment of undifferentiated BRD. Oxytetracycline and tilmicosin resulted in clinical improvement in calves with pneumonia that included a mycoplasma component.[82] In an industry-sponsored study, tulathromycin and florfenicol were effective treatments for BRD that included an *M. bovis* component.[83] For the treatment of *M. bovis*-associated diseases other than BRD, there are few data available. Cattle with *M. bovis*-associated arthritis have an especially poor response to treatment.[5,6] Aggressive early treatment before the development of extensive tissue necrosis seems most likely to be successful. Fluoroquinolones, tetracyclines, and macrolides tend to have good distribution into joints. Myringotomy with irrigation of the middle ear has been recommended for the treatment of otitis media in calves. However, to our knowledge the clinical efficacy or risks of this procedure have not been critically evaluated. There is 1 report of successful surgical treatment of a calf with *M. bovis*-associated otitis media-interna in which a bilateral tympanic bulla osteotomy was performed.[54]

Because improved efficacy is observed when treatment is initiated early in the course of experimental disease, early recognition and treatment of cases are likely to be

very important in successful therapy. To our knowledge there are no published studies that have critically evaluated the duration of therapy for *M. bovis*-associated disease. However, given that *M. bovis* disease often becomes chronic, continuing antimicrobial treatment until clinical resolution could be important and would involve extending treatment beyond most label recommendations. Research is needed to evaluate the effect of treatment duration on cost and outcome.

When Might Metaphylactic Antimicrobials be Indicated for *M. bovis*-Associated Disease?

In experimental *M. bovis* infections, response to treatment when antimicrobials are given early in the course of disease is often better than response rates reported for natural disease.[79,80] Metaphylaxis might therefore be more successful than treatment after clinical *M. bovis*-associated disease develops. There is little doubt that strategic treatment of cattle at high risk of developing undifferentiated BRD is beneficial in reducing the incidence and severity of disease, and some data support treatment of calves at high risk of *M. bovis*-associated disease. For example, in a blinded, randomized study, veal calves in a facility in which *M. bovis* was the predominant respiratory pathogen were treated with florfenicol or oral tilmicosin during a BRD outbreak.[84] Metaphylactic florfenicol resulted in higher weight gain, better clinical status, and reduced rates of BRD compared with tilmicosin or untreated controls. Tulathromycin is the only drug currently approved for metaphylactic use in the control of BRD associated with *M. bovis* in the United States. In an industry-sponsored, blinded, randomized field trial in high-risk cattle, significantly fewer cattle developed BRD after tulathromycin metaphylaxis than after no treatment or treatment with tilmicosin; *M. bovis* was isolated from affected cattle along with other BRD pathogens.[83] Given the limited data available, metaphylactic use of antimicrobials is probably justified when high levels of morbidity and mortality because of *M. bovis*-associated disease are being sustained or can be expected in high-risk cattle, although *M. bovis*-specific efficacy data and economic analyses are needed.

Can Vaccination Help Control *M. bovis*-Associated Disease?

In general, attempts to vaccinate cattle against *M. bovis*-associated disease have been unrewarding. However, several *M. bovis* bacterins are licensed for marketing in the United States for the control of *M. bovis*-associated respiratory disease or, in one case, mastitis. In addition, a number of US companies produce autogenous *M. bovis* bacterins. However, there is virtually no data demonstrating field efficacy of the available *M. bovis* vaccines. To our knowledge, no *M. bovis* vaccines are commercially available in Europe.

As discussed earlier, adaptive immune responses that are in place at the time of mycoplasma exposure can help control infection, so it is not surprising that vaccination can, in some instances, protect cattle from experimentally induced *M. bovis*-associated disease.[2,49] In a number of instances *M. bovis* vaccines have appeared promising in challenge studies but have been ineffective or resulted in increased severity of disease when applied in field trials. For example, an *M. bovis* bacterin prevented respiratory disease in calves that were challenged 3 weeks after vaccination.[2] However, when the same vaccine was used in a field trial, an increased rate and severity of respiratory disease was observed in the vaccinated group.[85] In another blinded, controlled field trial, a commercial *M. bovis* bacterin was no different to a placebo in preventing *M. bovis*-associated disease in high-risk dairy calves.[19] In both these studies a substantial proportion of calves were identified as infected with *M. bovis* before vaccination. Increased disease severity has also been observed in vaccinated, experimentally infected calves. For example, vaccination with *M. bovis* membrane proteins was associated with enhanced severity of respiratory disease following aerosol challenge, compared with control calves.[86] Increased severity of clinical mastitis was reported in cows vaccinated with an *M. bovis* bacterin compared with controls after intramammary inoculation of *M. bovis*.[87]

It is therefore apparent that vaccination against *M. bovis*-associated disease is sometimes possible in a controlled setting, but the vaccines critically evaluated to date are not protective in the field. The early age at which calves often become infected also presents a challenge to the development of a successful *M. bovis* vaccine. Ongoing research should lead to improved understanding of *M. bovis* antigens and might result in the development of more targeted vaccine approaches.

Wh Management Tools Can Be Used in the Control and Prevention of *M. bovis*-Associated Disease?

Biosecurity for M. bovis

The best way to prevent *M. bovis* infections is probably to maintain a closed herd or, if that is not possible, to screen and quarantine purchased animals.[4] Mycoplasma biosecurity practices targeted to the individual operation should be developed. For dairy herds, it is recommended that the bulk tank culture history of the herd of origin be examined when purchasing heifers or adults. If this history is unavailable, the bulk tank can be sampled at least 3 times spaced 3–4 days apart.[62] Where possible, calf health records should be examined to determine if *M. bovis*-associated diseases such as otitis media have been observed. When purchasing lactating cows, milk samples should be submitted for mycoplasma detection (culture, PCR, or sELISA), keeping in mind the low sensitivity of a single sample for detection of subclinical *M. bovis* mastitis.[62] Testing for *M. bovis* antibodies in milk might be useful to identify infected cows.[61] Testing purchased dry cows, purchased heifers, and heifers raised offsite at calving and isolating them until results are obtained has been recommended.[62] Serology has been used to help identify uninfected groups of cattle before purchase.[60]

Managing Mycoplasma Mastitis

Monitoring programs to detect *M. bovis* should be in place in herds that are attempting to remain mycoplasma-free, as well as in herds managing a mycoplasma problem. Herd level detection of *M. bovis* mastitis is usually achieved by testing of bulk tank milk by culture, PCR, or sELISA.[31,52,62] Bulk tank testing should be performed at least monthly, with more frequent sampling indicated for large herds, herds undergoing expansion, or when managing a mycoplasma problem. Sampling of clinical mastitis cases, high somatic cell count cows, and cows and heifers (especially new purchases) at calving is also important. Whole herd sampling is sometimes used when attempting to eliminate *M. bovis*, but low-test sensitivity means that repeated sampling is required. Mastitis records, including response to treatment, should be monitored.

The approach to management of *M. bovis* mastitis needs to be tailored to each operation and can range from culling of all *M. bovis*-infected cows to only culling cows with chronic clinical mastitis. *M. bovis* mastitis can be eliminated from dairy herds through aggressive surveillance and culling of infected cows,[31,88] and where a closed herd can be maintained and a small proportion of cows are infected this could be feasible. Conversely, for expansion herds or dairies where a large proportion of the lactating herd is infected, eradication of *M. bovis* might not be appropriate or economical. However, it should be emphasized that the worst outbreaks of clinical mycoplasma disease observed by some of the authors have occurred after mycoplasma mastitis was detected and not eliminated. Attempted elimination of all adult cows with intramammary mycoplasma infections remains the strongly recommended course of action. Economic analyses of the various approaches for managing mycoplasma mastitis in today's large dairy herds are critically needed to help guide veterinarians in recommending the most appropriate strategy.

In herds where the ultimate goal is eradication of *M. bovis* but not all infected cows can be immediately culled, strict segregation of infected cows has been used effectively to limit new infections.[31] As transmission might occur via routes other than the udder, cows should be segregated at all times, not just in the milking parlor. Strict milking parlor hygiene is recommended to reduce udder-to-udder transmission of *M. bovis*.[31,52] Cows with clinical mycoplasma mastitis should be culled. As discussed earlier, *M. bovis* can survive well in some bedding substrates. Ideally, bedding found to be mycoplasma positive, usually recycled bedding processed on the farm, should not be used to bed dairy animals of any age.

Managing M. bovis *Disease in Calves*

Surveillance for *M. bovis* in calf facilities should include monitoring of health records and the submission of appropriate samples from suspect cases for diagnostic testing. For the control of *M. bovis* infections in calves, general infectious disease control principles based on reducing exposure and maximizing host defenses can be used. *M. bovis* exposure via infected milk can be eliminated by pasteurization or by feeding milk replacer. Batch pasteurization of milk[7] at 65°C for 10 minute or 70°C for 3 minute or high-temperature (72°C) short-time (flash) pasteurization[89] will inactivate *M. bovis*. Other potential routes of exposure of calves to *M. bovis* include colostrum and respiratory secretions of infected animals. Exposure to infected colostrum could be reduced by pasteurization, by not pooling colostrum, and by not feeding colostrum from cows known to be infected with *M. bovis*. Exposure to airborne *M. bovis* could be reduced by good ventilation and low-stocking density. Calves with clinical mycoplasma disease shed very large numbers of organisms,[18,26] and moving sick calves to a separate hospital pen might reduce transmission in calf facilities. All-in, all-out practices or segregation of age groups might also limit transmission of *M. bovis* in multiage facilities. Removing fence-line contact with other cattle and limiting the time the calf spends in the maternity area will also reduce the potential for exposure. Proper sanitization of buckets, housing, and other equipment between uses, wearing gloves, and handling sick calves last could reduce fomite-mediated transmission. Although *M. bovis* survives surprisingly well in the environment, it is highly susceptible to heat and to most commonly used chlorine-, chlorhexidine-, acid-, or iodine-based disinfectants. Addressing nonspecific factors related to respiratory health such as air quality, colostrum management, and nutrition could also help limit the impact of *M. bovis*-related disease. Appropriate vaccination and control programs should be in place for respiratory viruses, as controlling other pathogens could decrease the risk of *M. bovis* coinfections.

Managing M. bovis *Disease in Stocker and Feeder Cattle*

Recommendations for the control and prevention of *M. bovis*-associated disease in stocker and feeder cattle focus on maximizing respiratory system health and immune function rather than *M. bovis*-specific measures. Strategic antibiotic treatment of high-risk animals on arrival or during an outbreak of BRD might be useful in reducing the incidence of mycoplasma disease. Segregating affected cattle and keeping the hospital pen separate from new arrivals could reduce exposure of high-risk animals to *M. bovis*. Using appropriate hygiene measures for handling sick cattle (use separate equipment or personnel or clean equipment among animals, feed last, etc) could reduce the chances of fomite-mediated *M. bovis* transmission.

Priority Areas for *M. bovis* Research

High-priority areas of basic *M. bovis* research that need to be addressed include identification of virulence factors, the nature of protective and harmful immune responses in *M. bovis* infections, the importance of coinfection with other pathogens in the progression of mycoplasma disease, and the potential of new vaccine technologies to protect from *M. bovis*-associated disease.

In applied research, a critical need is the development of cost-effective, sensitive, and specific diagnostic tests to

allow accurate identification of *M. bovis*-infected animals. Prevalence data are needed so that the true impacts of *M. bovis* infections can be determined and so that diagnostic-testing recommendations can be made. Epidemiological research is required to clarify risk factors for infection and disease, particularly those factors associated with severe outbreaks of clinical disease. Long-term studies would be helpful to determine the effect of calfhood *M. bovis* infection on disease and productivity later in life. Perhaps the most critical needs are evidence-based strategies to limit the clinical, welfare, and economic impacts of *M. bovis* infection. Identifying these strategies will require well-designed field studies to critically evaluate vaccines, antimicrobials for treatment, or metaphylaxis or both, and management strategies for the control of *M. bovis* infections.

Footnotes

[a] Helie P, Labrecque O, Babkine M, Francoz D. *Mycoplasma bovis* mural and valvular endocarditis in a heifer. In: Proceedings of the 58th Annual Meeting of the American College of Veterinary Pathologists, Savannah, GA, November 10–14, 2007 (abstract 45)

[b] Bio-X Diagnostics, Jemelle, Belgium

[c] AB BIODISK, Solna, Sweden

[d] Draxxin, Pfizer Animal Health, New York, NY

[e] Nuflor Gold, Intervet/Schering-Plough Animal Health, Summit, NJ

[f] Zactran Injectable Solution, Merial Canada, Baie d'Urfe, Quebec, Canada

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